Ekin Journal
July 2026, Volume 12, Issue 2
CROP BREEDING AND GENETICS

V. INTERNATIONAL PLANT BREEDING CONGRESS - 2025

www.ekinjournal.com
info@ekinjournal.com

bisab
Plant Breeders Union of Turkey
www.bisab.org.tr

Ekin Journal

International Journal of Crop Breeding and Genetics

Volume 12 Issue 2
12(2): 67-160, 2026
International biannual peer-reviewed journal

List of Referees/Reviewers

Abdullah Kahraman	Field Crops, Harran University, Türkiye
Ahmet Balkaya	Horticulture, Ondokuz Mayıs University, Türkiye
Ashok Kumar Yadav	ICAR-Indian Institute of Pulses Research, India
Atman Poonia	Plant Breeding, CCS HA University, India
Aylin Kabaş	Horticulture, Akdeniz University, Türkiye
Bayram Murat Asma	Horticulture, Turgut Özal University, Türkiye
Burak Akyüz	Horticulture, Ondokuz Mayıs University, Türkiye
Bülent Uzun	Field Crops, Akdeniz University, Türkiye
Cevdet Gümüş	Horticulture, Bartın University, Türkiye
Dilek Kandemir	Horticulture, Ondokuz Mayıs University, Türkiye
Erol Yalçınkaya	Alata Hortıcultural Research Institute (Retired), Türkiye
Ertan Sait Kurtar	Horticulture, Selçuk University, Türkiye
Ertan Yıldırım	Horticulture, Atatürk University, Türkiye
Evren Atmaca	Pulses Breeding, Eskişehir Transitional Zone A.R.I., Türkiye
Hatice Filiz Boyacı	Horticulture, Recep Tayyip Erdoğan University, Türkiye
İrfan Öztürk	Cereal Department, Trakya A.R.I., Türkiye
İsmail Demirtaş	Eğirdir Fruit Research Institute, Türkiye
Kenan Sönmez	Horticulture, Eskişehir Osmangazi University, Türkiye
Mehmet Emin Çalışkan	Field Crops, Niğde Ömer Halisdemir University, Türkiye
Mukesh Kumar	Horticulture Fruit Breeder), CCSHAU-RRS, India
Mukesh Saini	Plant Biotechnology, CCS HA University, India
Naresh K. Mehta	Plant Protection, CCS HA University, India
Oğuz Bilgin	Field Crops, Tekirdağ Namık Kemal University, Türkiye
Ömer SÖZEN	Field Crops, Sivas Science and Technology University, Türkiye
Pınar Çubukçu	Field Crops, Eastern Mediterranean A.R.I., Türkiye
Pim Lindhout	Potato Breeding, Solynta: Wageningen, NL
Praveen Sharma	Vegetable, MMDU, Mullana, Ambala, India
Rana Baytin Alacı	Horticulture, Van Yüzüncü Yıl University, Türkiye
Seda Sözer	Breeding and Genetics, Oil Seed Research Institute, Türkiye
Şebnem Kuşvuran	Horticulture, Çankırı Karatekin University, Türkiye
Virender Sardanavir	Oil Seed, Retired Professor at Punjab Agril University, India
Yasemin Evrenosoğlu	Horticulture, Eskişehir Osmangazi University, Türkiye
Zsolt Polgar	Potato Breeding Center, Hungarian University of Agricultural and Life Sciences, Hungary

Ekin Journal

International Journal of Crop Breeding and Genetics

Ekin Journal of Crop Breeding and Genetics is abstracted and indexed in

Centre for Agriculture and Bioscience International (CABI)

National Academy of Agricultural Sciences (NAAS) - ASOS Index

Eurasian Scientific Journal Index (ESJ) - Scientific Indexing Services (SIS)

Volume 12 Issue 2

12(2):67-160, 2026

International biannual peer-reviewed journal

ISSN 2149-1275 • e-ISSN 2459-069X

Ekin Journal

Owner
M. Sinan Berksan
On behalf of Plant Breeders Union of Türkiye (BISAB)

Editor-in-Chief
S. Ahmet Bağcı

Editors
Rishi K. Behl
Kadir Akan
Suren Pahuja

Associate Editor
Rajesh K. Arya

Managing Editor
Muzaffer Işık

Statistics Editor
Sinan Aydoğan

Language Editor
Prashant Luthra

ISSN Number
ISSN 2149-1275 • e-ISSN 2459-069X

Graphic Design
atölye KURUMSAL YAYINCILIK
Levent Karakas +90 533 656 1366

Published By
bisab Plant Breeders Union of Turkey
www.bisab.org.tr

Address Information
Plant Breeders Union of Türkiye
Adakale Street, No.: 22/12 Kızılay, 06420 Cankaya/Ankara - Türkiye
Phone: +90 312 433 3065-66 Fax: +90 312 433 3006
E-mail: bisab@bisab.org.tr • info@ekinjournal.com

Ekin Journal CONTENTS
Volume 12, Issue2, July 2026

Review Article:

Research Articles

www.ekinjournal.com
info@ekinjournal.com

EKIN

international biannual peer-reviewed crop breeding and genetics journal

Review Article

Ekin
Journal of Crop Breeding and Genetics

12(2):67-73, 2026

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Fruit Breeding in the Context of Climate Change Adaptation(§)

Pedro MARTÍNEZ-GÓMEZ

Department of Plant Breeding CEBAS-CSIC PO Box, 164, E-30100 Murcia, Spain

* Corresponding author e-mail: pmartinez@cebas.csic.es

Citation:
Martínez-Gómez P., 2026. Fruit Breeding in the Context of Climate Change Adaptation. Ekin J. 12(2):67-73.

Received: 23.06.2026 ***Accepted: 21.07.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

Spain is the world's leading exporter of fresh stone fruits belonging to the genus Prunus. This position has been favored by Spain's climate, which enables earlier production than in the rest of Europe, as well as and commercial use of high-quality, certified, virus-resistant new cultivars adapted to climate change the almond, apricot and plum by the development national in the different national breeding programs and particularly conducted at CEBAS-CSIC in Murcia in the case of almond, apricot and plum. In the context of the new climate, this communication aims to address the latest approaches being carried out by the CEBAS-CSIC of Murcia in the development of new Prunus varieties in the postgenomic scenario (once the complete sequence of the genomes of the main species is known). Furthermore, massive genome (Genotype by Sequencing, GBS) and transcriptome (RNA-Seq) sequencing is helping to better understand the biological and molecular processes associated with the expression of these traits of agronomic interest. Once these genes are identified, we can use this information to select seedlings that have these characteristics using classical Polymerase Chain Reaction (PCR) or quantitative Retrotranscribed PCR (qRT-PCR) with a potential application in marker-assisted selection. More recently, a new line of research has been initiated aimed at identifying epigenetic marks associated with characters, especially adaptation to the environment, such as flowering time. Epigenetics studies change in phenotype that are inherited, but are not due to alterations in the DNA sequence. This DNA methylation mechanism has been described as a regulator of dormancy breaking in fruit trees or fruit ripening.

Keywords: Stone fruits, *Prunus*, Breeding, Genetics, Genomics, Transcriptomics, Epigenetics

Introduction

Prunus production around the world was around 50 million tons being the peach the most important species, with an annual production of more than 27 Mt in 2023 (http://faostat.fao.org, updated June 2 2026). In Spain, *Prunus* production was of 2 Mt ranking this country in the second position behind China, and closely followed by Türkiye and Italy (Figure 1). In addition, Spain is the first exporter of peaches in the world, with more than 50% of the exported production (0.8 Mt) and an added value of this exportation of more than 900 million euros (M€) (www.fepex.es, Asociaciones de Productores Exportadores de Frutas, Hortalizas, Flores y Plantas vivas, FEPEX, updated June 2 2026). This leadership in the production and export of fruits has been marked by the climate compared to the rest of Europe, which allows early production, in addition to the development and exploitation of new quality, certified, virus-resistant and adapted varieties to the climate change obtained in the different national breeding programs.

Stone fruit (*Prunus*) breeding is an applied science of design where constantly new designs are performed to solve specific problems, such as productivity, resistance to biotic and abiotic stresses, fruit quality, postharvest performance and sensorial attributes (Martínez-Gómez et al., 2003). At this moment, the treating of breeding as science of design supposes to consider this activity closer to software development or the pharmaceutical industry than biology. The requirement of these new designs demands each time more complicated mechanisms to choose parental for crossing and select the new descendants (Martínez-Gómez, 2017). In addition, genetic transformation to develop Genetically Modified Organisms (GMOs),

(§) *This study was presented as an oral presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

including CRISPR (clustered regularly interspaced short palindromic repeats) application, is at this moment a far option from a consumer point of view (Martínez-Gómez, 2021; Martín-Valsaneda et al., 2023).

In this review, we have analyzed the new perspectives on fruit breeding in the context of climate change adaptation to ensure production and quality approaches being carried out by the CEBAS-CSIC of Murcia in Spain.

Development of new *Prunus* varieties

Plant breeding is thus a scientific discipline that contributes to the knowledge necessary to use a certain technology, which in turn applies that knowledge to produce new plant varieties and plant patents. Classical fruit breeding is a long and tedious process involving the generation of large populations through controlled crosses of previously selected progenitors and the final selection of top individuals, the future new varieties. This process can take between 5 years in the case of horticultural crops to 15 years in the case of perennial fruit crops (Figure 2) such as *Prunus* or 25 years in forest species (Martínez-Gómez et al., 2003).

Plant breeding differs from basic sciences in that it seeks not only to understand biological systems but also to modify them to achieve defined objectives. It therefore combines elements of natural science and design science, drawing on observation and experience to develop cultivars that as practical solve problems. When successful, this process transforms scientific knowledge into technological innovation through the breeding of new plant cultivars (Martínez-Gómez, 2020).

In addition, to develop suitable new cultivars, we need to know the relevant variables that influence our knowledge of the possible future in the success of new cultivars. It is thus necessary to examine the internal and external variables influencing the phenomenon in question. Both types of variables must be subjected to scientific evaluation. The first internal variables to consider in any almond breeding program are derived from plant traits that are considered as objectives. The knowledge of these internal variables of genetic type will indicate their suitability with respect to the proposed design objectives. Secondly, internal variables also include the current methodologies available for use in the selection of individuals. These methodologies are closely related to the level of knowledge available at the molecular levels especially in relation to the development and application of new markers for the selection of individuals and will give us an idea of the efficacy and feasibility of new designs or cultivars. Finally, we can include various economic factors within the production framework, i.e., the goals, processes and outcomes. The effectiveness and viability of the improvement program will depend on these methodological developments dedicated to the evaluation. These two terms (effectiveness and viability) are related in the economic context to the relationship between science and economics. To develop successful cultivars, breeders must identify the internal and external variables that influence the likelihood of achieving the breeding objectives. Internal variables include the genetic traits targeted by the programme and the methods available for selecting parents and progeny (Martínez-Gómez, 2017; 2020).

On the other hand, in plant breeding, it is necessary to consider a number of external variables including biotic and abiotic stress and interactions with the environment over a period of several years. There are many characteristics such as flowering time and floral compatibility which must be evaluated for at least three years, when trees produce the first flowers. Due to drawbacks, the use of molecular marker-assisted selection methods is of particular interest in breeding programs. These drawbacks make the use of methods of assisted selection by molecular markers already discussed. These environmental conditions will determine the feasibility of the objectives and the new variety in a given environment. The adaptation of new designs or new cultivars to specific climatic conditions is fundamentally conditioned by the winter chilling requirements and dormancy and by their adaptation to different soil conditions in the case of rootstocks. Its capacity for production depends largely on this character and on its interaction with the environment considered as an external variable. In addition, the knowledge of these external variables related to possible biotic stresses (pest and disease) will indicate their feasibility with respect to the proposed design objectives (Martínez-Gómez, 2017). The feasibility of developments is another important issue to consider. The technological feasibility will also depend on the existence of germplasm with the desired genes for resistance to different biotic or abiotic stresses (Figure 3).

Finally satisfying consumer demands is also an important external variable. New designs must have the appropriate characteristics to meet such demands. In addition, the availability of different techniques for analysis and selection is another important external variable. In addition, the social acceptance of these new cultivars will depend on criteria of environmental sustainability (Infante et al., 2008). On the other hand, we can include various factors of an economic nature, provided that they are considered within the framework of production: the phase of objectives, processes and results. This affects external economic variables, which are those that affect their market value. Because the

result of a new genetic variety presents durability (a life cycle) and involves a market price, which conditions the creative transformation carried out by technological innovation (García-Gómez et al., 2021).

The degree of knowledge of the variables determines the quality of the prediction in the design and development of new cultivars of *Prunus* in addition to its effectiveness, feasibility and suitability. New designs must meet the appropriate characteristics to meet those requirements. External variables are also the availability of the different analysis and selection contemporary techniques, since they have varied throughout history.

Application of molecular markers in the development and management of new *Prunus* varieties

The management, phenotyping and selection process of progenitors and seedlings are the main factors limiting the generation of new cultivars. In order to improve efficiency and viability of breeding programs in relation to parent and seedling selection, the implementation of molecular tools is an essential requirement, including development of new Marker Assisted Selection (MAS) strategies based on the molecular knowledge of the determination of the phenotypic traits. Molecular effort should be oriented in the analysis of the molecular basis of the target traits and the implementation of molecular tools to be incorporated together with the classical activity of the visual selection of good descendants (Martínez-Gómez et al., 2003). In this context, at this moment in the present Postgenomic era we are facing a new molecular-biological perspective based on new methodologies that are affecting the molecular analysis. This new molecular perspective opens new possibilities to improve the use of molecular tools in new more efficient and viable plant breeding (Martínez-Gómez, 2019).

At this moment, more than 650 plant species have been sequenced and their reference genomes are available including the most important crops in the world (https://www.ncbi.nlm.nih.gov/genome, accessed 1st July 2025). This whole sequenced work started in 2002 with the development of the reference genome of the rice. Last years for instance, several important crops were sequenced including hazelnut, mulberry, pistachio, poplar or almond in 2019. The development of complete genomes is making any organism accessible and amenable for many kinds of studies which will allow a precise reference of the molecular results obtained, and the development of high-throughput methods for genomic analysis involving the most abundant genetic variation (Single Nucleotide Polymorphisms, SNPs) and transcriptomic analysis at Differential Gene Expression (DEG) level in a new postgenomic perspective. At this moment, the new Big Data Biology harboring the development of DNA (and RNA as cDNA) high-throughput sequencing technologies together with bioinformatics analysis as well as the creation of databases with billions of data has made possible to access genetics knowledge at the level of each nucleotide. This new methodological perspective where millions of sequences are available in one single experiment with the detailed information of complete genomes (defined as the DNA organized into separate chromosomes inside the nucleus of a cell) and transcriptomes (described as the complete list of all types of RNA molecules). Several authors have even characterized this data-intensive biology as a new kind of science, a science of information management, different from traditional biology. Big data science is now being introduced in the development of molecular markers to assist breeding selection methodologies (Martínez-Gómez, 2019).

On the other hand, these molecular tools are also particularly useful when the evaluation of the complex traits such as biotic and abiotic stress and fruit quality including nutraceutical values. These complex traits are being recently introduced in the stone fruit breeding programs. Nutraceutical properties will add an important value to the new releases for industrial purposes. In these species nutraceutical value studies are focused on fiber contents; phenolic contents; carotenoid (correlated with vitamin A) and vitamin C and E contents; and mineral contents including magnesium, potassium, selenium and zinc (García-Gómez et al., 2021).

This new postgenomic perspective integrating available reference genomes and new sequencing and bioinformatic methodologies will allow the implementation of new Marker Assisted Selection (MAS) to accelerate breeding process. The application of these molecular tools will increase the viability and efficiency in the development of the new planned design. In this context, high-throughput sequencing technologies resulted in a great advance in the development and application of MAS strategies. Additionally, from a global point of view, the new post-genomics era is characterized by a change in perspective on trait expression derived from the Encyclopedia of DNA Elements (ENCODE) project, performed on humans, in which the study of RNA rather than DNA was determined as the linchpin of trait expression processes (Martínez-Gómez 2019). In fact, the genomes of eukaryotic organisms are almost entirely transcribed, giving birth to an enormous number of non-protein-

coding RNAs. In this new context, a recent concept, pervasive (interleaved) transcription, is being applied to the whole transcription process. These authors described pervasive transcription as the transcription of the interspersed genes that are embedded within the normal coding genes. The entire stretch of the genome (in other times called "junk DNA") is transcribed, whether it is coding for a particular protein or not. Therefore, not all coding sequences lie juxtaposed, and they may also overlap one another (Martínez-Gómez et al., 2012).

New integrated classical and molecular perspective in *Prunus* breeding

Plant productivity for feeding an increasing human population and the study of plant pest and diseases and their management in unpredictable context of global climatic changes related to different abiotic stress are particularly relevant aspects as well as genetic plant breeding from the point of view of the quality of fruits and vegetables. Therefore, classical breeding must be combined with MAS using the diverse molecular tools available in the postgenomic era in each time more complicated scenario.

The generation of large genetic and phenotypic information obtained in a massive way (through high-throughput sequencing and high throughput phenotyping approaches) and the creation of databases with billions of these data, result in what is called in research "Big Data", which also require the development of new mathematical algorithms (such as machine learning models) and a large set of bioinformatic tools. In addition, from a global perspective, the center of gravity of the molecular processes is focused on the expression of genes and the way in which such expression is regulated. These features have turned researcher's attention towards the study of gene regulatory networks, and in particular to the role of RNA and other non-derived DNA biomolecules (Martínez-Gómez, 2022). These new approaches will be of great help in the new challenges of the plant breeding.

This situation does not mean that MAS will replace conventional breeding; other ways is a necessary complement. The practical learning that conventional breeding gives is unparalleled with the molecular support. In this context, there has been a significant shift of conventional breeders to molecular breeding aspects. However, in this enjoyed strategy (conventional and molecular) the target must be well defined in a complete integrated work plan. While the ability of breeders to generate large new breeding populations is almost unlimited, the evaluation and selection of these promising seedlings is the main limiting factors due to the cost and time-consuming. In this context, genomic studies at DNA level are especially useful for the development of MAS strategies. In addition, proteomic (proteins and enzymes), transcriptomic (RNA) and epigenetic (DNA Methylation and histone modifications) studies are being applied to breeding programs. Finally, these strategies at genomic, epigenetic, transcriptomic and proteomic level should be all integrated for a better understanding of the molecular mechanisms involved in the most important plant breeding aspects, which will facilitate the development and optimization of molecular markers to apply in the field exploitation of the new varieties, offering and integrating complete technological offers increasing feasibility, effectiveness and success of the breeding programs (Figure 4).

In this new context DNA and RNA markers and epigenetic marks could be applied in the development (breeding) and exploitation (cultivation) of the newly developed *Prunus* varieties in Marker Assisted Selection (MAS), biological validation of DNA markers and monitoring flowering and ripening process (Figure 4).

Acknowledgements

This article represents an expanded and fully developed version of an initial abstract that was previously presented during the proceedings of the *5th International Plant Breeding Congress*, held in Antalya, Türkiye, from December 01 to 05, 2025.

This study was financially supported by the project "Stone fruit breeding for emerging challenges using new phenomic, genomic and modelling approaches" (23051/GERM/25) of the Seneca Foundation of the Region of Murcia (Spain).

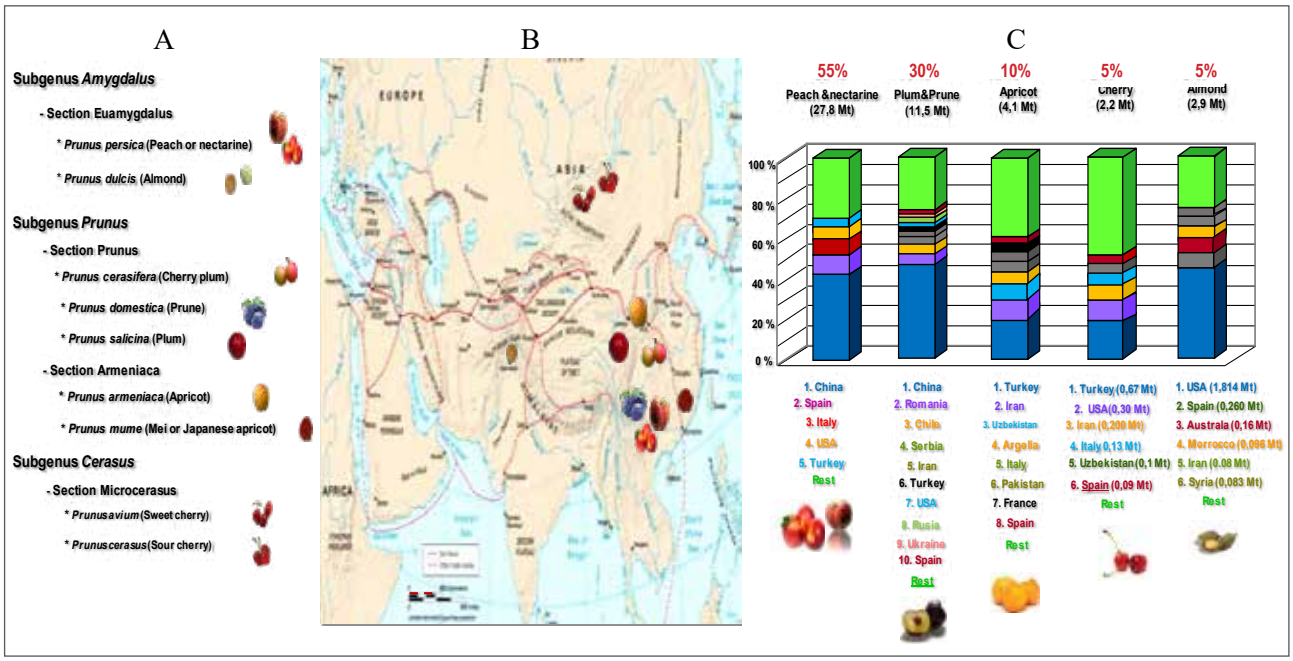


Figure 1. Botany of main cultivated *Prunus* species (A). Origin and dispersion of *Prunus* species through the silk road (B). Main *Prunus* production countries in the world (C).

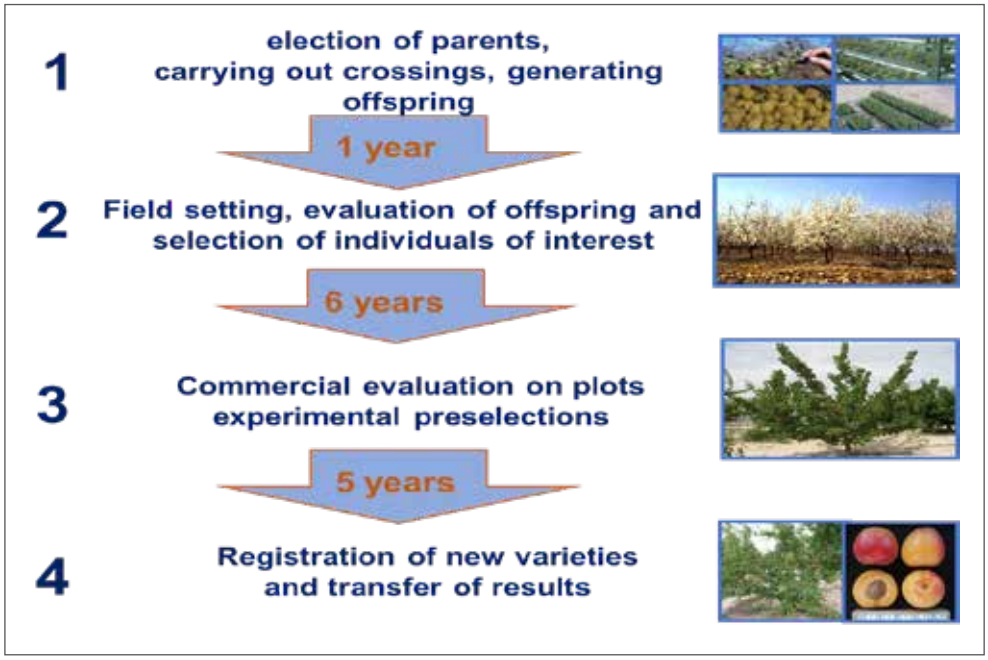


Figure 2. Representation of the most important steps in a *Prunus* breeding program.

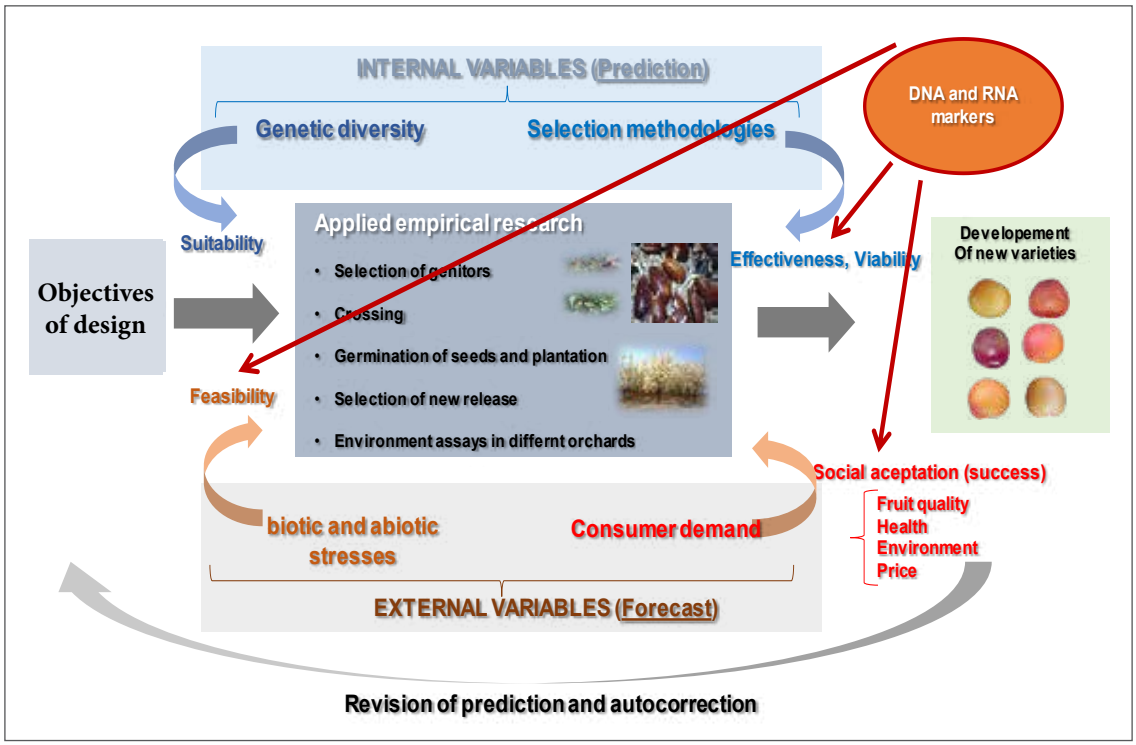


Figure 3. Schematic representation of *Prunus* breeding process as a science of design.

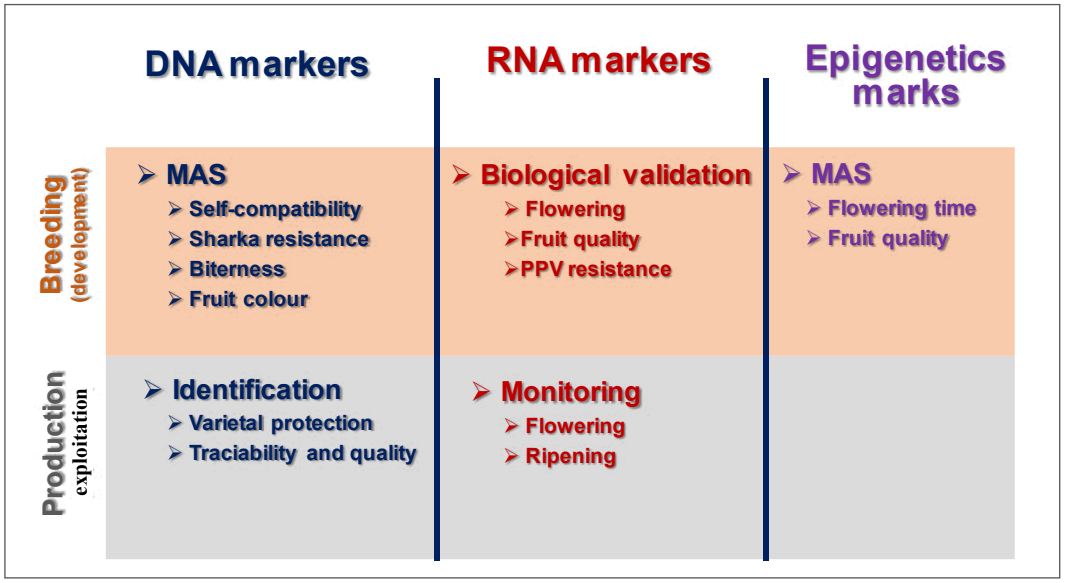


Figure 4. Application of molecular markers in the development and exploitation of new *Prunus* varieties.

References

García-Gómez, B., Salazar, J. A., Nicolás-Almansa, M., Razi, M., Rubio, M., Ruiz, D., and Martínez-Gómez, P. (2021). Molecular bases of fruit quality in *Prunus* species: An integrated genomic, transcriptomic, and metabolic review with a breeding perspective. *International Journal of Molecular Sciences*, *22*(1), Article 333. https://doi.org/10.3390/ijms22010333

Infante, R., Martínez-Gómez, P., and Predieri, S. (2008). Quality oriented fruit breeding: Peach [*Prunus persica* (L.) Batsch]. *Journal of Food, Agriculture* and *Environment*, *6*(2), 342-356.

Martínez-Gómez, P., Sozzi, G. O., Sánchez-Pérez, R., Rubio, M., and Gradziel, T. M. (2003). New approaches to *Prunus* tree crop breeding. *Journal of Food, Agriculture* and *Environment*, *1*(1), 52-63.

Martínez-Gómez, P., Sánchez-Pérez, R., and Rubio, M. (2012). Clarifying omics concepts, challenges and opportunities for *Prunus* breeding in the post-genomic era. *OMICS: A Journal of Integrative Biology*, *16*(5), 268-283. https://doi.org/10.1089/omi.2012.0002

Martínez-Gómez, P. (2017). Predicción científica y prescripción en mejora genética vegetal en cuanto a ciencia aplicada de diseño: El caso de la mejora de frutales del género *Prunus* [Scientific prediction and prescription in plant breeding as an applied science of design: The case of fruit tree breeding of the genus *Prunus*]. *Acta Agronómica*, *66*(1), 115-127. https://doi.org/10.15446/acag.v66n1.50801 (In Spanish).

Martínez-Gómez, P. (2019). Editorial for Special Issue "Plant Genetics and Molecular Breeding". *International Journal of Molecular Sciences*, *20*(11), Article 2659. https://doi.org/10.3390/ijms20112659

Martínez-Gómez, P. (2020). Scientific prediction and prescription in plant genetic improvement as an applied science of design. In W. J. González (Ed.), *Methodological prospects to scientific research: From pragmatism to pluralism* (pp. 167-182). Springer. https://doi.org/10.1007/978-3-030-51740-3_9

Martínez-Gómez, P. (2021). Desarrollo de nuevos diseños vegetales: Mejora genética vs transformación genética y edición génica [Development of new plant designs: Genetic breeding vs genetic transformation and gene editing]. *Magna Scientia Uceva*, *1*(1), 89-103. (In Spanish).

Martínez-Gómez, P. (2022). La nueva perspectiva molecular del gen en la era posgenómica [The new molecular perspective of the gene in the post-genomic era]. *Magna Scientia Uceva*, *2*(1), 65-74. (In Spanish).

Martín-Valmaseda, M., Rahimi-Devin, S., Ortuño-Hernández, G., Pérez-Caselles, C., Ehsan-Mahdavi, S. M., Bujdoso, G., Salazar, J. A., Martínez-Gómez, P., and Alburquerque, N. (2023). CRISPR/Cas as genome editing technique in fruit trees breeding. *International Journal of Molecular Sciences*, *24*(22), Article 16656. https://doi.org/10.3390/ijms242216656

Review Article

Ekin
Journal of Crop Breeding and Genetics

12(2):74-85, 2026

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Hybrid Potato Breeding, Seed Technology and Agronomy(§)

Paul C. Struik

Centre for Crop Systems Analysis, Plant Sciences Group, Wageningen University & Research, Bornsesteeg 48, 6708 PE Wageningen, The Netherlands

* Corresponding author e-mail: paul.struik@wur.nl

Citation:
Struik PC., 2026. Hybrid Potato Breeding, Seed Technology and Agronomy. Ekin J. 12(2):74-85.

Received: 23.06.2026 ***Accepted: 28.07.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

This article summarizes the advantages of hybrid diploid breeding of potato, with true potato seed as major propagule. Hybrid diploid breeding accelerates breeding of potato, allowing pyramiding resistance genes and introducing novel genes for maximum quality. The challenge is to compensate for the lack of positive effects of polyploidy by rapid progress in breeding and heterosis. Moreover, the use of true potato seed from such a breeding program makes it possible to produce large numbers of healthy seeds in a very short time that are very small, easy to store and easy to transport to remote areas. If successfully implemented, the use of such a new technology will revolutionize the breeding, seed systems, and seed and ware production systems of potato. Making maximum use of this technology requires identifying the best seed system model for different agro-ecological and socio-economic conditions and optimizing the production systems of seed tubers and ware potatoes based on the seed value chain selected and implemented.

Keywords: Diploid potato, hybrid breeding, seedling tuber, seed systems, transplant, true potato seed

Introduction

Potato (*Solanum tuberosum* L.) is commonly grown as an early crop, an intermediate short-cycle crop between two main crops, or as a main crop in many different cropping systems. It is also often grown as a temporary component crop in intercropping systems with perennial crops that have a long establishment phase, such as sugar cane or in intercropping systems in which the companion crop can provide some shelter (e.g., maize-potato intercropping in warm regions). In many parts of the world, it is a hunger-breaking crop because there are cultivars available with a very short crop cycle. Potato is very efficient in terms of resource use (land, water) and has a very high harvest index. Despite the international trade of ware and seed tubers, the potato crop is still very much a 'local for local' crop, because of the bulkiness of the seed tubers and the ware potatoes and because of the limited storability (Haverkort and Struik, 2015). As a crop for food security and income, the crop is developing rapidly, especially in Africa and Asia.

Despite its flexibility, potato also has some disadvantages. The most important ones are already mentioned: both the seed tubers and the ware potatoes are bulky and perishable. Moreover, the rate of multiplication is very low, making it necessary to use a large proportion of the area cropped with potato for seed production or save a large part of the harvest for seed. The crop is also affected by a plethora of pests and diseases, which are either air-borne (e.g., late blight), water-borne (e.g., bacterial wilt), vector-borne (e.g., viruses), or soil-borne (e.g., cyst nematodes), while many of these pests and diseases are (also) seed-tuber-borne, i.e., can be transmitted from one generation to the next when the crop is multiplied vegetatively, a phenomenon often called "seed degeneration". This seed degeneration puts a lot of challenges on the production of high-quality seed and the institutionalization of a functional seed

(§) *This study was presented as an oral presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

system. Several pests and diseases have quarantine status with zero tolerance, while others may result in declassification of the seed when tolerance levels are surpassed. The potato crop is also sensitive to abiotic stresses, including drought, excessive rainfall, heat, cold, salinity, contaminants, such as heavy metals and microplastics, and nutrient deficiencies. Finally, there are also physiological quality aspects for both seed tubers and ware potatoes that are very much influenced by environmental factors. The seed tubers may remain dormant for too long or may sprout too early, depending on conditions during production and storage conditions, while they might be ageing, losing vigour, when the temperature sum during storage gets too high; these aspects are very important in potato production regions with more than one growing season per calendar year (Struik and Wiersema, 1999). The potato crop may also show a wide diversity of physiological disorders of ware potatoes, such as secondary growth, knobby tubers, growth cracks, translucent ends, and hollow hearts.

This paper first deals with recent developments in global potato cultivation, seed systems, and quality of seed tubers. It then describes in detail the various aspects of breeding and seed production of potato, using hybrid diploid breeding and true potato seed production as a set of alternative technologies to create robust varieties with strong resistances against abiotic and biotic stresses and the best possible quality for a wide diversity of consumers as well as healthy types of propagules that can be used in adapted seed and ware production systems. I also highlight the potential use of true potato seed in Sub-Saharan Africa.

The paper is based on the hope that the new developments of hybrid diploid breeding and use of hybrid TPS will benefit global potato cultivation, especially in countries in which traditional seed production and ware production are challenging.

Global potato cultivation

The potato crop is widely cultivated because of its wide adaptability, high resource use efficiency, and high nutritional quality, amongst other factors. It is grown in more than 100 countries, processed into many different products (e.g., in Dutch supermarkets well over 150 products based on potato are on display; Haverkort et al., 2023), regularly consumed by more than 1 billion people, while there are strong international markets for seed potatoes, ware potatoes, and processed potatoes. Mordor Intelligence (2026) estimated that the value of the international potato market equalled USD 210 billion in 2025. The same source estimated that this value would grow from USD 124.46 billion in 2026 to USD 149.38 billion in 2031. This continued growth is remarkable given the increasing frequencies of weather shocks (heat, drought), increasing regulatory pressure (e.g., restrictions on the use of biocides), and strong price volatility. The causes of continued growth include rising demand for frozen products (such as frozen French fries) and the strong expansion of quick-service restaurants. In addition, farmers across the globe manage to adopt climate-smart cultural practices to cope with adverse weather conditions.

One of the most remarkable changes in potato cultivation over the last 75 years is the shift in production from the so-called developed countries to the so-called developing countries (Haverkort and Struik, 2015). In the last 20 years, more potatoes are being produced in developing countries than in developed countries. This trend is caused by an increase in acreage, with China and India currently having the largest areas cropped with potato, and an increase in productivity (i.e., yield per hectare). In Africa, there is also a substantial increase in production, but that is mainly caused by an increase in acreage, whereas in some countries, the potato yield per hectare is declining (e.g., in Kenya; Atieno et al., 2023; 2026). Major reasons for the yield decline are expansion to marginal areas because of land shortage, increase in cultivation by resource-poor farmers, and increased seed degeneration, because of limited seed renewal by smallholder farmers.

Informal versus formal seed systems

Although the potato plant is very versatile in its propagation and multiplication (Struik and Wiersema, 1999), the common method of propagation is through seed tubers. The rate of multiplication is very low, compared with other important food crops, such as cereals. Therefore, a large proportion of the world's potato acreage is devoted to seed production. Properly functioning seed tuber production systems are crucial to supply ware growers with high-quality seed tubers in a reliable way and against affordable prices.

A seed system can be defined as "*all activities of various stakeholders related to breeding and selection of varieties and the development, maintenance, bulking, storage, management, exchange, distribution and dissemination, and use of seed*" (Struik, 2023). The main purpose of a seed system is to provide affordable, high-quality seeds of all various varieties of all relevant crops at the right time. A seed system entails breeders, seed multipliers, and farmers, but also the informal and formal governance structures testing and controlling the quality of the seed.

In most countries, seed potato production is strictly regulated through certification schemes to prevent, or at least limit, transgenerational propagation of pests and diseases and guarantee true-to-type material of the right cultivar in this vegetatively propagated crop. Viroids, viruses, phytoplasmas, bacteria, fungi, nematodes, and insects can easily be transmitted from mother plants to progeny tubers or from the soil to the tubers. In formal seed systems, strict regulations for quarantine pests and diseases and strict tolerance levels for other pests and pathogens apply to guarantee that farmers can produce a good ware crop from healthy seed. Quality is maintained by testing fields, seed-tuber crops, and seed-tuber lots, and phasing out progeny after a number of field multiplications.

However, in many countries, for example in Asia, South America, and Sub-Saharan Africa, at least 90% of the seed tubers come from informal seed systems (Thomas-Sharma et al., 2016). Informal seed systems of potato have significant advantages. They provide local seed of preferred varieties when required and at relatively low cost. However, the phytosanitary quality is often sub-standard, especially in regions with little seed renewal and large pressure of air-borne (e.g., *Phytophthora infestans*) or water-borne (e.g., *Ralstonia solanacearum*) pathogens, vectors such as aphids, or endemic soil-borne pathogens, such as *Streptomyces scabies*.

Quality of seed tubers

Seed quality includes genetic quality (no off-types), physical quality (no damage, right size), physiological quality (desired level of vigour and number of sprouts), and phytosanitary quality (absence of seed-borne pests and pathogens). The latter quality characteristic is especially important for potato as seed health is very difficult to control.

How farmers perceive the quality of the seed in the case of potato has been investigated for many different socio-ecological environments (e.g., for Kenya, see Atieno et al., 2023; Kwambai et al., 2024; for Ecuador, see Navarrete et al., 2022a, b, 2023), and possible routes to alternative, sustainable seed systems bridging informal and formal systems have been described by Thomas-Sharma et al. (2016). Such seed systems aim to control seed degeneration. Seed degeneration is commonly defined as “the reduction in seed quality (and therefore the ultimate crop yield) due to pathogens and pests accumulating over successive cycles of propagation” (Struik, 2023). In some definitions, physiological ageing of the seed is also included in seed degeneration (Struik and Wiersema, 1999; Zou et al., 2024, 2025).

Seed degeneration of potato is common in areas where governance of seed quality is less strict. It is especially problematic in informal seed systems, although in such systems some level of tolerance to seed-borne pests and diseases in the commonly used varieties has been demonstrated. Simple techniques, performed by smallholder farmers, such as positive selection (Gildemacher et al., 2011; Schulte-Geldermann et al., 2012; Priegnitz et al., 2019, 2020), can slow down seed degeneration significantly. In extreme cases, these techniques might even reverse degeneration as some tubers of a secondarily infected plant do not contain a significant viral load (Bertschinger et al., 2017).

Physiological age of seed is especially problematic in regions with more than one growing season per calendar year. In such cases the seed can be either too young or too old for optimal performance. Lack of control of conditions during storage makes it difficult to manage physiological age and therefore seed vigour.

Breeding: from traditional breeding of tetrasomic cultivars to hybrid diploid breeding

Traditional breeding of potato is difficult. The tetrasomic inheritance and the associated complex genetics and huge linkage drag in traditional potato breeding systems make it very difficult to introduce desirable traits, such as resistance genes or quality traits. Introducing one gene through marker-assisted backcrossing takes 10 - 20 years, introducing two genes can easily take more than 25 years (Lindhout and Struik, 2023). The subsequent multiplication of seed tubers to commercialize the cultivar then takes another 5 - 10 years with traditional seed tuber multiplication.

In addition, there are many different traits to take into account (up to 50) as the crop is affected by many different abiotic and biotic stress factors, is very sensitive to genotype-by-environment interactions, and has many quality characteristics to be taken into account for the diverse outlets. Moreover, potato tubers need to be easy to store and process. Even when a new variety has been selected as promising, there is the problem of low multiplication rates: it takes many years to bulk up seed tuber lots to quantities that will allow wide testing in the field for performance under diverse practical conditions and subsequent commercialization.

Hybrid breeding is an advanced breeding system that might solve some of the problems with traditional potato breeding. Hybrid breeding involves generating inbred lines through repeated selfings over many generations followed by crossing inbred lines to generate hybrids. The inbred

lines make it possible for breeders to better control genetics and better predict the outcomes of their crossings based on marker-assisted techniques; this results in a much more efficient breeding process. Hybrid breeding is common in many important food crops, also because it serves the business model of the breeding companies well (ter Steeg et al., 2022).

Hybrid breeding in tetraploid potato is difficult because of the tetraploid nature of commercial potato cultivars and because inbreeding depression hampers the development of pure inbred lines, despite the fact that often tetraploid cultivars are (partially) self-compatible. While tetraploid potato cultivars are traditionally vegetatively propagated via seed tubers, the preferred method of propagation in hybrid potato is through true potato seed. This makes it necessary to develop specific seed systems and cultivation pathways for seed and ware. The multiplication rate is strongly increased resulting in fewer years between making a cross and producing enough seed for commercial use of a new variety.

The limitations of the complicated tetraploid breeding system mentioned above have been overcome by hybrid diploid breeding. Several universities and commercial companies in different countries, including the Netherlands, China, and the United States of America have started hybrid diploid breeding programs (Lindhout et al., 2011, 2018; Jansky et al., 2016; Zhang et al., 2021). This was only possible after the problem of self-incompatibility during self-pollination, common in diploid potato, was solved by the introduction of the *Sli* gene. This *Sli* locus allows the pollen tube to reach the ovary without the pollen tube growth being arrested in the style before reaching the ovary (de Vries et al., 2023). For an overview of the recent advances in the insights into the molecular mechanisms and genetic basis of potato self-incompatibility and key technologies to overcome this self-incompatibility, see Sood et al. (2024), Zhang et al. (2019, 2025) and Wang et al. (2026). Sood et al. (2024) also provided an overview of the achievements in diploid potato breeding, including advancements in genomic purging and phenotyping strategies.

Producing diploid inbreds of potato is associated with strong inbreeding depression, creating relatively weak inbred lines. Heterosis effects when crossing inbred lines are not as strong as hoped for. Nevertheless, the flexibility of the hybrid breeding system (ter Steeg et al., 2022) and the opportunities for marker-assisted breeding are very promising, especially in breeding for resistance and novel quality traits. Introducing one gene through marker-assisted backcrossing takes 10 - 20 years in conventional breeding but only takes 2 - 3 years with hybrid diploid breeding. Introducing 2 genes through marker-assisted backcrossing can easily take more than 25 years in traditional potato breeding but merely 3 - 4 years in hybrid diploid breeding. Producing enough true potato seed for commercialization only takes one season. Moreover, the resulting seed lots are much less likely to be contaminated with seed-borne pathogens and are easier to store and transport than conventional seed tuber lots. How the comparison between a tetrasomic breeding system with polyploidy benefits versus ease of breeding and heterosis benefits of hybrid diploid breeding finally works out is still a matter of debate, certainly if one considers the disruption of the entire seed and cultivation system associated with the use of hybrid diploid true potato seed.

A possible diploid hybrid potato breeding strategy is illustrated in Figure 1, based on Eggers (2025). Details on producing advanced inbred lines of diploid potato while coping with inbreeding depression and eliminating deleterious alleles, the use of genetic markers, genomic selection, and genomic prediction, implications of genotype × environment × management interactions, trait introgression, and the optimization of hybrid vigour are provided in the following sections.

Diploid hybrid breeding: from gene pool to advanced inbred lines

As in any breeding program, diploid potato breeding starts with the creation of a pool of crossable genetic resources that contains a wide set of useful genes, including genes that contribute to high yielding ability and quality, pest and disease resistance, resistance against abiotic stresses, and a wide diversity of other traits that are desirable in potato. With this germplasm, inbred lines are created making use of the *Sli* gene. An alternative could be to create knockout mutations in the incompatibility locus (Bradshaw, 2022). These inbred lines need to be fertile to enable their use in crossings, either as male or female plant, must have adequate vigour to produce adequate amounts of gametes, and must be homozygous (de Vries et al., 2023). Although the self-incompatibility issue has been solved, fertility might still be an issue, as it is associated with the vigour of the inbred lines. Moreover, homozygosity does not come easy due to the large heterozygosity: it requires a long, multi-annual process to achieve complete homozygosity (Bradshaw, 2022; de Vries et al., 2023), required to obtain a maximum level of uniformity in hybrids.

Strategies for reducing inbreeding depression, genomic selection, genomic design, and genomic prediction

Hybrid breeding also suffers from the presence of many deleterious alleles that are often hidden in tetraploid genotypes but become very harmful in homozygous diploids. Inbreeding depression is a major bottleneck in diploid potato breeding. Zhang et al. (2019) mapped the genome-wide incidence of constrained, deleterious mutations, obtained 344,831 predicted deleterious substitutions, and examined their phenotypic effects. They found that some deleterious recessive mutations indeed cause very large effects on vigour. They also demonstrated that lines that seemed to have high initial mutation burdens appeared to yield better elite inbred lines, a finding that is paradoxical. They also observed that most deleterious mutations were line specific. This suggests that a high degree of heterosis is possible when crossing inbred lines of different lineages. Zhang et al. (2021) used genome design to develop pure, fertile inbred lines by systematically purging the harmful mutations, selecting for genome complementarity, thus leveraging heterosis. They showed that using genetic design made purging of deleterious alleles through recombination not that difficult. They finally developed vigorous F1 hybrid seeds. In yet another study, Zhang et al. (2025) unravelled the regulatory mechanism underlying the male gamete-specific expression of the *Sli* gene, which could support diploid hybrid breeding.

It is clear from the above that in order to cope with very negative inbreeding effects, pedigree-based selection for fertility and vigour has to be conducted before selection for other performance traits, like yielding ability, can start. The common strategy in diploid breeding is therefore to start with selection processes to reduce inbreeding depression, thus creating a pool of fertile and vigorous inbred lines. This step is followed by recurrent selection to recombine favourable alleles to contribute to positive complex traits. For the identification and deletion of deleterious alleles (so-called marker-assisted purging; see Bradshaw, 2022) and finding QTLs for desired complex traits that contribute to the yielding ability, molecular marker techniques and genetic design approaches come in very useful. Marker technology can also be very useful for trait introgression, for example for pyramiding late-blight resistance genes.

Once inbred lines have been produced, creating genetic maps of useful traits and developing molecular markers can be conducted using common techniques that allow marker-assisted selection. However, as indicated by various research teams, the development of diploid breeding varieties involves recent, shallow pedigrees with only a few founders. Detection of QTLs of complex traits in diploid potato therefore requires alternative methods as, for example, proposed by Korontzis et al. (2020). These authors identified QTLs through a Genome Wide Association Study model, a tailored linkage approach, and by modelling multiallelic QTLs in complex pedigrees using identity-by-descent probabilities. They showed that all three approaches allowed them to detect QTLs, but they preferred the last approach as it gave a more direct and detailed interpretation.

Genotype × Environment × Management interactions

The genotype × environment × management interactions in potato are very strong, because growth and development of the potato crop are very sensitive to environmental conditions throughout its crop cycle with strong feedback and feedforward effects. These strong interactions reduce selection progress and make achieving consistent and rapid genetic improvement more difficult. Stockem et al. (2020) compared widely grown tetraploid cultivars with hybrid diploid genotypes under contrasting environmental conditions and crop management regimes. They found that the genotype × environment × management interactions were similar for both types of material, Moreover, they made a yield component analysis to assess what yield components contributed most to variation in yield across environments and crop management. Yield gain was strongest related to an increase in number of tubers per stem in both types of material.

Seed technology: towards novel diploid hybrid propagules

Figure 1 already showed that it is not just the creation of new cultivars but also the optimization of cultivation pathways for the production of true seed or seed tubers and the production of ware potatoes that require a lot of research and attention and new skills of practitioners.

Seed production systems are complex agronomic, social, and regulatory systems. This is certainly true for the complex seed potato systems (Struik and Wiersema, 1999). Potato is very versatile in methods of multiplication. Despite the intrinsic conservatism of the potato sector, tremendous changes have occurred over the past 50 years in terms of different multiplication technologies that have been developed, both in developing and developed countries. An overview of these developments is presented in

Figure 2. These techniques differ in the duration of each separate cycle of multiplication and in number of daughter propagules produced per mother propagule.

The last development is hybrid diploid potato breeding with potentially different end products. These might entail true potato seed (TPS), seedlings for transplanting (transplants), seedling tubers (i.e., progeny tubers of TPS-grown plants), and seed tubers (i.e., progeny tubers of crops grown from seedling tubers or their progeny).

Every seed system aims to multiply as fast as possible (either in terms of number of propagules, or in terms of weight, or a combination of the two), multiply as cheap as possible, keep the material free of diseases during multiplication, and produce material that is suitable for the next multiplication cycle or use in commercial ware production in terms of size, vigour, and physiological, genetic and phytosanitary quality. However, the traditional system of seed tuber production is notoriously slow in bulking up seed. About 10 - 15% of the total acreage of potato worldwide is used for seed (Struik and Wiersema, 1999). This is a much higher proportion than for most other crops, especially cereals, such as maize and barley.

Using hybrid true potato seed (HTPS) varieties has the following five advantages:

1- The multiplication system is very fast: one plant can easily produce 10,000 uniform seeds instead of 10 seed tubers.
2- The seeds of HTPS varieties are very light: one hectare requires 25 g of true seed instead of 2,000 kg of seed tubers.
3- The seed is free of almost all seed-borne pathogens (tuber spindle tuber viroid being one of the very few exceptions).
4- The seed can be easily stored for many years in simple, dry containers, whereas seed tubers require special facilities (diffuse light store, cold store) and can even then only be stored for 6 - 8 months at most.
5- Breeding is much faster, making it possible to incorporate resistance genes, pyramid different resistance genes in one variety and breed for yield and quality traits in years rather than in decades (see also the earlier section on breeding).

These advantages are especially important in remote areas with poor infrastructure and failing governance of seed systems, such as Eastern Africa. I therefore refer for further reading to den Braber et al. (2023) and the website of the Sepia Foundation (https://sepiapotato.nl/), but will also elaborate on using hybrid true potato seed in Sub-Saharan Africa in a separate section further below.

Physiology of the potato crop when grown from true seed or transplants

Kacheyo et al. (2021) published augmented descriptions of growth and development stages of potato (*Solanum tuberosum*) grown from different types of planting material. They showed that crops grown from true potato seed or seed tubers differ in the number of stems per plant, the presence of below-ground branches, the position of stolons on the main stem, the branching of the main stem, both at lower nodes and higher nodes, the sympodial branching intensity, and the number and uniformity of tubers. Despite these strong differences in below-ground and aboveground architectures, Gu et al. (2025) demonstrated that one fundamental aspect is very much the same for both types of crops: there is a very close relationship between rate of photosynthesis or stomatal conductance on the one hand and the leaf nitrogen content (in amount of nitrogen per unit area of leaf) on the other hand. This relationship holds across leaves from different origins (either from main stem or branch or from different orders of branches) and across leaf ages.

However, the different architectures will have consequences for tuber yield, number of tubers per stem, and tuber size distribution and therefore the cropping system for crops grown from propagules from hybrid diploid varieties needs to be newly designed.

Novel cultivation systems required

There are several different cultivation pathways possible for using true potato seed for the production of transplants, seed potatoes, and ware potatoes. The different options are illustrated in Figure 3.

van Dijk et al. (2025) showed that direct sowing is an option, but requires very special care, due to the small size of true potato seed, the low vigour of the seedlings, and the physiology of crops grown from TPS. They showed that plants can meet numerous challenges, from environmental factors (both abiotic and biotic, including weed) and associated with crop management such as hilling. Best results are obtained when the true potato seeds are pelleted, have received some additions in the pelleting material to stimulate growth, and are covered by plastic mulch during early growth. Nevertheless, the success rate of seed germination and seedling establishment can be relatively low and therefore sowing density must be high. There is a strong positive relation between sowing density and yield, especially when sowing takes place when conditions for emergence and plant establishment are not very conducive.

Success is more likely when sowing takes place in a nursery and vigorous transplants are produced

that can then be transplanted into the field after some hardening. Kacheyo et al. (2024a) showed that temperature and light intensity during raising of the seedlings are crucial to create enough leaf area per seedling and to produce compact seedlings (i.e., seedlings with a large amount of dry matter per unit of length of their stem) to make them vigorous. Stockem et al. (2023) indicated that not only light intensity, but also proportion of far-red light is important for increasing the number of tubers per seedling, and not only temperature, but also the difference between day and night temperature is important in determining the size of the individual tubers formed. Age of the transplant at transplanting is important but less critical than originally assumed, as long as progress to tuberization is not advanced.

By using different trays differing in plug size and volume, Kacheyo et al. (2025) showed that it is important to grow transplants at the right density and with a proper plug volume to give the transplants enough vigour and to make seedling production easier in terms of water management. There is a very strong trade-off between number of seedlings produced per unit area and individual seedling vigour, reflected by the seedlings' leaf area, leaf dry mass, stem dry mass, total dry mass, root: shoot ratio, height, and compactness.

Crops of the same genotype but grown from transplants or seedling tubers perform differently when used in the field to produce daughter tubers. Gu et al. (2024) showed that the branching pattern and the leaf area distribution over the different types of branches are different between TPS-grown crops and seedling-tuber grown crops with similar stem densities per unit area. Differences between the two types of crops depended on the year, most likely because of differences in soil tillage and environmental conditions during early growth; effects of year and propagule type interacted with the stem density effects created in the experiments. Most striking were the larger number of high-order branches in TPS grown plants. These architectural differences were also visible below-ground: crops grown from TPS produced many more tubers per stem, especially in the smaller size classes than crops grown from seedling tubers at the same stem density. Differences were most pronounced at low stem density but significant at all densities, with the higher densities producing fewer tubers per stem. Differences in number of tubers per stem had strong consequences for the tuber size distribution with more tubers obviously resulting in a shift to smaller tubers.

Kacheyo et al. (2024b) demonstrated that the shape and size of ridge or bed was extremely important for the number of tubers per unit area and the tuber yield per unit area of field crops produced from transplants. Especially planting in raised or flat beds gave more tubers and higher tuber yields than various ridge systems. Soil tillage is crucial to obtain the right number of tubers and the right yield but is also important for tuber quality as greening and other physiological disorders might also differ among various soil tillage systems.

Stockem et al. (2024) conducted a detailed yield component analysis on crops of different hybrids and grown from seedling tubers of the same size or weight but showing different numbers of eyes per seedling tuber. They found that within a hybrid, seedling tubers with fewer eyes produced more stems per eye resulting in almost the same number of stems per seedling tubers. Seedling tubers with fewer eyes also produced slightly more tubers per stem so that the number of tubers per plant was very similar for seedling tubers with more eyes and those with fewer eyes. The outcome was that crops from both types of seedling tubers provided the same yield, the same number of tubers, and similar tuber size distribution.

The results of our agronomic studies show that there is work to do to optimize a cropping system involving direct sowing of true potato seed. However, there are opportunities to grow potato crops from transplants and seedling tubers. Use of such propagules also requires adaptation of some of the general cultural practices, partly because of the differences in above- and below-ground architectures. A system producing normal seed tubers from a crop grown from seedling tubers is also an option in areas where seed degeneration is relatively slow.

Table 1 summarizes the characteristics required for high-quality propagules of the different types that might be produced in a diploid hybrid potato seed system.

Use of true potato seed in Sub-Saharan Africa

Lindhout et al. (2011) were the first to publish on the development of hybrid true potato seed. de Vries et al. (2016) reported that the first field trials with potato hybrids were conducted in 2015; These trials were repeated in Africa, in the Democratic Republic of Congo, in 2016 (see also Lindhout and Struik, 2023), a country where informal seed systems are dominant and seed tuber degeneration is fast. These experiments demonstrated the benefit of hybrid true potato seed in terms of seed health and resistance against late blight, but there were two problems. First, the material was still too heterozygous; second,

the regulatory legal framework did not permit hybrid true potato seed varieties to be grown (Lindhout and Struik, 2023).

den Braber et al. (2023) analyzed the potato sector in East Africa and concluded that for the introduction of hybrid true potato seed it was essential to develop a solid understanding of three important elements: a) how farmers evaluate innovations, b) what the local farm realities are, and c) the local seed systems in which the farmers operate. Using hybrid true potato seed requires a drastic change in the configurations of the local seed systems, and how the potato crop is grown, taking into account the agroecological and socio-economic context. They argued that in order to achieve that change, an iterative research cycle linking researchers, breeders, farmers, and other stakeholders should be designed and institutional embedding should be facilitated based on shared views on this innovation.

Gildemacher and ter Steeg (2023) argued that the use of hybrid true potato seed rather than seed tubers can greatly simplify the production system of early generation seed as it no longer requires a system of *in vitro* plantlets and minitubers that requires advanced technology, reliable infrastructure, and skilled labour. Additional advantages include, but are not limited to, absence of most seed-tuber borne pests and diseases, lack of seed dormancy, absence of physiological ageing, and long storability. But most of all: ease of logistics in storage, transport, and use. However, for smallholder farmers in Sub-Saharan Africa there are also major disadvantages, preventing instant success. These include factors related to their needs, economics, risk avoidance, and stressful environments, with large disease pressure and strong abiotic stresses. Under such conditions, plants from seed tubers are more robust than seedlings. Adoption of hybrid true potato seed will require a seed system based on decentralized seed tuber producers who can handle hybrid true potato seed of suitable varieties and produce seed tubers from it, that match the demands of the smallholder ware growers in Sub-Saharan Africa, including short dormancy, earliness, marketability, resistance to late blight, and tolerance to bacterial wilt.

Conclusions

We conclude that hybrid diploid true potato seed has great potential. The story of hybrid true potato seed is a story of hope that might revolutionize the potato sector, but in ways that are still largely unknown. Hybrid breeding might open new avenues for pyramiding resistance genes, introgressing novel traits such as heat tolerance and health promoting characteristics. The use of true potato seed opens new opportunities for tailor-made seed systems in Sub-Saharan Africa where the production of healthy seed is difficult and governance of seed systems complicated. However, this potential still requires validation in practice in different cropping systems and agro-ecologies. There are different models possible for producing propagules from hybrid diploid varieties. It is not yet fully crystalized what would be the best model for the use of hybrid varieties in developed and developing countries. Moreover, different propagules create crops that differ in crop physiology although some basics are very much shared. But because of the differences, crops from different propagules require different cultural practices and there is still a lot of research required to optimize seed systems and cropping systems of potato from hybrid varieties in different parts of the world. Capitalizing on the potential is therefore still a huge challenge. But in regions where the advantages are large and weigh heavily it is definitely worth trying.

Nevertheless, the technology of hybrid true potato seed could become a driver of change in the potato sector development, especially in Sub-Saharan Africa.

The technology addresses some fundamental challenges of the conventional vegetative multiplication system, including land requirement for seed production, availability of healthy early generation seed tubers, seed degeneration, and logistics of seed storage and transport.

Acknowledgements

This paper is an extended version of the abstract published in the Proceedings of the 5th International Plant Breeding Congress (December 01-05, 2025, Antalya, Türkiye).

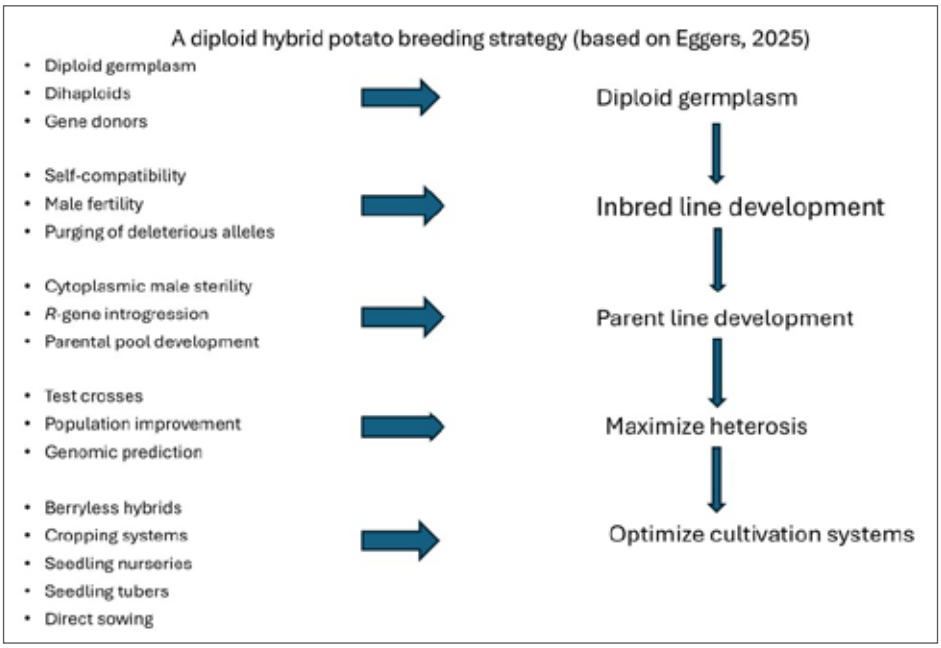


Figure 1. A strategy to create diploid hybrid cultivars, from collecting diploid germplasm through inbred line and parent line development to diploid cultivars with maximum heterosis and finally optimizing cultivation systems making use of novel propagules.

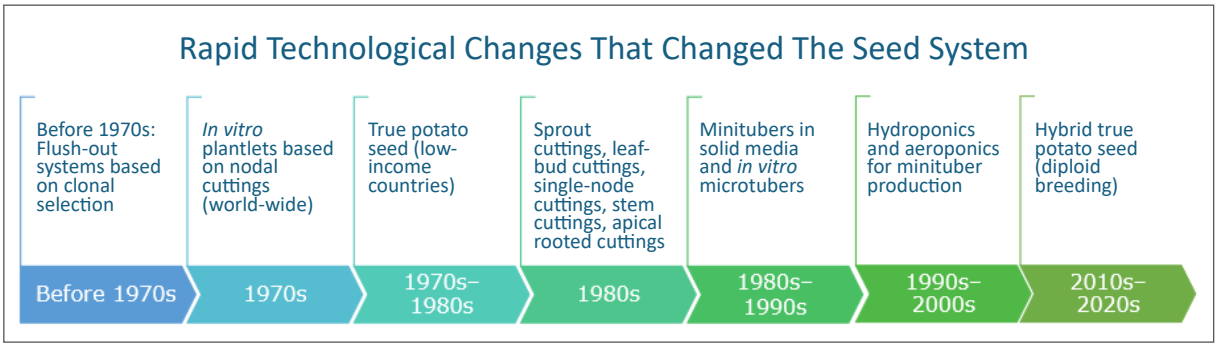


Figure 2. The rapid changes in multiplication of potato over the last 50 years. Based on Struik (2023).

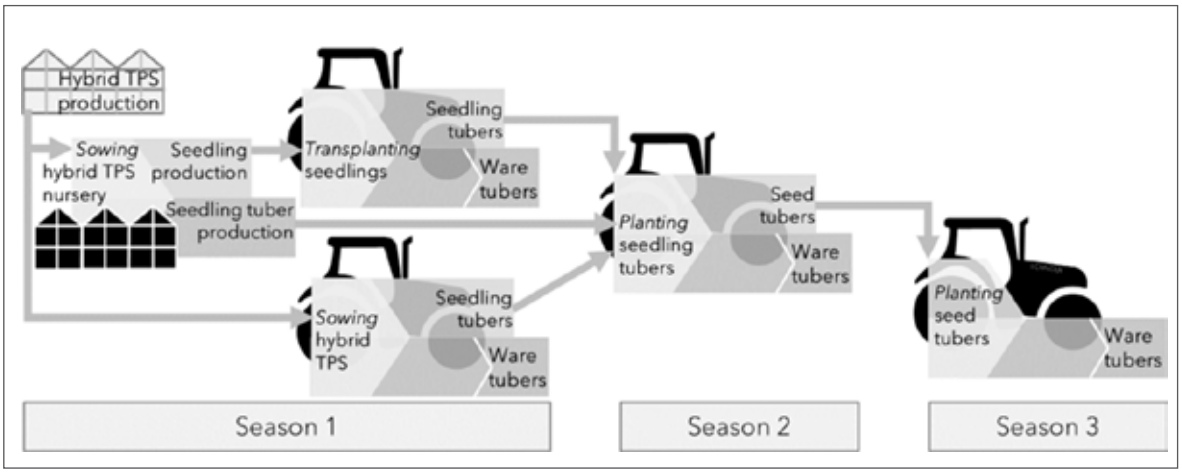


Figure 3. Different cultivation pathways starting from hybrid true potato seed (from van Dijk et al., 2021).

Table 1. Desired characteristics of the different types of propagules that can be produced in a diploid hybrid potato seed system.

Propagule Type	Size	Health	Physiology	Pre-Treatment	Other Characteristics
True Seed	Large and uniform	Disease-free (no viroids)	No dormancy; Maximum germination rate and seedling vigour	Seed priming	Properly enriched coating; Pellet size and shape should match sowing machine
Transplant	Right size; Proper leaf area; Compact; Sturdy; Vigorous	Disease-free	Proper age and status; No progress to tuberization	Properly hardened; Transplant-shock proof	Uniform
Seedling Tuber	Right size; Right number of eyes	Disease-free	Proper physiological age	Pre-sprouted	Proper number of sturdy sprouts after pre-sprouting
Normal Seed Tubers	Right size	Disease-free	Proper physiological age	Pre-sprouted	Proper number of sturdy sprouts after pre-sprouting

References

Atieno, E. O., Kilwinger, F. B. M., Almekinders, C. J. M., and Struik, P. C. (2023). How Kenyan potato farmers evaluate the seed: implications for the promotion of certified seed potato. *Potato Research, 66*, 811-829. https://doi.org/10.1007/s11540-022-09602-8

Atieno, E. O., Almekinders, C. J. M., and Struik, P. C. (2026). Developments and challenges of the Kenyan potato sector: Implications for smallholder farmers. *Potato Research, 69*, Article 98. https://doi.org/10.1007/s11540-026-10038-7

Bertschinger, L., Bühler, L., Dupuis, B., Duffy, B., Gessler, C., Forbes, G. A., Keller, E. R., Scheidegger, U. C., and Struik, P. C. (2017). Incomplete infection of secondarily infected potato plants - an environment dependent underestimated mechanism in plant virology. *Frontiers in Plant Science*, *8*, Article 74. https://doi.org/10.3389/fpls.2017.00074

Bradshaw, J. E. (2022). Breeding diploid F1 hybrid potatoes for propagation from botanical seed (TPS): Comparison with theory and other crops. *Plants*, *11*(9), Article 1121. https://doi.org/10.3390/plants11091121

de Vries, M. E., Adams, J. R., Eggers, E. J., Su, Y., Stockem, J. E., Kacheyo, O. C., van Dijk, L. C. M., Khera, P., Bachem, C. W., Lindhout, P., and van der Vossen, E. A. G. (2023). Converting hybrid potato breeding science into practice. *Plants*, *12*(2), Article 230. https://doi.org/10.3390/plants12020230

de Vries, M. E., Ter Maat, M., and Lindhout, P. (2016). The potential of hybrid potato for East Africa. *Open Agriculture*, *1*(1), 151-156. https://doi.org/10.1515/opag-2016-0020

den Braber, H., de Vries, M. E., Kacheyo, O. C., Struik, P. C., and Descheemaeker, K. (2023). How could hybrid true potato seed foster development in potato sectors in East Africa? In P. C. Struik, P. R. Gildemacher, D. Stemerding, and P. Lindhout (Eds.), *Impact of hybrid potato: The future of hybrid potato from a systems perspective* (pp. 95-114). Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-946-6_7

Eggers, E.-J. (2025). *Fertility on demand: An exploration of genes that control self-fertility in diploid potato* [Doctoral dissertation, Wageningen University]. Wageningen University Repository. https://doi.org/10.18174/630412

Gildemacher, P. R., Schulte-Geldermann, E., Borus, D., Demo, P., Kinyae, P., Mundia, P., and Struik, P. C. (2011). Seed potato quality improvement through positive selection by smallholder farmers in Kenya. *Potato Research*, *54*(3), 253-266. https://doi.org/10.1007/s11540-011-9190-5

Gildemacher, P., and ter Steeg, E. M. S. (2023). Potential impact of hybrid true potato seed in Sub-Saharan Africa. In P. C. Struik, P. R. Gildemacher, D. Stemerding, and P. Lindhout (Eds.), *Impact of hybrid potato: The future of hybrid potato from a systems perspective* (pp. 75-94). Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-946-6_6

Gu, J., Evers, J. B., Driever, S. M., Shan, K., and Struik, P. C. (2024). Branching response to stem density and its impact on yield in hybrid potato (*Solanum*

tuberosum) grown from true seeds and seedling tubers. *Field Crops Research*, *317*, Article 109548. https://doi.org/10.1016/j.fcr.2024.109548

Gu, J., Evers, J. B., Struik, P. C., and Driever, S. M. (2025). Architectural differences between hybrid potato plants grown from true seeds and seedling tubers do not influence leaf photosynthetic traits. *Potato Research*, *68*(4), 4653-4669. https://doi.org/10.1007/s11540-025-09752-x

Haverkort, A. J., and Struik, P. C. (2015). Yield levels of potato crops: Recent achievements and future prospects. *Field Crops Research*, *182*, 76-85. https://doi.org/10.1016/j.fcr.2015.06.002

Haverkort, A. J., Linnemann, A. R., Struik, P. C., and Wiskerke, J. S. C. (2023). On processing potato. 2. Survey of products, processes and operations in manufacturing. *Potato Research*, *66*(2), 339-383. https://doi.org/10.1007/s11540-022-09563-y

Jansky, S. H., Charkowski, A. O., Douches, D. S., Gusmini, G., Richael, C., Bethke, P. C., Spooner, D. M., Novy, R. G., De Jong, H., De Jong, W. S., Bamberg, J. B., Thompson, A. L., Bizimungu, B., Holm, D. G., Brown, C. R., Haynes, K. G., Sathuvalli, V. R., Veilleux, R. E., Creighton Miller Jr., J., ...Jiang, J. (2016). Reinventing potato as a diploid inbred line-based crop. *Crop Science*, *56*(4), 1412-1422. https://doi.org/10.2135/cropsci2015.12.0740

Kacheyo, O. C., Mhango, K. J., de Vries, M. E., Schneider, H. M., and Struik, P. C. (2024b). Bed, ridge and planting configurations for field-transplanted hybrid potato seedling crops. *Field Crops Research*, *317*, Article 109556. https://doi.org/10.1016/j.fcr.2024.109556

Kacheyo, O. C., Schneider, H. M., de Vries, M. E., and Struik, P. C. (2024a). Shoot growth parameters of potato seedlings are determined by light and temperature conditions. *Potato Research*, *67*(4), 1159-1186. https://doi.org/10.1007/s11540-023-09658-4

Kacheyo, O. C., Schneider, H. M., de Vries, M. E., and Struik, P. C. (2025). Growing vigorous potato seedlings in plug trays. *Potato Research*, *68*(2), 1767-1801. https://doi.org/10.1007/s11540-024-09799-w

Kacheyo, O. C., van Dijk, L. C. M., de Vries, M. E., and Struik, P. C. (2021). Augmented descriptions of growth and development stages of potato (*Solanum tuberosum* L.) grown from different types of planting material. *Annals of Applied Biology*, *178*(3), 549-566. https://doi.org/10.1111/aab.12669

Korontzis, G., Malosetti, M., Zheng, C., Maliepaard, C., Mulder, H. A., Lindhout, P., Veerkamp, R. F., and van Eeuwijk, F. A. (2020). QTL detection in a pedigreed breeding population of diploid potato. *Euphytica*, *216*, Article 145. https://doi.org/10.1007/s10681-020-02674-y

Kwambai, T. K., Griffin, D., Struik, P. C., Stack, L., Rono, S., Brophy, C., Nyongesa, M., and Gorman, M. (2024). Seed quality and variety preferences among potato farmers in north-western Kenya - lessons for the adoption of new varieties. *Potato Research*, *67*(1), 185-208. https://doi.org/10.1007/s11540-023-09626-8

Lindhout, P., and Struik, P. C. (2023). Hybrid potato breeding and production systems. In P. C. Struik, P. R. Gildemacher, D. Stemerding, and P. Lindhout (Eds.), *Impact of hybrid potato: The future of hybrid potato from a systems perspective* (pp. 17-30). Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-946-6_2

Lindhout, P., De Vries, M., Ter Maat, M., Su, Y., Viquez-Zamora, M., and Van Heusden, S. (2018). Hybrid potato breeding for improved varieties. In G. Wang-Pruski (Ed.), *Achieving sustainable cultivation of potatoes: Volume 1: Breeding, nutritional and sensory quality* (pp. 53-78). Burleigh Dodds Science Publishing. https://doi.org/10.19103/AS.2017.0016.05

Lindhout, P., Meijer, D., Schotte, T., Hutten, R. C. B., Visser, R. G. F., and Van Eck, H. J. (2011). Towards F1 hybrid seed potato breeding. *Potato Research*, *54*(4), 301-312. https://doi.org/10.1007/s11540-011-9196-z

Mordor Intelligence (2026). https://www.mordorintelligence.com/industry-reports/potato-market. Accessed 24 July 2026.

Navarrete, I., Andrade-Piedra, J., López, V., Yue, X., Herrera, J., Barzallo, M., Quimbiulco, K., Almekinders, C. J. M., and Struik, P. C. (2023). Farmers experiencing potato seed degeneration respond but do not adjust their seed replacement strategies in Ecuador. *American Journal of Potato Research*, *100*(1), 39-51. https://doi.org/10.1007/s12230-022-09893-0

Navarrete, I., López, V., Andrade-Piedra, J. L., Almekinders, C. J. M., Kromann, P., and Struik, P. C. (2022b). Agroecological settings and seed recycling account only partially for potato seed degeneration in Ecuador. *Agronomy for Sustainable Development*, *42*(6), Article 109. https://doi.org/10.1007/s13593-022-00840-1

Navarrete, I., López, V., Borja, R., Oyarzún, P., Garrett, K. A., Almekinders, C. J. M., Xing, Y., Struik, P. C., and Andrade-Piedra, J. L. (2022a). Variety and on-farm seed management practices affect potato seed degeneration in the tropical highlands of Ecuador.

Agricultural Systems, *198*, Article 103387. https://doi.org/10.1016/j.agsy.2022.103387

Priegnitz, U., Lommen, W. J. M., van der Vlugt, R. A. A., and Struik, P. C. (2019). Impact of positive selection on incidence of different viruses during multiple generations of potato seed tubers in Uganda. *Potato Research*, *62*(1), 1-30. https://doi.org/10.1007/s11540-018-9394-z

Priegnitz, U., Lommen, W. J. M., van der Vlugt, R. A. A., and Struik, P. C. (2020). Potato yield and yield components as affected by positive selection during several generations of seed multiplication in southern Uganda. *Potato Research*, *63*(4), 507-543. https://doi.org/10.1007/s11540-020-09455-z

Schulte-Geldermann, E., Gildemacher, P. R., and Struik, P. C. (2012). Improving seed health and seed performance by positive selection in three Kenyan potato varieties. *American Journal of Potato Research*, *89*(6), 429-437. https://doi.org/10.1007/s12230-012-9264-1

Sood, S., Mangal, V., Thakur, A. K., Buckseth, T., Chaudhary, B., Kumar, V., and Singh, B. (2024). Diploid inbred-based hybrids: Fast-forward breeding approach in potatoes. *Physiology and Molecular Biology of Plants*, *30*(12), 1955-1968. https://doi.org/10.1007/s12298-024-01544-4

Stockem, J., Bus, M. D., de Vries, M. E., and Struik, P. C. (2024). Opening eyes on seedling tuber quality in potato: Size matters. *Potato Research*, *67*(4), 1691-1715. https://doi.org/10.1007/s11540-023-09681-5

Stockem, J., de Vries, M., and Struik, P. C. (2023). Shedding light on a hot topic: Tuberization in potato. *Annals of Applied Biology*, *183*(3), 170-180. https://doi.org/10.1111/aab.12845

Stockem, J., de Vries, M., van Nieuwenhuizen, E., Lindhout, P., and Struik, P. C. (2020). Contribution and stability of yield components of diploid hybrid potatoes. *Potato Research*, *63*(3), 345-366. https://doi.org/10.1007/s11540-019-09444-x

Struik, P. C. (2023). *From photons to farmers: Consilience in crop physiology.* Wageningen University & Research. https://doi.org/10.18174/640011

Struik, P. C., and Wiersema, S. G. (1999). *Seed potato technology*. Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-759-2

ter Steeg, E., Struik, P. C., Visser, R. G. F., and Lindhout, P. (2022). Crucial factors for the feasibility of commercial hybrid breeding in food crops. *Nature Plants*, *8*(5), 463-473. https://doi.org/10.1038/s41477-022-01142-7

Thomas-Sharma, S., Abdurahman, A., Ali, S., Andrade-Piedra, J. L., Bao, S., Charkowski, A. O., Crook, D., Kadian, M., Kromann, P., Struik, P. C., Torrance, L., Garrett, K. A., and Forbes, G. A. (2016). Seed degeneration in potato: The need for an integrated seed health strategy to mitigate the problem in developing countries. *Plant Pathology*, *65*(1), 3-16. https://doi.org/10.1111/ppa.12439

van Dijk, L. C. M., Kacheyo, O. C., Lieftink, W. C., Lommen, W. J. M., and Struik, P. C. (2025). Is field-sowing of hybrid true potato seeds feasible for seed or ware potato production under Dutch conditions? *Potato Research*, *68*(4), 3705-3732. https://doi.org/10.1007/s11540-025-09731-2

van Dijk, L. C. M., Lommen, W. J. M., de Vries, M. E., Kacheyo, O. C., and Struik, P. C. (2021). Hilling of transplanted seedlings from hybrid true potato seeds does not enhance tuber yield but can affect tuber size distribution. *Potato Research*, *64*(2), 353-374. https://doi.org/10.1007/s11540-020-09476-8

Wang, Y., Lin, T., Wang, Z., Fan, L., Wang, Y., Jiao, X., Wang, W., Zong, X., Chen, J., and Yin, Y. (2026). Breaking self-incompatibility for diploid hybrid potato breeding: Advances, mechanisms, and emerging technologies. *Frontiers in Plant Science*, *17*, Article 1848562. https://doi.org/10.3389/fpls.2026.1848562

Zhang, C., Wang, P., Tang, D., Yang, Z., Lu, F., Qi, J., Tawari, N. R., Shang, Y., Li, C., and Huang, S. (2019). The genetic basis of inbreeding depression in potato. *Nature Genetics*, *51*(3), 374-378. https://doi.org/10.1038/s41588-018-0319-1

Zhang, C., Yang, Z., Tang, D., Zhu, Y., Wang, P., Zhou, G., Xiong, X., Shang, Y., Li, C., and Huang, S.-W. (2021). Genome design of hybrid potato. *Cell*, *184*(15), 3873-3883. https://doi.org/10.1016/j.cell.2021.06.006

Zhang, S., Liao, Q., Zhang, Z., Zhu, X., Jia, Y., Shang, Y., et al. (2025). Origin of a self compatibility associated MITE in *Petota* and its application in hybrid potato breeding. *New Phytologist*, *246*(4), 1647-1659. https://doi.org/10.1111/nph.70093

Zou, C., van der Putten, P. E. L., Datema, M., Mossink, L., Lommen, W. J. M., Struik, P. C., and van Ittersum, M. K. (2025). Field performance of seed tubers of potato (*Solanum tuberosum* L.) of different physiological age. *Potato Research*, *68*(1), 187-217. https://doi.org/10.1007/s11540-024-09731-2

Zou, C., van der Putten, P. E. L., Mossink, L., Lommen, W. J. M., van Ittersum, M. K., and Struik, P. C. (2024). Using sprout behaviour to quantify physiological ageing of seed tubers of potato (*Solanum tuberosum* L.). *Environmental and Experimental Botany*, *219*, Article 105648. https://doi.org/10.1016/j.envexpbot.2024.105648

Research Article

Ekin
Journal of Crop Breeding and Genetics

12(2):86-95, 2026

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Evaluation of Low Temperature Tolerance in Grafted Cucumber Seedlings with Interspecific Hybrid *Cucurbita* Rootstocks (*C. maxima* × *C. moschata*) Using Morphological and Biochemical Indicators(§)

Seda ATASOY[1*] EMRE İPEK[1] Sibel BAHADIR[1] Büşra ALTUNTAŞ[1] Ahmet BALKAYA[2] Süleyman KAVAK[3] Şeküre Şebnem ELLİALTIOĞLU[4]

[1] Petektar Seed R&D Center, Kurşunlu Mah. 12081. Sk. B Blok No:19, Aksu, Antalya, Türkiye

[2] Department of Horticulture, Faculty of Agriculture, Ondokuz Mayıs University, Samsun, Türkiye

[3] Department of Horticulture, Faculty of Agriculture and Agricultural Technologies, Isparta University of Applied Sciences, Isparta, Türkiye

[4] Doqutech Academy Ltd. Co., Ankara University Technopark, Ankara, Türkiye

[*] Corresponding author e-mail: bensedaatasoy@gmail.com

Citation:
Atasoy S., İpek E., Bahadır S., Yapıcı B., Balkaya A., Kavak S., Ellialtıoğlu ŞS., 2026. Evaluation of Low Temperature Tolerance in Grafted Cucumber Seedlings with Interspecific Hybrid *Cucurbita* Rootstocks (*C. maxima* × *C. moschata*) Using Morphological and Biochemical Indicators. Ekin J. 12(2):86-95.

Received: 18.04.2026 ***Accepted: 18.06.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

The use of grafted seedlings enhances water and nutrient uptake under abiotic stress conditions, such as high or low temperatures and drought, by promoting a stronger root system. Additionally, grafting improves tolerance to diseases and pests, thereby positively affecting both yield and quality. Hybrids of *C. moschata* × *C. moschata* and *C. maxima* × *C. moschata* are currently the most commonly used rootstocks for cucumber (*Cucumis sativus* L.) seedling production. In this study, the cucumber cultivar PTK40 F1 was used as the scion, while non-grafted PTK40 F_1 seedlings served as the control group. In this study, 11 promising interspecific hybrid Cucurbita rootstocks tolerant of low temperatures were used. The performance of the promising rootstock candidates was compared with that of commercial rootstocks such as Shintoza F_1, Nun 9075 F_1, Cobalt F_1, Cremna F_1, and Max Crom F_1. Grafted and non-grafted seedlings were acclimated at 15°C/10°C (day/night) after reaching the 3-4 true-leaf stage. Subsequently, they were subjected to a low-temperature treatment at 5°C (day and night) for two days. Plant damage was evaluated visually using a scale ranging from 0 (no damage) to 5 (dead plant). The results showed that the rootstock/scion combinations KB20/PTK40 F_1, KB17/PTK40 F_1, KB19/PTK40 F_1, and KB22/PTK40 F_1 exhibited high visual tolerance to low temperatures with low damage scores (0-1). However, biochemical analyses revealed significant physiological differences. Under low-temperature stress, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels generally increased. Contrary to visual observations, KB22 exhibited high lipid peroxidation (MDA: 20.39 nmol g^{-1} FW). The physiologically most tolerant genotypes were identified as KB5/PTK40 F_1 (MDA: 9.99 nmol g^{-1} FW) and KB4/PTK40 F_1 (MDA: 10.73 nmol g^{-1} FW), which maintained the lowest oxidative damage and did not require excessive proline accumulation. KB20/PTK40 F_1 also demonstrated a balanced tolerance mechanism. Based on these findings, the combinations KB20, KB4, and KB5, identified as tolerant, will be further evaluated for plant growth and yield performance under cold-season greenhouse conditions in future studies.

Keywords: *Cucurbita* rootstock, Grafted seedling, Low-temperature stress, Oxidative stress, MDA, Proline

(§) *This study was presented as an oral presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important and widely cultivated vegetable crops worldwide. According to the Food and Agriculture Organization (2025) records, the annual global production of cucumber exceeds 97 million tons, with a substantial share produced in Asian and Mediterranean countries. Türkiye is among the leading countries in the world in terms of total cucumber production. It has a significant share, particularly in greenhouse cultivation. In Türkiye, 1,468,437 tons of fresh-market cucumbers were produced on 219,968 decares. Of this total production, 1,028,122 tons were obtained from protected cultivation covering 73,592 decares (Turkish Statistical Institute, 2024).

Environmental stress conditions constitute one of the primary challenges in cucumber production. Low-temperature stress is recognized as a critical factor that limits seedling development, reduces photosynthetic capacity, and induces oxidative damage, particularly during the early spring and late autumn periods (Li et al., 2015). Although the plants have developed various morphological and physiological adaptive mechanisms to cope with low-temperature stress, many commercial cucumber cultivars remain highly sensitive to this stress (Karaağaç et al., 2017). Therefore, in addition to the adoption of alternative cultivation techniques, the use of qualified, low-temperature-tolerant genotypes has become essential for successful cultivation.

In recent years, grafted seedlings have emerged as one of the most effective cultivation strategies to mitigate abiotic stresses, including low and high temperatures and drought. Grafted seedlings enhance stress tolerance through a strong root system, improve nutrient and water uptake, increase yield, and reduce quality losses (Lee and Oda, 2003; Schwarz et al., 2010; Balkaya, 2014). The production and use of grafted seedlings have rapidly increased in Türkiye over the past 20 years, with the widespread adoption of interspecific *C. maxima* × *C. moschata* and intraspecific *C. moschata* × *C. moschata* rootstocks in grafted cucumber seedling production (Yücel et al., 2022; İpek et al., 2025). Interspecific *Cucurbita* hybrids are particularly favored because of their strong root systems and greater tolerance of environmental stresses.

Biochemical markers play a pivotal role in elucidating plant responses to abiotic stress conditions. Reactive oxygen species (ROS) generated under low-temperature conditions induce oxidative damage at the cellular level, resulting in lipid peroxidation. Malondialdehyde (MDA), a major product of lipid peroxidation, is a widely utilized marker for assessing stress severity (Heath and Packer, 1968; Hasanuzzaman et al., 2020). Similarly, proline accumulation plays a critical role in mechanisms such as maintaining osmotic balance, stabilizing cellular structures, and scavenging free radicals (Szabados & Savouré, 2010). Furthermore, hydrogen peroxide (H_2O_2) holds central importance in stress responses, acting as both an indicator of oxidative stress and a signaling molecule (Slesak et al., 2007).

Currently, assessing low-temperature tolerance of interspecific hybrid *Cucurbita* rootstock candidates using both morphological and biochemical parameters represents a significant area of research. This study aimed to compare the tolerance to low-temperature stress of cucumber seedlings grafted onto locally developed hybrid *Cucurbita* rootstocks (from the breeding program) and commercial rootstocks with that of non-grafted seedlings, using morphological (visual assessment) and biochemical (MDA, proline, and H_2O_2 content) analyses. The findings of this study are expected to contribute to the development of new low-temperature-tolerant local rootstocks suitable for grafted cucumber cultivation.

We hypothesized that locally developed interspecific hybrid *Cucurbita* rootstocks would exhibit higher low-temperature tolerance than commercial rootstocks by minimizing oxidative stress and maintaining cell membrane stability. The primary novelty of this research is the physiological profiling of promising local *Cucurbita* rootstock candidates against the other commercial varieties. By combining visual scoring with key biochemical indicators (MDA, H_2O_2, and proline), this study overcomes the limitations of morphological observation alone and elucidates the underlying oxidative defense mechanisms for cold tolerance.

Materials and Methods

Genetic Materials

The plant genetic materials for this study consisted of interspecific hybrid *Cucurbita* rootstock candidates (*C. maxima* × *C. moschata*) derived from a project supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK), which aimed to develop rootstocks for grafted cucumber seedling production. The *Cucurbita* rootstock candidates, which exhibit resistance to biotic stress factors such as Fusarium wilt (*Fusarium oxysporum* f. sp. *cucumerinum*) and root-knot nematodes (*Meloidogyne* spp.), have been self-pollinated to the S_5-S_6 breeding generations, ensuring their genetic homogeneity.

Eleven of these rootstock candidates (KB2, KB3, KB4, KB5, KB6, KB13, KB17, KB18, KB19, KB20, and KB22), identified as tolerant to low-temperature stress and exhibiting no graft incompatibility when

used for grafted cucumber (*C. sativus* L., cv. PTK40 F_1) seedling production, were utilized. The cucumber cultivar PTK40 F_1 (Petektar Seed Company) was used as the scion, with non-grafted PTK40 F_1 plants serving as the control. Additionally, PTK40 F_1 was grafted onto commercial rootstocks such as Shintoza F_1, Nun 9075 F_1, Cobalt F_1, Cremna F_1, and Max Crom F_1, and these were compared with the local *Cucurbita* rootstock candidates.

Grafting Cucumber Cultivar onto *Cucurbita* Rootstocks

The grafting studies were conducted in a private seedling production facility in Antalya, Türkiye. In the grafting experiment, seeds of the scion and rootstock were sown on April 15, 2025, and April 17, 2025, respectively. Grafting was performed on May 2, 2025, when the rootstocks had their cotyledons parallel to the soil surface and the first true leaf emerging, and the scions were at the first true-leaf stage. Prior to the procedure, all equipment and hands were disinfected with a 1% dipotassium peroxodisulfate solution, and grafting was performed using the one-cotyledon method. Following grafting, seedlings were maintained in a healing chamber for seven days and subsequently transferred to the R&D center of Petektar Seed Company on May 20, 2025, for low-temperature stress testing.

Determination of Low-Temperature Tolerance in Non-Grafted and Grafted Cucumber Seedlings with Different Hybrid *Cucurbita* Rootstocks Using Morphological Indicators

The experiment was conducted in a completely randomized design with three replications, each consisting of 30 plants. Plants reaching the three or four true leaf stage were grown in a growth chamber with 70% relative humidity under a 12/12 h light/dark photoperiod with a light intensity of 100 µmol m^{-2} s^{-1}. As part of the cold acclimation and stress process, the cucumber seedlings were maintained at 15°C/10°C (day/night) for two days, followed by 5°C/5°C for an additional two days (Li et al., 2015). Following the 2-day low-temperature treatment, visual scoring and leaf sampling for biochemical analyses were performed immediately, without a post-stress recovery period, to evaluate the acute stress response.

Following the low-temperature treatments, grafted and non-grafted seedlings were visually evaluated for cold injury symptoms. Evaluations were conducted using a 0-5 scale (**0:** No symptoms/damage, **1:** Desiccation on 10% of the leaf area (trace injury, **2:** 10-30% slight injury, **3:** 30-50% moderate injury, **4:** >50% advanced/severe injury, **5:** dead plant) developed by Smeets and Wehner (1997) and modified in this study.

Determination of Low-Temperature Tolerance in Non-Grafted and Grafted Cucumber Seedlings with Different Hybrid *Cucurbita* Rootstocks Using Biochemical Analyses

Leaf samples from grafted and non-grafted cucumber seedlings were collected and subjected to biochemical analyses at the R&D laboratory of Petektar Seed Company.

Determination of Oxidative Stress Markers (H_2O_2 and MDA)

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels were assessed as oxidative stress markers. H_2O_2 content was determined according to the method described by Slesak et al. (2007). Absorbance was measured at 436 nm, and results were expressed on a fresh-weight (FW) basis. MDA content, as an indicator of lipid peroxidation, was analyzed using the method described by Ohkawa et al. (1979). Absorbance readings were taken at 532 and 600 nm. Calculations were performed using the formula provided by Güneri Bağcı (2010), and the results were expressed as nmol g^{-1} FW.

Determination of Proline Levels

The proline content, which acts as a crucial osmoprotectant under stress conditions, was determined following the methodology described by Kaur and Asthir (2015). Fresh leaf samples were homogenized in an extraction solution, and the resulting supernatant was reacted with acid ninhydrin and glacial acetic acid. After incubation in a hot water bath, the absorbance of the colored reaction product was measured spectrophotometrically at 520 nm. The proline concentration was quantified using a standard curve and the results were expressed as µmol g^{-1} fresh weight (FW).

Statistical Analyses

Prior to the analysis of variance, the normality of the data distribution and homogeneity of variances were evaluated using the Shapiro-Wilk and Levene's tests, respectively. In plant stress physiology, acute treatments such as low temperature often induce extreme biochemical responses compared to non-stressed controls, naturally leading to deviations from strict homoscedasticity and normality. However, a two-way analysis of variance (Anova) was performed on the original data, as this analysis is highly robust to such deviations in completely balanced experimental designs commonly used in agricultural research (Gomez and Gomez, 1984). Furthermore, recent methodological evaluations in crop sciences emphasize that ANOVA maintains its reliability and Type I error control even when data deviate from normal distribution, especially when sample sizes are equal across treatments (Kozak and Piepho, 2018). Therefore, a two-way Anova was confidently used to

evaluate the main effects of temperature, genotype, and their interaction (temperature × genotype). Differences among the interaction means were determined using Tukey's multiple comparison test at a significance level of $P < 0.05$.

Results and Discussion

Low-Temperature Tolerance of Non-Grafted and Grafted Cucumber Seedlings on Different Hybrid *Cucurbita* Rootstocks Using Morphological Indicators

Following the low-temperature test, visual evaluations of grafted and non-grafted cucumber seedlings revealed notable differences among seedlings grafted onto different candidate hybrid *Cucurbita* rootstocks (Fig.1). In the study, when local *Cucurbita* rootstock candidates were evaluated alongside commercial rootstocks, the lowest cold damage score was observed in candidate KB20/PTK40 F_1 (0.0), followed by KB17/PTK40 F_1 (0.67), KB22/PTK40 F_1 (0.78), and KB19/PTK40 F_1 (0.89). These rootstocks exhibited high visual tolerance, performing statistically comparable to the commercial rootstock Shintoza F_1/PTK40 F_1 (0.67). The superior performance of interspecific hybrids is often attributed to their vigorous root systems, which maintain better water status and nutrient uptake under stress conditions (Lee et al., 2010; Huang et al., 2013). However, as noted in previous studies, visual assessment alone may not always reflect the instantaneous physiological status of the plant, especially under short-term stress conditions, as cellular damage may precede visible symptoms (Zhou et al., 2007). Therefore, these visual findings were cross-referenced with biochemical markers to determine the mean low-temperature tolerance levels.

Oxidative Stress Responses (H_2O_2 and MDA) in Non-Grafted and Grafted Cucumber Seedlings on Hybrid *Cucurbita* Rootstocks

Low-temperature stress induces oxidative stress by triggering the accumulation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), which can damage cellular components (Mittler, 2002). In this study, physiological data on low-temperature tolerance revealed critical differences among the rootstocks in tolerance mechanisms.

Analysis of H_2O_2 content revealed that low-temperature-sensitive genotypes, including KB6 and PTK40 F_1 (non-grafted), showed dramatic increases (up to 404%), while the highest ROS-scavenging capacity was recorded in rootstock candidates KB4 (731.62 nmol g^{-1} FW) and KB5 (1029.39 nmol g^{-1} FW) (Table 1). Both of these *Cucurbita* rootstock candidates exhibited significantly lower H_2O_2 levels than the commercial Shintoza F_1 rootstock (2164.49 nmol g^{-1} FW) and the visually tolerant KB22 rootstock (1721.04 nmol g^{-1} FW) (Table 1). Low H_2O_2 accumulation in tolerant *Cucurbita* rootstocks under low temperature stress has been widely linked to an efficient antioxidant enzyme system capable of scavenging ROS prior to the onset of oxidative damage (Slesak et al., 2007; Gill and Tuteja, 2010).

Under low-temperature stress, lipid peroxidation (MDA) levels, which serve as a direct indicator of cell membrane damage, exhibited a similar pattern (Hodges et al., 1999). Grafted cucumber seedlings on rootstocks KB5 (9.99 nmol g^{-1} FW), KB4 (10.73 nmol g^{-1} FW), and KB13 (10.73 nmol g^{-1} FW) showed the highest membrane stability (Table 2), suggesting that the cell membranes of these rootstocks were efficiently protected against oxidative damage.

A notable discrepancy was observed in the KB22 rootstock. Although KB22 appeared tolerant in visual assessments (Score: 0.78), it exhibited the highest MDA content (20.39 nmol g^{-1} FW) among all rootstocks, statistically matching the sensitive groups. This result indicates that while the KB22 rootstock successfully maintained leaf turgor and water status in the short term, avoiding visible wilting, it experienced severe lipid peroxidation at the cellular level. This confirms that biochemical damage often precedes visible morphological symptoms under acute cold stress, making cell membrane markers like MDA more reliable than visual scoring for accurate tolerance screening (Campos et al., 2003). In contrast, the KB20 rootstock candidate exhibited a balanced tolerance strategy, maintaining moderate yet stable levels of both H_2O_2 (1218.96 nmol g^{-1} FW) and MDA (12.38 nmol g^{-1} FW), thereby confirming its physiological resilience in line with its visual performance (Table 2, Fig. 1).

Changes in Proline Content in Non-Grafted and Grafted Cucumber Seedlings on Hybrid *Cucurbita* Rootstocks

Proline accumulation constitutes a widespread response to osmotic stress in plants; its role is dual, functioning both as an osmoprotectant and as a signaling molecule indicative of stress-induced cellular damage (Ashraf and Foolad, 2007).

In this study, the highest proline accumulation was observed in the rootstocks KB6 (617% increase) and KB2 (577% increase) (Table 3). This pronounced accumulation reflects a highly active osmoprotective defense mechanism aimed at mitigating low-temperature-induced cellular damage under stress pressure (Szabados and Savouré, 2010; Zulfiqar et al., 2020; Ghosh et al., 2022).

The rootstocks KB4, KB5, and KB20, identified as tolerant to low temperature, exhibited smaller increases in proline levels than the other rootstocks (Table 3). The absence of massive proline accumulation in these rootstocks may be attributed to more effective oxidative stress management, as indicated by lower levels of ROS and MDA. In plants, the maintenance of cellular integrity under stress conditions is closely associated with efficient antioxidant defense mechanisms.

In this study, cucumber seedlings grafted onto the KB22 rootstock exhibited low proline levels (6.43 µmol g^{-1} FW) despite showing high membrane damage, as indicated by elevated MDA levels (Table 3). This lack of an adequate biochemical response, combined with severe cellular damage, suggests that the stress signaling mechanism in the KB22 rootstock may be impaired or insufficient to trigger defense pathways effectively. This response pattern contrasts with that observed in rootstocks such as KB5 and KB20, which exhibit traits associated with improved tolerance to low-temperature stress.

When the biochemical responses are evaluated as an integrated physiological mechanism, a clear cascade from ROS generation to osmoprotectant emerges. In highly tolerant rootstocks like KB4 and KB5, the prevention of excessive H_2O_2 accumulation mitigates downstream lipid peroxidation, evidenced by minimal MDA levels. Because early-stage oxidative stress is efficiently managed by intrinsic ROS-scavenging systems, these genotypes maintain cellular integrity without needing massive, energy-consuming proline synthesis. Conversely, susceptible rootstocks (e.g., KB6, KB2) attempt to compensate for high oxidative damage through massive proline accumulation (over 500% increase). Notably, the KB22 genotype represents an impaired physiological response model; despite severe membrane damage (highest MDA), it fails to trigger an adequate proline response, indicating a decoupled stress-signaling pathway. These integrated findings suggest that true low-temperature tolerance in Cucurbita rootstocks relies not on reactive osmoprotection (proline), but on proactive ROS suppression (low H_2O_2) and subsequent membrane stability (low MDA).

Conclusions

Cucumber (*Cucumis sativus* L.) is a thermophilic crop highly sensitive to low-temperature stress. During unheated greenhouse cultivation in winter and early spring, low temperatures act as a major abiotic stress factor that impairs physiological functions, restricts vegetative growth, and ultimately causes significant reductions in both yield and fruit quality (Blum and Jordan, 1985; Amin et al., 2022; Sun et al., 2024). Currently, one of the most effective strategies to improve cucumber yield under these adverse conditions is the use of cold-tolerant rootstocks. *Cucurbita* species used as rootstocks exhibit a higher capacity for water and nutrient uptake, as well as greater cytokinin production under low-temperature stress compared to cucumbers (Tachibana, 1988; Xiao et al., 2025). Consequently, numerous studies have reported that grafted cucumbers demonstrate significantly higher tolerance to cold stress than non-grafted varieties (Den Nijs, 1981; Tachibana, 1982; Bulder et al., 1991; Fu et al., 2024).

In this study, low-temperature stress tolerance in cucumber seedlings grafted onto interspecific *Cucurbita* rootstock candidates was comprehensively assessed by integrating morphological and biochemical indicators. Overall, the results indicate that visual morphological observations alone are insufficient and should be supported by physiological evidence to accurately determine tolerance to low-temperature stress. Based on the morphological responses observed in the classical low-temperature test and biochemical analyses, cucumber rootstock-scion combinations with *Cucurbita* rootstocks KB4 and KB5 were identified as the most physiologically tolerant. These combinations maintained cellular integrity by exhibiting the lowest levels of oxidative stress (H_2O_2 and MDA) without requiring excessive proline accumulation. Furthermore, KB20 rootstock demonstrated a highly stable, well-balanced performance, with strong concordance between superior visual scores and biochemical markers, indicating considerable potential for use as a commercial rootstock. Similarly, cucumber seedlings grafted onto the KB13 rootstock exhibited notable physiological stability, suggesting its reliability as a rootstock candidate.

While commercial rootstocks (particularly Shintoza F_1, Cremna F_1, and Cobalt F_1) exhibited high levels of H_2O_2 and MDA (indicating cell membrane damage) under low temperatures, several hybrid rootstock candidates, such as KB20, KB4, KB5, and KB13, successfully maintained these stress markers at significantly lower levels. Conversely, susceptible rootstock candidates such as KB6 (617%) and KB2 (577%) exhibited massive proline accumulation as a reactive metabolic response to severe cold stress. Although this indicates a strong activation of osmoprotective defense mechanisms, it reflects an effort to compensate for high oxidative damage rather than true low-temperature tolerance, distinguishing them from proactively tolerant genotypes like KB4 and KB5.

Non-grafted PTK 40 F_1 seedlings exhibited a markedly higher increase in H_2O_2 (404%) under low-temperature stress compared with most grafted combinations, whereas their stress-protective proline accumulation (24%) remained notably low. These findings indicate that non-grafted cucumber plants are highly susceptible to cold stress and that grafting onto rootstocks effectively mitigates oxidative stress (H_2O_2 and MDA) while enhancing plant defense mechanisms.

Based on the findings of this study, KB20, KB4, and KB5 appear to be the most promising rootstocks for grafted cucumber production under low-temperature conditions (Fig. 2). In the near future, these local *Cucurbita* rootstocks are expected to be registered and introduced into commercial cucumber seedling production.

Acknowledgements

This study was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) under the TEYDEB Grant Programme (Project No: 3230319). The financial support and valuable contributions of TÜBİTAK are gratefully acknowledged. The findings of this study were presented orally at the V. International Plant Breeding Congress, held December 1-5, 2025.

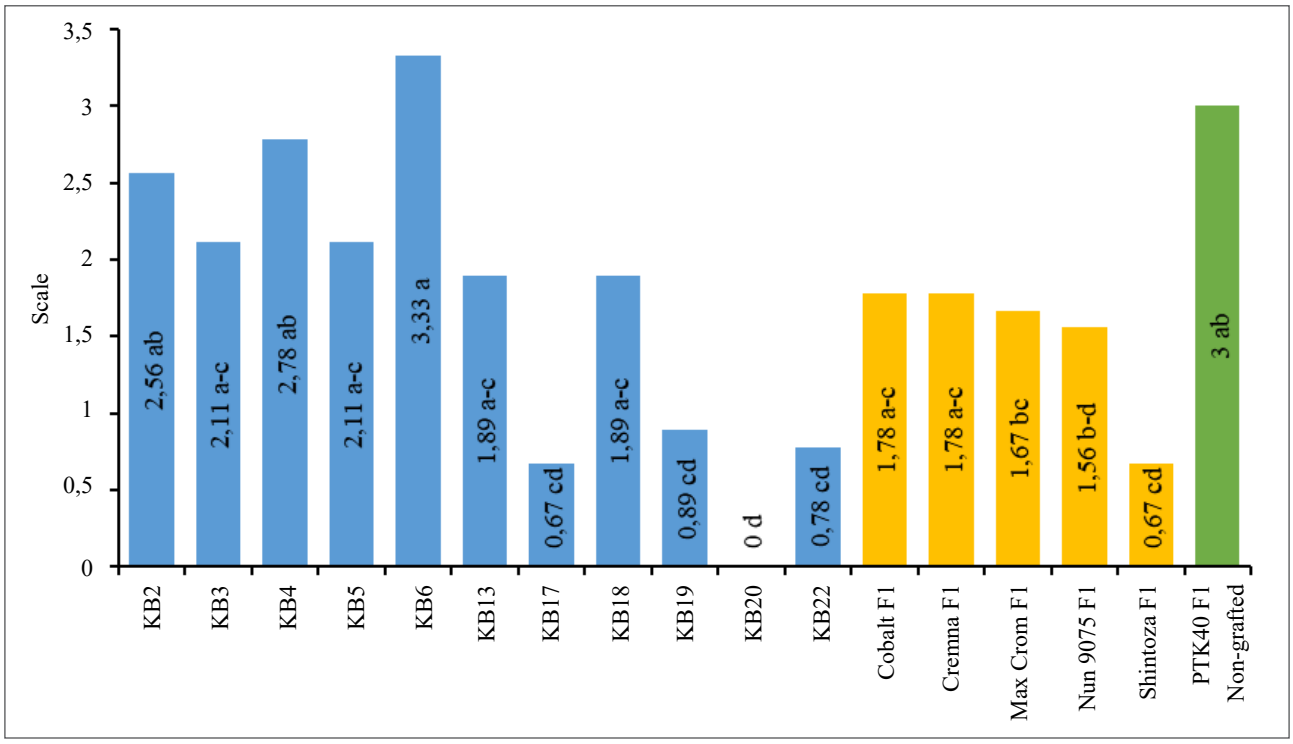


Figure 1. Scale scores representing low-temperature tolerance levels of interspecific hybrid rootstock candidates and commercial rootstocks (Different letters indicate significant differences at $P < 0.05$).

Figure 2. Morphological appearance of grafted cucumbers using KB20, KB5, and KB4 rootstocks under low temperature stress.

Table 1. Effects of low-temperature stress on hydrogen peroxide (H_2O_2) content of different rootstocks and cucumber seedlings (nmole g^{-1} FW).

	Treatment			
Rootstocks/Cultivars	**Low Temperature**	**Control**	**Mean**	**Increase (%)**
KB2	1369.32 d-h	808.73 ı-m	1089.03 g-ı	69
KB3	1165.13 g-j	437.06 lm	801.09 ı-k	167
KB4	731.62 j-m	581.32 k-m	656.47 k	26
KB5	1029.39 g-k	805.18 ı-m	917.28 h-k	28
KB6	2533.38 a	1128.35 g-j	1830.86 b	125
KB13	1147.01 g-j	902.89 g-l	1024.95 g-j	27
KB17	1752.49 c-e	586.29 k-m	1169.39 e-h	199
KB18	1751.42 c-e	1240.81 e-j	1496.12 c-e	41
KB19	1871.88 b-d	1414.57 d-g	1643.22 b-d	32
KB20	1218.96 f-j	1058.53 g-k	1138.74 f-h	15
KB22	1721.04 c-f	882.64 h-l	1301.84 e-g	95
Cobalt F_1	1058.88 g-k	753.30 j-m	906.09 h-k	41
Cremna F_1	1137.94 gj	352.49 m	745.22 jk	223
Max Crom F_1	2311.07 ab	1280.61 e-ı	1795.84 bc	80
Nun 9075 F_1	2449.11 a	2394.21 a	2421.66 a	2
Shintoza F_1	2164.49 a-c	832.89 ı-m	1498.69 c-e	160
PTK40 F_1 (Non-grafted)	2392.61 a	474.37 lm	1433.49 d-f	404
P	0.0001		0.0001	

Means followed by different letters in the same column are significantly different according to Tukey's test ($P < 0.05$). Analysis of variance (ANOVA) showed high significance for treatments ($P < 0.001$).

Table 2. Effects of low-temperature stress on malondialdehyde (MDA) content of different rootstocks and cucumber seedlings (nmole g^{-1} FW).

	Treatment			
Rootstocks/Cultivars	**Low Temperature**	**Control**	**Mean**	**Increase (%)**
KB2	12.40 c-f	8.39 f-h	10.40 ef	48
KB3	10.91 c-h	8.17 f-h	9.54 ef	34
KB4	10.73 d-h	8.30 f-h	9.52 ef	29
KB5	9.99 d-h	7.74 f-h	8.87 f	29
KB6	15.27 a-d	8.33 f-h	11.80 c-f	83
KB13	10.73 d-h	8.34 f-h	9.54 ef	29
KB17	16.26 a-c	7.94 f-h	12.10 b-f	105
KB18	11.04 c-h	7.10 f-h	9.07 ef	56
KB19	11.87 c-h	7.05 gh	9.46 ef	68
KB20	12.38 c-g	10.47 d-h	11.42 d-f	18
KB22	20.39 a	9.26 e-h	14.82 a-d	120
Cobalt F_1	18.85 ab	10.40 d-h	14.62 a-d	81
Cremna F_1	19.78 a	11.19 c-h	15.49 ab	77
Max Crom F_1	18.74 ab	11.58 c-h	15.16 a-c	62
Nun 9075 F_1	14.08 b-e	10.71 d-h	12.39 a-e	31
Shintoza F_1	20.25 a	11.20 c-h	15.73 a	81
PTK40 F_1 (Non-grafted)	11.58 c-h	6.58 h	9.08 ef	76
P	0.0001		0.0001	

Means followed by different letters in the same column are significantly different according to Tukey's test ($P < 0.05$). Analysis of variance (ANOVA) showed high significance for treatments ($P < 0.001$).

Table 3. Effects of low-temperature stress on proline content of different rootstocks and cucumber seedlings (nmole g^{-1} FW).

	Treatment			
Rootstocks/Cultivars	**Low Temperature**	**Control**	**Mean**	**Increase (%)**
KB2	41.22 a	6.09 e-k	23.65 a	577
KB3	6.12 e-k	3.31 h-k	4.71 f	85
KB4	9.87 d-g	3.86 h-k	6.86 d-f	156
KB5	15.87bc	10.60 c-f	13.23 b	50
KB6	38.07 a	5.31 f-k	21.69 a	617
KB13	11.26 c-e	7.12 e-k	9.19 cd	58
KB17	8.34 d-ı	5.88 e-k	7.11 c-f	42
KB18	17.68 b	3.40 h-k	10.54 bc	420
KB19	8.80 d-h	5.38 f-k	7.09 c-f	64
KB20	6.79 e-k	5.52 f-k	6.16 d-f	23
KB22	6.43 e-k	4.40 g-k	5.42 f	46
Cobalt F_1	6.87 e-k	2.09 jk	4.48 f	229
Cremna F_1	6.75 e-k	1.88 k	4.31 f	259
Max Crom F_1	7.60 d-j	1.91 k	4.76 f	298
Nun 9075 F_1	13.06 b-d	5.03 f-k	9.04 c-e	159
Shintoza F_1	6.13 e-k	3.08 ı-k	4.61 f	99
PTK40 F_1 (Non-grafted)	6.10 e-k	4.92 f-k	5.51 ef	24
P	0.0001		0.0001	

Means followed by different letters in the same column are significantly different according to Tukey's test ($P < 0.05$). Analysis of variance (ANOVA) showed high significance for treatments ($P < 0.001$).

References

Amin, B., Atif, M. J., Meng, H., Ali, M., Li, S., Alharby, H. F., Majrashi, A., Hakeem, K. R., and Cheng, Z. (2022). Melatonin rescues photosynthesis and triggers antioxidant defense response in *Cucumis sativus* plants challenged by low temperature and high humidity. *Frontiers in Plant Science*, *13*, 855900. https://doi.org/10.3389/fpls.2022.855900

Ashraf, M., Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, *59*(2), 206-216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Balkaya, A. (2014). Aşılı sebze üretiminde kullanılan anaçlar. *TÜRKTOB Türkiye Tohumcular Birliği Dergisi*, *3*(10), 4-7. (In Turkish)

Blum, A., Jordan, W. R. (1985). Breeding crop varieties for stress environments. *Critical Reviews in Plant Sciences*, *2*(3), 199-238. https://doi.org/10.1080/07352688509382196

Bulder, H. A. M., den Nijs, A. P. M., Speek, E. J., Hasselt, P. R., and Kuiper, P. J. C. (1991). The effect of low root temperature on growth and lipid composition of low temperature tolerant rootstock genotypes for cucumber. *Journal of Plant Physiology*, *38*(6), 661-666. https://doi.org/10.1016/S0176-1617(11)81312-X

Campos, P. S., Quartin, V., Ramalho, J. C., and Nunes, M. A. (2003). Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. plants. *Journal of Plant Physiology*, *160*(3), 283-292. https://doi.org/10.1078/0176-1617-00833

den Nijs, A.P. M. (1981). Adaptation of the glasshouse cucumber to lower temperatures in winter by breeding. *Acta Horticulturae*, *118*, 65-72. https://doi.org/10.17660/ActaHortic.1981.118.8

Food and Agriculture Organization of the United Nations. (2025). *FAOSTAT* [Data set]. Retrieved January 15, 2025, from https://www.fao.org/faostat

Fu, X., Feng, Y., Zhang, Y., Bi, H., and Ai, X. (2024). Salicylic acid improves chilling tolerance via CsNPR1–CsICE1 interaction in grafted cucumbers. *Horticulture research*, *11*(10), uhae231. https://doi.org/10.1093/hr/uhae231

Ghosh, U. K., Islam, M. N., Siddiqui, M. N., Cao, X., and Khan, M. A. R. (2022). Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. *Plant Biology*, *24*(2), 227-239. https://doi.org/10.1111/plb.13363

Gill, S. S., Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*(12), 909-930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gomez, K. A., Gomez, A. A. (1984). Statistical procedures for agricultural research. John Wiley and Sons.

Güneri Bağcı, E. (2010). Determination of oxidative stress induced by drought in chickpea (*Cicer arietinum* L.) cultivars by physiological and biochemical parameters [Doctoral dissertation, Ankara University]. (In Turkish)

Hasanuzzaman, M., Bhuyan, M. H. M. B., Zulfiqar, F., Raza, A., Mohsin, S. M., Mahmud, J. A., Fujita, M., and Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*, *9*(8), 681. https://doi.org/10.3390/antiox9080681

Heath, R. L., Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, *125*(1), 189-198. https://doi.org/10.1016/0003-9861(68)90654-1

Hodges, D. M., DeLong, J. M., Forney, C. F., and Prange, R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, *207*(4), 604-611. https://doi.org/10.1007/s004250050524

Huang, Y., Li, J., Hua, B., Liu, Z., Fan, M., and Bie, Z. (2013). Grafting onto different rootstocks as a means to improve watermelon tolerance to low potassium stress. *Scientia Horticulturae*, *149*, 80-85. https://doi.org/10.1016/j.scienta.2012.02.009

İpek, E., Bahadır, S., Yapici, B., Atasoy, S., and Balkaya, A. (2025). Düşük sıcaklığa toleranslı türler arası hibrit kabak anaçlarının aşılı hıyar fide üretimindeki kullanılabilirliğinin değerlendirilmesi. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 30*(3), 692-702. https://doi.org/10.37908/mkutbd.1695923

Karaağaç, O., Kar, H., Murat Doğru, Ş., Özbakır Özer, M., and Demir, E. (2017). *Örtüaltı hıyar (Cucumis sativus L.) yetiştiriciliğine uygun düşük sıcaklığa toleranslı yerli hibrit kabak (Cucurbita spp.) anaçlarının geliştirilmesi ve düşük sıcaklığa dayanıklılığın fizyolojik ve biyokimyasal düzeyde incelenmesi* (Project No. 114O843). TÜBİTAK.

Kaur, G., Asthir, B. (2015). Proline: a key player in plant abiotic stress tolerance. *Biologia Plantarum*, *59*(4), 609-619. https://doi.org/10.1007/s10535-015-0549-3

Kozak, M., Piepho, H. P. (2018). What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. *Journal of Agronomy and Crop Science*, *204*(1), 86-98.

Lee, J. M., Oda, M. (2003). Grafting of herbaceous vegetable and ornamental crops. *Horticultural Reviews*, *28*, 61-124.

Lee, J. M., Kubota, C., Tsao, S. J., Bie, Z., Echevarria, P. H., Morra, L., and Oda, M. (2010). Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Scientia Horticulturae*, *127*(2), 93-105. https://doi.org/10.1016/j.scienta.2010.08.003

Li, Y., Tian, X., Wei, M., Shi, Q., Yang, F., and Wang, X. (2015). Mechanisms of tolerance differences in cucumber seedlings grafted on rootstocks with different tolerance to low temperature and weak light stresses. *Turkish Journal of Botany*, *39*(4), 606-614. https://doi.org/10.3906/bot-1404-115

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, *7*(9),405-410.

Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351-358. https://doi.org/10.1016/0003-2697(79)90738-3

Schwarz, D., Rouphael, Y., Colla, G., and Venema, J. H. (2010). Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. *Scientia Horticulturae*, *127*(2), 162-171. https://doi.org/10.1016/j.scienta.2010.09.016

Slesak, I., Libik, M., Karpinska, B., Karpinski, S., and Miszalski, Z. (2007). The role of hydrogen peroxide in regulation of plant metabolism and cellular signalling in response to environmental stresses. *Acta Biochimica Polonica*, *54*(1), 39-50.

Smeets, L., Wehner, T. C. (1997). Environmental effects on genetic variation of chilling resistance in cucumber. *Euphytica*, *97*(2), 217-225. https://doi.org/10.1023/A:1003084821178

Sun, S., Yang, Y., Hao, S., Liu, Y., Zhang, X., Zhang, X., and Luo, Y. (2024). Comparison of transcriptome and metabolome analysis revealed cold-resistant metabolic pathways in cucumber roots under low-temperature stress in root zone. *Frontiers in Plant Science*, *15*, 1413716. https://doi.org/10.3389/fpls.2024.1413716

Szabados, L., Savouré, A. (2010). Proline: a multifunctional amino acid. *Trends in Plant Science*, *15*(2), 89-97.

Tachibana, S. (1982). Comparison of effects of root temperature on growth and mineral nutrition of cucumber cultivars and figleaf gourd. *Journal of the Japanese Society for Horticultural Science*, *51*(3), 299-308. https://doi.org/10.2503/jjshs.51.299

Tachibana, S. (1988). Cytokinin concentrations in roots and root xylem exudate of cucumber and figleaf gourd as affected by root temperature. *Journal of the Japanese Society for Horticultural Science*, *56*(4), 417-425. https://doi.org/10.2503/jjshs.56.417

Turkish Statistical Institute. (2024). *Crop production statistics* [Data set]. Retrieved January 15, 2025, from https://data.tuik.gov.tr/

Xiao, H., Deng, W., Ahmad, B., Guo, C., Shi, S., He, S., and Yang, Z. A. (2025). *Cucurbita ficifolia* rootstock enhances resistance to low-temperature stress in cucumber. *Horticulturae*, *11*(3), 242. https://doi.org/10.3390/horticulturae11030242

Yücel, Ş., Karaağaç, O., Balkaya, A., and Kandemir, D. (2022). Aşılı fide üretiminde kullanılan anaçlar. In H. Yetişir and Ş. Ş. Ellialtıoğlu (Eds.), *Sebzelerde Fide Yetiştiriciliği-2* (pp. 399-490). Gece Kitaplığı.

Zhou, Y. H., Huang, L. F., Zhang, Y. L., Shi, K., Yu, J. Q., and Nogués, S. (2007). Chill-induced decrease in capacity of RuBP carboxylation and associated H_2O_2 accumulation in cucumber leaves are alleviated by grafting onto figleaf gourd. *Annals of Botany*, *100*(4), 839-848. https://doi.org/10.1093/aob/mcm181

Zulfiqar, F., Akram, N. A., and Ashraf, M. (2020). Osmoprotection in plants under abiotic stresses: new insights into a classical phenomenon. *Planta*, *251*(1), 3. https://doi.org/10.1007/s00425-019-03293-1

Research Article

Ekin
Journal of Crop Breeding and Genetics

12(2):96-102, 2026

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Genotype-dependent *In Vitro* Propagation Responses of Newly Registered Turkish Hazelnut Cultivars(§)

Merve ŞEKERLİ[1*] **Dilek BAYRAMİN[1]** **Hüseyin DOKU[1]**

[1] Hazelnut Research Institute, Giresun, Türkiye

[*] Corresponding author e-mail: mervesekerli@gmail.com

Citation:
Şekerli M., Bayramin D., Doku H., 2026. Genotype-dependent *In Vitro* Propagation Responses of Newly Registered Turkish Hazelnut Cultivars. Ekin J. 12(2):96-102.

Received: 09.06.2026 ***Accepted: 15.07.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

Hazelnut (*Corylus avellana* L.) genetic resources are essential for biodiversity conservation and breeding programs. Recently registered cultivars with valuable agronomic traits require efficient clonal propagation systems to ensure the production of disease-free planting material and the establishment of uniform orchards. Although micropropagation offers a promising alternative to conventional propagation methods, hazelnut remains a recalcitrant species due to low regeneration capacity and frequent latent contamination. In this study, four newly registered Turkish hazelnut cultivars (Okay 28, Allahverdi, Çetiner, and Süslü) were evaluated for their *in vitro* propagation responses. Axillary nodal explants collected between May and August 2025 were surface-sterilized and cultured on Nas and Read Medium (NRM) supplemented with 5 mg L^{-1} BA and 0.01 mg L^{-1} IBA. After four weeks, contamination and shoot induction rates were recorded. The highest contamination levels were observed in 'Okay 28' (84%) and 'Çetiner' (85%), whereas 'Süslü' (23%) and 'Allahverdi' (39%) showed the lowest contamination. Correspondingly, 'Süslü' and 'Allahverdi' exhibited the highest shoot induction rates (56% and 44%, respectively). During multiplication, 'Süslü' produced the highest number of shoots per explant (2.3) and the greatest shoot length (4.6 cm). Rooting on NRM supplemented with 2 mg L^{-1} IBA revealed significant genotype-dependent variation. This study represents the first report on the *in vitro* propagation of these newly registered hazelnut cultivars and highlights the importance of genotype-specific optimization for efficient micropropagation and germplasm conservation.

Keywords: *Corylus avellana,* hazelnut, micropropagation

Introduction

Hazelnut (*Corylus avellana* L.) is among the most economically significant nut crops worldwide, with Türkiye serving as the leading producer and exporter, supplying approximately 61% of global hazelnut production (FAOSTAT, 2024). Hazelnut cultivation is the principal livelihood and an essential economic pillar of the Black Sea region. Türkiye's diverse ecological conditions have facilitated the accumulation of rich *Corylus* germplasm, resulting in extensive genetic variability and numerous locally adapted landraces. This genetic wealth forms the backbone of breeding programs aimed at improving yield, kernel quality, and resilience to biotic and abiotic stress factors.

The Hazelnut Research Institute (HRI) in Giresun maintains the national hazelnut germplasm collection, comprising 459 *C. avellana* genotypes originating from different production zones (Şekerli, 2024). These genotypes represent a valuable reservoir for conservation, breeding, and biotechnological studies. However, conventional propagation method by suckers, it is slow, seasonal, and susceptible to pathogen transmission, while also showing considerable variability among propagules (Beyhan et al., 2018). Consequently, there is a growing need for efficient *in vitro* propagation systems that enable the rapid and reliable multiplication of elite cultivars while preserving genetic fidelity.

(§) *This study was presented as an oral presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

Plant tissue culture provides a powerful platform for the clonal propagation, conservation, and improvement of woody species. Although several studies have reported *in vitro* propagation of Turkish cultivars such as 'Tombul', 'Çakıldak', and 'Foşa' (Şekerli, 2024; Elçiçek 2020), no data are available regarding the micropropagation responses of recently registered cultivars developed through national breeding efforts. Currently, 23 hazelnut cultivars are officially registered in Türkiye, each with distinct agronomic properties and adaptation capacities (HRI, 2026). Among these, four recently registered cultivars have not previously been evaluated under *in vitro* conditions. Understanding the morphogenic responses of these newly released cultivars under controlled culture conditions is therefore critical for establishing robust propagation protocols and enhancing the management of hazelnut genetic resources.

In this study, four recently registered Turkish hazelnut cultivars with distinct agronomic traits were evaluated. 'Okay 28', developed through breeding programs at HRI, is noted for its larger nut and kernel size compared with the widely cultivated 'Tombul' (Balık et al., 2015). The 'Allahverdi' cultivar, obtained through selection breeding and registered in 2015, displays a leafing time that is on average 10-15 days later than that of 'Tombul' (Balık et al., 2016). 'Çetiner' registered in 2022, is the earliest leafing cultivar among all examined varieties and exhibits a comparable kernel ratio along with reduced susceptibility to biennial bearing (Bektaş and Çil 2023). The most recently registered cultivar, 'Süslü' (TTSM, 2025), is a red-leaved ornamental hazelnut with distinctive husk coloration, contributing both genetic and aesthetic diversity to Turkish hazelnut germplasm.

The aim of this study was to evaluate the *in vitro* shoot induction and proliferation responses of these four new Turkish hazelnut cultivars cultured on Nas and Read medium (NRM) (Nas and Read, 2004). Genotype-specific differences were assessed to determine optimal conditions for high-quality shoot production. The findings provide a foundation for large-scale propagation of these cultivars and contribute to the long-term conservation and utilization of Türkiye's hazelnut genetic resources.

Materials and Methods

Plant Materials

One-year-old shoots were collected from approximately 10-year-old field-grown mother trees of the hazelnut cultivars 'Okay 28', 'Allahverdi', 'Çetiner', and 'Süslü' maintained in the germplasm collection orchard of the Hazelnut Research Institute (HRI), Giresun, Türkiye. Explant collection was carried out during the first weeks of June, July, and August 2025. During each sampling period, shoots from all four cultivars were collected and used as biological replicates for the study. The mother trees were pruned in March 2025 to promote the development of vigorous current-season shoots suitable for *in vitro* culture. No fungicides, plant growth regulators, or other chemical treatments were applied to the mother trees prior to explant collection.

Surface Sterilization and Culture Initiation

After the leaves had been removed, the shoots were rinsed under running tap water for 1 hour. The shoots were cut into 2-3 cm nodal segments and surface-sterilized under a laminar flow hood by immersion in 70% ethanol for 30 seconds followed by 1% (v/v) sodium hypochlorite (NaOCl). Preliminary sterilization trials were conducted to optimize the exposure time, and a treatment time of 5 min was selected as the optimal condition for all subsequent experiments. The explants were then rinsed three times with sterile distilled water and blotted dry on sterile filter paper.

Following the surface sterilization procedure, the explants were cultured individually in 16 × 100 mm culture tubes containing 5 mL of Nas and Read Medium (NRM; Nas and Read, 2004) supplemented with 5 mg L^{-1} 6-benzyladenine (BA), 0.01 mg L^{-1} indole-3-butyric acid (IBA), 30 g L^{-1} sucrose, and 0.6% (w/v) plant agar (Duchefa). The pH of the medium was adjusted to 5.7 before the addition of agar, after which the medium was autoclaved. Cultures were maintained at 23 ± 2°C under a 16 h photoperiod with a light intensity of 40 µmol m^{-2} s^{-1}. After four weeks of culture, the percentages of contaminated explants and shoot induction were recorded (Figure 1).

Shoot Multiplication

At the end of the initiation stage, the original woody explants were discarded, and only newly developed, contamination-free microshoots approximately 2 cm in length and containing two to three nodes were selected for the multiplication stage. The basal leaves of the selected microshoots were removed prior to transfer to fresh culture medium. The microshoots were then transferred to fresh NRM with the same composition as described above.

Microshoots were cultured in culture jars containing 50 mL of medium, with five microshoots per jar. Three biological replicates were used for each cultivar, and each replicate consisted of four culture jars. The cultures were maintained under the same environmental conditions described for the initiation stage. Microshoots were transferred onto fresh medium of the same composition at four-week intervals without shoot excision or division.

After a total culture period of eight weeks, the number of shoots per explant and the mean shoot length were measured and recorded (Figure 2).

Root Induction

Microshoots longer than 4 cm obtained after two consecutive four-week subcultures in the multiplication stage were transferred to fresh NRM supplemented with 2 mg L^{-1} IBA, 30 g L^{-1} sucrose, and 0.6% (w/v) plant agar for root induction. The pH of the medium was adjusted to 5.7 before the addition of agar and autoclaving. After autoclaving, filter-sterilized IBA (0.22 µm membrane filter, Isolab) was aseptically added to the cooled medium.

Microshoots were cultured in culture jars containing 50 mL of medium, with five microshoots per jar. Three biological replicates were used for each cultivar, and each replicate consisted of four culture jars. Cultures were maintained under the same environmental conditions described above. After six weeks of culture, the rooting percentage, the mean number of roots per microshoot, and the length of the longest root were recorded (Figure 3).

Acclimatization

Rooted plantlets cultured for six weeks on rooting medium were transferred into paper pots containing a substrate mixture of peat:perlite (2:1:1, v/v/v). The paper pots were placed in transparent plastic containers with closed lids to maintain high relative humidity and kept in a growth chamber at 23 ± 1°C, 70% relative humidity, and a 16 h photoperiod. After four weeks, humidity was gradually reduced by progressively opening the container lids, which were completely removed after 30 days. Plantlets were fertilized weekly with Hoagland nutrient solution (Hoagland and Arnon, 1938). Following acclimatization, the plantlets were transferred to a temperature-controlled greenhouse maintained at 25 ± 2°C and equipped with an automated misting system**.** The plantlets were grown in seedling trays under low plastic tunnels to maintain high humidity and promote successful establishment.

Data Collection and Analysis

The experiments were conducted using a completely randomized design. For culture initiation, each explant was cultured individually in a separate culture tube, and each tube was considered an experimental unit. The initiation experiment consisted of three independent experimental runs, conducted during the first weeks of June, July, and August 2025, with 100 explants per cultivar in each run. For the multiplication and rooting experiments, five microshoots were cultured in each jar, and each culture jar was considered an experimental unit. For each cultivar, the experiment consisted of three independent experimental runs, with four jars per run, resulting in a total of 12 jars and 60 microshoots per cultivar. Percentage data, including contamination, shoot induction, and rooting percentages, were subjected to arcsine square-root transformation before analysis of variance. All data were analyzed by analysis of variance (ANOVA) using IBM SPSS Statistics version 18.0. Differences among cultivar means were compared using Tukey's honestly significant difference test at $p < 0.05$. Untransformed percentage means are presented in the tables and figures for ease of interpretation.

Results

Microbial contamination and shoot induction rates varied significantly among the cultivars tested ($p < 0.05$) (Figure 4). The highest contamination levels were observed in 'Okay 28' (84%) and 'Çetiner' (85%), while 'Süslü' (23%) and 'Allahverdi' (39%) exhibited the lowest levels. Consistent with this trend, 'Süslü' and 'Allahverdi' showed the highest shoot induction frequencies, 56% and 44%, respectively. In contrast, 'Okay 28' (7%) and 'Çetiner' (10%) demonstrated the lowest shoot formation rates.

Contamination-free explants were subsequently transferred to multiplication medium. Among the cultivars, 'Süslü' produced the highest mean number of shoots per explant (2.3) and the greatest shoot length (4.6 cm). 'Allahverdi', 'Okay 28', and 'Çetiner' generated 1.31, 1.02, and 0.78 shoots per explant, respectively (Figure 5). Shoot elongation followed a similar pattern: 'Allahverdi' reached an average shoot length of 3.51 cm, followed by 'Okay 28' (2.15 cm), whereas 'Çetiner' exhibited the shortest mean shoot length (2.05 cm) (Figure 5).

Rooting performance demonstrated substantial variation among the cultivars (Figure 6). 'Süslü' achieved the highest rooting success (90%), producing an average of 4.56 roots per micro-cutting. This was followed by 'Allahverdi' with 83% rooting and a mean of 3.51 roots per micro-cutting, and 'Okay 28' with 72% rooting and an average of 2.15 roots. 'Çetiner' exhibited the lowest rooting success (56%), with a mean of 2.05 roots per micro-cutting.

Discussion

Extensive breeding and selection programs in hazelnut have led to the development of several superior cultivars with improved agronomic traits. Rapid, disease-free, and clonal propagation of these cultivars is essential for their widespread adoption and for establishing uniform orchards. In Türkiye,

where propagation is largely carried out through suckers, conventional methods remain insufficient to meet the growing demand for high-quality planting material and are unsuitable for sustainable large-scale production. In contrast, micropropagation enables continuous, controlled, and clonal plant production throughout the year, making it a valuable tool for modern hazelnut cultivation.

In the present study, mature field-grown trees served as the source of explants. Consequently, the observed microbial contamination rates were higher than those reported in studies using juvenile material or plants grown under controlled conditions. This outcome is consistent with previous work demonstrating that the use of potted or greenhouse-grown donor plants significantly reduces both bacterial and fungal contamination during culture initiation (Messeguer and Mele 1983; Yu and Reed 1995; Bacchetta et al., 2008; Şekerli, 2024). Similarly, explants derived from mature trees often show lower establishment success compared with juvenile tissues, as reported in other woody species and in hazelnut (Bassil et al., 1991; Diaz-Sala et al., 1990; Messeguer and Mele, 1987). Studies comparing juvenile and mature nodal explants of *C. avellana* have consistently shown higher culture establishment and regeneration capacity in juvenile plant material, supporting the results of the current study.

Regeneration capacity in hazelnut is well known to be highly genotype-dependent, and previous studies have reported considerable variability among cultivars in both shoot induction and rooting responses (Hand et al., 2014; Akin et al., 2017). The findings of the present study are consistent with these observations. Among the cultivars evaluated, 'Süslü' exhibited the highest shoot and root induction capacity, whereas 'Okay 28' and 'Çetiner' displayed the lowest performance. 'Okay 28', one of the two Turkish cultivars developed through breeding programs (Balık et al., 2015), is primarily recognized for its large nut size but appears to possess limited regenerative capacity *in vitro*. The 'Allahverdi' cultivar, a registered variety developed through selection breeding, is distinguished by leaf emergence occurring approximately two weeks later than that of the reference cultivar 'Tombul' (Balık et al., 2016), and exhibited an intermediate regeneration performance among the four cultivars tested. Although the physiological basis of the superior regeneration performance of 'Süslü' remains unclear, genotypic differences in endogenous biochemical and hormonal profiles may contribute to the observed variation (Lupo et al., 2025). In line with this, other red-leaved hazelnut types, such as *Corylus maxima* Mill., have also been reported to synthesize substantial levels of flavonoids and diarylheptanoids with notable bioactive properties (Riethmüller et al., 2015; Felegyi-Tóth et al., 2022). The red-leaved phenotype and ornamental value of 'Süslü' further underscore its potential as a promising candidate for both horticultural and biotechnological applications. Overall, the genotype-dependent variation observed in this study highlights the necessity of cultivar-specific optimization when developing micropropagation protocols for hazelnut.

Conclusions

This study presents the first report on the *in vitro* propagation responses of the newly registered Turkish hazelnut cultivars 'Okay 28', 'Allahverdi', 'Çetiner', and 'Süslü'. The successful regeneration of contamination-free plant material from these cultivars provides a valuable foundation for future tissue culture and biotechnology research. The ability to obtain clean, clonal stock material is essential for breeding programs, conservation strategies, and large-scale propagation efforts aimed at supporting Türkiye's hazelnut industry. Further optimization studies focusing on medium composition, growth regulators, and rooting conditions will help enhance the efficiency and reliability of propagation protocols for these important cultivars.

Acknowledgements

An earlier version of this work was published as an abstract in the *Abstract Book* of the 5th International Plant Breeding Congress**,** held on December 1-5, 2025**,** in Antalya, Türkiye**.**

Funding

The authors received no specific funding for this work. The study was conducted using the institutional facilities and resources of the General Directorate of Agricultural Research and Policies (TAGEM), Türkiye.

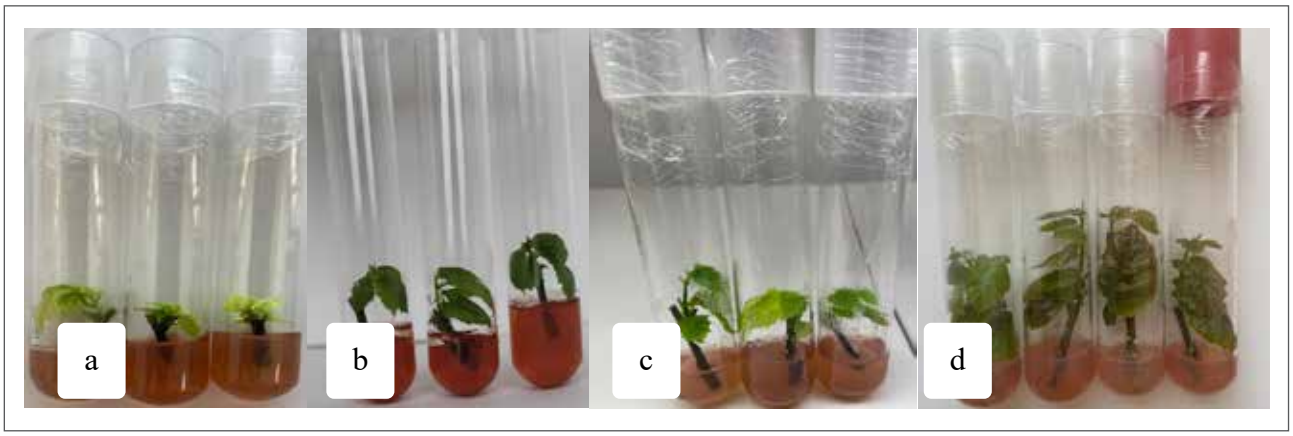


Figure 1. Culture initiation from axillary nodal explants. a) 'Okay 28' b) 'Allahverdi' c) 'Çetiner' d) 'Süslü'.

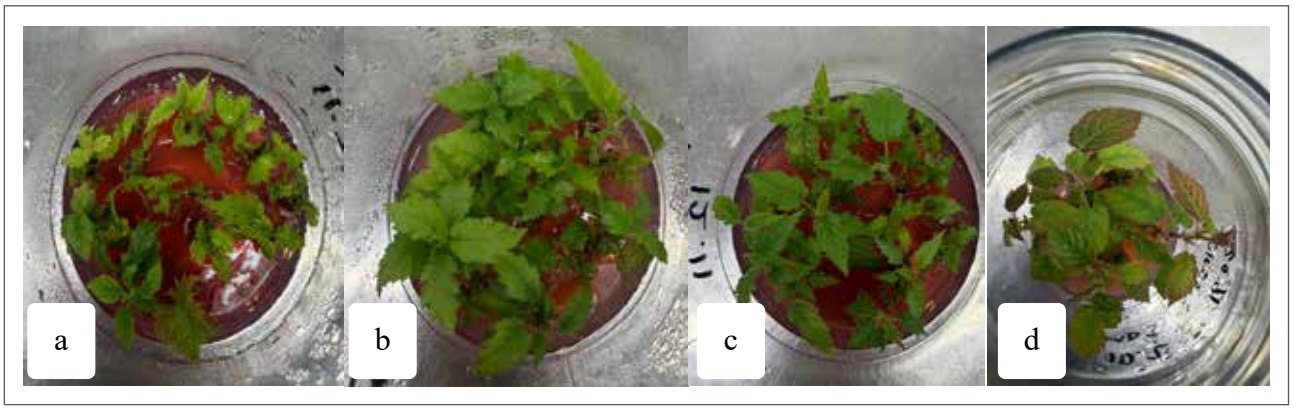


Figure 2. Shoot multiplication of four cultivars. a) 'Okay 28' b) 'Allahverdi' c) 'Çetiner' d) 'Süslü'

Figure 3. *In vitro* rooting of 'Süslü' cultivar.

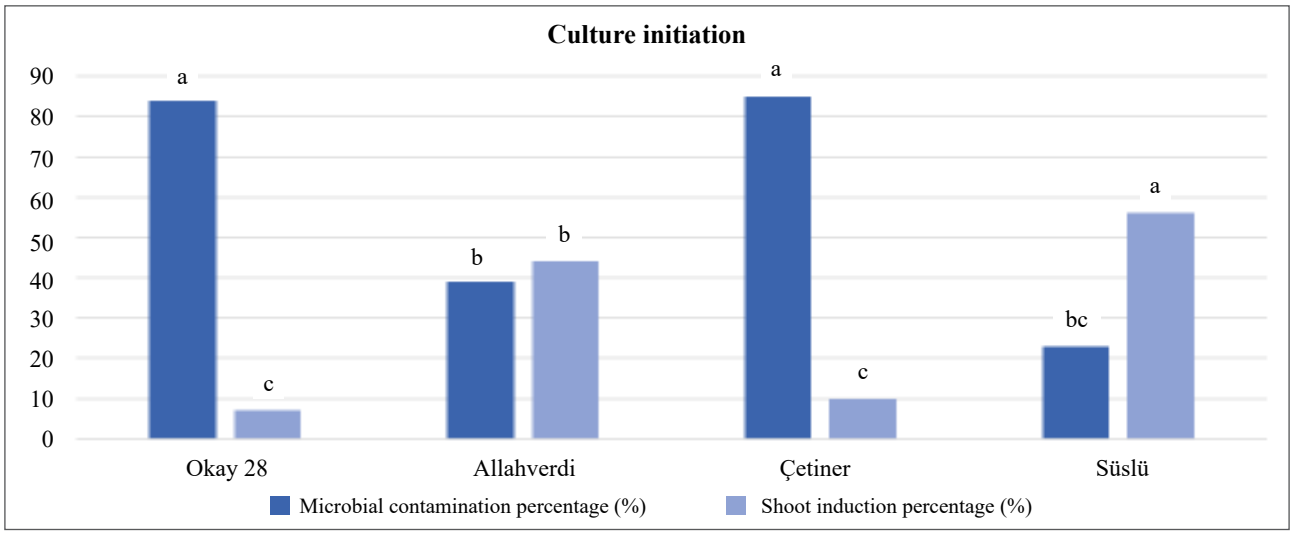


Figure 4. Visual microbial contamination and shoot induction percentage of four cultivars. Different uppercase letters indicate significant differences between genotypes (Tukey's HSD test at $p < 0.05$).

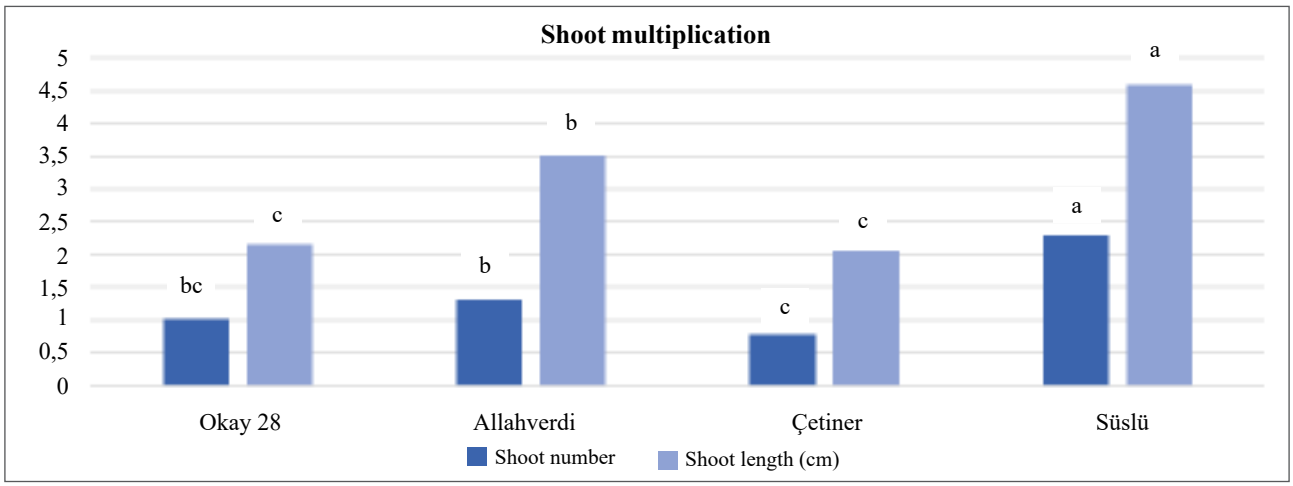


Figure 5. Average shoot number and shoot length of four cultivars in multiplication medium for eight weeks. Different uppercase letters indicate significant differences between genotypes (Tukey's HSD test at $p < 0.05$).

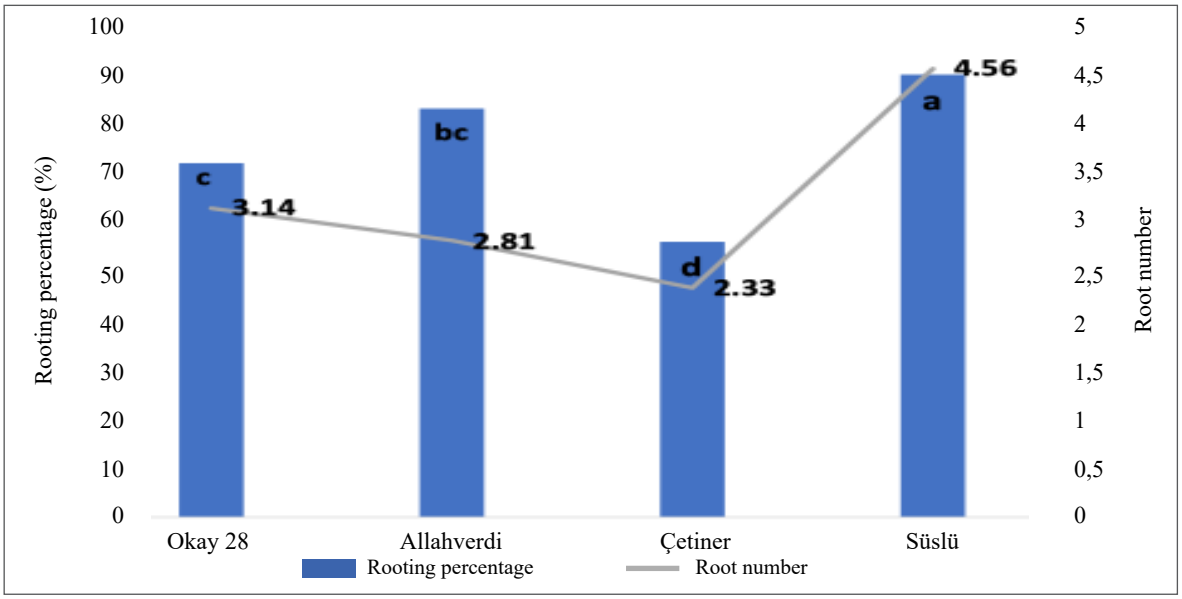


Figure 6. Rooting percentage and average root numbers of four cultivars in rooting medium for six weeks. Different uppercase letters indicate significant differences between genotypes (Tukey's HSD test at $p < 0.05$).

References

Akin, M., Eyduran, E., Niedz, R. P., and Reed, B. M. (2017). Developing hazelnut tissue culture medium free of ion confounding. *Plant Cell, Tissue and Organ Culture*, *130*(3), 483-494. https://doi.org/10.1007/s11240-017-1238-z

Bacchetta, L., Aramini, M., Bernardini, C., and Rugini, E. (2008). *In vitro* propagation of traditional Italian hazelnut cultivars as a tool for the valorization and conservation of local genetic resources. *HortScience*, *43*(2), 562-566.

Balık, H. I., Balık, S. K., Beyhan, N., and Erdoğan, V. (2016). *Hazelnut cultivars*. Republic of Turkey Ministry of Food, Agriculture and Livestock, General Directorate of Agricultural Research and Policies.

Balık, H., Balık, S. K., and Okay, A. (2015). Yeni fındık çeşitleri (Okay 28 ve Giresun Melezi) [New hazelnut cultivars (Okay 28 and Giresun Melezi)]. *Harran Tarım ve Gıda Bilimleri Dergisi*, *19*(2), 104-109. [in Turkish]

Bassil, N. V., Mok, D. W. S., Mok, M. C., and Rebhuhn, B. J. (1991). Micropropagation of the hazelnut, *Corylus avellana* L. *Acta Horticulturae*, *300*, 137-140.

Bektaş, A., and Çil, D. (2023). 'Çetiner' fındık (*Corylus avellana* L.) çeşidinin fenolojik, pomolojik ve morfolojik özellikleri. *Akademik Ziraat Dergisi*, *12*(Special Issue), 153-158. https://doi.org/10.29278/azd.1366750 [in Turkish]

Beyhan, N., Aci, F., and Balık, H. I. (2018). Propagation of some Turkish hazelnut cultivars by stooling. *Acta Horticulturae*, *1281*, 15-22. https://doi.org/10.17660/ActaHortic.2020.1281.3

Diaz-Sala, C., Rey, M., and Rodriguez, R. (1990). *In vitro* establishment of a cycloclonal chain from nodal segments and apical buds of adult hazel (*Corylus avellana* L.). *Plant Cell, Tissue and Organ Culture*, *23*(3), 151-157.

Elçiçek, M. (2020). *Foşa, çakıldak ve tombul fındık çeşitlerinin in vitro koşullarda kitlesel üretimi* (Publication No. 633688) [Master's thesis, Çukurova University]. Council of Higher Education Thesis Center. [in Turkish]

FAOSTAT. (2024). *Food and Agriculture Organization of the United Nations*. http://faostat.fao.org/ (Retrieved November 15, 2025)

Felegyi-Tóth, C. A., Tóth, Z., Garádi, Z., Boldizsár, I., Nedves, A. N., Simon, A., and Riethmüller, E. (2022). Membrane permeability and aqueous stability study of linear and cyclic diarylheptanoids from *Corylus maxima*. *Pharmaceutics*, *14*(6), Article 1250.

Hand, C., Maki, S., and Reed, B. M. (2014). Modeling optimal mineral nutrition for hazelnut micropropagation. *Plant Cell, Tissue and Organ Culture*, *119*(2), 411-425. https://doi.org/10.1007/s11240-014-0544-y

Hoagland, D. R., and Arnon, D. I. (1938). *The water-culture method for growing plants without soil* (Circular No. 347). California Agricultural Experiment Station, University of California.

HRI (Hazelnut Research Institute). (2026). *Fındık çeşitlerimiz*. https://arastirma.tarimorman.gov.tr/findik/Menu/47/Findik-Cesitlerimiz (Retrieved July 14, 2026) [in Turkish]

Lupo, M., Alfieri, G., Filippi, S., Modesti, M., Brunori, E., Pacchiarelli, A., and Silvestri, C. (2025). Red-leaf hazelnut: Biotechnological approaches for secondary metabolite production and potential biological activities. *Plant Physiology and Biochemistry*, *229*, Article 110329.

Messeguer, J., and Melé, E. (1983). Clonal propagation of *Corylus avellana* L. *in vitro*. In *Atti del Convegno Internazionale sul Nocciuolo* (pp. 293-295). Avellino, Italy.

Messeguer, J., and Melé, E. (1987). *In vitro* propagation of adult material and seedlings of *Corylus avellana*. *Acta Horticulturae*, *212*, 499-504. https://doi.org/10.17660/ActaHortic.1987.212.76

Nas, M. N., and Read, P. E. (2004). A hypothesis for the development of a defined tissue culture medium of higher plants and micropropagation of hazelnuts. *Scientia Horticulturae*, *101*(1-2), 189-200. https://doi.org/10.1016/j.scienta.2003.10.004

Riethmüller, E., Tóth, G., Alberti, Á., Végh, K., Burlini, I., Könczöl, Á., and Kéry, Á. (2015). First characterisation of flavonoid- and diarylheptanoid-type antioxidant phenolics in *Corylus maxima* by HPLC-DAD-ESI-MS. *Journal of Pharmaceutical and Biomedical Analysis*, *107*, 159-167.

Şekerli, M. (2024). Season, thermotherapy and surface sterilization play important roles in microbial contamination of hazelnut *in vitro* cultures. *Plant Cell, Tissue and Organ Culture*, *157*(3), Article 70.

TTSM (Tohumluk Tescil ve Sertifikasyon Merkez Müdürlüğü). (2025). *Meyve ve asma tescil raporu* [Fruit and vine registration report]. https://www.tarimorman.gov.tr/BUGEM/TTSM/Sayfalar/Detay.aspx?SayfaId=212 [in Turkish]

Yu, X., and Reed, B. M. (1995). A micropropagation system for hazelnuts (*Corylus* species). *HortScience*, *30*(1), 120-123.

Research Article

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Ekin
Journal of Crop Breeding and Genetics

12(2):103-109, 2026

Molecular Resistance Determination of Different Eggplant (*Solanum melongena* L.) Genetic Resources and Breeding lines against Fusarium Wilt(§)

Ayşe KAHRAMAN[1*] **Seyfullah BİNBİR**[1] **Tamer BAYTIN**[1]

[1] Horticulture Department, Vegetable Section Aegean Agricultural Research Institute, PO Box 9 35661, İzmir, Türkiye

[*] Corresponding author e-mail: aysekahraman2@gmail.com

Citation:
Kahraman A., Binbir S., Baytın T., 2026. Molecular Resistance Determination of Different Eggplant (*Solanum melongena* L.) Genetic Resources and Breeding lines against Fusarium Wilt. Ekin J. 12(2):103-109.

Received: 30.06.2026 ***Accepted: 23.07.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

Eggplant (*Solanum melongena* L.) is a major vegetable crop with great potential for genetic improvement owing to its large and mostly untapped genetic diversity. Türkiye is the 4th country with the highest production amount among the world's eggplant producing countries. The most important factor limiting eggplant production is its susceptibility to soil-borne diseases such as Fusarium and Verticillium wilt. Resistance to Fusarium wilt in eggplant is monogenic. In the Aegean Agricultural Research Institute, vegetable genetic resources are used extensively in both open-pollinated and hybrid variety breeding studies. In the present study, a total of 48 eggplant genotypes, including F_4 breeding lines and landraces from the institute's gene pool, were screened for resistance to Fusarium wilt using the PCR-based SCAR426 molecular marker. All landraces and 23 breeding lines lacked the resistance-associated allele and were classified as susceptible based on marker analysis. Among the tested materials, only one inbred line ETA5 was found to carry the resistance allele. As classical phenotypic screening was not performed in the present study, the resistance indicated by the SCAR426 marker for ETA5 requires confirmation through classical disease resistance assays. This genotype could represent an alternative source of resistance and may be valuable for future eggplant breeding programs.

Keywords: SCAR426, molecular evaluation, *Solanum melongena*, Fusarium wilt, sources

Introduction

Eggplant (*Solanum melongena* L.) is one of the four most important *Solanaceous* crops and is widely cultivated and consumed in Asia, the Mediterranean basin, and Southeast Europe. From the 1970s onward, traditional summer open-field eggplant production progressively shifted toward year-round intensive cultivation systems. In Turkiye, a total of 115 eggplant varieties were officially registered as of 2025. These registered varieties include both open-pollinated and hybrid types; however, the majority are hybrids, while only 8% are open-pollinated varieties. Additionally, 13% of the registered varieties are rootstock types, which are mainly interspecific hybrids or hybrids involving *Solanum torvum* (Gökseven and Akbudak 2022; TTSM, 2026).

Eggplant is susceptible to various biotic stresses, including nematodes, wilt diseases, eggplant fruit and shoot borer, two-spotted spider mites, whiteflies, and aphids. These factors cause significant reductions in yield, fruit quality, shelf life, and nutritional content (Arafa et al., 2022). One of the most important factors limiting eggplant production is its susceptibility to soil-borne diseases, particularly Fusarium and Verticillium wilts. Fusarium wilt of eggplant was firstly reported and described in Japan by Japanese Plant Pathologist Matuo and Ishigami during 1958. It is caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *melongenae*, which induces wilt disease and leads to severe yield losses, especially in Asian, European, and Mediterranean countries.

(§) *This study was presented as an oral presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

Soil pathogenic eggplant fungus described for the first time in Japan, and found in abundance in Asia: China, Korea (Cho and Shin, 2004; Altınok, 2005; Zhuang, 2005; Dong et al., 2017). Also, *Fusarium oxysporum* f. sp. *melongenae* has been reported in several European countries, including the Netherlands (Van Steekelenburg, 1976), Greece, Spain, and Italy, as well as in Türkiye where it was first documented in 2005 (Van Steekelenburg, 1976; Stravato and Cappelli, 1990; Holevas et al., 2000; Urrutia et al., 2004; Altınok, 2005; Cappelli et al., 2013).

Three eggplant germplasms, *LS1934*, *LS174*, and *LS2436*, have been found to be highly resistant to *Fusarium oxysporum* f. sp. *melongenae* in multiple studies based on genetic mapping and inoculation tests (Sakata et al., 1996; Mochizuki et al., 1997; Monma et al., 1997; Miyatake et al., 2016) mapped a major resistance locus (*FM1*) for Fusarium wilt at the end of chromosome 2, with two allelic forms Fm1(L) from LS1934 and Fm1(E) from LS174.

Mutlu et al. (2008) developed tightly linked SRAP (Me8/Em5) and SRAPRGA markers that flank the Fusarium wilt resistance gene at approximately 1.2 cM, along with a RAPD marker at 2.6 cM. These markers were converted to SCAR markers that were confirmed across F_2, BC_1, and BC_3 generations, demonstrating high reliability for markerassisted selection of resistant eggplant genotypes. Boyacı et al. (2011) confirmed by classical genetics that resistance in LS1934 and LS2436 follows a single dominant gene inheritance pattern. Sulu et al. (2022), evaluated three molecular markers (SCAR426, CAPs_903, and SIVR844) that are known to be linked with major disease resistance genes in eggplant. The study used breeding materials, including the resistant accessions *Solanum melongena* LS1934 and LS2436, and assessed resistance both with markers and classical inoculation tests (rootdip) against *Fusarium oxysporum* f. sp. *melongenae* (FOM) and other pathogens. Especially SCAR426 marker for Fusarium resistance is useful tools for markerassisted selection in disease resistance breeding of eggplant.

Molecular testing was conducted using the SCAR426 marker on 77 eggplant genotypes selected for yield and quality traits developed by the Alata Horticulture Research Institute (AL/Mersin/Türkiye). LS2434, LS1934, and Köksal were used as resistant controls, while Kemer, Yula, and NSFB-99 served as susceptible controls. PCR analyses revealed that four eggplant genotypes (P11, P29, P49, and P52) were resistant to *Fusarium oxysporum* f. sp. *melongenae* (FOM), producing the expected 426 bp band, whereas the remaining 73 genotypes were identified as susceptible (Çolak-Ateş et al., 2018).

At the Aegean Agricultural Research Institute, vegetable genetic resources are extensively utilized in both open-pollinated and hybrid eggplant breeding programs. Currently, eight eggplant varieties with diverse fruit characteristics-including reddish-purple and black-purple skin color, fruit lengths ranging from very long to short, and cylindrical to pear-shaped forms-have been registered (Haytaoğlu et al.2023; TTSM 2026).

In the present study, F_4 eggplant breeding lines and germplasm, including landraces from the institute's gene pool, were screened for resistance to Fusarium wilt using the PCR-based SCAR426 molecular marker. This is the first study to evaluate Turkish local eggplant landraces for Fusarium wilt resistance.

Materials and Methods

The genetic material of eggplant used in this study was obtained from advanced breeding lines maintained in the eggplant gene pool of the Aegean Agricultural Research Institute (AARI), Vegetable Department, Türkiye. 48 genotypes were tested totally. 21 eggplant landraces (Manisa, Muğla, Kayseri and Niğde provinces) 24 F_4 stage inbred lines and one registered local variety (Yamula). The 'Köksal' cultivar was used as the resistant control, whereas 'Aydın Siyahı-55' was used as the susceptible control. Detailed information regarding the genotypes used in the study is provided in Table 1.

Genomic DNA was isolated from fresh young leaf tissues of resistant and susceptible controls and breeding lines using the GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific). DNA concentration and quality were determined using a spectrophotometer, and DNA integrity was confirmed on a 1% agarose gel. All control genotypes and breeding lines were screened for the presence of the *FOM* resistance gene using the dominant Sequence Characterized Amplified Region marker SCAR426 (Mutlu et al., 2008). Sequence of SCAR426 was shown in Table 2.

PCR amplifications were carried out in a total reaction volume of 20 µL, containing 5 µL PCR master mix (DreamTaq Green Master Mix, Thermo Fisher Scientific), 1 µL each of forward and reverse primers (10 pmol), 2 µL genomic DNA (15-20 ng), and 11 µL nuclease-free water. The PCR conditions consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at the primer-57°C specific temperature for 60 s, and extension at 72°C for 60 s, with a final extension at 72°C for 7 min. The PCR products were separated on a 2% agarose gel and visualized using a GelCapture MiniBIS UV imaging system. The Gene

Ruler 100 bp Plus DNA Ladder (Thermo Scientific) was used as a molecular standard to confirm the marker SCAR426. The SCAR426 dominant primer provided a single 426 bp band for the homozygote resistant (R) genotypes, a single 800 bp band for the susceptible (S) genotypes. The SCAR426 marker screening was repeated twice to confirm the reliability of the results.

Results and Discussion

All 48 eggplant genotypes, including landraces, advanced inbred lines, and control varieties, were successfully amplified using the SCAR426 molecular marker linked to Fusarium wilt resistance. The marker produced clear and reproducible banding patterns, confirming the reliability of the PCR amplification and gel electrophoresis procedures. The SCAR426 marker differentiated the genotypes into two distinct classes based on banding patterns: homozygous resistant genotypes exhibiting a single 426 bp band, susceptible genotypes showing a single 800 bp band. The susceptible control variety Aydın Siyahı-55 produced the expected 800 bp band, while the resistant control Köksal showed the resistant allele, validating the effectiveness of the marker system used in this study (Figure 1).

Among the tested materials, resistance-associated bands were detected in only one inbred line, indicating the presence of the Fusarium wilt resistance gene in the AARI eggplant gene pool. In contrast, a considerable proportion of landraces and breeding lines lacked the resistance-associated allele and were classified as susceptible based on marker analysis. The resistance allele was detected exclusively in the inbred line ETA5.

The SCAR426 marker was originally developed from a cross between the resistant parent 'LS2436' and the susceptible parent 'NSFB99' (Mutlu et al., 2008). However, unlike the mapping population used for marker development, the present study evaluated genetically diverse eggplant materials, including open pollinated registered cultivar, landraces from different geographical regions, and F_4 stage inbred lines (derived from cultivated x wild crosses). The heterogeneous genetic backgrounds of these materials, particularly the landraces, may have reduced the predictive accuracy of the marker. Furthermore, as SCAR426 is a dominant marker, it cannot discriminate heterozygous individuals from homozygous resistant genotypes, which may have contributed to the inconsistencies between marker genotypes and resistance phenotypes.

Çolak-Ateş et al. (2018) evaluated the resistance of 77 eggplant genotypes to Fusarium wilt (*Fusarium oxysporum* f. sp. *melongenae*, FOM), Potato virus Y (PVY), and root-knot nematode (*Meloidogyne incognita*, RN) using marker-assisted selection and classical inoculation test. The SCAR426 marker was used to screen for FOM resistance, with 'Köksal' and 'LS2436' serving as resistant controls and 'NSFB99' as the susceptible control. Molecular marker analysis identified four genotypes (P11, P29, P49, and P52) as heterozygous resistant to FOM. The resistance of these genotypes was subsequently confirmed through classical inoculation testing evaluation. Furthermore, conventional screening for PVY and RN resistance showed that all genotypes were susceptible to PVY, whereas only P29 and P52 exhibited resistance to both FOM and RN.

In another study, Şimşek et al. (2020) initially screened 120 eggplant inbred lines and four commercial hybrid cultivars previously reported as resistant using the SCAR426 molecular marker. All inbred lines were identified as susceptible, whereas all four commercial hybrid cultivars exhibited the resistance-associated marker profile. Subsequently, eggplant breeding materials (F_1-F_8 generations) were screened using the SCAR426 marker linked to the *FOM* resistance gene, and 533 seedlings were evaluated by root-dip inoculation with a *Fusarium oxysporum* f. sp. *melongenae* (FOM) isolate. All seedlings identified as resistant by the marker survived the inoculation assay. However, six hybrids lacking the SCAR426 resistance-associated marker were also found to be resistant in the classical inoculation test. These findings indicate that SCAR426 is a useful marker for developing eggplant lines resistant to Fusarium wilt, but they also suggest the existence of an additional source of resistance independent of the resistance gene derived from the genotype 'LS2436'. Among the genotypes evaluated in this study, only the inbred line ETA5 carried the resistance-associated allele detected by the SCAR426 marker. ETA5 exhibited pear-shaped fruits, slightly striped green skin, and white fruit flesh characteristics (Figure 2). The remaining breeding lines and all landrace genotypes were classified as susceptible according to the marker analysis.

Conclusions

In this study, 48 eggplant genotypes, including landraces, advanced inbred lines, and registered varieties, were screened for Fusarium wilt resistance using the SCAR426 molecular marker. The marker effectively distinguished resistant and susceptible genotypes, producing clear 426 bp bands in homozygous resistant lines and 800 bp bands in susceptible ones. Several advanced inbred lines and selected landraces were found to carry the resistance allele, while a considerable portion of the tested germplasm remained susceptible. As classical

phenotypic screening was not performed in the present study, the resistance indicated by the SCAR426 marker for ETA5 requires confirmation through classical disease resistance assays. Nevertheless, the resistant control 'Köksal' consistently produced the expected resistant band, in agreement with previous studies, indicating stable marker performance in this genotype. Similarly, the genotypes evaluated in the present study may exhibit resistance when subjected to classical phenotypic screening, even if they are not identified as resistant by the SCAR426 marker. Considering the possibility of alternative resistance sources independent of the LS2436-derived resistance gene, confirmation through classical phenotypic evaluation would provide a more reliable assessment of resistance. If the resistance of ETA5 is confirmed through classical phenotypic screening, this genotype could represent an alternative source of resistance and may be valuable for future eggplant breeding programs. In the next steps, susceptible genotypes identified in this study should be further evaluated through classical resistance assays and screened with newly developed resistance-associated markers to better understand their genetic background.

Acknowledgments

This article represents an expanded and fully developed version of an initial abstract that was previously presented during the proceedings of the 5th International Plant Breeding Congress, held in Antalya, Türkiye, from December 01 to 05, 2025.

The authors gratefully acknowledge the financial support provided by TAGEM through Project No. TAGEM/BBAD/E/123A1/P1/6079, entitled *Determination of Disease and Nematode Resistance in Tomato, Pepper, and Eggplant Breeding Materials Using Molecular Markers III*. The authors also thank Yüksel Seeds for providing the seeds of the 'Köksal' eggplant variety.

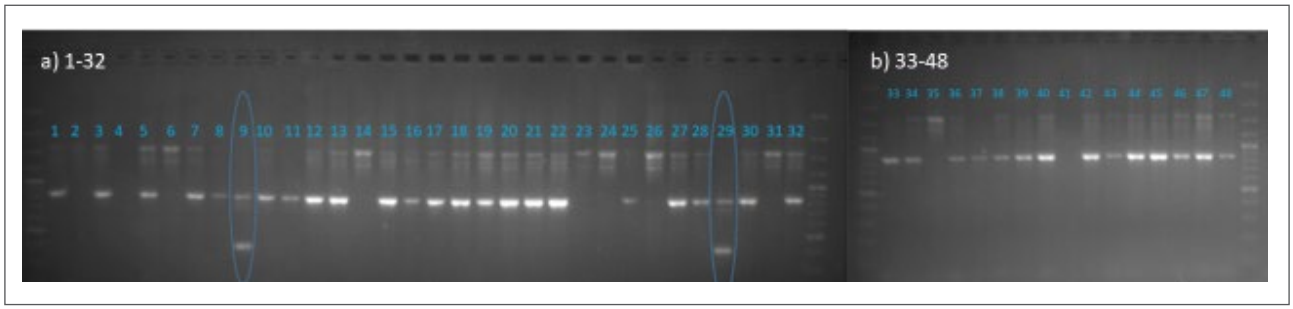


Figure 1. The genotypes used in the study. a) Genotypes 1-32 genotypes 1.Aydın Siyahı-55 (Susceptible Control) 9. Köksal (Resistant Control). 29. ETA 5 Inbred Line b) Genotypes 33-48 homozygous resistant genotype =426 bp band susceptible control variety Aydın Siyahı-55 produced 800 bp band. The Gene Ruler 100 bp Plus DNA Ladder (Thermo Scientific).

Figure 2. Fruit Shape of Susceptible control Aydin Siyahı-55 variety,Resistant Control Köksal, Resistance inbred line ETA5 and Yamula local variety (Original).

Table 1. The genotypes used in the study. The 'Aydın Siyahı-55' and 'Köksal' cultivars were used as susceptible and resistant controls, respectively.

No	Genotype Name	Material Type / Origin	Source Institution
1	Aydın Siyahı-55	Registered Variety (Susceptible Control)	Aegean Agricultural Research Institute
2	GK1	Landrace, Province Manisa	Aegean Agricultural Research Institute
3	GK2	Landrace, Province Manisa	Aegean Agricultural Research Institute
4	GK3	Landrace, Province Manisa	Aegean Agricultural Research Institute
5	GK4	Landrace, Province Manisa	Aegean Agricultural Research Institute
6	GK5	Landrace, Province Manisa	Aegean Agricultural Research Institute
7	GK6	Landrace, Province Manisa	Aegean Agricultural Research Institute
8	Yamula patlıcanı	Registered Variety	Registered local variety
9	Köksal	Registered hybrid Variety (Resistant Control)	Yüksel Seed Company
10	GK7	Landrace, Province Nigde	Aegean Agricultural Research Institute
11	GK8	Landrace, Province Nigde	Aegean Agricultural Research Institute
12	GK9	Landrace, Province Nigde	Aegean Agricultural Research Institute
13	GK10	Landrace, Province Nigde	Aegean Agricultural Research Institute
14	GK11	Landrace, Province Nigde	Aegean Agricultural Research Institute
15	GK12	Landrace, Province Mugla	Aegean Agricultural Research Institute
16	GK13	Landrace, Province Mugla	Aegean Agricultural Research Institute
17	GK14	Landrace, Province Mugla	Aegean Agricultural Research Institute
18	GK15	Landrace, Province Mugla	Aegean Agricultural Research Institute
19	GK16	Landrace, Province Manisa	Aegean Agricultural Research Institute
20	GK17	Landrace, Province Manisa	Aegean Agricultural Research Institute
21	GK18	Landrace, Province Manisa	Aegean Agricultural Research Institute
22	GK19	Landrace, Province Kayseri	Aegean Agricultural Research Institute
23	GK20	Landrace, Province Kayseri	Aegean Agricultural Research Institute
24	GK21	Landrace, Province Kayseri	Aegean Agricultural Research Institute
25	ETA1	Inbred Line	Aegean Agricultural Research Institute
26	ETA2	Inbred Line	Aegean Agricultural Research Institute
27	ETA3	Inbred Line	Aegean Agricultural Research Institute
28	ETA4	Inbred Line	Aegean Agricultural Research Institute
29	ETA5	Inbred Line	Aegean Agricultural Research Institute
30	ETA6	Inbred Line	Aegean Agricultural Research Institute
31	ETA7	Inbred Line	Aegean Agricultural Research Institute
32	ETA8	Inbred Line	Aegean Agricultural Research Institute
33	ETA9	Inbred Line	Aegean Agricultural Research Institute
34	ETA10	Inbred Line	Aegean Agricultural Research Institute
35	ETA11	Inbred Line	Aegean Agricultural Research Institute
36	ETA12	Inbred Line	Aegean Agricultural Research Institute
37	ETA13	Inbred Line	Aegean Agricultural Research Institute
38	ETA14	Inbred Line	Aegean Agricultural Research Institute
39	ETA15	Inbred Line	Aegean Agricultural Research Institute
40	ETA16	Inbred Line	Aegean Agricultural Research Institute
41	ETA17	Inbred Line	Aegean Agricultural Research Institute
42	ETA18	Inbred Line	Aegean Agricultural Research Institute
43	ETA19	Inbred Line	Aegean Agricultural Research Institute
44	ETA20	Inbred Line	Aegean Agricultural Research Institute
45	ETA21	Inbred Line	Aegean Agricultural Research Institute
46	ETA22	Inbred Line	Aegean Agricultural Research Institute
47	ETA23	Inbred Line	Aegean Agricultural Research Institute
48	ETA24	Inbred Line	Aegean Agricultural Research Institute

Table 2. Sequence of SCAR 426 (Mutlu et al., 2008).

Primer SCAR 426	Sequences 5'- 3'
Forward	TGA GTC CAA ACC GGA CTA CAA G
Reverse	GAC TGC GTA CGA ATT AAC TCT ACG

References

Altınok, H. (2005). First report of Fusarium wilt of eggplant caused by *Fusarium oxysporum* f. sp. *melongenae* in Turkey. *Plant Pathology*, *54*(6), Article 846. https://doi.org/10.1111/j.1365-3059.2005.01228.x

Arafa, R. A., Prohens, J., Solberg, S. Ø., Plazas, M., and Rakh, M. (2022). Breeding and genome mapping for resistance to biotic stress in eggplant. In C. Kole (Ed.), *Genomic designing for biotic stress resistant vegetable crops* (pp. 115-140). Springer, Cham. https://doi.org/10.1007/978-3-030-97785-6_4

Boyacı, H. F., Ünlü, A., and Abak, K. (2011). Genetic analysis of resistance to wilt caused by *Fusarium oxysporum* f. sp. *melongenae* in eggplant (*Solanum melongena*). *Indian Journal of Agricultural Sciences*, *81*(9), 799-802.

Cappelli, C., Stravato, V., Rotino, G., and Buonaurio, R. (1995). Sources of resistance among *Solanum* spp. to an Italian isolate of *Fusarium oxysporum* f. sp. *melongenae*. *EUCARPIA: IXth Meeting on Genetics and Breeding on Capsicum and Eggplant*, 218-221.

Cho, W. D., and Shin, H. D. (2004). *List of plant diseases in Korea* (4th ed.). Korean Society of Plant Pathology.

Çolak Ateş, A., Fidan, H., Özarslandan, A., and Ata, A. (2018). Determination of the resistance of certain eggplant lines against Fusarium wilt, Potato Y potyvirus and root-knot nematode using molecular and classic methods. *Fresenius Environmental Bulletin*, *27*(11), 7446-7453.

Dong, Z., Hsiang, T., Luo, M., and Xiang, M. (2017). Draft genome sequence of an isolate of *Fusarium oxysporum* f. sp. *melongenae*, the causal agent of Fusarium wilt of eggplant. *Genome Announcements*, *5*(7), Article e01597-16. https://doi.org/10.1128/genomeA.01597-16

Ephytia. (2026). *Fusarium oxysporum* f. sp. *melongenae*. INRA. https://ephytia.inra.fr/en/C/7321/Eggplant-Fusarium-oxysporum-f-sp-melongena

Gökseven, A., and Akbudak, N. (2022). Influence of grafting on fruit quality traits in eggplant grafted onto *Solanum torvum* and interspecific rootstocks. *Journal of Biological and Environmental Sciences*, *16*(46), 35-47. https://izlik.org/JA39RC38LB

Haytaoğlu, M. A., Mutlu, S., Kahraman, A., and Binbir, S. (2023). Registration of "Fener" eggplant (*Solanum melongena* L.) variety. *Ekin Journal of Crop Breeding and Genetics*, *9*(2), 172.

Holevas, C., Chitzanidis, A., Pappas, A., Tzamos, E., and Elena, K. (2000). Disease agents of cultivated plants observed in Greece from 1981 to 1990. *Annales de l'Institut Phytopathologique Benaki*, *19*, 1-96.

Matsuo, T., and Ishigami, K. (1958). On the wilt of eggplant and its causal fungus *Fusarium oxysporum* f. *melongenae* n.f. *Annals of the Phytopathological Society of Japan*, *23*(4), 189-192. https://doi.org/10.3186/jjphytopath.23.189

Miyatake, K., Saito, T., Negoro, S., Yamaguchi, H., Nunome, T., Ohyama, A., and Fukuoka, H. (2016). Detailed mapping of a resistance locus against Fusarium wilt in cultivated eggplant (*Solanum melongena*). *Theoretical and Applied Genetics*, *129*(2), 357-367. https://doi.org/10.1007/s00122-015-2632-8

Mochizuki, H., Sakata, Y., Yamakawa, K., Nishio, T., Komochi, S., Nariakawa, S., and Monma, S. (1997). Eggplant parental line and breeding line resistant to Fusarium wilt. *Bulletin of the National Research Institute of Vegetables, Ornamental Plants and Tea: Series A (Vegetables and Ornamental Plants)*, *12*, 85-90.

Monma, S., Sato, T., and Matsunaga, H. (1996). Evaluation of resistance to bacterial, Fusarium and Verticillium wilt in eggplant and eggplant-related species collected in Ghana. *Capsicum & Eggplant Newsletter*, (15), 71-72.

Mutlu, N., Boyacı, H. F., Göçmen, M., and Abak, K. (2008). Development of SRAP, SRAP-RGA, RAPD, and SCAR markers linked with a Fusarium wilt resistance gene in eggplant. *Theoretical and Applied Genetics*, *117*(8), 1303-1312. https://doi.org/10.1007/s00122-008-0864-4

Sakata Y, Monma S, Narikawa T, Komochi S. (1996). Evaluation of resistance to bacterial wilt and Verticillum wilt in eggplants (Solanum melongena L.) collected in Malaysia. J Jpn Soc Hort Sci. 65:81-88.

Simsek, D., Samur, D., Mutlu, N., and Pinar, H. (2020). Marker assisted backcross breeding for *Fusarium* wilt (*Fusarium oxysporum* Schlecht. f. sp. *melongenae*) in eggplant. *International Journal of Agricultural and Natural Sciences*, *13*(2), 91–100.

Sulu, G., Polat, I., Boyacı, H. F., Yildirim, A., and Gümrükcü, E. (2022). Screening and validation

of three molecular markers for disease resistance in eggplant. *Czech Journal of Genetics and Plant Breeding*, *58*(2), 83-92. https://doi.org/10.17221/105/2021-CJGPB

TTSM. (2026). *Turkish Seed Registration and Certification Center official statistics*. Republic of Türkiye Ministry of Agriculture and Forestry. https://www.tarimorman.gov.tr/BUGEM/TTSM

Urrutia Herrada, M., Gomez Garcia, V., and Tello Marquina, J. (2004). Fusarium wilt on eggplant in Almeria (Spain). *Boletín de Sanidad Vegetal - Plagas*, *30*(1), 85-92.

Van Steekelenburg, N. (1976). Fusarium wilt of eggplant in the Netherlands. *Netherlands Journal of Plant Pathology*, *82*(5), 191-192. https://doi.org/10.1007/BF01981446

Zhuang, W. Y. (Ed.). (2005). *Fungi of northwestern China*. Mycotaxon, Ltd.

Research Article

Ekin
Journal of Crop Breeding and Genetics

12(2):110-116, 2026

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

In Vitro Regeneration Studies in Turkish Tomato Cultivars(§)

Sibel BAHADIR[1*] Petek YAPICI[2] Süleyman KAVAK[3] Ş. Şebnem ELLİALTIOĞLU[4]

[1] Department of Agricultural Biotechnology, Faculty of Agriculture, Ondokuz Mayıs University, Samsun, Türkiye

[2] Petektar Seed Co., Antalya, Türkiye

[3] Isparta University of Applied Science, Faculty of Agriculture, Department of Horticulture, Isparta, Türkiye

[4] Doqutech Academy Llc. Co., Ankara University Technopolis, Ankara, Türkiye

[*] Corresponding author e-mail: bhdr.sibel@gmail.com

Citation:
Bahadır S., Yapıcı P., Kavak S., Ellialtıoğlu ŞŞ., 2026. *In Vitro* Regeneration Studies in Turkish Tomato Cultivars. Ekin J. 12(2):110-116.

Received: 22.06.2026 ***Accepted: 26.07.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

Tomato is among the most extensively studied plant species in global breeding and genetic research. In recent years, CRISPR-Cas9 technology offers extensive opportunities for integrating traits difficult to transfer through classical breeding methods into qualified genotypes or for generating precise mutations. Furthermore, the regenerative capacity of the studied plant species or genotype in tissue culture is a prerequisite for advancing genetic studies. This study aimed to induce *in vitro* regeneration from cotyledon and hypocotyl explants of four different tomato varieties (PTK-254, PTK-273, Şekerkız, Yelpare 82) developed by Petektar Seed Co. Organogenesis was achieved in MS medium using different doses and combinations of the growth regulators TDZ, BAA, IAA, and Zeatin. In this study, where the interaction of nutrient medium and varieties was found to be important, the highest shoot formation was obtained from MS-1, MS-8, MS-5, and MS-6 media containing the Şekerkız variety. These combinations, in which more than 10 shoots were obtained from each explant, were followed by the Yelpare 82, PTK-254, and PTK-273 varieties cultured in MS-1 medium. The combination of 1.0 mg/L Zeatin and 0.1 mg/L IAA (MS-1) provided regeneration in all varieties, albeit to varying degrees. The use of TDZ alone at doses of 2 and 3 mg/L can also yield positive results depending on the variety. Developing shoots were sub-cultured and grown. For rooting, hormone-free MS medium was used, and tomato plantlets were transferred to peat pots and acclimatized. In this study, the *in vitro* regeneration system for advanced genetic applications was optimized on experimental material.

Keywords: Tomatoes, organogenesis, *Solanum lycopersicum* L., Zeatin, TDZ

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically valuable members of the Solanaceae family and ranks as the world's second most produced vegetable after potato. Recent statistics indicate that global tomato production has reached nearly 192 million tons, with Türkiye contributing about 13.3 million tons annually. This production volume places Türkiye third worldwide, following China and India (Anonymous, 2025a). While most tomatoes are consumed domestically, approximately 590 thousand tons are exported, accounting for nearly half of the country's total revenue from fresh vegetable exports (Anonymous, 2025b).

It is widely accepted that tomato originated in the American continent. The primary center of origin is considered to be South America, particularly the western coastal highlands spanning parts of Chile, Bolivia, Ecuador, Colombia, and Peru (Bai and Lindhout, 2007). Wild tomato species occur naturally in these regions (Taylor, 1986). The cultivated tomato (*S. lycopersicum*) was introduced from Mexico to Spain during the early sixteenth century, from where it gradually spread throughout Europe (Caicedo and Peralta, 2013). According to Diez and Nuez (2008),

(§) *This study was presented as an poster presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

Turkish merchants played an important role in its dissemination to Mediterranean and Middle Eastern countries. Bayraktar (1953) and Oraman (1968) reported that tomato was introduced into Türkiye around 1770. Initially cultivated in the Adana province and other Mediterranean regions, tomato production subsequently expanded to the Marmara region, Istanbul, and eventually throughout Anatolia (Abak and İlbi, 2022). Over time, locally adapted genotypes and landraces emerged, giving rise to numerous traditional local varieties. As in many other vegetable crops, Türkiye represents an important diversification center for tomato, with a rich collection of local populations and traditional varieties (Sönmez, 2014).

Today, tomato is consumed in a wide range of fresh and processed products and is increasingly recognized as a functional food. As one of the richest natural sources of lycopene, tomato also contains carotenoids and polyphenolic compounds that contribute not only to its nutritional value but also to its sensory and functional characteristics, including flavor and aroma (Martí et al., 2016; Sönmez and Ellialtıoğlu, 2014). Although local varieties and other open-pollinated genotypes cultivated in Türkiye exhibit considerable genetic diversity and desirable quality traits, many of them require further improvement for use in modern breeding programs. Consequently, efficient biotechnological approaches have become increasingly important to accelerate breeding and facilitate the incorporation of valuable agronomic and quality traits into elite germplasm.

Among the advanced biotechnological tools currently used in crop improvement, genetic transformation and CRISPR/Cas9-mediated genome editing have opened new opportunities for precise modification of economically important traits. However, the successful application of these technologies depends primarily on the ability to regenerate whole plants from cultured tissues. Therefore, the development of efficient and reproducible *in vitro* regeneration systems is considered a fundamental prerequisite for both functional genomics and modern breeding applications.

Extensive studies have been conducted on tomato *in vitro* regeneration through organogenesis using various explants, including cotyledons, hypocotyls, leaves, stem segments, pedicels, petioles, and inflorescences. A comprehensive review of these studies was presented by Senapati (2016). Regeneration efficiency is influenced by several factors, particularly genotype, explant source, culture medium composition, and the type and concentration of plant growth regulators. In regeneration systems, the balance between cytokinins and auxins governs organogenesis, callogenesis, and somatic embryogenesis through synergistic, antagonistic, or additive interactions depending on the plant species and tissue type. Generally, *in vitro* regeneration consists of an induction phase, a shoot elongation phase, and a rooting phase. In tomato, zeatin and BA are the most frequently used cytokinins, whereas IAA and NAA are commonly employed auxins to promote shoot regeneration (Rai et al., 2013; Seçgin, 2021; Sherkar and Chavan, 2014; Wayase and Shitole, 2014).

Since regeneration capacity is highly genotype-dependent, optimization of regeneration protocols remains essential for newly developed breeding materials. Therefore, the objective of the present study was to determine the most suitable regeneration medium for four Turkish tomato cultivars developed by Petektar Seed Co. by evaluating different explant types and combinations of plant growth regulators. The optimized regeneration protocol established in this study is expected to provide a reliable platform for future applications in genetic transformation, genome editing, and tomato breeding.

Materials and Methods

Plant material

The experiments were conducted at the Petektar Seed R&D Center, which comprises a research greenhouse and plant biotechnology laboratories located in the Kurşunlu district of Antalya. Four tomato varieties PTK-254, PTK-273, Şekerkız, and Yelpare 82 were used as plant material.

Seed sterilization and *in vitro* germination

Seeds of four tomato (*S. lycopersicum*) varieties were placed in 50 mL Falcon tubes and first immersed in 70% (v/v) ethanol for 1 min. This was followed by surface sterilization in 20% (v/v) sodium hypochlorite (NaOCl) solution containing 2-3 drops of Tween-20 for 20 min. The seeds were then rinsed three times with sterile distilled water, each wash lasting 5 min. After washing, seeds were transferred onto sterile filter papers inside a laminar flow cabinet to remove excess moisture and then placed on germination medium in glass jars. All procedures were carried out under aseptic conditions, and cultures were incubated at 25°C under a 16/8 h light/dark photoperiod in a growth chamber.

Explant preparation and culture conditions

In all four tomato varieties, explants were prepared and transferred to regeneration media when the cotyledon leaves were positioned horizontally and the first true leaf had just emerged. For this purpose, hypocotyl tissues and longitudinally bisected cotyledon leaves were used as explant sources.

Murashige and Skoog (MS) mineral salts and vitamins (Murashige and Skoog, 1962) were used

as the basal medium. The media were supplemented with 3% (w/v) sucrose and solidified with 0.7% (w/v) agar. The pH of all media was adjusted to 5.7 before autoclaving. Media were sterilized at 121°C for 15 min in an autoclave. The explants were cultured on MS medium containing eight different combinations of plant growth regulators (PGRs). For each treatment, 10 Petri dishes were prepared, and six cotyledon explants were placed per Petri dish. In addition, approximately 30 hypocotyl explants (approximately 1 cm in length) were placed in two Petri dishes per treatment. The concentrations and combinations of PGRs used in the experiment, along with their respective code numbers, are presented in Table 1.

Rooting and acclimatization

Once regeneration had initiated, shoots approximately 2-3 cm in length were transferred to RM medium for root induction. All cultures were incubated in a growth chamber at 26 ± 2 °C under a 16/8 h light/dark photoperiod. Rooted plantlets were then transferred into pots containing peat-based substrate. The plants were gradually acclimatized and subsequently transferred to the greenhouse under healthy growth conditions (Okutan et al., 2025).

Statistical analysis

One-way analysis of variance (ANOVA) was performed to evaluate differences between media and varieties. The general linear model procedure of MINITAB 17.0 Statistical Software was used for data analysis. All main effects were considered as fixed factors. Tukey's multiple range post hoc test was employed for multiple comparisons of the means with an alpha level of 0.05.

Results and Discussion

To determine the optimal regeneration medium for tomato, the effects of explant source and plant growth regulators were evaluated using four different tomato varieties. Cotyledon and hypocotyl explants were cultured on MS media containing various combinations and concentrations of plant growth regulators. No regeneration was observed from hypocotyl tissues. The effects of eight different MS media compositions on plant regeneration from cotyledon explants are presented in Table 2.

The interaction between medium compositions and varieties was found to be statistically significant. The highest regeneration rate (82%) was obtained in the 'MS-1 × Şekerkız' and 'MS-1 × Yelpare 82' combinations. Since the mean values were subjected to angular transformation before statistical analysis, these transformed values were also used for interpretation. The next highest regeneration rates were observed in the 'MS-5 × Şekerkız' and 'MS-6 × Şekerkız' combinations (55.2%). Although numerical differences were observed among treatments, regeneration occurred to varying degrees in almost all combinations, except for 'MS-3' medium and the 'PTK-254' variety, which showed no response. Considering the fundamental principle of tissue culture that regeneration frequency is highly genotype-dependent, the interaction effect was further analyzed separately. Under this approach, statistically significant differences were also observed both among varieties and among media compositions.

The composition of the culture medium, especially the growth regulators it contains, has a decisive effect on the *in vitro* morphogenesis and shoot regeneration capacity of plants. Among the tested media compositions, 'MS-1' medium containing '1.0 mg/L Zeatin + 0.1 mg/L IAA' provided by far the highest regeneration rate (71.4%) compared with the others. This evaluation represents the mean regeneration response across all varieties (Table 2). Similarly, several researchers have also reported that the use of Zeatin has a positive effect on regeneration efficiency in tomato. According to Gubiš et al. (2005), not only the combination of zeatin and IAA but also the TDZ and IAA combination promoted shoot regeneration in tomato explants. In addition, 'MS-5' medium, containing 3.0 mg/L TDZ, achieved the second-highest average regeneration rate (approximately 27%). Cao et al. (2020) also demonstrated that trans-zeatin, a cytokinin hormone, plays an important role in the *in vivo* regeneration of tomato. This medium (MS5), together with 'MS-8' (3.0 mg/L BA + 0.5 mg/L IAA), showed statistically similar results, falling within the same significance group. Seçgin et al. (2021) also reported that the combination of 3.0 mg/L BA + 0.1 mg/L IAA (corresponding to MS-8) was suitable for regeneration from cotyledon explants in the tomato cultivar 'Crocker'. The authors noted, however, that the use of kinetin resulted in better regeneration performance in the 'Bobcat' cultivar.

Genotype had a pronounced effect on regeneration success in tomato. It has been well documented that certain cultivars are recalcitrant and highly challenging for regeneration. Among the varieties used in this study, 'Şekerkız' exhibited the highest average regeneration rate, as 41.9% (Figure 1), followed by 'Yelpare 82' (23.3%). 'PTK-273' ranked third with 16.3%, showing no statistically significant difference from 'Yelpare 82' (Table 2). 'PTK-254' displayed the lowest regeneration response (Figure 2). For this variety, further optimization using alternative PGR combinations and concentrations appears necessary. The influence of genotype on regeneration capacity is well documented in tomato tissue culture studies. Grozeva et al. (2006)

reported significant differences among tomato cultivars in their response to *in vitro* regeneration, emphasizing that genotype plays a decisive role in morphogenic competence. Similarly, El-Shafey et al. (2017) observed that variations in shoot regeneration frequency were strongly associated with the genetic background of the tested cultivars, regardless of identical culture conditions. These findings indicate that regeneration potential is an inherent, genotype-dependent trait rather than solely a result of external culture factors. Therefore, the selection of a responsive genotype is crucial for the successful establishment of an efficient regeneration system in tomato. It is difficult to propose a single universal regeneration formula suitable for all tomato varieties. In light of the findings from this study, Zeatin combined with a low level of IAA proved to be the most effective cytokinin treatment for promoting regeneration in tomato explants. All obtained shoots were successfully acclimatized and subsequently transplanted into soil (Figure 3).

Conclusions

In this study, nutrient media supplemented with Zeatin, TDZ, and BA (cytokinins) in combination with IAA (auxin) were tested for their ability to induce shoot regeneration from cotyledon explants of four tomato varieties. The results indicated that genotype was the key factor affecting regeneration efficiency, though the composition of PGRs in the medium also contributed to the observed differences. Among the tested varieties, 'Şekerkız' demonstrated superior regenerative potential, whereas 'PTK-254' showed limited response, confirming its recalcitrant nature. Zeatin, together with BA and TDZ, promoted regeneration to different extents across varieties, highlighting the genotype-dependent nature of morphogenic response. Furthermore, cotyledon explants proved to be markedly more efficient in regeneration compared to hypocotyl explants.

The outcomes of this research provide a solid basis for future investigations, particularly for applications involving genetic transformation and accelerated breeding in tomato. This study underscores the importance of genotypic variation in tissue culture responses, suggesting that a tailored optimization of regeneration protocols for each genotype is indispensable for achieving consistent and reproducible results.

Acknowledgements

The authors used OpenAI ChatGPT solely to improve the English language, grammar, and readability of the manuscript. The AI tool was not used to generate scientific content, analyze data, interpret results, or draw conclusions. All scientific content and conclusions are entirely the responsibility of the authors.

Ethical Statement

A preliminary version of this study was presented as a poster at the "Vth International Plant Breeding Congress, 1-5 December 2025, Sherwood Exclusive Lara, Antalya, Türkiye". The present manuscript is an expanded and revised version prepared for full publication.

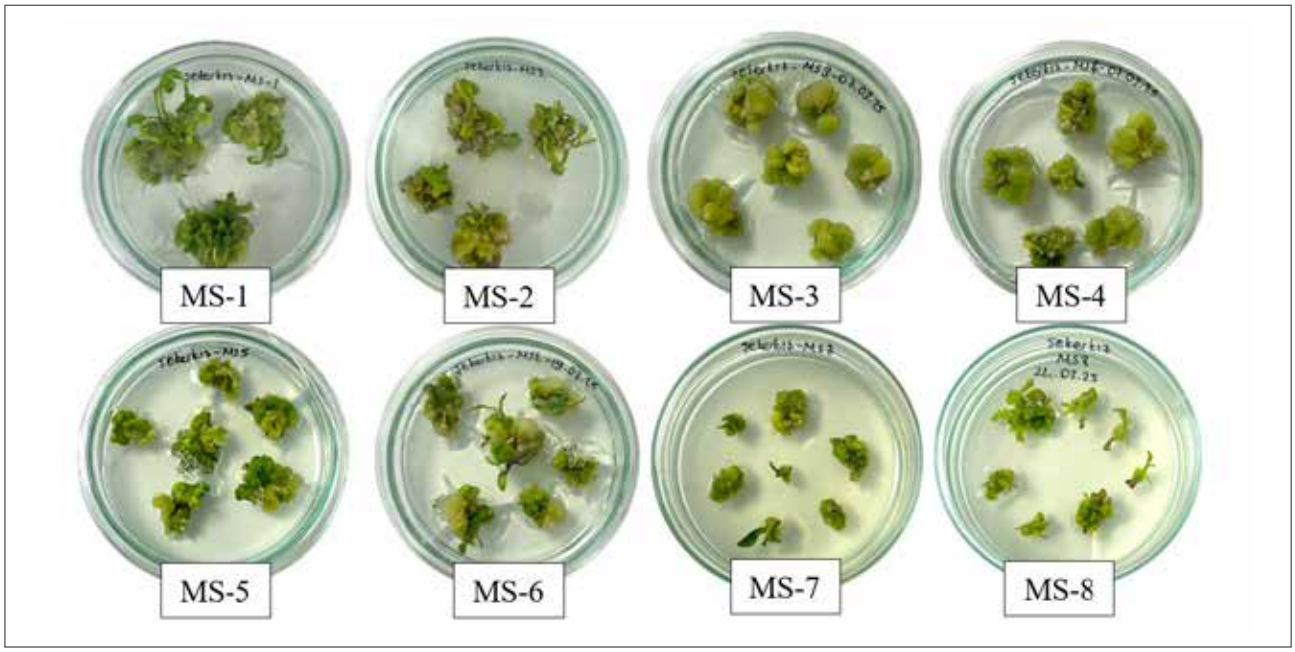


Figure 1. Shoot regeneration of 'Şekerkız' tomato cotyledon explants in eight media compositions.

Figure 2. Healthy shoots obtained from the 'PTK-254' tomato variety, which showed the lowest regeneration capacity among the tested varieties were cultured on MS-1 medium.

Figure 3. Tomato regenerants exhibiting healthy growth following the acclimatization process.

Table 1. The codes, types, and concentrations of plant growth regulators (PGRs) used in the regeneration media.

PGRs (mg/L)	Medium Code							
	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8
Zeatin	1.0	2.0						
IAA	0.1	0.1						0.5
TDZ			1.0	2.0	3.0	0.5	1.0	
BA						0.5	1.0	3.0

Table 2. *In vitro* regeneration percentages of four tomato varieties cultured on eight different MS media compositions.

Medium Code	Varieties				
	Şekerkız	Yelpare 82	PTK-273	PTK-254	Means*
MS-1	94.4 ***82.0 a***	94.4 ***82.0 a***	61.1 ***51.5 abcde***	83.3 ***70.2 ab***	***71.4 A***
MS-2	38.9 ***38.0 abcde***	27.8 ***26.8 bcde***	22.2 ***23.0 bcde***	0 ***0 e***	***22.0 BC***
MS-3	0 ***0 e***	5.56 ***8.0 de***	0 ***0 e***	0 ***0 e***	***2.0 C***
MS-4	22.2 ***23.5 bcde***	5.56 ***8.0 de***	5.56 ***8.0 de***	0 ***0 e***	***9.9 BC***
MS-5	66.7 ***55.2 abcd***	16.7 ***19.8 bcde***	22.2 ***23.5 bcde***	0 ***0 e***	***24.6 B***
MS-6	66.7 ***55.2 abcd***	11.1 ***11.8 de***	5.56 ***8.0 de***	0 ***0 e***	***18.6 BC***
MS-7	16.7 ***15.0 cde***	0 ***0 e***	11.1 ***16.1 cde***	0 ***0 e***	***7.8 BC***
MS-8	77.8 ***67.0 abc***	33.3 ***30.0 abcde***	0 ***0 e***	0 ***0 e***	***24.2 B***
Means**	***41.9 A***	***23.3 B***	***16.3 BC***	***8.8 C***	

Variety ($p < 0.05$, Tukey's multiple comparison test)
Medium ($p < 0.05$, Tukey's multiple comparison test)
Variety × Medium ($p < 0.05$, Tukey's multiple comparison test)

* Means followed by different letters within the same column are significantly different at the 0.05 probability level.
** Means followed by different letters within the same row are significantly different at the 0.05 probability level.
Italic and bold values represent angular transformed data.

References

Abak, K. and İlbi, H. (2022). Domates Islahı [in Turkish]. In: K. Abak, A. Balkaya, Ş. Ş. Ellialtıoğlu and E. Düzyaman (Eds.), *Sebze Islahı Cilt III: Solanaceae (Patlıcangiller)*, pp. 19-192. BİSAB Yayınları, Gece Kitaplığı Yayınevi, Ankara.

Anonymous (2025a). FAOSTAT (Food and Agriculture Organization of the United Nations.) *crop production data: Tomato production quantity (tons)* [Data set]. https://www.fao.org/faostat/en/#data/QCL (Accessed: 05.11.2025).

Anonymous (2025b). İstanbul Ticaret Borsası (Istanbul Commodity Exchange) *Türkiye yaş sebze-meyve ihracat verileri* [Fresh fruit and vegetable export data of Turkey]. https://www.itb.org.tr (Accessed: 7 November 2025).

Bai, Y. and Lindhout, P. (2007). Domestication and breeding of tomatoes: What have we gained and what can we gain in the future? *Annals of Botany*, *100*(5), 1085-1094. https://doi.org/10.1093/aob/mcm150

Bayraktar, K. (1953). *Sebze bahçelerinde yetiştirilen yerli ve Amerikan domates çeşitlerinin özellikleri*

ve teknolojik değerleri üzerinde mukayeseli araştırmalar [in Turkish]. Ankara Üniversitesi Ziraat Fakültesi Yayınları: 42, Ankara.

Caicedo, A. and Peralta, I. (2013). Basic information about tomatoes and the tomato group. In: *Genetics, Genomics, and Breeding of Tomato* (1st ed., pp. 1-36). CRC Press.

Cao, H., Zhang, X., Ruan, Y., Zhang, L., Cui, Z., Li, X. and Jia, B. (2020). miRNA expression profiling and zeatin dynamic changes in a new model system of *in vivo* indirect regeneration of tomato. *PLoS ONE*, *15*(12), e0237690. https://doi.org/10.1371/journal.pone.0237690

Díez, M. J. and Nuez, F. (2008). Tomato. In: J. Prohens and F. Nuez (Eds.), *Vegetables II: Fabaceae, Liliaceae, Solanaceae, and Umbelliferae* (Handbook of Plant Breeding, Vol. 2), pp. 249-323. Springer, New York, NY. https://doi.org/10.1007/978-0-387-74110-9_8

El-Shafey, N. M., Hassan, N., Khodary, S. E. A., and Badr, A. (2017). Differential *in vitro* direct regeneration of tomato genotypes on various combinations of growth regulators. *Biotechnology*, *16*(4-6), 155-164. https://doi.org/10.3923/biotech.2017.155.164

Grozeva, S., Rodeva, V., and Danailov, Z. (2006). Effect of genotype, explant type and culture medium on shoot regeneration in tomato (*Lycopersicon esculentum* Mill.) *in vitro*. *Bulgarian Journal of Agricultural Science*, *12*(3), 435-439. https://www.agrojournal.org/12/03-11.htm

Gubiš, J., Lajchová, Z., Klčová, L., and Jureková, Z. (2005). Influence of growth regulators on plant regeneration in tomato. *Horticultural Science*, *32*(3), 118-122. https://doi.org/10.17221/3777-HORTSCI

Martí, R., Roselló, S., and Cebolla-Cornejo, J. (2016). Tomato as a source of carotenoids and polyphenols targeted to cancer prevention. *Cancers*, *8*(6), Article 58. https://doi.org/10.3390/cancers8060058

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Okutan, E., Akyüz Çağdaş, E., Polat, M., Sarıtoprak, O., Aktaş, H., and Ellialtıoğlu, Ş. Ş. (2025). Doku kültürüyle çoğaltılan maviyemiş (*Vaccinium corymbosum* L.) sürgünlerinin köklendirilmesi üzerinde çalışmalar [in Turkish]. *Research Journal of Biology Sciences*, *18*(1), 12-23.

Oraman, M. N. (1968). *Sebze İlmi* [in Turkish]. Ankara Üniversitesi Basımevi.

Rai, A. C., Singh, M., and Shah, K. (2013). Engineering drought tolerant tomato plants over-expressing *BcZAT12* gene encoding a C_2H_2 zinc finger transcription factor. *Phytochemistry*, *85*, 44-50. https://doi.org/10.1016/j.phytochem.2012.09.007

Seçgin, Z. (2021). *Domateste doku kültürü, genetik transformasyon ve CRISPR/CAS9 sistemi ile genom işleme optimizasyonu* [in Turkish] [Doctoral dissertation, Ondokuz Mayıs University]. YÖK National Thesis Center. (Thesis No. 696166).

Seçgin, Z., Kavas, M., and Yıldırım, K. (2021). Optimization of *Agrobacterium*-mediated transformation and regeneration for CRISPR/Cas9 genome editing of commercial tomato cultivars. *Turkish Journal of Agriculture and Forestry*, *45*(6), 704-716. https://doi.org/10.3906/tar-2009-49

Senapati, S. K. (2016). A review on research progress on *in vitro* regeneration and transformation of tomato. *Annual Research & Review in Biology*, *9*(6), 1-9. https://doi.org/10.9734/ARRB/2016/22300

Sherkar, H. D., and Chavan, A. M. (2014). Studies on callus induction and shoot regeneration in tomato. *Science Research Reporter*, *4*(1), 89-93.

Sönmez, K. (2014). *Likopen, ß-karoten ve morfolojik özellikler bakımından yerel sofralık domateslerde genotip x çevre interaksiyonu* [Doctoral dissertation, Ankara University] [in Turkish]. YÖK National Thesis Center. (Thesis No. 386368).

Sönmez, K., and Ellialtıoğlu, Ş. Ş. (2014). Domates, karotenoidler ve bunları etkileyen faktörler üzerine bir inceleme [in Turkish]. *Derim*, *31*(2), 107-130. https://doi.org/10.16882/derim.2014.32662

Taylor, I. B. (1986). Biosystematics of the tomato. In J. G. Atherton & J. Rudich (Eds.), *The Tomato Crop: A Scientific Basis for Improvement* (pp. 1-34). Chapman and Hall. https://doi.org/10.1007/978-94-009-3137-4_1

Wayase, U. R., and Shitole, M. G. (2014). Effect of plant growth regulators on organogenesis in tomato (*Lycopersicon esculentum* Mill.) cv. Dhanashri. *International Journal of Pure and Applied Sciences and Technology*, *20*(2), 65-71.

Research Article

Ekin
Journal of Crop Breeding and Genetics

12(2):117-127, 2026

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Factors Influencing Plant Height in Lentil (*Lens culinaris* M.) for Mechanical Harvesting(§)

Abdulkadir AYDOĞAN[1] **Selin GÜNDÜZ[1]** **Elif ATASAYAR[1]** **Havva Vildan KILINÇ[1]**

Ersin KAVLAK[1] **Yusuf AYDOĞAN[2]**

[1] Field Crops Central Research Institute, Republic of Türkiye Ministry of Agriculture and Forestry, Ankara, Türkiye

[2] Ecoseed Tohumculuk Sanayi ve Ticaret Limited Şti. Beytepe, Çankaya, Ankara

* Corresponding author e-mail: kadirtyc@gmail.com

Citation:
Aydoğan A., Gündüz S., Atasayar E., Kılınç HV., Kavlak E., Aydoğan Y., 2026. Factors Influencing Plant Height in Lentil *(Lens culinaris* M.) for Mechanical Harvesting. Ekin J. 12(2):117-127.

Received: 24.06.2026 ***Accepted: 28.06.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

The primary goal of agricultural activities is profitability as in every sector. A product that is economically unviable is not adopted by farmers. Profitable crop production is associated with yield and production costs. In legumes, the harvesting method plays a role in determining costs. Morphologically, lentil is relatively short and does not grow upright compared to other plants, especially cereals. This affects the harvesting method and can lead to additional and significant costs. Therefore, the suitability of the lentil plant for mechanical harvesting is more important than for other plants. When varieties unsuitable for mechanical harvesting due to their short height are grown, profitability decreases, and farmers abandon production.

The experimental trials were conducted at the İkizce Research and Application Station, affiliated with the Field Crops Research Institute, during the 2020/21 and 2021/22 growing seasons. Seven different varieties were used in the trials during each growing season. Experimental materials were planted as summer and autumn sowings in both years. This study aimed to determine the characteristic(s) that most affect the suitability of the lentil plant for mechanical harvesting and its height in terms of growing season. In the combined analysis, statistically significant differences in plant height were found between year, variety, growing season, year*growing season, and growing season*variety. Furthermore, plant height was correlated with days to 50% flowering, days to maturity, yield, and first pod height.

***Keywords*:** Lentil, plant height, variety, growing season, autumn planting, summer planting

Introduction

Lentil (*Lens culinaris* Medic.), as other edible leguminous crops, is high in protein and contain vitamins and minerals that are vital for a healthy diet (Aydoğan et al, 2020; Irmak and Sözen, 2024;). Due to the feature of extended storage stability, it is crucial to prevent food wasting. According to FAO, 2025, lentil is an important element of crop rotation systems while due to its resilience to the climate change it can contribute ecosystem and biodiversity. It can boost the soil biodiversity and fertility by fixing atmospheric nitrogen into the soil. In soils with low nitrogen content (Gustafson, 2017).

Economic sustainability is the priority of farmers and agricultural businesses (Pannell et al. 2006; Tey et al. 2012). They can continue to cultivate the crop when it makes a profit. If the crop is profitable, it also means that it is economically sustainable. The profitability of a crop is determined by yield and cost. There are numerous factors that could affect the yield of the crop production. It is possible to divide that factors into three main categories. Which are technical

(§) *This study was presented as an oral presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

factors (crop management practices, decision making), biological factors (insects, weeds, pests and diseases,) and environmental factors (climatic conditions, soil properties, topography, and water availability etc.) (Metclfe and Elkins, 1980). As like other crops, many of the factors affecting lentil yield are influenced by uncontrollable climate. Compared to cereals, legumes have lower yields under the same growing conditions (Aydoğan et al. 2002; Karadavut and Sözen, 2019). This is one of the most crucial factors that affect the increases in the production cost of the lentil.

An integral component of every economic activity is the inevitably arising cost structure. In general, in legume production, and specifically in lentil cultivation, the most decisive factor increasing production costs under Turkish conditions is the harvesting method. Lentil harvesting is carried out using three methods: 1. Manual harvesting; 2. Two-stage harvesting including machine harvesting, field drying, and finally threshing; 3. Machine (combine harvester) harvesting (Figure 1). Both manual and two-stage harvesting methods are performed when the plants turn from green to golden yellow (grain moisture content around 30%) (Khayrallah, 1981). In suitable fields without stones, combine harvesters are used directly when the lentils are completely dry during the harvesting and threshing process (Karadas et al., 2018). Methodologically, manual harvesting is the method that causes the least grain loss compared to other methods (Mennad et al., 2017).

In West Asia and North Africa (WANA) countries, lentil harvesting is mostly done by hand (Nannish, 2000). However, in many countries, hand harvesting practices stand out as one of the main factors limiting the sustainability of lentil production due to increasing labour costs and difficulties in securing labour in rural areas. In Türkiye, lentil harvesting is largely carried out through mechanisation. Nevertheless, in seasons when the plants exhibit a morphological structure unsuitable for machine harvesting due to climatic conditions, producers are forced to resort to hand harvesting methods. A study conducted in Jordan revealed that hand harvesting of lentils constituted 17% of the production cost (Nanish, 2000), while in Mardin, this harvesting method accounted for 9.6% of the total cost (Karadaş et al. 2018). Ghimire et al. (2024) reported that in Nepal, the highest cost in lentil production is labour, accounting for 40.3%, followed by harvesting costs at 16.7%. A study conducted in Mexico between 2019 and 2020 revealed that the labour cost of manual harvesting and subsequent threshing accounted for an average of 40% of the total production cost (Sáenz-Reyes et al., 2022). Another study reported that variable costs constituted 76.50% of lentil production costs, with 26.04% of these variable costs being harvesting and threshing expenses (Karadaş et al., 2018). It has been determined that for lentil production to be profitable, labour and harvesting costs need to be reduced by 30.1% and 23.6%, respectively, and a 1% increase in labour costs increases total production costs by 0.42% (Ghimire et al., 2024). Although harvesting costs are relatively low in mechanized harvesting, one of the significant disadvantages of this method is grain loss. Indeed, Mennad et al. (2017) reported that grain loss rates in mechanized harvesting reached 25% in Syria and ranged from 13-23% in Balkan countries.

The most determining factor in harvesting method is plant height. Suitability for mechanical harvesting depends primarily on the plant's structure, but also on its condition at harvest time. The morphological characteristics of lentils do not support a strong, upright stem form, which creates significant difficulties in mechanical harvesting. Furthermore, lentil stalks are thin and weak (Saxsena, 2009), and although stalk length is reported to vary between 15-75 cm (Malhotra et al., 1974), the plant height does not reach a level suitable for machine harvesting in most planting seasons. In addition, the widespread and prostrate growth of the above-ground parts of the plant is among the biggest obstacles to mechanized harvesting.

Kuzbakova et al., (2022) listed the factors related to plant height/first pod height as follows; (1) morphological characteristics: growth type, earliness, lateness, erect, prostrate and semi-prostrate, distribution of pods on the stem, (2) environmentally: planting time, photoperiod (long and short days), temperature, abiotic stress, (3) agronomically: planting time, planting density. In lentils, the heritability of first pod height is defined as low-moderate (h^2:0.28; h^2: 0.55) (Tabti et al., 2018; Ahmad et al., 2021). Plant height during harvest is influenced by both genetic factors and environmental conditions (Tullu et al., 2001). Abiotic stresses such as drought and heat, and disease infections significantly affect plant growth and reduce plant height (Parihar et al., 2020; Pratap et al., 2021). Furthermore, it has been reported that early abiotic stress factors (extreme temperatures and drought) further reduce the height of the first pod in lentils, while late-stage stress factors have less effect on plant height (Kuzbakova et al., 2022). Within the scope of agronomic practices, the row spacing used in planting significantly affects plant height and the height of the first pod. It has been reported that the height of the first pod increases as the row spacing decreases (Togay, 2000). Studies have shown that harvesting lentils from a height of 5 cm above the soil surface results in the least loss compared to hand harvesting, with a reduction of 10.1% in grain

yield and 16.1% in straw yield (Silim et al. 1989). A harvesting height of 5 cm above the soil surface is claimed as the most appropriate level. In lentils, tall plant stature is a desirable characteristic because it allows for mechanised harvesting. Additionally, lentil straw, with its high protein content ranging from 4.2% to 7.8% (Erskine et al., 1989), is widely used in animal feeding. In many places, lentil straw is called "kes", and its price is almost the same as the price of the grain.

The research was conducted to determine the effect of growing season (autumn and spring sowing) and selected agronomic traits on plant height in red and green lentil genotypes for mechanised harvesting.

Materials and Methods

The experimental material consisted of the following varieties: Fırat 87, Şakar, Çağıl, Tigris, Şanlıbey, Çiftçi, and Evirgen (red; autumn-sown, winter-grown lentils); Meyveci 2001, Gümrah, Karagül, Bozok, Sultan 1; and the Canadian varieties Richlea and Laird (green; summer-sown lentils). The trials were established in a randomized complete block design (RCBD) with four replications. The trials were conducted over two years, in 2021 and 2022, as autumn and summer- sown at the Gölbaşı-İkizce Research and Application Farm of the Field Crops Central Research Institute. For autumn and summer sowing, 300 and 250 seeds per square meter were used, respectively. At sowing, 12 kg da^{-1} of DAP (diammonium phosphate) fertilizer was applied in all experiments.

Autumn trials were planted on October 26, 2020 and October 12, 2021, respectively, during the 2020/21 and 2021/22 seasons. Summer trials were planted on March 10, 2021 and March 29, 2022. During the trial period, observations were made on first pod height (cm) and plant height (cm), number of days to 50% flowering and number of days to maturity (d), plot yield (g plot-1), and 1000-seed weight (g/1000 seeds). Square root transformation was applied to first pod height and plant height. Plant height and first pod height were square root transformed. Plot yields were converted to kg da^{-1}. Plant height was subjected to variance analysis in terms of genotype, growing season, and years. Furthermore, correlations between the obtained observation values and plant height were examined. Statistical analyses were performed with the JMP 11 software. Climate data for the 2020/21 and 2021/22 growing seasons are given in Figures 2 and 3.

When climate data were examined, during the 2020/21 season autumn-sown and summer-sown plots received 190 mm and 113.4 mm of rainfall, respectively. During the following season, total rainfall 202 mm for summer-sown plots, whereas 83 mm was recorded for autumn-sown plots. Accordingly, the amount of rainfall differs in each season while autumn-sown plots received higher rainfall in 2020/21, summer-sown plots received higher rainfall in the 2021/22 growing season.

Results

Plant height and first pod height analysis

Autumn sowing

In the 2020/21 autumn-sown season, the average plant height was 22 cm. The difference in plant height between varieties was found to be statistically significant at the 1% level. The highest plant height was 25 cm in the Fırat 87 variety, while the lowest was 18 cm in the Evirgen variety. However, the season in 2021/22, the average plant height was determined to be 24 cm, and the differences in plant height between varieties were not statistically significant. The lowest height was 23 cm in the Tigris variety, and the highest was 25 cm in the Fırat 87, Çağıl, Şakar, and Şanlıbey varieties.

In the two-year average of autumn sowing conditions, the difference in plant height between varieties and years was found to be statistically significant at the level of 5% for varieties and 1% for years. The average plant height was 23.1 cm in autumn sowing over the experimental period (Table 1).

Summer sowing

The average plant height in the summer-sown season was 26 cm and 33.9 cm in 2021 and 2022, respectively. In both years, the highest plant heights were observed in the Canadian varieties Richlea and Laird. The difference in plant height between the varieties in the plantings carried out in the summer seasons of 2021 and 2022 was found to be statistically significant at the 1% level. The average for summer-sown over the two years was 30.2 cm. According to the combined analysis, the difference in plant height between the varieties and years was found to be statistically significant at the 1% level. The highest plant height over the two years was reached in the Laird variety with 36.5 cm (Table 2).

The overall evaluation revealed that the plant height of the varieties tested in 2022 was 29 cm higher than the previous year. The average plant height was 30 cm in summer sowing conditions and 23 cm in autumn sowing conditions. Specifically, the Fırat 87 variety reached 25 cm in autumn-sown, while the Laird variety reached 33 cm in summer-sown. The differences in plant height observed for the year and growing season were statistically significant at the 1% level (Figure 4). Plant height and first pod height (cm) values according to growing season and year are shown in Figure 5.

The height of the first pod varied between 9.6-13.0 cm in autumn-sown and between 11.6-15.0 cm in summer. The averages were 10.9 cm in autumn-sown and 13.6 cm in summer-sown. The highest first pod height was observed in the Şakar variety (autumn) at 14 cm, and in the Laird variety (summer) at 15.8 cm. Overall, an increase in plant height was associated with a corresponding increase in first pod height.

Morphological Observations Related to Plant Height

Correlation analysis was carried out to determine the relevance between morphological observations and plant height (Table 3). A statistically significant correlation detected at the 1% level between plant height and 50% flowering days, days to maturity, yield, and first pod height. An insignificant but negative relationship was also found with 1000-seed weight.

Yield

Statistical analysis values for combined yield (kg/da) for two years in autumn and summer seasons are given in Table 4. The average yield for two years in autumn-sown plots was 112 kg/da, whereas it was 153 kg/da in summer. Yields in summer-sown plots were 36.6% higher than those in autumn-sown plots. The highest yield under autumn sowing was observed for the Çiftçi variety (169 kg /da), whereas under summer sowing, the highest yield was recorded for the Karagül variety (179.8 kg/ da). The difference in yield between varieties and years in autumn-sown was found to be statistically significant at the 1% level. However, while the difference in yield between varieties in summer-sown was not statistically significant, the difference between years was found to be statistically significant at the 1% level.

Discussion

For mechanized harvesting of lentils, not only the height of the plant but also its upright growth habit is also a priority. When analysing the study data, the plant's growth patterns were not considered in either autumn or summer planting. In autumn planting, the plant height of the genotypes varied between 18 and 25 cm. In red lentils, the average plant height after two years of autumn planting was found to be 23.1 cm. The highest height was found in the Fırat 87 variety, described as "Commando" due to its spots, in the Southeastern Anatolia Region, at 24.9 cm. A study conducted in Konya showed that when planting was done on October 10th, the average plant height was 26.42 cm, while on October 20th it was 26.57 cm (Hakkoymaz and Önder, 2021). In other words, the difference in planting dates for autumn sowing did not affect plant height. Similarly, a study conducted in Southeastern Anatolia in 2014 used both red and autumn varieties, and plant height ranged from 44.3-55.0 cm, with first pod height varying between 10-24 cm (Koç and Akdeniz, 2019).

In our study, as well as in studies conducted in Konya and Ceylanpınar, although red varieties and autumn sowings in October, the plant height in Ceylanpınar was found to be higher compared to autumn sowings in the Central Anatolia Region. This is because in Central Anatolia, the cold winters prevent vegetative growth and halt plant development. In contrast, winters in Southeastern Anatolia are milder than in Central Anatolia, allowing plants to continue growing and making them more suitable for mechanised harvesting. A study reported that when harvesting with a machine at a speed of 5 km/h and a cutting height of 59 mm from the ground, the losses during manual harvesting were statistically insignificant (Sidahmed and Jaber, 2004). From this perspective, it has been observed that in a stone-free field, where mechanised planting has been done, and a roller has been passed over it after planting, the initial pod length should be at least 10 cm for mechanised harvesting when the machine head touches the ground.

Moreover, in summer-sowing, green lentil genotypes were preferred. As a result, plant heights ranged from 27.0 to 36.5 cm, and the average was 30.2 cm. The difference in plant height between the varieties was found to be statistically significant at the 1% level. Compared with the autumn-sown, the average plant height was recorded as 13.4 cm taller. It was observed that the highest height was obtained from two Canadian varieties: Richlea (35.1 cm) and Larid (36.5 cm). The Canadian varieties achieved the highest height due to their registered characteristics. Canada's geographical location plays a significant role in the climatic influence on plant morphology. Canadian varieties (registered at 60 degrees North latitude) experience longer summer days in their country of origin. When these varieties are brought to Türkiye (42 degrees North latitude), they complete their growing cycle in Türkiye with shorter daylight hours and more days. Therefore, in Türkiye, the Canadian variety fulfils its temperature/sunshine requirements over a longer period, resulting in even greater height growth.

Summerfield et al. (1985) stated that long days and high temperatures accelerate the flowering process, but genotypes show relative sensitivity to each of these factors, resulting in differences in flowering time under the same inductive environment. According to the results of our study, the plant height of other local summer varieties remained below 30 cm. According to the results of a study conducted in Yozgat by Erbaş

Köse et al. (2017), the longest plant height of 41.79 cm was observed in the Local-3 population among 5 registered (Sultan 1, Meyveci 2001, Gümrah, Bozok and Karagül) and 4 local lentil varieties. It is estimated that this is due to the intensive use of imported green lentil seeds in the region. In a study conducted in Kırşehir in 2013-14, the plant height of green lentils was found to be between 18 and 21.3 cm (Sözen and Karadavut, 2017). Although the varieties used in that study were the same as some of the genotypes used in our study, it is predicted that climate and soil conditions probably affected the obtaining of different plant heights.

A correlation study between morphological characters revealed a strong and positive relationship between plant height and 50% flowering days, maturation days, yield, and first pod height. Kang et al. (2017) reported a strong correlation between first pod height and plant height with plant density. Similarly, Kuzbakov et al. (2022) reported a strong correlation (r=0.83-0.89) between the height of the first pod and plant height. A study conducted in Pakistan to determine the suitability of 36 lentil genotypes for mechanized harvesting showed a negative relationship between yield and the minimum pod height, and a highly significant correlation between the minimum pod height and plant height (Jawad et al., 2019). Plant height and first pod height are also affected by abiotic stress factors such as drought and high temperatures, as well as diseases, leading to a decrease in height (Sözen and Karadavut, 2017; Parihar et al., 2020; Pratap et al., 2021). Indeed, in the 2021/22 growing season, plant height was greater in both autumn and summer crops compared to the 2020/21 season. This is because rainfall in April and May, which are crucial months for cultivation, was higher than in the previous year.

A study indicated that early-flowering lentil genotypes yielded higher harvests, while late-flowering genotypes grew taller (Buekert et al., 2020). In our study, the number of days to 50% flowering ranged from 65-75 days for summer varieties and 195-199 days for autumn varieties. In summer varieties, the early-flowering varieties Meyveci 2001 and Sultan 1, with 65 days of flowering, had plant heights of 27.6 and 27 cm, respectively. The late-flowering varieties were Richlea and Larid, with 75 days of flowering. However, in terms of yield, the early-flowering varieties Meyveci and Sultan 1 yielded 160.5 kg/da and 155.9 kg/da, respectively; while the late-flowering varieties Richlea yielded 144.7 kg/da and Larid yielded 134.1 kg/da. Although many studies indicate that preferring to sow in autumn over summer results in at least a 50% increase in yield (Andrews, 1987; Sakar et al., 1988; Aydoğan et al., 2004), in our study, the yield recorded from summer-sowing genotypes was 36.8 kg/da higher than that obtained from autumn-sowing. This difference is attributed to bird damage observed under autumn sowing conditions.

In conclusion, it has been observed that factors affecting plant height in lentils are influenced by both the growing season and the genotype. Accordingly, the study recommend that in lentil breeding studies, selection should be based on to prioritize on medium-late maturing genotypes rather than very early or very late varieties to achieve optimal plant height development.

Acknowledgment

This study was produced from National and Regional Lentil Breeding Projects of Türkiye. The projects were supported by the General Directorate of Agricultural Research and Policies (TAGEM).

This article represents an expanded and fully developed version of an initial abstract that was previously presented during the proceedings of the 5th International Plant Breeding Congress, held in Antalya, Türkiye, from December 01 to 05, 2025.

Figure 1. Harvesting methods of lentil cultivation.

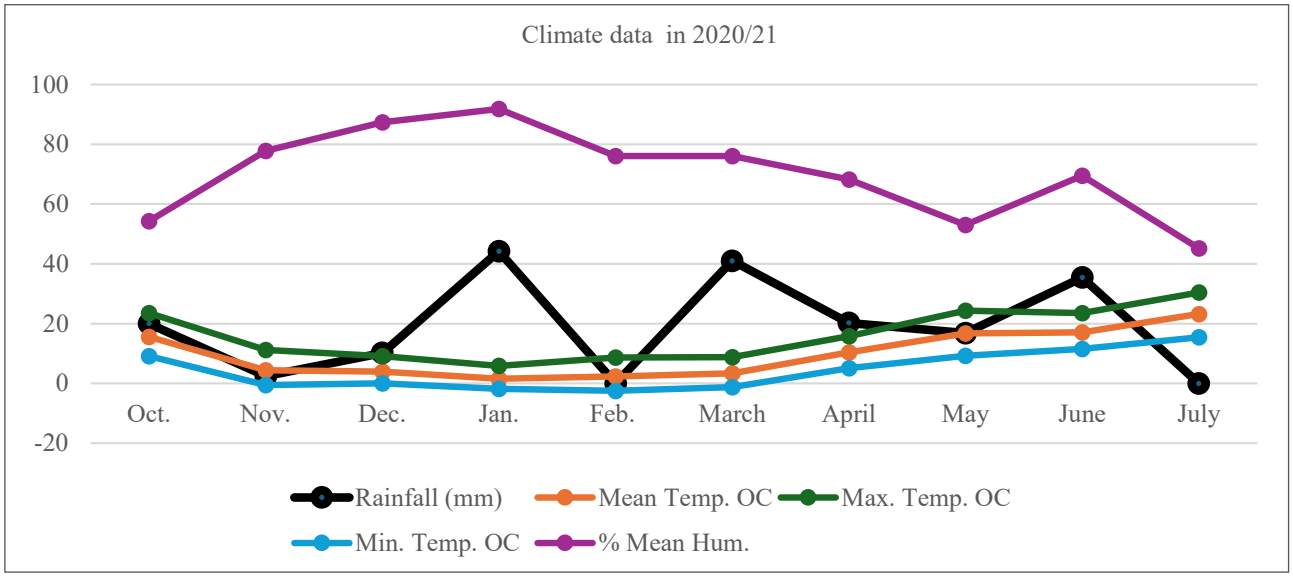


Figure 2. 2020/21 Climate data of Gölbaşı İkizce • Source: Turkish State Meteorological Service (TSMS), 2023

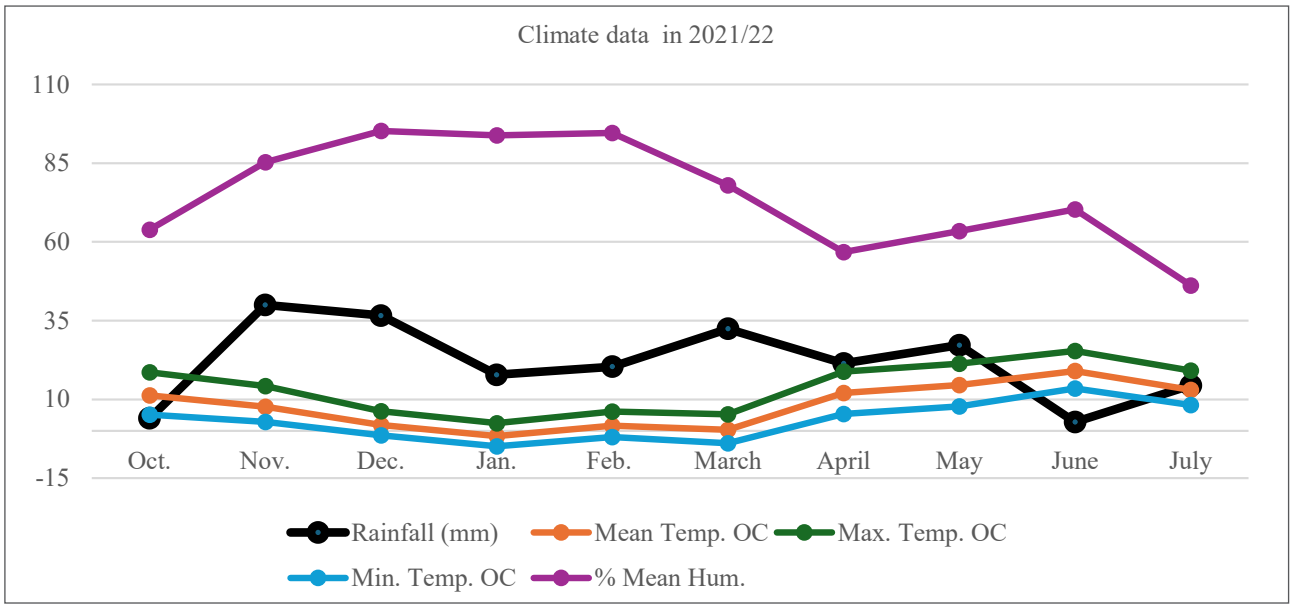
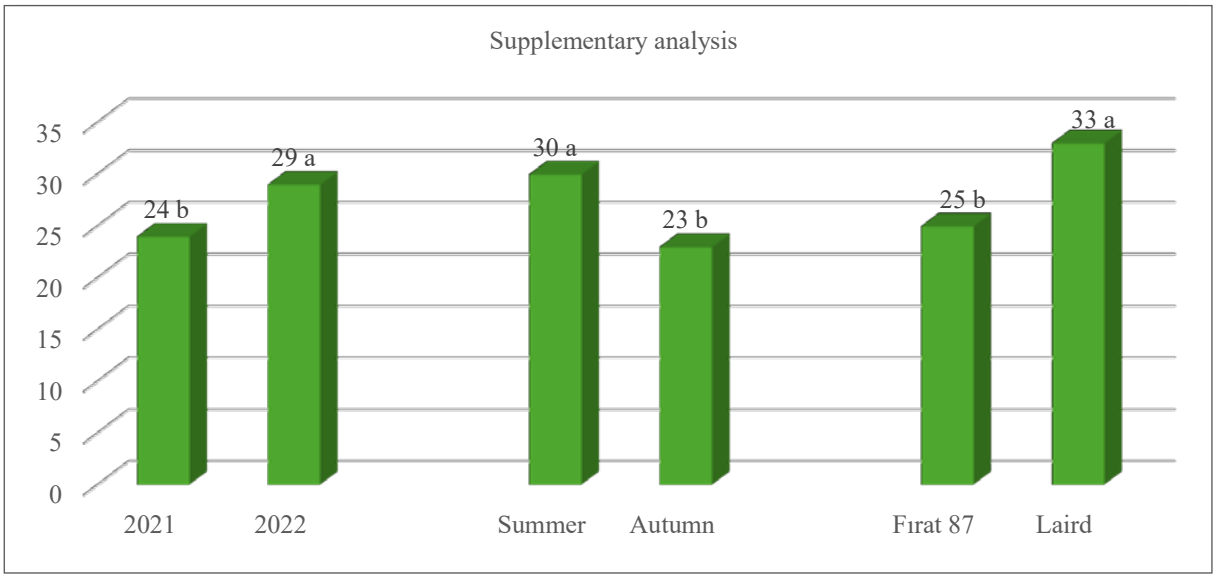
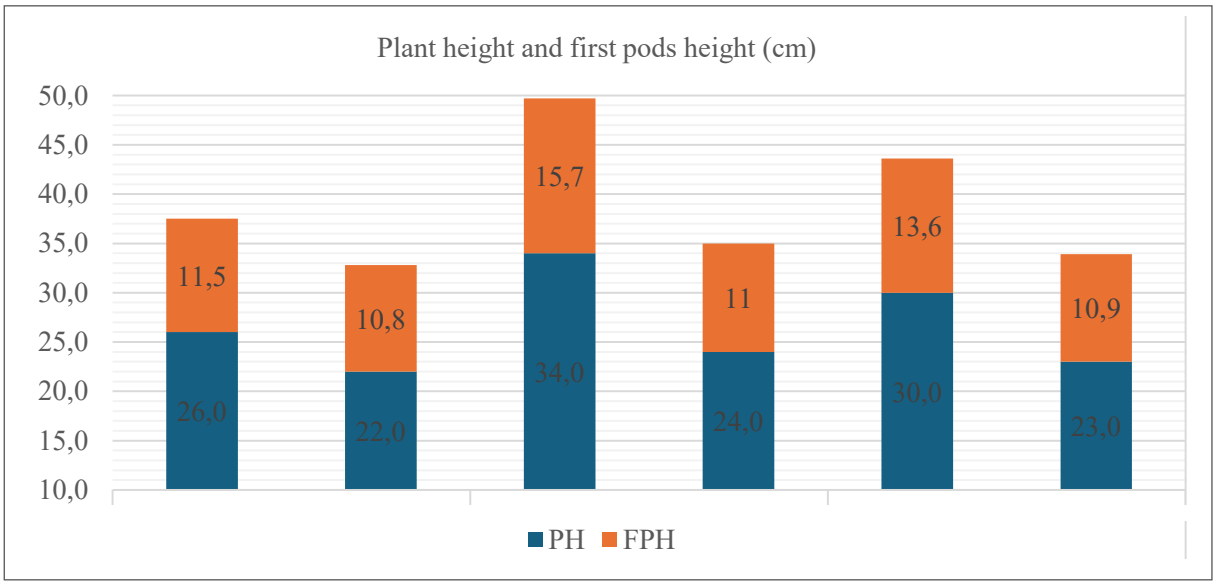


Figure 3. 2021/22 Climate data of Gölbaşı İkizce • Source: Turkish State Meteorological Service (TSMS), 2023



$P_{(Year)}$:**; P(Season):**; P(Season*Cultivar):*; CV (%): 7,56935

Figure 4. Plant height (cm) by year, growing season, and cultivars.



PH: Plant High; FPH: First Pod High

Figure 5. Variation in plant height and first pod height (cm) across growing seasons and years.

Table 1. Statistical analysis for plant height (cm) in Autumn sowing.

Cultivars	2020/21	2021/22	Mean of years for plant height
Fırat 87	25 a	25	24,9 a
Şakar	24 ab	25	24,4 ab
Çağıl	23 abc	25	23,9 ab
Çiftçi	22 bc	24	22,9 abc
Şanlıbey	20 cd	25	22,5 bc
Tigris	22 bc	23	22,3 bc
Evirgen	18 d	24	21,1 c
Mean	22	24	23,1
$F_{(Year)}$			18.10**
$F_{(Cultivar)}$	6.427**	0,352ns	3.12*
$F_{(Year*Cultivar)}$			2.44*
CV (%)	4.277	4,98	4,66
LSD	0,3	0,36	0,23

*:< %5; **: <%1, ns: not significant

Table 2. Statistical analysis for plant height (cm) in summer planting.

Cultivars	Years		Mean of Years for Plant Height
	2020/21	2021/22	
Meyveci 2001	23 c	32,3 b	27,6 b
Gümrah	26 bc	31,0 b	28,5 b
Karagül	24,5 bc	30,0 b	27,3 b
Bozok	25,8 bc	32,5 b	29,1 b
Sultan-1	22,0 c	32,0 b	27,0 b
Richlea	30,3 ab	40,0 a	35,1 a
Laird	33,5 a	39,5 a	36,5 a
Mean	26	33,9	30,2
$F_{(Year)}$			52,95**
$F_{(Cultivar)}$	4,42**	4,565**	8,13**
$F_{(Year*Cultivar)}$			0,75ns
CV (%)	7,42	4,98	6,47
LSD	0,56	0,48	0,36

*:< %5; **: <%1, ns: not significant

Table 3. Correlation between plant height and selected traits.

Traits	Days to 50% Flowering	Maturity duration (days)	Thousand grain weight	Yield	First pod height (cm)	Plant height (cm)
Days to 50% flowering		0.965**	−0.552**	0.320*	0.112	0.463**
Maturity duration (days)			−0.481**	0.348**	0.060	0.466**
Thousand grain weight				−0.003	−0.038	−0.067
Yield					0.192	0.533**
First pod height (cm)						0.402**
Plant height (cm)						

**p>0,01

Table 4. Combined analysis of yield values autumn- and summer-sown trials.

Autumn Sown		Summer Sown	
Cultivars	**Yield (kg/da)**	**Cultivars**	**Yield (kg/da)**
Çiftçi	169 a	Karagül	179,8 a
Şakar	136,5 b	Meyveci 2001	160,5 ab
Çağıl	133,6 b	Bozok	158,9 ab
Fırat 87	113,5 b	Sultan 1	155,9 ab
Tigris	81,1 c	Richlea	144,8 b
Şanlıbey	79,9 c	Gümrah	138,4 b
Evirgen	70,4 c	Laird	134,1 b
Mean	112,0		153,2
F (Cultivar)	14,773**	4,565**	ns
F (Year)	12,506**		46,38**
LSD (Cultivar)	27,32	4,98	34,779
CV (%)	24,05	0,48	22,39

*:< %5; **: <%1, ns: not significant

References

Ahmad, N. S., Moradi, N., Rafaat, J. G., and Mohammed, D. J. (2021). Genetic variability and heritability estimates of agronomic traits in lentil (*Lens culinaris* Medik.). *Romanian Agricultural Research*, *38*, 1-12.

Andrews, C. J. (1987). Low-temperature stress in field and forage crop production - An overview. *Canadian Journal of Plant Science*, *67*(4), 1121-1133. https://doi.org/10.4141/cjps87-152

Aydogan, A., Ates, D., Karagul, V., Keçeli, A., Sanal, T., Kaplan Evlice, A., Cinkaya, N., and Yuce, M. (2020). Grain yield, grain weight and protein, Fe, Zn, Se and phytic acid concentrations of lentil under dryland conditions. *International Journal of Agriculture and Biological Sciences*, *4*(6), 8-25.

Aydoğan, A., Karagül, V., and Bozdemir, Ç. (2002). Orta Anadolu Bölgesi kışlık mercimek (*Lens culinaris* Medik.) ıslah çalışmaları (In Turkish). *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi*, *11*(1-2), 1-8. https://izlik.org/JA23LF33UP

Bueckert, R., Zakeri, H., Pritchard, J., and Lafond, G. (2020). First versus last born: Flowers, pods, and yield formation in no-tillage lentil. *Crop Science*, *60*(3), 1634-1647. https://doi.org/10.1002/csc2.20141

Erskine, W., Rihawi, S., and Capper, B. S. (1990). Variation in lentil straw quality. *Animal Feed Science and Technology*, *28*(1-2), 61-69. https://doi.org/10.1016/0377-8401(90)90068-J

FAO (Food and Agriculture Organization). (2025). *World Pulses Day*. https://www.fao.org/world-pulses-day/en/ (Accessed: October 3, 2025).

Ghimire, B., Dhakal, S. C., Marahatta, S., and Bastakoti, R. C. (2024). Are lentil (*Lens culinaris*) farms productive, profitable, and efficient in resource allocation? A cross-sectional study from Nepal. *Legume Science*, *6*(1), e217. https://doi.org/10.1002/leg3.217

Gustafson, D. I. (2017). Greenhouse gas emissions and irrigation water use in the production of pulse crops in the United States. *Cogent Food & Agriculture*, *3*(1), 1334750. https://doi.org/10.1080/23311932.2017.1334750

Hakkoymaz, O., and Önder, M. (2021). Kışlık kırmızı mercimek (*Lens culinaris* Medic.) genotiplerinin fenolojik ve morfolojik özellikleri üzerine ekim

zamanlarının etkisi (In Turkish). *Journal of Bahri Dagdas Crop Research*, *10*(2), 155-160. https://dergipark.org.tr/tr/pub/bdbad

Jawad, M., Malik, S. R., Sarwar, M. A., Asadullah, M., Hussain, I., and Khalid, R. (2019). Genetic analysis of lentil (*Lens culinaris*) exotic germplasm to identify genotypes suitable for mechanical harvesting. *Pakistan Journal of Agricultural Research*, *32*(1), 152-158. https://doi.org/10.17582/journal.pjar/2019/32.1.152.158

JMP11. (2014). *JSL syntax reference*. SAS Institute.

Kang, B. K., Kim, H. T., Choi, M. S., Koo, S. C., Seo, J. H., Kim, H. S., et al. (2017). Genetic and environmental variation of first pod height in soybean [*Glycine max* (L.) Merr.]. *Plant Breeding and Biotechnology*, *5*(1), 36-44. https://doi.org/10.9787/PBB.2017.5.1.36

Karadaş, K., Bakçı, C., and Kadirhanoğulları, İ. H. (2018). Tarım işletmelerinde mercimek üretim maliyetinin hesaplanması (In Turkish). *Journal of Agricultural Faculty of Atatürk University*, *49*(2), 118-123. https://doi.org/10.17097/ataunizfd.384604

Irmak, M., and Sözen, Ö. (2024). Kırşehir ekolojik koşullarında bazı yeşil mercimek genotiplerinin tarımsal özelliklerinin belirlenmesi (In Turkish). In V. Saruhan and B. Bayhan (Eds.), *Tarımda Güncel Araştırma Konuları II* (1st ed., pp. 50). Iksad Publishing House.

Karadavut, U., and Sözen, Ö. (2019). Yerel mercimek genotiplerinin verime etki eden bazı karakterleri için genotipik ve çevresel etkilerin belirlenmesi (In Turkish). *Türk Tarım ve Doğa Bilimleri Dergisi*, *6*(4), 870-877. https://doi.org/10.30910/turkjans.633623

Khayrallah, W. A. (1979). Mechanization of lentil harvesting. In C. Webb & G. Hawtin (Eds.), *Lentils* (pp. 131-141). Commonwealth Agricultural Bureaux.

Koç, A., and Akdeniz, H. (2019). Bazı kırmızı mercimek (*Lens culinaris* Medik.) genotiplerinin, Beyazkule-Ceylanpınar sulu koşullarında verim ve teknolojik özelliklerinin belirlenmesi (In Turkish). *Erciyes Tarım ve Hayvan Bilimleri Dergisi*, *2*(2), 15-20. https://izlik.org/JA99PY38CX

Kuzbakova, M., Khassanova, G., Oshergina, I., Ten, E., Jatayev, S., Yerzhebayeva, R., Bulatova, K., Khalbayeva, S., Schramm, C., Anderson, P., Sweetman, C., Jenkins, C. L. D., Soole, K. L., and Shavrukov, Y. (2022). Height to first pod: A review of genetic and breeding approaches to improve combine harvesting in legume crops. *Frontiers in Plant Science*, *13*, Article 948099. https://doi.org/10.3389/fpls.2022.948099

Erbaş Köse, Ö. D., Bozoğlu, H., and Mut, Z. (2017). Yozgat koşullarında yetiştirilen yeşil mercimek genotiplerinin verimine ekim sıklığının etkisi (In Turkish). *KSÜ Doğa Bilimleri Dergisi*, *20*(Özel Sayı), 351-355. https://doi.org/10.18016/ksudobil.349244

MGM (Meteoroloji Genel Müdürlüğü). (2023). *Meteoroloji Genel Müdürlüğü*. https://mgm.gov.tr/ (Accessed: December 10, 2023).

Malhotra, R. S., Singh, K. B., and Singh, J. K. (1974). Genetic variability and genotype-environmental interaction studies in lentil. *Journal of Research, Punjab Agricultural University*, *10*(1), 17-21.

Metcalfe, D. S., Elkins, D. M., and Hughes, H. D. (1980). *Crop production: Principles and practices* (4th ed.). Macmillan.

Mennad, T., Taklit, A., Houari, A., Khelili, S., and Bedjaoui, A. (2017). Harvesting loss: Solutions for the challenges faced in the production and harvesting of lentil (*Lens culinaris*) in Algeria. *American-Eurasian Journal of Sustainable Agriculture*, *11*(2), 36-42.

Nannish, N. (2000). *Lentil and chickpea production in Jordan*. National Centre of Agricultural Research and Technology Transfer (NCARTT).

Pannell, D. J., Marshall, G. R., Barr, N., Curtis, A., Vanclay, F., and Wilkinson, R. (2006). Understanding and promoting adoption of conservation practices by rural landholders. *Australian Journal of Experimental Agriculture*, *46*(11), 1407-1424. https://doi.org/10.1071/EA05037

Parihar, A. K., Dixit, G. P., Bohra, A., Gupta, D. S., Singh, A. K., Kumar, N., et al. (2020). Genetic advancement in dry pea (*Pisum sativum* L.): Retrospect and prospect. In S. S. Gosal and S. H. Wani (Eds.), *Accelerated plant breeding, volume 3: Food legumes* (pp. 283-341). Springer, Cham. https://doi.org/10.1007/978-3-030-47306-8_10

Pratap, A., Das, A., Kumar, S., and Gupta, S. (2021). Current perspectives on introgression breeding in food legumes. *Frontiers in Plant Science*, *11*, Article 589189. https://doi.org/10.3389/fpls.2020.589189

Sakar, D., Durutan, N., and Meyveci, K. (1988). Factors which limit the productivity of cool season food legumes in Turkey. In R. J. Summerfield (Ed.),

World crops: Cool season food legumes (pp. 137-145). Kluwer Academic Publishers. https://doi.org/10.1007/978-94-009-2764-3_14

Saxena, M. C. (2009). Plant morphology, anatomy and growth habit. In W. Erskine, F. J. Muehlbauer, A. Sarker, & B. Sharma (Eds.), *The lentil: Botany, production and uses* (pp. 34-46). CABI.

Sáenz-Reyes, J. T., Muñoz-Flores, H. J., Ruíz-Rivas, M., Rueda-Sánchez, A., Castillo-Quiroz, D., and Castillo-Reyes, F. (2022). Diagnosis of lentil cultivation in family production units in Michoacán. *Revista Mexicana de Ciencias Agrícolas*, *13*(27), 23-35. https://doi.org/10.29312/remexca.v13i27.3160

Sidahmed, M. M., and Jaber, N. S. (2004). The design and testing of a cutter and feeder mechanism for the mechanical harvesting of lentils. *Biosystems Engineering*, *88*(3), 295-304. https://doi.org/10.1016/j.biosystemseng.2004.04.002

Silim, S. N., Saxena, M. C., and Erskine, W. (1989). Effect of cutting height on the yield and straw quality of lentil and on a succeeding wheat crop. *Field Crops Research*, *21*(1), 49-58. https://doi.org/10.1016/0378-4290(89)90040-3

Sözen, Ö., and Karadavut, U. (2017). Bazı yeşil mercimek genotiplerinde dane verimi ve verim komponentleri arasındaki ilişkilerin belirlenmesi (In Turkish). *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi*, *26*(1), 104-110. https://doi.org/10.21566/tarbitderg.323605

Sözen, Ö., and Karadavut, U. (2017). Determination of the relationship between yield and yield components of winter red lentil genotypes under the conditions of Amik Plain. *Türk Tarım ve Doğa Bilimleri Dergisi*, *4*(4), 468-476.

Summerfield, R. J., Roberts, E. H., Erskine, W., and Ellis, R. H. (1985). Effects of temperature and photoperiod on flowering in lentils (*Lens culinaris* Medic.). *Annals of Botany*, *56*(5), 659-671. https://doi.org/10.1093/oxfordjournals.aob.a087055

Tabti, D., Laouar, M., Rajendran, K., Kumar, S., and Abdelguerfi, A. (2018). Analysis of gamma rays induced variability in lentil (*Lens culinaris* Medik.). *Agronomy Research*, *16*(5), 2169-2178. https://doi.org/10.15159/AR.18.202

Tey, Y. S., and Brindal, M. (2012). Factors influencing the adoption of precision agricultural technologies: A review for policy implications. *Precision Agriculture*, *13*(6), 713-730. https://doi.org/10.1007/s11119-012-9273-6

Togay, N. (2000). Van koşullarında sıra aralığı ve serpme ekimin mercimek (*Lens culinaris* Medic) çeşitlerinde verim ve verim öğelerine etkisi (In Turkish). *Journal of Agricultural Sciences*, *6*(4), 125-130. https://doi.org/10.1501/Tarimbil_0000000989

Tullu, A., Kusmenoglu, I., McPhee, K. E., and Muehlbauer, F. J. (2001). Characterization of core collection of lentil germplasm for phenology, morphology, seed and straw yields. *Genetic Resources and Crop Evolution*, *48*(2), 143–152. https://doi.org/10.1023/A:1011254629628

Research Article

Ekin
Journal of Crop Breeding and Genetics

12(2):128-141, 2026

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Agronomic Characteristics and Cold Tolerance of Some Wheat Genotypes Under Different Ecological Locations(§)

Berrin DUMLU[1*] Ümran KÜÇÜKÖZDEMİR[1] Halit KARAGÖZ[2] Orçun YILMAZ[3]

[1] Eastern Anatolia Agricultural Research Institute, Erzurum, Türkiye

[2] Ege University, Faculty of Agriculture, Department of Field Crops, İzmir, Türkiye

[3] Aegean Agricultural Research Institute, İzmir, Türkiye

[*] Corresponding author e-mail: berrin.dumlu@tarimorman.gov.tr

Citation:
Dumlu B., Küçüközdemir Ü., Karagöz H., Yılmaz O., 2026. Agronomic Characteristics and Cold Tolerance of Some Wheat Genotypes under Different Ecological Locations. Ekin J. 12(2):128-141.

Received: 26.06.2026 ***Accepted: 22.07.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

This study aimed to evaluate the agronomic characteristics and cold tolerance of 18 wheat genotypes (11 advanced lines and 7 standard cultivars) under different ecological conditions of Eastern Anatolia. Field experiments were conducted during the 2021-2022 growing season in four locations (Erzurum-Aziziye, Erzurum-Pasinler, Erzincan, and Muş) using a randomized complete block design with three replications. Agronomic traits such as grain yield, protein content, thousand grain weight, and hectoliter weight were measured. Cold tolerance was tested under controlled conditions at -15, -17, and -19 °C. The results revealed significant ($P< 0.01$) genotypic differences for all parameters. The average survival rates were 67.07%, 54.74%, and 38.08% at -15, -17, and -19 °C, respectively, indicating a clear decline with decreasing temperature. Among the genotypes, Parla and genotype 10 displayed the highest cold tolerance, while Esperia and Palandöken 97 exhibited superior protein and hectoliter weights. Lines 1 and 5 are thought to be suitable candidates for registration due to their high yield and large grains. PCA separated the genotypes based on yield, quality, and cold response, showing that the traits are largely independent. These results indicate a wide genetic variation for cold tolerance and quality traits among the tested materials, suggesting that genotypes such as Parla and Palandöken 97 could serve as valuable parents in breeding programs targeting cold tolerance and yield stability in harsh winter conditions.

Keywords**:** Wheat, cold tolerance, grain yield, protein content, thousand grain weight, genotype × environment interaction, principal component analysis (PCA)

Introduction

The sustainability of global cereal production depends on the adaptation of genotypes to environmental stress conditions for yield and quality. Particularly in major crops such as wheat, not only agronomic traits like grain yield but also quality components such as protein content, hectolitre weight, and thousand-grain weight have gained increasing importance (Han et al., 2014). Different genotypes may variably perform for quality traits under the same growing conditions. Moreover, exposure to low-temperature stresses such as frost and freezing is known to adversely affect wheat development, survival, and grain formation. For example, based on the LT_{50} criterion used in frost tolerance assessment, genotypic differences were observed, and this trait was found to be associated with thousand-grain weight (Dumlu et al., 2025). In this context, elucidating the relationships between quality, yield characteristics, and cold tolerance is critical for genotype selection, especially in high-altitude or extremely cold environments. In this study, 18 genotypes were evaluated across four different locations (Aziziye, Erzincan, Muş, and Pasinler), and for each genotype, yield, thousand-

(§) *This study was presented as an oral presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

grain weight, hectolitre weight, protein content, and cold tolerance test results were analysed. Thus, the aim was to compare the genotypes not only in terms of yield potential but also regarding their quality and cold adaptation capacities.

Materials and Methods

Plant Material and Experimental Design

In this study, eighteen wheat genotypes, 11 advanced lines and 7 standard cultivars (Ayyıldız, Alturna, Alparslan, Doğu 88, Esperia, Palandöken 97, and Parla), were used as genetic material. The trials were conducted in the 2021-2022 growing season at four locations representing different altitudes and climates. Experiments followed a randomised complete block design (RCBD) with three replications to minimise environmental variation. The research was conducted on 7,2 square meter plots for two years during the 2021-2022 growing seasons. In both years, wheat was sown as a winter crop using a seed drill on October 15th. Diammonium phosphate and nitro power (26%) were used as fertilizers in the experiments. All of the phosphorus fertilizer was applied with sowing, half of the nitrogen fertilizer immediately after sowing, and the remaining half during the stem elongation period, at a rate of 6 kg N and 6 kg P_2O_5 per decare.

Data Collection

Grain yield per decare (kg/da), protein content (%), hectoliter weight (kg hL^{-1}), and thousand-grain weight (g) were measured. Cold tolerance tests were conducted under controlled environmental conditions. The experiment involved applying three different low temperature treatments to the plants: -15, -17, and -19°C (Dumlu et al., 2025). After completing the cold treatments in a climate chamber and cold room, the pots were transferred to a greenhouse environment. To determine the effects of low temperature treatments on plant viability, the plants were grown in the greenhouse for three weeks. At the end of this period, the number of surviving plants was recorded (Figure 2).

Statistical Analysis

Data were analyzed using analysis of variance (JMP 7). Differences among genotypes averages for the traits were tested by the LSD method at the 1% level. Principal Component Analysis (PCA) was used to identify associations among traits and to group genotypes according to their performance.

Results and Discussion

The results of this study demonstrate that cold stress has a strong impact on survival and yield-related traits. The superior performance of Parla and Alturna under cold conditions suggests the presence of effective physiological and molecular mechanisms, such as the activation of the CBF/COR gene family, which enhances freezing tolerance (Dhillon et al., 2013; Knox et al., 2010).

The wide variation among genotypes agrees with findings from Fowler and Gusta (1979), who reported LT_{50} values ranging from -18°C to -24°C among winter wheat cultivars. The PCA results further confirmed that cold tolerance and yield traits are controlled by distinct but complementary mechanisms.

A- Yield Performance Across Locations

The grain yields of the 18 wheat genotypes across the experimental locations are presented in Table 1. Analysis of variance revealed highly significant differences ($P < 0.01$) among locations and in the combined dataset for grain yield. The mean yield at the Aziziye was 674.4 kg da^{-1}, with the highest yield obtained from line 4 (761.1 kg da^{-1}) and the lowest from the cultivar Palandöken 97 (530.6 kg da^{-1}). These findings indicate clear differences among genotypes and suggest that certain lines are better adapted to the environmental conditions of Aziziye. Similar observations have been reported in previous studies, where genotypes exhibited differential yield responses within the same environment (Öztürk et al., 2021). In Erzincan, the mean yield was 630.8 kg da^{-1}. The highest yield was recorded for genotype 1 (698.4 kg da^{-1}), while the lowest was obtained from the cultivar Doğu 88 (556.7 kg da^{-1}). Compared with Aziziye, yield levels in Erzincan were slightly lower and varied within a narrower range. This pattern reflects a genotype × environment interaction, which is frequently used to explain yield variability among environments (Kara et al., 2018). At the Muş location, the mean yield was 380.6 kg da^{-1}, with the highest yield recorded for Palandöken 97 (474.6 kg da^{-1}) and the lowest for genotype 1 (263.4 kg da^{-1}). The relatively low mean yield indicates that the environmental conditions of Muş were more limiting and less favourable for wheat production. Consistent with this, several studies have reported wider yield gaps among genotypes under environmentally constrained conditions (Yılmaz et al., 2024). In Pasinler, the average yield was 515,3 kg da^{-1}. The highest yield was obtained from the Alturna genotype (652,8 kg da^{-1}), and the lowest from genotype 6 (461.1 kg da^{-1}). This location provided an intermediate yield environment, allowing for a balanced expression of genotypic variation. Similar findings have been reported by Demir, (2000), who observed that moderate environments tend to reduce extreme yield differences among genotypes. Across all locations, the overall mean yield was 543,5 kg da^{-1}. Aziziye and Erzincan emerged as high-yielding sites, Muş

represented a low-yielding environment, and Pasinler occupied an intermediate position (Table 1). These results confirm the strong influence of genotype x environment interaction and demonstrate that genotype performance is environment-dependent. Hence, it is unrealistic to expect high-yielding genotypes to perform uniformly across diverse growing conditions (Kara et al., 2018; Yılmaz et al., 2024).

B- Protein Content and Genotype × Environment Interactions

The grain protein contents of the 18 wheat genotypes determined across four different locations are presented in Table 2. According to the variance analysis results, the effects of locations and the combined analysis were found to be highly significant ($p < 0.01$) for protein content. The mean protein content in the Aziziye location was calculated as 13.17%. The highest protein content was obtained from the genotype Doğu 88 (16.14%), while the lowest was recorded in genotype 8 (9.61%). This indicates clear differences among genotypes, suggesting that certain genotypes adapted more effectively to the environmental conditions of Aziziye. Similarly, Öztürk et al. (2021) reported that different wheat genotypes exhibited variable protein performance within the same location due to genotype × environment (G×E) interactions. In Erzincan, the average protein content was found to be 13.33%. The highest value was again observed in Doğu 88 (16.39%), while the lowest was in genotype 8 (9.77%). These results indicate that protein levels in Erzincan were slightly higher than in Aziziye, although the variation among genotypes followed a similar trend. Kara et al. (2018) emphasised that environmental factors such as soil structure and temperature exert a considerable influence on protein synthesis, and that differences among genotypes are shaped by these environmental effects. In the Muş location, the mean protein content was determined as 12.12%. The highest protein percentage was once again obtained from Doğu 88 (14.85%), while the lowest was found in genotype 8 (8.84%). The lower mean protein value in Muş suggests that the region's environmental conditions-particularly temperature fluctuations and limited soil nitrogen availability-restricted protein accumulation. In line with this, Yılmaz et al. (2024) reported that under environments with low yield potential, greater variations in protein content tend to occur among genotypes. In Pasinler, the mean protein content was measured as 12.65%, with the highest value recorded in Doğu 88 (15.49%) and the lowest in genotype 8 (9.23%). This location represented a moderately favourable environment, enabling distinct yet balanced differences among genotypes. Demir, (2000) similarly noted that in environments with moderate productivity, genotypic differences tend to be more homogeneous and less extreme. When all locations were combined, the overall mean protein content was calculated as 12.83%. The genotype Doğu 88 had the highest mean value (15.74%), while genotype 8 exhibited the lowest (9.37%). These findings clearly demonstrate that the G×E interaction significantly influenced grain protein content. Aziziye and Erzincan were identified as high-protein environments, Muş as a low-protein site, and Pasinler as an intermediate environment. Generally, the fact that high-protein genotypes (such as Doğu 88 and Esperia) did not show consistent performance across all environments indicates that genetic potential interacts dynamically with environmental conditions. Consistent with the literature, it is well established that protein content in wheat is determined by both genetic and environmental factors. In particular, nitrogen availability, temperature, and rainfall play a major role in determining protein accumulation (Kara et al., 2018; Yılmaz et al., 2024; Demir, 2000). Therefore, in breeding programmes aimed at achieving higher protein content, not only genotype selection but also the specific environmental characteristics of each location must be carefully considered (Table 2). This result confirms the generally negative relationship between protein content and yield, which tends to be strengthened under variable environmental conditions (Triboi et al., 2003; Hernandez et al., 2018).

C- Hectolitre Weight and Genotype × Environment Interaction

The hectolitre weight (HLW) values of the 18 wheat genotypes evaluated across four different locations revealed that the genotypes responded differently to environmental conditions, indicating a significant genotype × environment (G×E) interaction effect. According to the variance analysis results, differences among locations and the combined analysis were statistically highly significant ($p < 0.01$) for hectolitre weight. In the Aziziye location, the mean HLW was determined as 79.59 kg hl^{-1}, with the highest value recorded in genotype 1 (85.4 kg hl^{-1}) and the lowest in genotype 11 (74.6 kg hl^{-1}). In Erzincan, the average HLW was slightly higher (80.44 kg hl^{-1}), where the highest value was observed in Esperia (85.72 kg hl^{-1}) and the lowest again in genotype 11 (75.24 kg hl^{-1}). The Pasinler and Muş locations exhibited lower mean HLW values compared with the other environments. The average HLW in Pasinler was 76.41 kg hl^{-1}, whereas in Muş it was 73.25 kg hl^{-1}. These values are considerably lower than those obtained in Aziziye and Erzincan. In Muş, the highest HLW was obtained from genotype 1 (78.57 kg hl^{-1}), while the lowest was

found in genotype 10 (65.5 kg hl^{-1}). In Pasinler, the highest value was again recorded in genotype 1 (81.98 kg hl^{-1}), and the lowest in genotype 10 (68.35 kg hl^{-1}). These findings demonstrate that genotypes, particularly under Muş and Pasinler conditions, experienced notable reductions in grain density and filling capacity. This can largely be attributed to the climatic characteristics of these regions, such as lower temperatures, shorter and cooler vegetation periods, or suboptimal grain filling conditions associated with low radiation and high humidity. Evers and Millar (2002) reported that grain filling and endosperm density are closely related to environmental stress, while Williams et al. (2008) found that hectolitre weight may vary by up to 10% depending on temperature and water stress. Similarly, Hernandez et al., (2018) and Sharma et al. (2020) observed that low temperature, high humidity, and shortened grain-filling duration negatively affected HLW in wheat genotypes grown under different climatic regions. In the combined analysis, the mean HLW across all locations was calculated as 77.47 kg hl^{-1}. The highest average value was obtained from genotype 1 (82.74 kg hl^{-1}), and the lowest from genotype 10 (71.96 kg hl^{-1}). These results indicate that some genotypes exhibit more stable performance under varying environmental conditions. Finlay and Wilkinson (1963) and Yan and Kang (2002) emphasised that genotypes with high mean performance and low environmental sensitivity in multi-environment trials tend to possess broad adaptation ability. In this context, genotype 1 and Esperia can be considered promising materials with broad environmental adaptability and stable performance in terms of hectolitre weight, whereas genotypes 10, 11, and 8, which consistently exhibited lower HLW values, appear to be more susceptible to environmental stress. These findings are consistent with those reported by Peterson et al. (1992), confirming that hectolitre weight is determined by the combined effects of both genetic structure and environmental factors (Table 3).

D- Thousand Grain Weight and Genotype × Environment Interaction

The thousand grain weight (TGW) values of the 18 wheat genotypes evaluated across four different locations revealed significant differences both among genotypes and between environments. According to the results of the variance analysis, the effects of location and the combined analysis were found to be highly significant ($p < 0.01$) for thousand grain weight. In the Aziziye location, the mean TGW was determined as 34.98 g, with the highest value recorded in genotype 1 (41.21 g) and the lowest in genotype 4 (29.35 g). In Erzincan, the average TGW was calculated as 35.57 g; genotype 5 exhibited the highest value (44.91 g), while genotype 4 showed the lowest (29.64 g). In the Muş and Pasinler locations, mean TGW values were 31.83 g and 33.59 g, respectively. These comparatively lower values, particularly in Muş, can be attributed to the adverse effects of low temperatures and the shorter growing season during the grain-filling stage. Similarly, Liu et al. (2016) and Kumar et al. (2019) reported that thousand grain weight in wheat is negatively affected by environmental stresses, particularly low temperature and drought conditions. Moreover, the variation in genotype performance across locations highlights the importance of genotype × environment (G×E) interaction. Finlay and Wilkinson (1963) and Yan and Kang (2002) emphasised that genotypes showing high mean performance combined with low environmental sensitivity in multi-environment trials possess broad adaptation capacity. In line with this, Öztürk et al. (2021) and Kara et al. (2018) observed that different wheat genotypes exhibited variable TGW values within the same environment, resulting from the interaction between genetic structure and environmental factors. Similarly, Liu et al. (2016) and Kumar et al. (2019) reported that grain weight in wheat is adversely affected by low temperatures and water stress. According to the combined analysis, the mean TGW across all locations was calculated as 34.06 g. The highest value was obtained from genotype 5 (40.03 g), while the lowest was recorded in genotype 4 (28.47 g). These findings confirm that both genetic potential and environmental conditions play a determining role in thousand grain weight, and that some genotypes possess broad environmental adaptability (Table 4). This trait is closely associated with grain density and plumpness and is strongly influenced by environmental stress factors (Evers and Millar, 2002; Williams et al., 2008).

E- Cold Tolerance and LT_{50} Evaluation under Controlled Conditions

In this controlled study, the winter hardiness levels of advanced breeding lines and standard cultivars were evaluated at -15, -17, and -19°C in three replications, and significant differences among genotypes were observed at all temperature levels ($p < 0.01$). According to the results, the mean survival rate was 67.07% at -15°C, 54.74% at -17°C, and 38.08% at -19°C. This indicates that as temperature decreases, the survival rate of the genotypes declines, clearly defining the threshold of cold tolerance. Based on LT_{50} (the temperature at which 50% of plants are killed), 15 genotypes maintained more than 50% survival at -15°C, while at -17°C, 13 genotypes exhibited survival above 50% and five genotypes showed regeneration between 30-50%. At -19°C, however, the average survival rate decreased

to 38.08%. At this lowest temperature, significant differences were detected among genotypes in terms of survival rate: the highest survival was observed in the cultivar Parla (63.33%) and genotype 10 (53.48%), whereas the lowest survival was recorded in genotype 11 (17.22%). This finding suggests that Parla exhibits superior cold tolerance by maintaining metabolic activity and preserving cellular membrane integrity under low temperatures. Such performance is consistent with mechanisms reported in highly cold-tolerant winter wheat cultivars, which are associated with strong cold acclimation capacity and elevated expression of the *CBF/COR* gene family (Dhillon et al., 2013; Knox et al., 2010). Conversely, the low survival rate of genotype 11 (17.22%) can be attributed to membrane disruption, osmotic imbalance, and insufficient synthesis of protective proteins under cold stress. Similarly, Fowler and Gusta (1979) reported that genotypes with lower survival rates at sub-zero temperatures typically exhibit higher LT_{50} values, indicating weaker cold tolerance. The results obtained at -19°C therefore demonstrate a wide range of variation in winter hardiness among the tested genotypes, highlighting that genotypes such as Parla possess superior adaptation capacity to extremely low temperatures and could serve as valuable genetic material in future breeding programmes for winter-hardy wheat. These findings are in agreement with previously reported LT_{50} values - for instance, -24°C in the cultivar *Norstar* (Fowler and Gusta, 1979) - and confirm that there is broad genetic variation in cold tolerance among wheat genotypes. Similarly, Knox et al., (2010) reported that LT_{50} in winter wheats varies between -18°C and -24°C depending on genotype, acclimation duration, and *CBF/COR* gene expression. Overall, this study demonstrates that most of the tested genotypes possess moderate to high levels of winter hardiness and show strong potential for local adaptation. The significant genotypic differences observed further indicate that these materials represent an important genetic resource for breeding programmes aimed at improving winter survival in wheat (Table 5).

The 3D scatter plot illustrates the survival rates of 18 wheat genotypes measured at -15, -17, and -19°C, thereby visualising the differences in their levels of cold tolerance. As observed, the survival rate of the genotypes decreases with declining temperature, clearly demonstrating the physiological impact of cold stress on plant vitality. In particular, the cultivar Parla, with a survival rate of 63.33%, and line 10, with 53.48%, exhibited superior performance under low-temperature conditions, indicating a strong capacity for cold acclimation. This observation aligns with previous findings in the literature, where enhanced cold tolerance in winter wheat has been associated with increased expression of *CBF* (C-repeat Binding Factor) and *COR* (Cold Regulated) gene families (Dhillon et al., 2013; Knox et al., 2010). Conversely, genotype 11, which recorded a low survival rate of 17.22%, likely suffered from an inability to maintain cell membrane integrity, low membrane lipid saturation, and inadequate antifreeze protein synthesis. Fowler and Gusta (1979) similarly reported that genotypes exhibiting low survival rates under freezing stress tend to have higher LT_{50} values, indicating weaker cold tolerance. The findings of this study are consistent with LT_{50} values previously reported in the literature- for example, -24°C in the cultivar *Norstar* (Fowler and Gusta, 1979), thereby confirming the presence of wide genetic variation in cold tolerance among the tested genotypes. Consequently, genotypes such as Parla and line 10, which maintain high survival rates under extremely low temperatures, can be considered valuable genetic materials for advanced breeding programmes targeting improved winter hardiness in wheat (Figure 1).

F-Principal Component Analysis (PCA) of Yield, Quality, and Cold Tolerance Traits

A Principal Component Analysis (PCA) was conducted to examine the multivariate relationships between yield and quality traits (hectolitre weight, thousand grain weight, and protein content) and the cold tolerance values determined at different temperature levels (-15, -17, and -19°C). The first two principal components explained the majority of the total variance, indicating that the variables were effectively summarised within a two-dimensional plane (Figure 3). According to the PCA biplot, hectolitre weight and thousand grain weight were positioned in the same region-specifically in the upper-right quadrant-indicating a strong and positive association between these two traits. This finding suggests that genotypes with larger and denser grains tend to have higher hectolitre weights. In contrast, protein content was located in the opposite direction of the first principal component, revealing a negative relationship between this trait and the yield-related variables. This observation aligns with previous studies reporting a general inverse correlation between protein content and grain yield in wheat (Triboi et al., 2003; Hernandez et al., 2018). The cold tolerance variables (-15, -17, and -19°C) clustered together in the lower-right quadrant, indicating that they were positively correlated with each other but weakly associated with yield and quality traits. Among these, the -19°C variable most clearly distinguished genotypes in terms of cold tolerance. Similarly, studies by Dhillon et al. (2013) have shown

that, under extreme low-temperature conditions, the physiological mechanisms determining genotype selection are largely independent of yield components. Overall, the PCA results demonstrated that quality-related traits (hectolitre weight and protein content) and stress-tolerance traits (cold tolerance) were clearly separated, suggesting that these two groups of traits are controlled by distinct genetic and physiological mechanisms. This finding emphasises the importance of simultaneously evaluating yield stability and cold adaptation in breeding programmes conducted in high-altitude regions with a high risk of frost.

G - Cluster Analysis Based on Yield, Quality, and Cold Tolerance Traits

According to the heatmap, the combined analysis results illustrate the clustering of 18 wheat genotypes based on yield, protein content, thousand grain weight, hectolitre weight, and cold tolerance traits under different low-temperature conditions (-15, -17, and -19°C). In the colour scale, red tones represent higher values, while green tones indicate lower ones. Overall, varieties such as Esperia and Palandöken 97 stood out in certain quality parameters-particularly in protein content and hectolitre weight-whereas Alturna and several breeding lines (notably lines 4, 5, and 6) exhibited higher grain yield and thousand grain weight. In the cold tolerance tests, lines showing greener tones at -17 and -19°C (lines 3, 4, 5, and 6) were identified as being more resistant to low temperatures. This observation supports the potential genetic trade-off between cold tolerance and high yield or grain weight reported in the literature (Li et al., 2018). Moreover, a generally negative correlation between protein content and yield-commonly described in wheat (Triboi et al., 2003)-was also evident here, as high-protein genotypes (Esperia and Palandöken 97) tended to show comparatively lower yields. Thus, the clustering pattern in the heatmap indicates that the genotypes can be grouped into genetically distinct subclusters based on their cold tolerance and quality characteristics. These differences could be effectively exploited in breeding programmes aimed at optimising both yield performance and environmental adaptation potential (Figure 4).

H- Venn diagram illustrating the overlap of wheat genotypes across four key traits

The Venn diagram illustrates the comparative distribution of 11 advanced wheat lines and 7 standard cultivars (Alparslan, Alturna, Ayyıldız, Doğu 88, Esperia, Palandöken 97, and Parla) based on protein content, cold tolerance (-19°C), grain yield, and hectolitre weight, according to the combined analysis results. The analysis revealed that the cultivar Alparslan was located in the overlapping region of both the protein and cold tolerance categories, indicating a genetic advantage combining high protein potential with tolerance to low temperatures. Similarly, line 1, which intersected with the cultivar Ayyıldız in the same region, exhibited a comparable combination of high protein content and cold tolerance. Esperia was positioned at the intersection of the yield and hectolitre weight groups, together with line 5, suggesting that these two genotypes stand out with their high grain plumpness and yield potential. This indicates that Esperia and line 5 could serve as valuable parental materials in breeding programmes targeting both physiological resilience and agronomic productivity. Likewise, line 8, together with the cultivar Parla, was positioned exclusively within the hectolitre weight region, highlighting their superiority in terms of grain density and quality attributes. The absence of triple or quadruple intersections suggests that these traits are largely independent, being controlled by distinct genetic mechanisms. In agreement with previous reports indicating weak correlations between yield, protein content, and cold tolerance in wheat (Kurt et al., 2022; Tadesse et al., 2019), the present findings demonstrate that some advanced lines share close trait similarities with specific standard cultivars. In particular, the Ayyıldız-Line 1, Esperia-Line 5, and Parla-Line 8 pairings emerge as promising candidates for future breeding programmes aimed at developing cold-tolerant, high-yielding, and high-quality wheat genotypes, and could therefore be prioritised as potential parental genitors in hybridisation efforts (Figure 5).

The results indicated highly significant ($p < 0.01$) genotypic differences for all traits. Parla and Alturna displayed superior cold tolerance and yield stability across environments, while Esperia and Palandöken 97 exhibited high protein and hectoliter values. The mean survival rate declined with decreasing temperature, with 67.07% at -15°C, 54.74% at -17°C, and 38.08% at -1°C.

At -19°C, Parla had the highest survival rate (63.33%), followed by genotype 10 (53.48%), whereas genotype 11 had the lowest (17.22%). This pattern indicates wide variation in cold tolerance among genotypes. The correlation matrix showed that yield and cold tolerance were positively related, while protein content was relatively independent of yield performance.

Conclusions

In this study, eighteen wheat genotypes (11 advanced breeding lines and 7 standard cultivars) were evaluated across four different locations (Aziziye,

Erzincan, Muş, and Pasinler) in terms of yield, quality, and cold tolerance characteristics. The findings revealed significant variation among genotypes and demonstrated that environmental conditions exerted a strong influence on these traits. In terms of grain yield, the Aziziye (674.4 kg da^{-1}) and Erzincan (630.8 kg da^{-1}) locations were the most productive. The highest yield was obtained from line 4 (761.1 kg da^{-1}), while the lowest was recorded for the cultivar Palandöken 97 (530.6 kg da^{-1}). Regarding protein content, the cultivars Doğu 88 and Esperia stood out, with an overall mean of 12.83%. The highest protein content (15.74%) was observed in Doğu 88, while the lowest (9.37%) was recorded in line 8. For hectolitre weight, line 1 (82.74 kg hl^{-1}) and the cultivar Esperia (82.66 kg hl^{-1}) exhibited the highest values, whereas line 10 recorded the lowest. The thousand grain weight (TGW) was highest in line 5 (40.03 g) and line 1 (39.82 g). These genotypes, with their larger kernels, reflected high yield potential and stable performance under stress conditions. In cold tolerance tests, the mean survival rates were 67.07% at -15°C, 54.74% at -17°C, and 38.08% at -19°C. The highest survival rate was observed in the cultivar Parla (63.33%), whereas the lowest was found in line 11 (17.22%). This demonstrates that Parla possesses a superior cold acclimation capacity.

The PCA biplot analysis revealed a clear structural variation among the examined wheat genotypes. The first two principal components together explained 70.4% of the total variance, indicating that the agronomic and physiological traits analysed effectively captured the genotypic differences. The PC1 axis primarily demonstrated an inverse relationship between yield and the cold tolerance levels (-15°C, -17°C, -19°C). This finding indicates that high-yielding genotypes tended to be more sensitive to low temperatures, whereas lower-yielding genotypes exhibited greater cold tolerance. This is consistent with previous studies reporting increased stress sensitivity in high-yielding genotypes (Poudel et al., 2020; Ding et al., 2020). On the PC2 axis, protein content and hectolitre weight showed positive loadings, forming the main component for distinguishing quality-related traits. The cold tolerance variables were positioned in the same direction, confirming their high correlation and indicating that genotypes responded similarly to low-temperature stress. In the PCA biplot, Parla, line 1, and Palandöken 97 clustered within the cold-tolerant group, whereas lines 2, 4, and 11 were grouped as high-yielding but cold-sensitive genotypes. These results highlight PCA as an effective tool for evaluating adaptation and stress tolerance across different environments. The findings suggest that the use of cold-tolerant genotypes as parents in breeding programmes could enhance yield stability in winter wheat cultivation. Conversely, high-yielding but less tolerant genotypes could be better utilised in mild-climate production systems. Future integration of PCA results with genomic selection and marker-assisted breeding data is expected to facilitate more accurate and durable genetic gains for stress tolerance. The results obtained from this study provide important insights for advanced wheat breeding programmes: Parla, line 10, and Palandöken 97 can be considered as winter-hardy parental materials due to their combination of high cold tolerance and moderate-to-high yield potential. Doğu 88 and Esperia, with their high protein content and hectolitre weights, are promising for quality-oriented breeding. Lines 1 and 5, with high yield and large grain size, are strong candidates for varietal registration**.** According to the PCA findings, selecting genotypes that balance yield, quality, and cold tolerance will provide more efficient genetic progress. The results offer valuable phenotypic foundations for application in marker-assisted and genomic selection strategies (Figure 6).

This study revealed substantial genetic diversity at both agronomic and physiological levels and identified promising genotypes combining high yield potential with strong winter hardiness and grain quality traits. The findings provide a solid scientific basis for the development of cold-tolerant, high-quality, and stable-yielding wheat cultivars adapted to the Eastern Anatolia region. The combined use of field performance data, cold tolerance tests and PCA analysis enabled a comprehensive evaluation of wheat genotypes under contrasting ecological conditions.

Acknowledgements

This article represents an expanded and fully developed version of an initial abstract that was previously presented during the proceedings of the 5th International Plant Breeding Congress, held in Antalya, Türkiye, from December 01 to 05, 2025.

This study was conducted at the Eastern Anatolia Agricultural Research Institute and was produced from the project “Eastern Anatolia Wheat Breeding Studies” number TAGEM/17/A07/P01/002.

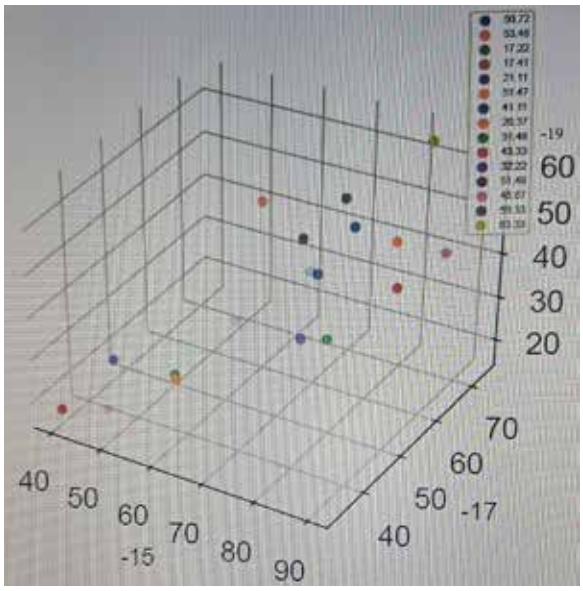


Figure 1. 3D scatter plot of cold tolerance degrees.

Figure 2. Climate cabin and greenhouse images from cold resistance studies.

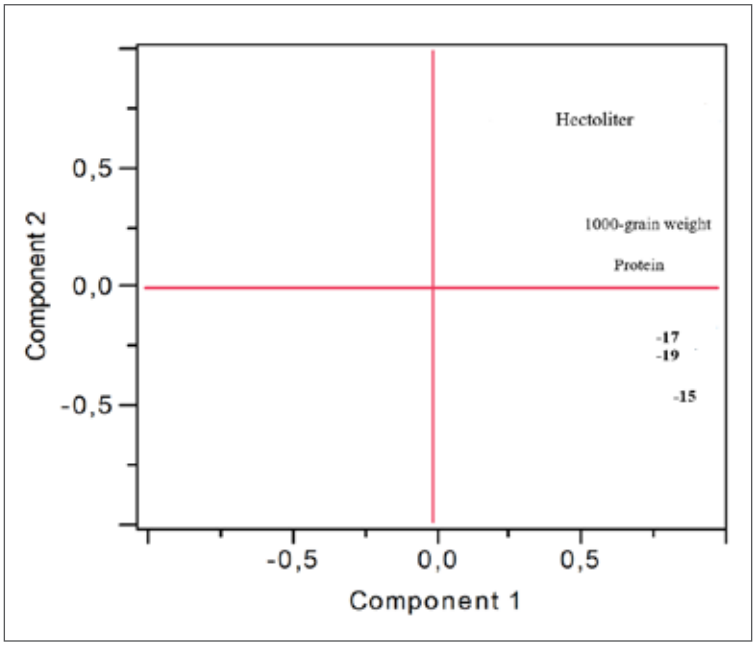


Figure 3. Multivariate Evaluation of Genotypic Relationships (PCA) chart.

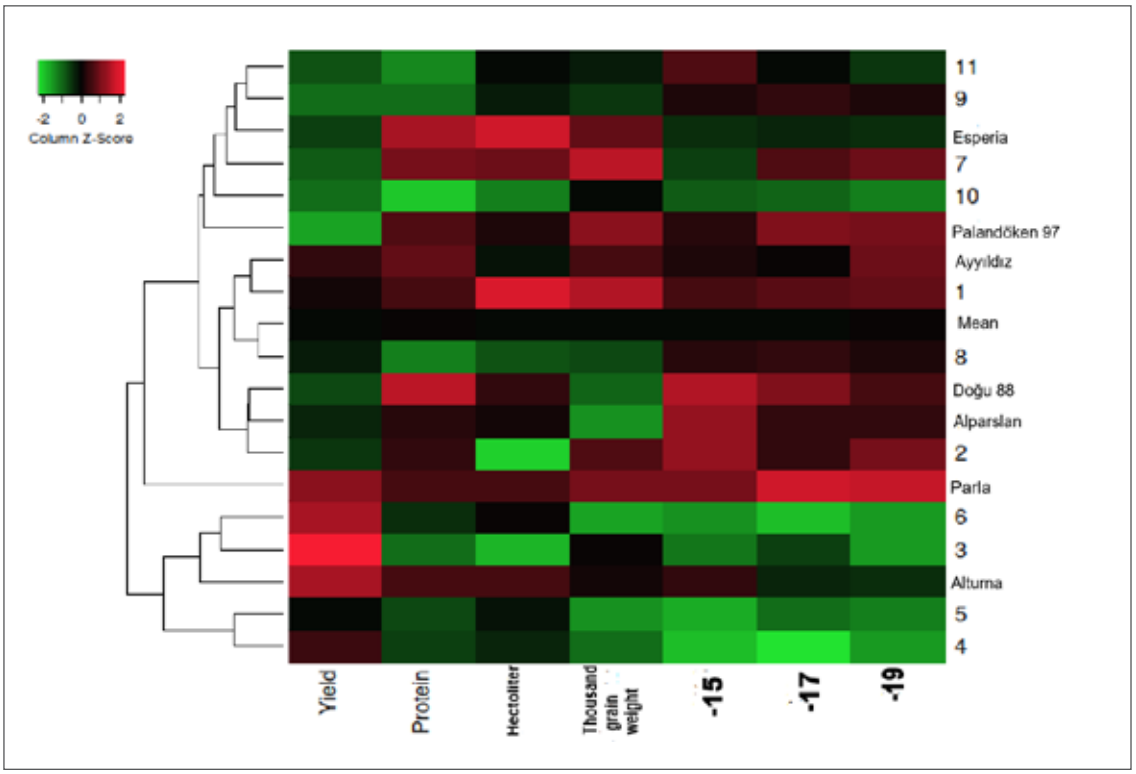


Figure 4. Heat Mapper Cluster Analysis Based on Yield, Quality, and Cold Tolerance Traits Chart.

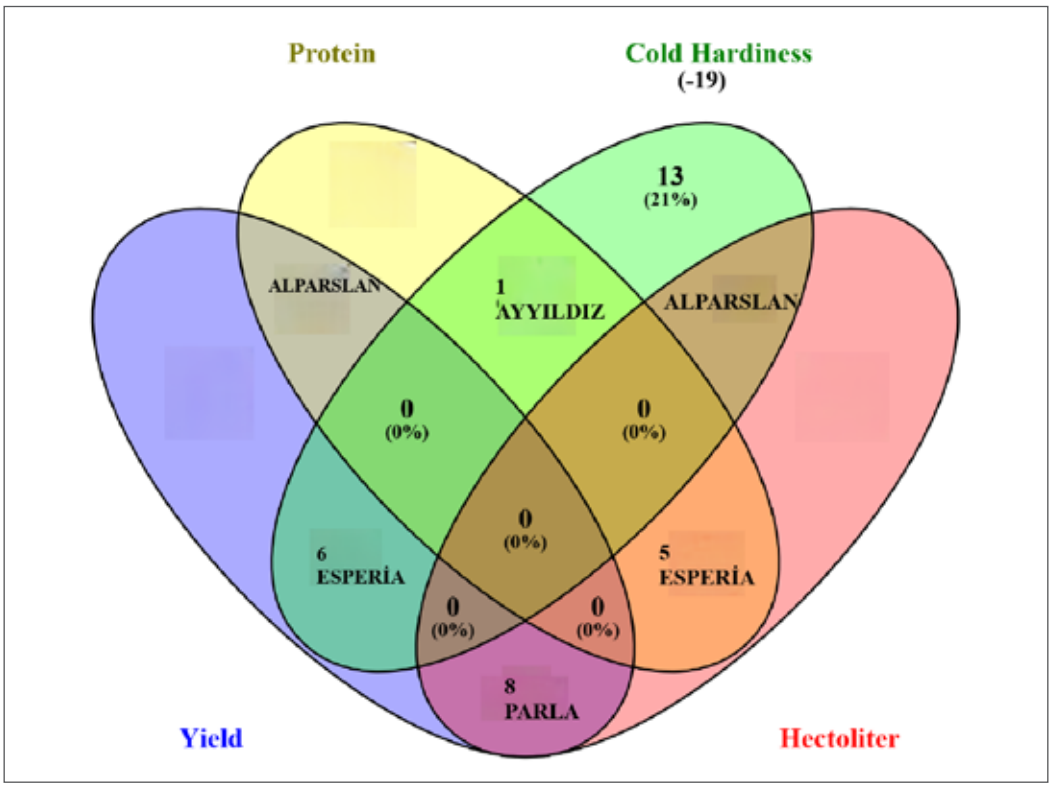


Figure 5. Venn diagram illustrating the overlap of wheat genotypes across four key traits.

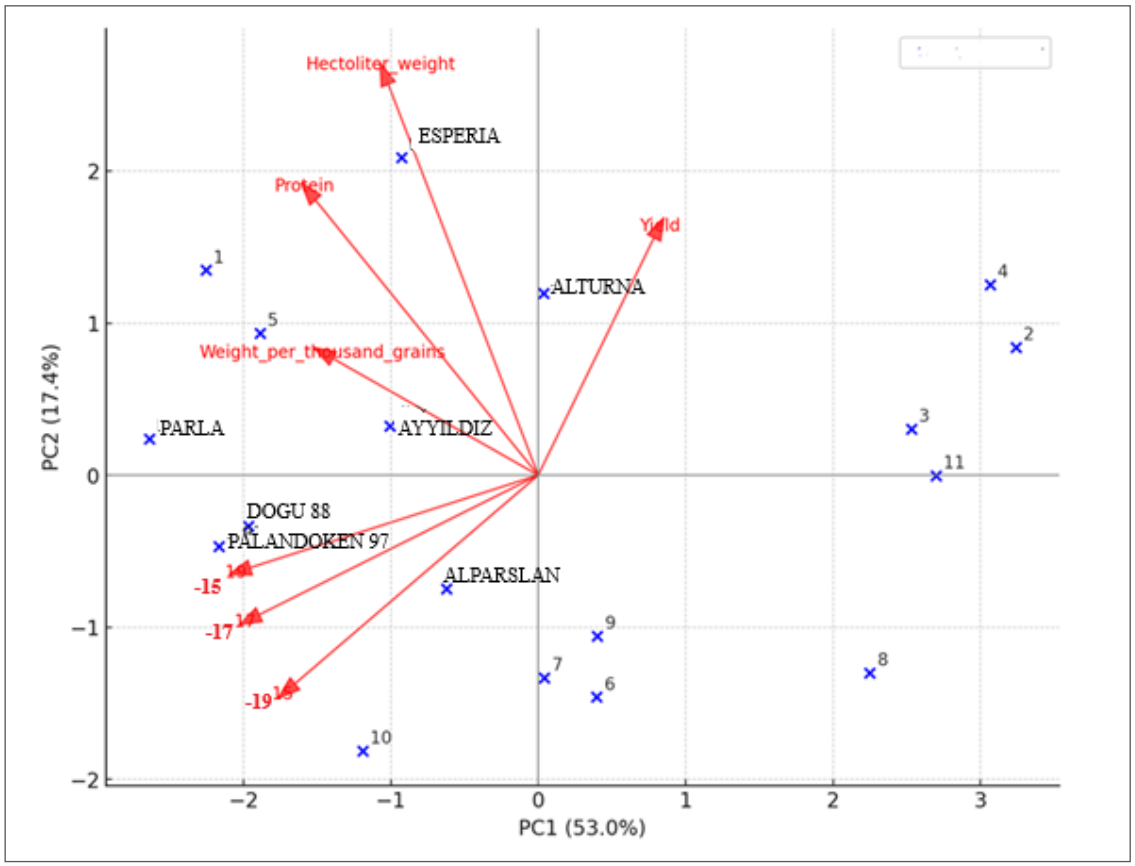


Figure 6. Principal component biplot analysis of the wheat genotypes evaluated..

Table 1. Yield performance of genotypes by location.

Genotype	Aziziye	Erzincan	Muş	Pasinler	Combined
11	716,7 a-c	677,8 ab	472,4 a	573,6 bc	610,1 a
4	761,1 a	672,0 a-c	390,9 b-e	529,4 c-e	588,3 ab
Alturna	752,2 ab	638,9 c-e	304,5 fg	652,8 a	587,1 ab
Parla	711,9 a-d	637,7 de	425,0 a-d	540,0 b-d	578,7 bc
2	696,1 b-e	683,1 ab	332,4 e-g	525,0 c-e	559,2 cd
Ayyıldız	645,3 e-g	658,3 b-e	328,8 e-g	594,2 ab	556,6 c-e
1	658,9 d-g	698,4 a	263,4 g	571,7 bc	548,1 d-f
3	632,5 fg	666,4 a-d	324,0 e-g	543,9 b-d	541,7 d-g
6	644,2 e-g	602,3 fg	438,2 a-c	461,1 f-h	536,5 d-h
Alparslan	605,6 g-ı	638,0 de	360,7 d-f	529,4 c-e	533,4 d-h
10	558,1 ıj	597,2 g	450,7 a-c	514,4 c-f	530,1 e-h
Esperia	687,2 c-f	663,0 b-e	276,6 g	479,7 e-h	526,6 f-h
Doğu 88	572,8 h-j	556,7 h	452,9 ab	508,9 d-f	522,8 f-ı
9	631,9 fg	629,9 e-g	380,9 c-e	441,4 gh	521,0 f-ı
5	615,8 gh	560,9 h	406,6 a-d	493,9 d-g	519,3 g-ı
8	608,9 g-ı	633,3 d-f	381,3 c-e	431,7 h	513,8 hı
7	622,8 gh	599,5 fg	386,4 b-e	440,8 gh	512,4 hı
Palandöken 97	530,6 j	541,7 h	474,6 a	443,3 gh	497,5 ı
Mean	674,4 A	630,8 B	380,6 D	515,3 C	543,5
CV	5,0	4,2	11,0	7,3	6,0
F Value	10,24**	15,14**	7,04**	8,29**	8,67**
LSD	51,3	33,8	70,6	59,9	55,3

Table 2. Protein ratios of genotypes by locations (%).

Genotype	Aziziye	Erzincan	Muş	Pasinler	Combined
Doğu 88	16,14 a	16,39 a	14,85a	15,49a	15,74a
Esperia	15,94 b	16,19 a	14,66b	15,3b	15,54b
5	15,19 c	14,89 bc	13,97c	14,58c	14,61c
Ayyıldız	14,74 d	14,97 b	13,56d	14,15d	14,37d
Palandöken 97	14,32 e	14,55 cd	13,17e	13,75e	13,96e
1	13,98 f	14,89 bc	12,86f	13,42f	13,94ef
Parla	14,29 e	14,52 cd	13,15e	13,72e	13,94ef
Alturna	14,24 e	14,46 d	13,1e	13,67e	13,88f
10	14,21 e	13,48 f	13,07e	13,64e	13,54g
Alparslan	13,78 g	14,00 e	12,68g	13,23g	13,44h
4	12,47 h	12,64 g	11,47h	11,97h	12,16ı
2	12,05 ı	12,22 h	11,09ı	11,57ı	11,75j
3	11,86 j	12,03 h	10,91j	11,39j	11,57k
7	11,27 k	11,44 ı	10,37k	10,82k	10,99l
11	11,25 k	11,44 ı	10,35k	10,8k	10,97l
6	10,98 l	11,15 ıj	10,1l	10,54l	10,71m
9	10,80 m	10,97 j	9,94m	10,37m	10,53n
8	9,61 n	9,77 k	8,84n	9,23n	9,37o
Mean	13,17 B	13,33 A	12,12 D	12,65 C	12,83
CV	6,0	10,2	6,1	5,0	7,0
F Value	1168,2**	214,5**	1214,9**	1210,7**	19,5**
LSD	0,14	0,35	0,14	0,15	0,14

Table 3. Hectolitre weight of genotypes by location.

Genotype	Aziziye	Erzincan	Muş	Pasinler	Combined
1	85,4 a	85,15 a	78,57 a	81,98 a	82,74 a
Esperia	85,0 b	85,72 a	78,20 b	81,60 b	82,66 a
5	83,6 c	81,21 b-d	76,91 c	80,26 c	80,09 b
Alturna	81,2 d	81,89 b	74,70 d	77,95 d	78,97 c
Parla	81,2 d	81,89 b	74,70 d	77,95 d	78,97 c
Doğu 88	80,8 e	81,49 bc	74,34 e	77,57 e	78,58 d
Palandöken 97	80,3 f	80,98 b-e	73,88 f	77,09 f	78,09 e
Alparslan	80,0 g	80,68 b-e	73,60 g	76,80 g	77,80 f
4	79,8 gh	80,33 b-e	73,42 gh	76,61 gh	77,61 g
9	79,6 h	80,13 b-e	73,23 h	76,42 h	77,41 h
3	79,3 ı	79,83 c-f	72,96 ı	76,13 ı	77,12 ı
Ayyıldız	79,2 ı	79,87 b-f	72,86 ı	76,03 ı	77,02 ı
7	78,9 j	79,43 d-f	72,59 j	75,74 j	76,73 j
2	78,7 j	79,24 ef	72,40 j	75,55 j	76,54 k
6	77,5 k	78,04 fg	71,30 k	74,40 k	75,37 l
8	76,3 l	76,84 gh	70,20 l	73,25 l	74,20 m
11	74,6 m	75,24 h	68,63 m	71,62 m	72,55 n
10	71,2 n	79,98 b-e	65,5 n	68,35 n	71,96 o
Mean	79,59 B	80,44 A	73,25 D	76,41 C	77,47
CV	3,0	15,2	3,3	4,0	3,1
F Value	1200,0**	13,5**	1190,2**	1202,8**	130,9**
LSD	0,26	1,89	0,24	0,26	0,33

Table 4. Thousand grain weights of genotypes by location.

Genotype	Aziziye	Erzincan	Muş	Pasinler	Combined
5	38,72 c	44,91 a	35,24 c	37,17 c	40,03 a
1	41,21 a	41,08 b	37,50 a	39,56 a	39,82 b
Palandöken 97	39,58 b	39,92 bc	36,02 b	38,00 b	38,40 c
Parla	38,94 c	39,27 b-d	35,44 c	37,38 c	37,77 d
Esperia	38,32 d	38,65 cd	34,87 d	36,79 d	37,17 e
10	37,82 e	38,00 c-e	34,42 e	36,31 e	36,64 f
Ayyıldız	37,46 f	37,78 de	34,09 f	35,96 f	36,34 g
Alturna	35,73 g	36,04 ef	32,51 g	34,40 g	34,66 h
11	35,18 h	35,48 f	32,01 h	33,77 h	34,12 ı
8	35,09 h	35,36 f	31,93 h	33,69 h	34,04 ı
9	34,30 ı	34,57 fg	31,21 ı	32,93 ı	33,27 j
7	33,15 j	33,43 gh	30,17 j	31,82 j	32,16 k
6	32,61 k	32,89 g-ı	29,68 k	31,31 k	31,64 l
Doğu 88	31,52 l	31,80 h-j	28,68 l	30,26 l	30,58 m
2	31,06 m	31,35 ı-k	28,26 m	29,82 m	30,13 n
Alparslan	29,83 n	30,10 jk	27,15 n	28,64 n	28,94 o
3	29,78 n	30,07 jk	27,1 n	28,59 n	28,89 o
4	29,35 o	29,64 k	26,71 o	28,18 o	28,47 p
Mean	34,98 B	35,57 A	31,83 D	33,59 C	34,06
CV	5,0	4,2	5,0	6,4	3,1
F Value	1196,35**	40,21**	1210,30**	1190,10**	101,52**
LSD	0,30	1,96	0,26	0,29	0,33

Table 5. Cold Resistance Degrees of Genotypes (%).

Genotype	-15	-17	-19
1	75,93 d	62,22 c	50,72 c
2	40,00 n	33,33 k	17,41 ı
3	41,67 m	44,44 ı	21,11 h
4	46,67 l	36,67 j	17,41 ı
5	58,89 ı	61,55 c	51,47 c
6	71,48 f	58,89 d	41,11 f
7	70,00 g	58,99 d	41,11 f
8	53,70 j	45,19 ı	20,37 h
9	76,67 d	54,17 f	31,48 g
10	85,93 b	58,52 d	53,48 b
11	50,53 k	49,05 h	17,22 ı
Alparslan	86,30 b	58,52 d	43,33 e
Alturna	73,33 e	51,85 g	32,22 g
Ayyıldız	70,95 fg	55,56 e	51,48 c
Doğu 88	90,00 a	65,93 b	46,67 d
Esperia	61,11 h	51,48 g	32,22 g
Palandöken 97	71,90 f	65,56 b	53,33 b
Parla	82,22 c	73,33 a	63,33 a
Mean	67,07 A	54,74 B	38,08 C
CV	10,0	9,3	14,2
F value	1197,9**	1194,8**	1199,6**
LSD	1,28	0,83	1,22

References

Demir, Y. (2000). Growth and proline content of germinating wheat genotypes under ultraviolet light. *Turkish Journal of Botany*, *24*(1), 67-70.

Dhillon, T., and Stockinger, E. J. (2013). Cbf14 copy number variation in the A, B, and D genomes of diploid and polyploid wheat. *Theoretical and Applied Genetics*, *126*(11), 2777-2789. https://doi.org/10.1007/s00122-013-2171-0

Ding, Y., Wang, W., Zhuang, Q., and Luo, Y. (2020). Adaptation of paddy rice in China to climate change: The effects of shifting sowing date on yield and irrigation water requirement. *Agricultural Water Management*, *228*, Article 105890. https://doi.org/10.1016/j.agwat.2019.105890

Dumlu, B., Tosun, M., Karagoz, H., Kucukozdemir, U., Bocianowski, J., Alipour, H., and Türkoglu, A. (2025). Assessment of genetic diversity in wheat (*Triticum aestivum*) genotype for cold tolerance, agronomic, and quality traits. *Crop and Pasture Science*, *76*(6), Article CP25047. https://doi.org/10.1071/CP25047

Evers, T., and Millar, S. (2002). Cereal grain structure and development: Some implications for quality. *Journal of Cereal Science*, *36*(3), 261-284. https://doi.org/10.1006/jcrs.2002.0435

Finlay, K. W., and Wilkinson, G. N. (1963). The analysis of adaptation in a plant-breeding programme. *Australian Journal of Agricultural Research*, *14*(6), 742-754. https://doi.org/10.1071/AR9630742

Fowler, D.B. and Gusta L.V., 1979. Selection for winterhardiness in wheat. I Identification of genotypic variability. Crop Sci. 19: 769-772. https://doi.org/10.2135/cropsci1979.0011183X001900060005x

Han, R., Jian, C., Lv, J., Yan, Y., Chi, Q., Li, Z., Wang, Q., Zhang, J., Liu, X., and Zhao, H. (2014). Identification and characterization of microRNAs in the flag leaf and developing seed of wheat (*Triticum aestivum* L.). *BMC Genomics*, *15*, Article 289. https://doi.org/10.1186/1471-2164-15-289

Hernandez-Ochoa, I. M., Asseng, S., Kassie, B. T., Xiong, W., Robertson, R., Pequeno, D. N. L., Sonder, K., Reynolds, M., Babar, M. A., Milan, A. M., and Hoogenboom, G. (2018). Climate change impact on Mexico wheat production. *Agricultural and Forest Meteorology*, *263*, 373-387. https://doi.org/10.1016/j.agrformet.2018.09.008

Kara, K., Rached-Kanouni, M., Mezghani, N., Mnasri, S., and Ben Naceur, M. B. (2018). Molecular characterization of bread wheat genotypes *Triticum aestivum* L. through microsatellites SSR markers. *International Multidisciplinary Scientific GeoConference: SGEM*, *18*(6.2), 377-384. https://doi.org/10.5593/sgem2018/6.2/S25.050

Knox, A. K., Dhillon, T., Cheng, H., Tondelli, A., Pecchioni, N., and Stockinger, E. J. (2010). CBF gene copy number variation at Frost Resistance-2 is associated with levels of freezing tolerance in temperate-climate cereals. *Theoretical and Applied Genetics*, *121*(1), 21-35. https://doi.org/10.1007/s00122-010-1288-7

Kumar, A., Kapoor, P., Chunduri, V., Sharma, S., and Garg, M. (2019). Potential of *Aegilops* sp. for improvement of grain processing and nutritional quality in wheat (*Triticum aestivum*). *Frontiers in Plant Science*, *10*, 308. https://doi.org/10.3389/fpls.2019.00308

Kurt, M., and Dizlek, H. (2022). The effects of two-step tempering treatment on the physical, chemical and technological properties of flour in bread wheats (*Triticum aestivum* L.). *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *25*(1), 181-190. https://doi.org/10.18016/ksutarimdoga.vi.878636

Li, C., Zong, Y., Wang, Y., Jin, S., Zhang, D., Song, Q., Zhang, R., and Gao, C. (2018). Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. *Genome Biology*, *19*, Article 59. https://doi.org/10.1186/s13059-018-1443-3

Liu, B., Asseng, S., Müller, C., Ewert, F., Elliott, J., Lobell, D. B., Martre, P., Ruane, A. C., Wallach, D., Jones, J. W., Rosenzweig, C., Aggarwal, P. K., Alderman, P. D., Anothai, J., Basso, B., Biernath, C., Cammarano, D., Challinor, A., Deryng, D., … Zhu, Y. (2016). Similar estimates of temperature impacts on global wheat yield by three independent methods. *Nature Climate Change*, *6*(12), 1130-1136. https://doi.org/10.1038/nclimate3115

Öztürk, A., Ekinci, S. A., Kodaz, S., and Aydın, M. (2021). Agronomic performance of the alternative cereal species in the highest plain of Turkey. *Journal of Agricultural Sciences*, *27*(2), 195-203. https://doi.org/10.15832/ankutbd.630466

Peterson, C. J., Graybosch, R. A., Baenziger, P. S., and Grombacher, A. W. (1992). Genotype and environment effects on quality characteristics of hard red winter wheat. *Crop Science*,

32(1), 98-103. https://doi.org/10.2135/cropsci1992.0011183X003200010022x

Poudel, M. R., Ghimire, S., Pandey, M. P., Dhakal, K., Thapa, D. B., and Poudel, H. K. (2020). Yield stability analysis of wheat genotypes at irrigated, heat stress and drought condition. *Journal of Biology and Today's World*, *9*(5), Article 220.

Sharma, N., Bhatia, S., Chunduri, V., Kaur, S., Sharma, S., Kapoor, P., Kumari, A., and Garg, M. (2020). Pathogenesis of celiac disease and other gluten related disorders in wheat and strategies for mitigating them. *Frontiers in Nutrition*, *7*, Article 6. https://doi.org/10.3389/fnut.2020.00006

Tadesse, W., Sanchez-Garcia, M., Assefa, S. G., Amri, A., Bishaw, Z., Ogbonnaya, F. C., and Baum, M. (2019). Genetic gains in wheat breeding and its role in feeding the world. *Crop Breeding, Genetics and Genomics*, *1*, e190005. https://doi.org/10.20900/cbgg20190005

Triboï, E., Martre, P., and Triboï-Blondel, A.-M. (2003). Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content. *Journal of Experimental Botany*, *54*(388), 1731-1742. https://doi.org/10.1093/jxb/erg183

Williams, R. M., O'Brien, L., Eagles, H. A., Solah, V. A., and Jayasena, V. (2008). The influences of genotype, environment, and genotype × environment interaction on wheat quality. *Australian Journal of Agricultural Research*, *59*(2), 95-111. https://doi.org/10.1071/AR07185

Yan, W., and Kang, M. S. (2002). *GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists*. CRC Press.

Yilmaz, H., Karatas, R., Demirel, F., Soysal, S., Türkoğlu, A., Yilmaz, A., and Ciftci, V. (2024). Variations in protein, gluten, Zeleny sedimentation and yield of certain wheat (*Triticum aestivum* L.) cultivars under different climatic conditions. *Euphytica*, *220*(12), Article 190. https://doi.org/10.1007/s10681-024-03446-8

Research Article

Ekin
Journal of Crop Breeding and Genetics

12(2):142-152, 2026

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Applications of Chromosome Doubling in Haploid Squashes and Pumpkins (*Cucurbita* spp.)(§)

Emre İPEK[1*] Büşra ALTUNTAŞ[2] Süleyman KAVAK[3] Ş. Şebnem ELLİALTIOĞLU[4]

[1] Petektar Seed R&D Center, Kurşunlu Mah. 12081. Sk. B Blok No:19, Aksu, Antalya, Türkiye

[2] Petektar Seed R&D Center, Kurşunlu Mah. 12081. Sk. B Blok No:19, Aksu, Antalya, Türkiye

[3] Isparta University of Applied Sciences, Faculty of Agronomics and Agricultural Technologies,

[4] Doqutech Academy Llc. Co., Ankara University Technopark, Ankara, Türkiye

[*] Corresponding author e-mail: emre.ipek@petektar.com

Citation:
İpek E., Altuntaş B., Kavak S., Ellialtıoğlu ŞŞ, 2026. Applications of Chromosome Doubling in Haploid Squashes and Pumpkins (*Cucurbita* spp.). Ekin J. 12(2):142-152.

Received: 07.07.2026 ***Accepted: 27.07.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

Squashes and pumpkins are important vegetable species belonging to the genus *Cucurbita* of the family Cucurbitaceae. The genus Cucurbita (2n=40) comprises five cultivated species, the most economically important of which are *Cucurbita pepo* L., *C. maxima* Duch., and *C. moschata* Duch. The doubled haploidization technique, which allows the rapid and efficient obtaining of pure lines in variety breeding programs in many plant species, is also successfully applied in the *Cucurbita* genus. The irradiated pollen technique yields successful results in all three squash species in obtaining haploid plants. Successful applications have been made in projects conducted at our company to obtain haploid plants in squash. However, it is not possible to propose a practical protocol for chromosome doubling in squash and obtaining doubled haploid (DH) plants from haploid plants. Although the literature contains successful applications and reports of pure-line seeds, the doubling step remains a key challenge in the production of pure lines in squash. For this purpose, studies were conducted on chromosome doubling in haploid squash plants under *in vitro* and *in vivo* conditions. Concentration and duration of colchicine applications were tested, and stomatal examinations revealed diploid plants. The most effective method was determined to be a 3-hour application period using a 0.5% colchicine dose used in acclimated young haploid seedlings at the 3-4 leaf stage. Plants grown from this treatment were self-pollinated to obtain pure-line seeds. However, optimization studies on chromosome doubling in squash varieties are still needed.

Keywords**:** Squashes and pumpkins, haploid, *Cucurbita pepo, C. moschata, C. maxima*, colchicine, chromosome doubling

Introduction

Squashes and pumpkins, belonging to the genus *Cucurbita* of the Cucurbitaceae family, are among the most important vegetable crops. Native to the Americas, their domestication coincides with the beginnings of settled agriculture. Among the five cultivated species, *Cucurbita pepo* L., *C. maxima* Duch., and *C. moschata* Duch. are the most economically significant (Whitaker and Davis, 1962; Robinson and Decker-Walters, 1997). *C. pepo* and *C. maxima* are generally adapted to temperate climates, whereas *C. moschata* thrives better in tropical and subtropical regions. Globally, squash is cultivated on approximately 2 million hectares, producing around 27 million tons annually. China, India, and Russia are the leading producers, while Türkiye ranks eighth with an average annual production between 700,000 and 800,000 tons (Anonymous, 2025a). Approximately 14% of Türkiye's total squash production is allocated to foreign trade, reflecting the crop's considerable contribution to the country's agricultural exports (Anonymous, 2025b).

(§) *This study was presented as an poster presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

The domestication of *Cucurbita pepo*, native to Mexico and Central America, dates back approximately 10,000 years (Smith, 1997), and it is regarded as one of the earliest cultivated crops of the New World (Nee, 1990). Historical evidence suggests that *C. moschata* was domesticated around 3000 BC in Peru and 2000 BC in Guatemala (Smith, 2005), while *C. maxima* originated along the Peruvian coast around 2000 BC (Ferriol and Picó, 2008). Cultivated *Cucurbita* species subsequently spread to the Old World and became widely grown after the 16th century (Paris, 1989). Although no wild *Cucurbita* species are found in Türkiye, the genus exhibits substantial genetic diversity within the region (Balkaya and Karaağaç, 2006). According to Harlan (1951), Anatolia serves as a micro-center of diversity for several Cucurbitaceae species, including *C. melo* L., *C. sativus* L., *C. moschata* Duch., and *C. pepo* L.

Due to their monoecious floral structure, cucurbits exhibit a high degree of cross-pollination, with rates typically ranging from 60% to 80%, depending on pollinator activity and the genetic background of the population (Abbey, 2016). This high outcrossing rate considerably prolongs the time required to achieve homozygosity during the development of parental inbred lines for F1 hybrid breeding. Even after several generations of selfing, residual genetic segregation may persist. While self-pollinated species usually reach acceptable homozygosity within 4-6 generations, this process may extend to 8-10 generations in cucurbits (Kurtar et al., 2024). Kesici et al. (2000) carried out 11 successive generations of selfing to develop parental lines for hybrid breeding in summer squash. While inbreeding depression was not evident, the authors noted a slight reduction in plant habit and fruit size, which can complicate the characterization and stabilization of desirable line traits.

Within plant tissue culture, the doubled haploid (DH) approach, also known as gametic embryogenesis, has become increasingly important in modern plant breeding. It is particularly valuable for rapidly developing high-performing hybrid varieties in both field crops and vegetable seed production. The doubled haploidization process significantly shortens the time required for line purification—a critical step in F1 hybrid development that would otherwise necessitate 6-8 generations of selfing in highly outcrossing species—reducing it to just 1-2 years. This method not only conserves time, labor, and resources but also accelerates the introduction of new varieties to the market. Moreover, DH technology enables the production of fully homozygous "pure lines," which cannot be achieved through conventional repeated selfing (Kurtar et al., 2020).

Several factors influence, and at times constrain, the successful application of the DH technique in plant breeding. Beyond the limitations encountered during haploid plant production, a major challenge lies in restoring fertility via chromosome doubling. Achieving doubled haploid plants with a diploid chromosome complement represents a crucial and indispensable step in the process. Chromosome doubling is carried out in haploid (n) plants, as determined through stomatal observations and flow cytometry analyses, either under *in vitro* or *in vivo* conditions (Kurtar et al., 2024).

Colchicine is the most commonly employed antimitotic agent for chromosome doubling in cucurbits (Lim and Earle, 2009; Galazka and Niemirowicz-Szczytt, 2013; Aslam et al., 2017). Recent comprehensive reviews have highlighted that chromosome doubling remains one of the major bottlenecks in doubled haploid (DH) technology and polyploid breeding, particularly in recalcitrant vegetable species. Fomicheva et al. (2024) emphasized that the success of chromosome doubling is strongly influenced by species, genotype, developmental stage, colchicine concentration, exposure time, and application method, making it difficult to establish a universal protocol. Focusing specifically on the Cucurbitaceae family, Ermolaev et al. (2026) reported that colchicine remains the principal antimitotic agent for chromosome doubling in *Cucurbita*, *Cucumis*, and *Citrullus* species, although alternative compounds such as oryzalin and other dinitroaniline herbicides have also been investigated. Nevertheless, chromosome doubling efficiency is still highly variable, and the frequent occurrence of mixoploid and chimeric plants indicates that reliable, species-specific protocols are still lacking, particularly in *Cucurbita*. Likewise, Singh et al. (2025) concluded that although colchicine continues to be the standard chemical for induced polyploidization because of its effectiveness in disrupting spindle formation during mitosis, alternative antimitotic agents, including oryzalin, trifluralin, and amiprophos-methyl, may improve chromosome doubling efficiency or reduce phytotoxic effects in certain species. Collectively, these recent studies demonstrate that further optimization of chromosome doubling protocols remains essential for efficient DH production in cucurbit breeding programs.

Various chromosome duplication strategies have been developed, including immersion of entire plantlets or nodal explants (Sari and Abak, 1996; Caglar and Abak, 1997), treatments applied directly to apical meristems (Nikolova and Niemirowicz-Szczytt, 1996), shoot tips (Yetisir and Sari, 2003; Solmaz et al., 2011), as well as lateral shoots. These approaches have

been widely adopted in cucurbit breeding programs to induce doubled haploidy efficiently.

Despite considerable progress in haploid production techniques, chromosome doubling remains one of the least optimized steps in doubled haploid (DH) technology for *Cucurbita* species. Most previous studies have focused on haploid induction, whereas practical and reproducible protocols for obtaining fertile DH plants through chromosome doubling are still limited. Therefore, the development of efficient chromosome doubling strategies is essential for accelerating DH-based breeding in cucurbit crops. In the present study, haploid squash plants derived from parthenogenetic embryos induced by irradiated pollen of *C. pepo*, *C. maxima*, and *C. moschata* were subjected to both *in vitro* and *in vivo* colchicine treatments at different developmental stages. The novelty of this study lies in the comparative evaluation of chromosome doubling approaches under both *in vitro* and *in vivo* conditions across economically important *Cucurbita* species, with the aim of identifying a practical protocol for obtaining fertile doubled haploid plants.

Materials and Methods

The experiments were carried out at the Petektar Seed R&D Center, which comprises a research greenhouse and plant biotechnology laboratories located in the Kurşunlu district of Antalya. Plant materials consisted of breeding lines and experimental genotypes of *Cucurbita pepo*, *C. maxima*, and *C. moschata* maintained in the breeding program of Petektar Seed Co. For the *in vitro* doubled haploid (DH) experiments, breeding lines from ongoing breeding projects were used. These materials consisted of segregating F_3 populations selected based on desirable fruit characteristics and cold tolerance. Haploid plants were obtained from parthenogenetic embryos induced by irradiated pollen.

Obtaining haploid plants in Cucurbita spp.

Male flowers were collected one day before anthesis and irradiated with 150 Gy using a Cobalt-60 gamma source (Baktemur et al., 2014). Pollen, although generatively inactive, retained its germination capacity and was used to pollinate female flowers isolated one day earlier. Fruits were harvested at 3-4 weeks of age, before reaching full maturity, following successful pollination. The harvested fruits were dissected under sterile conditions, and individual seeds were carefully examined. Embryos excised aseptically were cultured on nutrient media and successfully regenerated into plants (Kurtar ve Seymen, 2021; Yiğit et al., 2023). Further details and results concerning this stage have been previously described by Yapıcı et al. (2023).

In vitro colchicine treatments and ploidy estimation

Parthenogenetic embryos were directly cultured on nutrient media supplemented with colchicine (Figure 1). Three colchicine concentrations (0.3%, 0.5%, and 0.6%) were incorporated into MS (Murashige and Skoog, 1962) media (MS salts and vitamins + 0.01 mg/L IAA + 0.01 mg/L GA_3 + 3% sucrose + 0.7% agar, pH 5.8). Following autoclaving and placement in the culture cabinet, colchicine was aseptically incorporated into the nutrient media via filter sterilization. For each treatment, 200 haploid embryos of *C. pepo* were cultured. After germination and one month of growth (Figure 1), the embryos were acclimatized and successfully developed into healthy seedlings in the incubation room during 21 days (Figure 2).

Chloroplast numbers in stomatal guard cells were determined from leaf sections. Fresh leaves were initially cleared in Carnoy's solution (3 parts ethanol: 1 part glacial acetic acid), rinsed in sterile water for 2-5 minutes, and subsequently stained with 1% I-KI solution for 30 seconds. For each sample, chloroplast counts were performed on 30 stomata, and observations were made under a microscope at 400× magnification (Doğan et al., 2023).

In vivo colchicine treatments

Haploid *C. maxima* and *C. moschata* plantlets obtained through irradiated pollen-induced parthenogenetic embryogenesis and subsequently acclimatized were treated with colchicine in the incubation room. *In vivo* application of antimitotic agents was carried out following modified protocols of Nikolova and Niemirowicz-Szczytt (1996) and Kurtar (2018). Small cotton pieces, soaked in the doubling solutions, were placed on the apical regions of seedlings at the 4-5 true-leaf stage. The cotton was then covered with aluminum foil and stretch film to prevent light exposure and evaporation (Figure 3). For this purpose, a 0.5% colchicine solution was applied for 3 hours, after which the shoot tips were rinsed with sterile water. The treatment was repeated a second time following a one-day interval. Treated seedlings were maintained under a daily photoperiod at 25 ± 2°C during the day and 18 ± 2°C at night. After 15 days, the plants were transferred to the greenhouse and established in their designated positions, where they were grown under controlled conditions.

Selfing, fruit set, and seed set

Fruit and seed set observations were used to assess the reproductive potential of the doubled haploids (DHs). One day before anthesis, both female and male flowers were isolated using cloth bags. The following morning, female flowers were self-pollinated and re-

isolated to prevent unintended pollen contamination. Cloth bags were removed on the second or third day after pollination. For each DH plant, at least one female flower was pollinated. However, self-pollination was not always possible, as in some cases, both male and female flowers were not present on the same plant at the same time. Fruit set was recorded as the number of plants bearing fruit, while seed set was evaluated based on the average number of fully developed seeds per fruit.

Results and Discussion

In vitro colchicine treatments and ploidy estimation

In treatments with 0.3% colchicine, 8% of the embryos failed to develop into complete plants and could not be transferred to soil. Similarly, 12% and 14% of the embryos in 0.5% and 0.6% colchicine treatments, respectively, did not survive to full plantlets. All other plants were successfully acclimatized and grown in the growth chamber until they reached the 4-5 leaf stage.

As a result of the treatments applied to obtain doubled haploid plants directly from haploid individuals through colchicine supplementation in the nutrient media, mixoploid *C. pepo* plants were obtained in all treatments without statistically significant differences. The stomata and chloroplast numbers in the leaves of these plants were examined following the protocol of Doğan et al. (2023). Even within three different regions of a single leaf, variations in chloroplast numbers were observed. When sampling was increased across leaves on the same plant, similar variation was consistently observed throughout the entire plant.

Antimitotic agents are commonly recognized as metaphase inhibitors, exerting their effects primarily during the metaphase stage of cell division. Compounds such as colchicine, colcemid, vinblastine, acenaphthene, dinitroanilines, phosphoroamidates, pyridines, benzamides, and benzoic acid derivatives interfere with metaphase progression and induce chromosome doubling. In particular, colchicine disrupts microtubule dynamics, thereby preventing cell division and leading to chromosome duplication (Dewitte and Murray, 2003; Vaughn, 2006; Dhooghe et al., 2011). Exposure of haploid plants to colchicine, oryzalin, trifluralin, and other antimitotic chemicals *in vitro* or *in vivo* results in chromosome doubling (Kurtar, 2018; Vural et al., 2019). Colchicine acts by inhibiting microtubule formation, thus altering the cell division process in plant cells. Recent reviews have confirmed that colchicine remains the most widely used antimitotic agent in doubled haploid technology because of its effectiveness in disrupting spindle formation during mitosis. However, chromosome doubling efficiency is strongly influenced by species, genotype, developmental stage, treatment duration, and application method. Alternative antimitotic agents, including oryzalin, trifluralin, and amiprophos-methyl, have also been investigated to improve chromosome doubling efficiency or reduce phytotoxic effects, although no universally applicable protocol has yet been established for vegetable crops, particularly for recalcitrant members of the Cucurbitaceae (Fomicheva et al., 2024; Ermolaev et al., 2026; Singh et al., 2025). Marangoz et al. (2025) applied colchicine at concentrations of 0.3%, 0.4%, and 0.6% in both semi-solid and double-layer media for 14 and 21 days in pepper anther cultures. They reported that colchicine significantly enhances the production of spontaneous doubled haploid plants, with the most effective treatment being 0.6% colchicine for 21 days in semi-solid medium.

The size of stomatal guard cells and the number of chloroplasts within these cells show a strong correlation with the ploidy level of plants, indicating whether they are haploid, diploid, or polyploid (Ellialtıoğlu et al., 2002; Tepe et al., 2002; Bat et al., 2021). Therefore, chloroplast numbers in the stomatal guard cells were examined in plantlets obtained following *in vitro* colchicine treatment of summer squash (*C. pepo*). Based on reference values established for *C. pepo*, the average number of chloroplasts per stoma was 5.2 ± 1.3 in haploids and 9.8 ± 0.7 in diploids. In acclimatized plantlets, chloroplast counts were recorded from five different leaf regions. The mean chloroplast number per stoma increased from 6.6 ± 1.2 in the 0.3% colchicine treatment to 8.3 ± 0.9 and 10.7 ± 1.6 in the 0.5% and 0.6% treatments, respectively. This dose-dependent increase indicates the presence of cells with duplicated chromosomes and, particularly at the highest colchicine concentration, cells with even higher ploidy levels. However, the coexistence of cells exhibiting both haploid and diploid characteristics clearly indicates the presence of mixoploidy. Cytological observations confirmed that none of the regenerated plants were entirely haploid or fully diploid; instead, all regenerated plantlets exhibited varying degrees of mixoploidy. As illustrated in Figures 4 and 5, microscopic observations revealed chloroplast distributions characteristic of haploid, diploid, and mixoploid tissues within the same regenerated plants.

Nevertheless, the occurrence of mixoploid plants following colchicine treatment is not unique to *Cucurbita* and has been reported in several crop species. Chinnathambi et al. (2023) obtained regenerated marigold plants with mixed ploidy following colchicine

treatment of androgenic haploids, while Klíma et al. (2024) reported a high proportion of mixoploid regenerants after colchicine application in microspore-derived swede plants. Similarly, Fomicheva et al. (2025) observed the formation of mixoploid regenerants during chromosome doubling of carrot haploid embryos using colchicine and trifluralin. Collectively, these studies demonstrate that chromosome doubling induced by antimitotic agents is rarely uniform throughout the entire plant because different meristematic regions or cell layers may respond differently to treatment. Consequently, the formation of mixoploid plants should be regarded as a common biological consequence of chromosome doubling rather than a species-specific phenomenon. The results obtained in the present study are therefore consistent with recent findings across several plant families and with the conclusions of Fomicheva et al. (2024) and Ermolaev et al. (2026), who identified incomplete chromosome doubling and the frequent occurrence of mixoploid plants as major bottlenecks in doubled haploid production, particularly in the Cucurbitaceae. Further optimization of chromosome doubling protocols will therefore be essential to increase the frequency of doubled, fertile plants in *Cucurbita*.

When the mixoploid squash plants were grown in the greenhouse and induced to flower, frequent occurrences of male flowers lacking pollen and morphologically abnormal flowers with malformed stigmas were observed (Figure 6). In cases where pollen-producing male flowers were used for self-pollination or for pollinating female flowers of control diploid plants, no fruit set was achieved. Moreover, in many plants, male and female flowers did not develop simultaneously, which further limited successful fertilization. Indeed, Yuan et al. (2015) reported in cabbage and broccoli, and Fomicheva et al. (2025) in carrot, that colchicine treatments applied to haploid plants obtained via androgenesis can result in the development of mixoploid individuals, and that obtaining seeds from these plants is often a limiting factor. Therefore, under the experimental conditions of this study, *in vitro* colchicine treatments were not sufficient to achieve successful DH seed formation in squash.

***In vivo* colchicine treatments**

In vivo chromosome doubling of winter squash and pumpkin haploid lines was performed through colchicine treatments applied to the apical parts of acclimatized haploid plantlets. Following the protocol of Kurtar (2018) and considering all experimental results obtained throughout the study, the colchicine treatment was optimized as 0.5% colchicine applied for 3 h and repeated twice at one-day intervals. This treatment successfully produced doubled haploid (DH) plants. Three fertile DH plants with viable seeds were obtained from each self-pollinated *C. maxima* and *C. moschata* genotype. Out of 20 acclimatized haploid plants treated per species, a 15% chromosome doubling rate was achieved (Figure 7), which is comparable to the values previously reported by Kurtar (2018).

The successful recovery of fertile DH plants in the present study confirms that chromosome doubling efficiency depends not only on the antimitotic agent itself but also on genotype, developmental stage, treatment duration, and application strategy. Recent reviews have emphasized that these factors interact to determine chromosome doubling success, making protocol optimization more critical than colchicine concentration alone (Fomicheva et al., 2024). The use of acclimatized haploid seedlings at the 4-5 leaf stage in the present study appears to have contributed to the successful recovery of fertile DH plants, highlighting the importance of selecting an appropriate developmental stage for chromosome doubling.

Cucurbita species have long been regarded as recalcitrant crops with respect to both plant regeneration and chromosome doubling (Kathiravan et al., 2006; Ananthakrishnan et al., 2007; Ebrahimzadeh et al., 2018). These challenges are reflected in the generally low responsiveness of cucurbits to spontaneous chromosome doubling and *in vitro* polyploid induction (Hooghvorst and Nogués, 2020). Consequently, obtaining fertile doubled haploid plants remains considerably more difficult in *Cucurbita* than in many other vegetable crops.

Previous studies have successfully applied colchicine for chromosome doubling in several cucurbit and solanaceous species, including *Cucumis melo* (Sauton and Dumas de Vaulx, 1987; Lim and Earle, 2009), *Citrullus lanatus* (Sari and Abak, 1996), *Cucumis sativus* (Caglar and Abak, 1997), pepper (Vural et al., 2019; Marangoz et al., 2025), and other vegetable crops. However, because of the particular recalcitrance of *Cucurbita*, alternative antimitotic agents such as oryzalin and trifluralin have also been evaluated (Kurtar, 2018; Ebrahimzadeh et al., 2018). Recent reviews indicate that although these compounds may reduce phytotoxicity or improve chromosome doubling efficiency in certain species, colchicine remains the principal antimitotic agent in cucurbit breeding because of its broad applicability and consistent performance (Ermolaev et al., 2026; Singh et al., 2025). Therefore, future improvements in chromosome doubling efficiency are more likely to be achieved through optimization of treatment protocols than by replacing colchicine itself.

Colchicine has been applied at concentrations ranging from 0.05 to 5 g L^{-1}, depending on species and application protocol (Fomicheva et al., 2024). The present study demonstrated that a 0.5% colchicine treatment for 3 h, repeated twice, was sufficient to recover fertile DH plants in both *C. maxima* and *C. moschata*. Although additional optimization involving different concentrations, exposure periods, or repeated applications may further improve chromosome doubling efficiency (Kurtar, 2018), the present protocol provides a practical and reproducible approach for obtaining fertile DH plants in economically important *Cucurbita* species.

The number of seeds harvested per DH *Cucurbita* plant ranged from 32 to 166. Despite the well-known recalcitrance of cucurbits to chromosome doubling, fertile DH plants were successfully obtained. The resulting DH seed populations are currently being evaluated under protected cultivation for genetic uniformity and phenotypic stability. These results demonstrate that optimized *in vivo* colchicine treatment can effectively overcome one of the major bottlenecks in doubled haploid technology and provide a reliable strategy for accelerating breeding programs in *Cucurbita*.

Conclusions

This study demonstrated that chromosome doubling efficiency in *Cucurbita* is strongly influenced by the method and developmental stage at which colchicine is applied. While *in vitro* colchicine treatments resulted predominantly in mixoploid plants that failed to produce fertile seeds, *in vivo* application to acclimatized haploid plantlets successfully generated fertile doubled haploid plants in both *C. maxima* and *C. moschata*. The optimized protocol, consisting of two applications of 0.5% colchicine for 3 h at one-day intervals, proved to be an effective and reproducible approach for chromosome doubling under the conditions of this study. These findings confirm that optimization of treatment strategy, rather than increasing colchicine concentration alone, is critical for improving chromosome doubling efficiency in recalcitrant *Cucurbita* species. The developed protocol provides a practical tool for accelerating doubled haploid production and may contribute to the development of pure lines and the shortening of breeding cycles in cucurbit improvement programs. Future studies should evaluate the applicability of this protocol to a broader range of genotypes and investigate alternative antimitotic agents and genotype-specific optimization strategies to further enhance chromosome doubling efficiency.

Acknowledgements

A part of this study was prepared as part of the TÜBİTAK TEYDEB Project No. 3230319. We would like to express our gratitude to TÜBİTAK for their support.

The authors used OpenAI ChatGPT solely to improve the English language, grammar, and readability of the manuscript. The AI tool was not used to generate scientific content, analyze data, interpret results, or draw conclusions. All scientific content and conclusions are entirely the responsibility of the authors.

Ethical Statement

A preliminary version of this study was presented as a poster at the "Vth International Plant Breeding Congress, 1-5 December 2025, Sherwood Exclusive Lara, Antalya, Türkiye". The present manuscript is an expanded and revised version prepared for full publication.

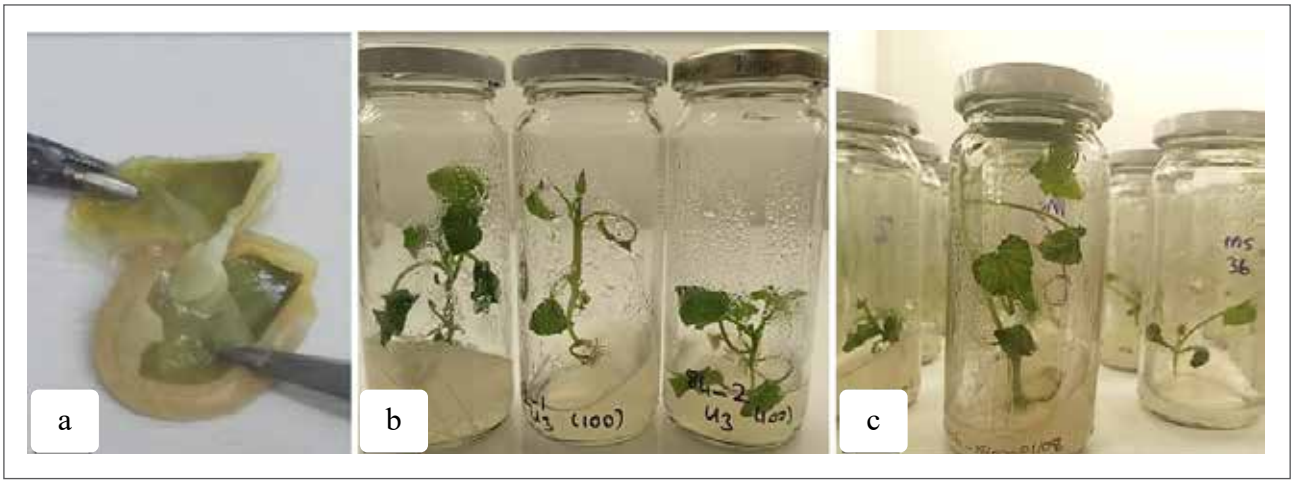


Figure 1. *In vitro* colchicine treatments. (a) Isolation and culture of parthenogenetic embryos, (b) Haploid squash plantlets grown for 21 days on media containing different colchicine concentrations, (c) Plantlets transferred to MS medium and further developed.

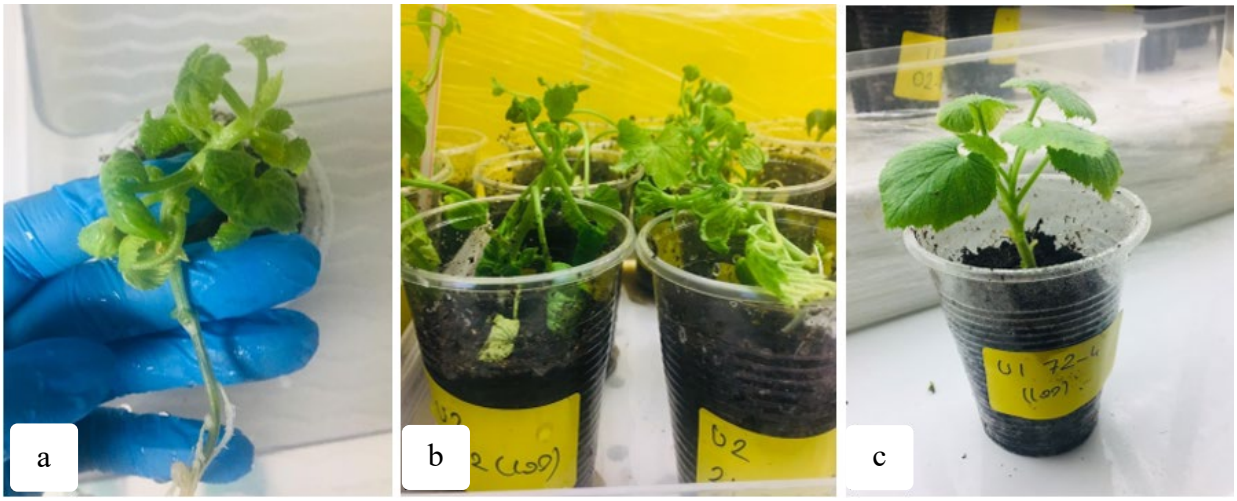


Figure 2. Acclimatization of squash plantlets obtained after colchicine treatment. (a) A plantlet was removed from the glass jar and cleaned of agar, (b) Plantlets transplanted into sterile soil and placed in a mini greenhouse for acclimatization, (c) A fully acclimatized squash plantlet showing healthy growth.

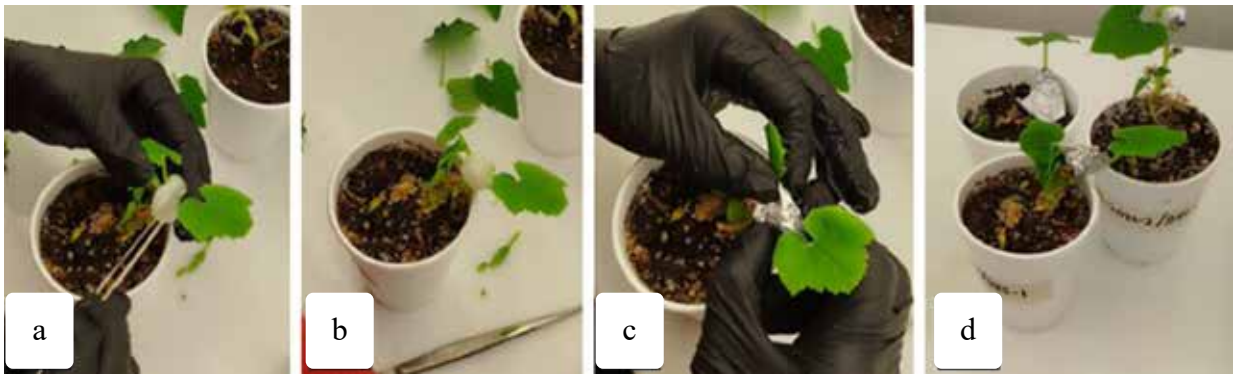


Figure 3. *In vivo* colchicine treatments. (a, b) Placement of cotton imbibed with colchicine solution on the shoot apex, (c, d) Covering the cotton with aluminum foil and leaving it for 3 hours.

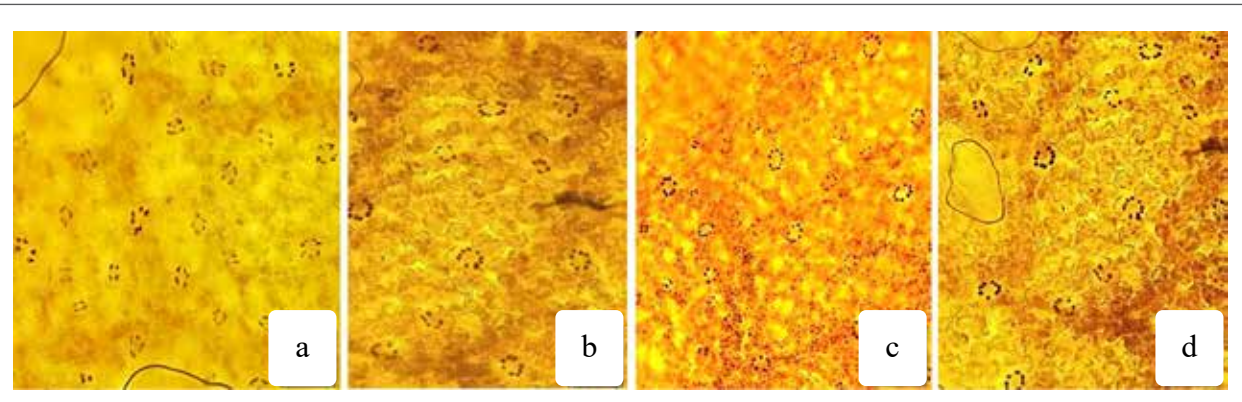


Figure 4. Chloroplast patterns associated with ploidy levels in different plants. (a) Chloroplasts in stomatal cells of a haploid squash leaf, (b-d) Chloroplasts in stomatal cells of a diploid squash leaf.

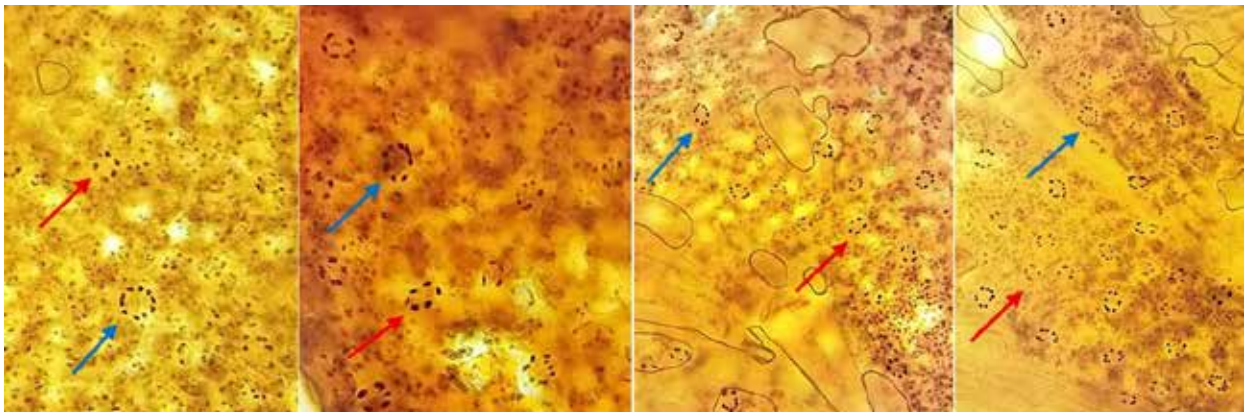


Figure 5. Chloroplast distribution in stomatal cells of mixoploid squash plants (blue arrows: chloroplasts in diploid cells; red arrows: chloroplasts in haploid cells).

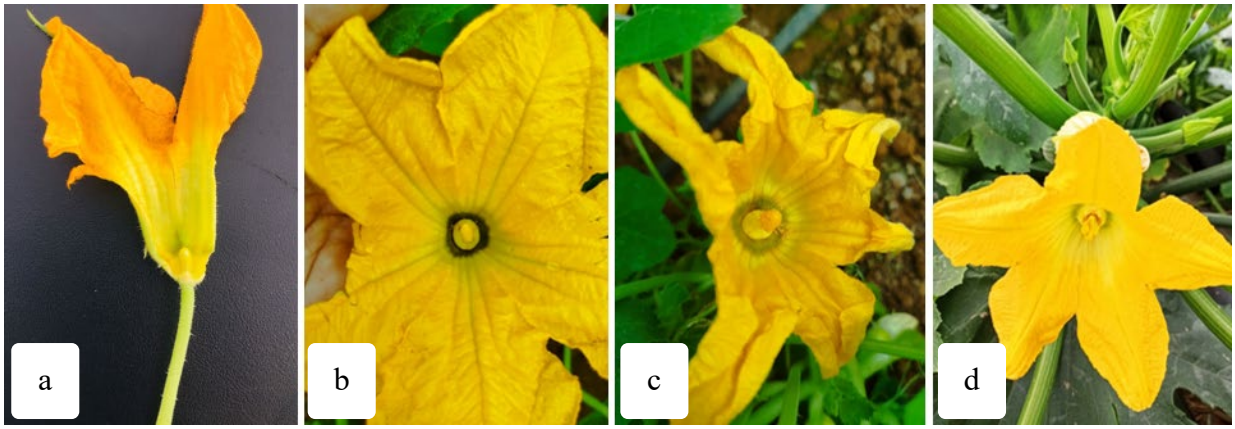


Figure 6. Abnormal flower structures observed in mixoploid squash plants (a, b) Male flowers without pollen, (c) Male flower with pollen but morphologically abnormal and incapable of fertilization, (d) Female flower exhibiting stigma anomalies.

Figure 7. Fruits obtained from self-pollination of healthy DH plants derived from *in vivo* colchicine treatments.

References

Abbey, L. (2016). Cucurbits physiological stages of growth. In M. Pessarakli (Ed.), *Handbook of cucurbits: Growth, cultural practices, and physiology* (pp. 151-170). CRC Press.

Ananthakrishnan, G., Xia, X., Amutha, S., Singer, S., Muruganantham, M., Yablonsky, S., Fischer, E., and Gaba, V. (2007). Ultrasonic treatment stimulates multiple shoot regeneration and explant enlargement in recalcitrant squash cotyledon explants *in vitro*. *Plant Cell Reports*, *26*(3), 267-276. https://doi.org/10.1007/s00299-006-0235-1

Anonymous. (2025a). *FAOSTAT: Squash and pumpkin production quantity (tons)* Food and Agriculture Organization [Data set]. United Nations. https://www.fao.org/faostat/en/#data/QCL (Accessed: 07.11.2025).

Anonymous. (2025b). *Fresh fruit and vegetable export data of Türkiye. Türkiye yaş sebze-meyve ihracat verileri.* İstanbul Ticaret Borsası (İTB). https://www.itb.org.tr (Accessed: 11 November 2025).

Aslam, M., Farid, B., Khakwani, K., Maqbool, M. A., and Zou, H. (2017). *In vivo* maternal haploid seed production and chromosome doubling with different anti-microtubular agents in maize. *International Journal of Agricultural and Biology*, *19*(1), 114-120. https://doi.org/10.17957/IJAB/15.0251

Baktemur, G., Yücel, N. K., Taşkın, H., Çömlekçioğlu, S., and Büyükalaca, S. (2014). Effects of different genotypes and gamma ray doses on haploidization using irradiated pollen technique in squash. *Turkish Journal of Biology*, *38*(3), 318-327. https://doi.org/10.3906/biy-1309-5

Balkaya, A., and Karaağaç, O. (2006). Vegetable genetic resources of Turkey. *Journal of Vegetable Science*, *11*(4), 81-102. https://doi.org/10.1300/J484v11n04_08

Bat, H., Altındağ, F. N., Yiğit, M. A., Ellialtıoğlu, Ş. Ş., and Çömlekçioğlu, N. (2021). Ploidy estimation in pepper and eggplant via stomata characteristics. *International Journal of Agriculture Forestry and Life Sciences*, *5*(2), 139-146. https://izlik.org/JA25KP23EU

Caglar, G., and Abak, K. (1997). *In vitro* colchicine application of haploid cucumber plants. *Cucurbit Genetics Cooperative Report*, *20*, 21-23. https://www.ars.usda.gov/ARSUserFiles/60800500/CGC/CGC%2020%20(1997).pdf#page=33

Chinnathambi, V., Panwar, S., Singh, K. P., Namita, Lekshmy, S., and Mallick, N. (2023). Studies on *in vitro* chromosome doubling of haploid derived through androgenesis in marigold. *Indian Journal of Horticulture*, *80*(4), 333-338. https://doi.org/10.58993/ijh/2023.80.4.5

Dewitte, W., and Murray, J. A. (2003). The plant cell cycle. *Annual Review of Plant Biology*, *54*(1), 235-264. https://doi.org/10.1146/annurev.arplant.54.031902.134836

Dhooghe, E., Van Laere, K., Eeckhaut, T., Leus, L., and Van Huylenbroeck, J. (2011). Mitotic chromosome doubling of plant tissues *in vitro*. *Plant Cell, Tissue and Organ Culture*, *104*(3), 359-373. https://doi.org/10.1007/s11240-010-9786-5

Doğan, O., Kara, Z., Yazar, K., and Ekinci, H. (2023). Diploit ve tetraploit asma genotiplerinde poliploidi doğrulama yöntemlerinin testi. *Bahçe*, *52*(Özel Sayı 1), 10-17. (In Turkish)

Ebrahimzadeh, H., Soltanloo, H., Shariatpanahi, M. E., Eskandari, A., and Ramezanpour, S. S. (2018). Improved chromosome doubling of parthenogenetic haploid plants of cucumber (*Cucumis sativus* L.) using colchicine, trifluralin, and oryzalin. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *135*(3), 407-417. https://doi.org/10.1007/s11240-018-1473-y

Ellialtıoğlu, Ş., Sarı, N., and Abak, K. (2002). Haploid bitki üretimi. M. Babaoğlu, E. Gürel, and S. Özcan (Ed.), *Bitki Biyoteknolojisi I* içinde (ss. 137-189). Selçuk Üniversitesi Vakfı Yayınları.

Ermolaev, A., Fomicheva, M., and Domblides, E. (2026). Advances in polyploid breeding of Cucurbitaceae crops: From polyploidy research to triploid seedless hybrid breeding. *Crops*, *6*(1), 5. https://doi.org/10.3390/crops6010005

Ferriol, M., and Picó, B. (2008). Pumpkin and winter squash. In J. Prohens and F. Nuez (Eds.), *Vegetables I: Asteraceae, Brassicaceae, Chenopodicaceae, and Cucurbitaceae* (pp. 317-349). Springer. https://doi.org/10.1007/978-0-387-30443-4_10

Fomicheva, M., Kozar, E., and Domblides, E. (2025). Carrot (*Daucus carota* L.) haploid embryo genome doubling with colchicine and trifluralin. *Horticulturae*, *11*(5), 505. https://doi.org/10.3390/horticulturae11050505

Fomicheva, M., Kulakov, Y., Alyokhina, K., and Domblides, E. (2024). Spontaneous and chemically induced genome doubling and polyploidization in

vegetable crops. *Horticulturae*, *10*(6), 551. https://doi.org/10.3390/horticulturae10060551

Gałązka, J., and Niemirowicz-Szczytt, K. (2013). Review of research on haploid production in cucumber and other cucurbits. *Folia Horticulturae*, *25*(1), 67-78. https://doi.org/10.2478/fhort-2013-0008

Harlan, J. R. (1951). Anatomy of gene centers. *The American Naturalist*, *85*(821), 97-103. https://doi.org/10.1086/281657

Hooghvorst, I., and Nogués, S. (2020). Opportunities and challenges in doubled haploids and haploid inducer-mediated genome-editing systems in cucurbits. *Agronomy*, *10*(9), 1441. https://doi.org/10.3390/agronomy10091441

Kathiravan, K., Vengedesan, G., Singer, S., Steinitz, B., Paris, H. S., and Gaba, V. (2006). Adventitious regeneration *in vitro* occurs across a wide spectrum of squash (*Cucurbita pepo*) genotypes. *Plant Cell, Tissue and Organ Culture*, *85*(3), 285-295. https://doi.org/10.1007/s11240-006-9079-1

Kesici, S., Abak, K., and Tunar, M. (2000). Yazlık kabaklarda erkencilikte ve verimde heterozis etkisi. In *III. Sebze Tarımı Sempozyumu* (pp. 167-172). Isparta, Türkiye.

Klíma, M., Shmeit, Y. H., Kopecký, P., Vítámvás, P., Kosová, K., Prášil, I. T. ve Fernández-Cusimamani, E. (2024). Impact of selected antimitotic substances on doubled haploid and polyploid regeneration in microspore cultures of swede (*Brassica napus* ssp. *napobrassica* (L.) Hanelt). *Czech Journal of Genetics and Plant Breeding*, *60*(2), 79-85. https://doi.org/10.17221/84/2023-CJGPB

Kurtar, E. S. (2018). The effects of anti-mitotic agents on dihaploidization and fertility in winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.) androgenic haploids. *Acta Scientiarum Polonorum Hortorum Cultus*, *17*(5), 3-14. https://doi.org/10.24326/asphc.2018.5.1

Kurtar, E. S. and Seymen, M. (2021). Gynogenesis in *Cucurbita* species. J. M. Seguí-Simarro (Ed.), *Doubled haploid technology: Volume 3: Emerging tools, cucurbits, trees, other species* içinde (s. 123-133). Humana Press. https://doi.org/10.1007/978-1-0716-1335-1_9

Kurtar, E. S., Ellialtıoğlu, Ş. Ş. and Metin, D. (2024). Doku kültürü tekniklerinin hızlandırılmış ıslah amaçlı olarak sebze tohumculuğunda kullanımı. A. Balkaya ve L. Arın (Ed.), *Sebze tohum üretimi ve teknolojisinde güncel ve yenilikçi uygulamalar* (pp. 12–30). IKSAD Publishing House. https://doi.org/10.5281/zenodo.13822756 (In Turkish)

Kurtar, E. S., Seymen, M. ve Kal, Ü. (2020). An overview of doubled haploid plant production in *Cucurbita* species. *Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi*, *30*(3), 510-520. https://doi.org/10.29133/yyutbd.741087

Lim, W. and Earle, E. D. (2009). Enhanced recovery of doubled haploid lines from parthenogenetic plants of melon (*Cucumis melo* L.). *Plant Cell, Tissue and Organ Culture*, *98*(3), 351-356. https://doi.org/10.1007/s11240-009-9563-5

Marangoz, B. N., Çömlekçioğlu, N., Gürsoy, E., Altuntaş, B. and Ellialtıoğlu, Ş. Ş. (2025). Effects of colchicine applications on embryo yield and spontaneous chromosome doubling in pepper (*Capsicum annuum* L.) anther culture. *Manas Journal of Agriculture, Veterinary and Life Sciences*, *15*(1), 76-90. https://doi.org/10.53518/mjavl.1583740

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473-497.

Nikolova, V. and Niemirowicz-Szczytt, K. (1996). Diploidization of cucumber (*Cucumis sativus* L.) haploids by colchicine treatment. *Acta Societatis Botanicorum Poloniae*, *65*(3-4), 311-317. https://doi.org/10.5586/asbp.1996.048

Paris, H. S. (1989). Historical records, origins, and development of the edible cultivar groups of *Cucurbita pepo* (Cucurbitaceae). *Economic Botany*, *43*(4), 423-443. https://doi.org/10.1007/BF02935916

Robinson, R. W., and Decker-Walters, D. S. (1997). *Cucurbits*. CAB International. Wallingford, UK. 226 p. (Crop Production Science in Horticulture n.6).

Sari, N., and Abak, K. (1996). Effect of colchicine treatment with different doses and periods on *in vitro* chromosome doubling in haploid watermelon. *Turkish Journal of Agriculture and Forestry*, *20*(6), 555-559.

Sauton, A., and Dumas de Vaulx, R. (1987). Production of haploid plants in melon (*Cucumis melo* L.) as a result of gynogenesis induced by irradiated pollen. *Agronomie*, *7*(2), 141-147. https://doi.org/10.1051/agro:19870209

Singh, B., Yun, S., Gil, Y., and Park, M. H. (2025). The role of colchicine in plant breeding. *International*

Journal of Molecular Sciences, *26*(14), 6743. https://doi.org/10.3390/ijms26146743

Smith, B. D. (1997). The initial domestication of *Cucurbita pepo* in the Americas 10,000 years ago. *Science*, *276*(5314), 932-934. https://doi.org/10.1126/science.276.5314.932

Smith, B. D. (2005). Reassessing Coxcatlan cave and the early history of domesticated plants in Mesoamerica. *Proceedings of the National Academy of Sciences*, *102*(27), 9438-9445. https://doi.org/10.1073/pnas.0502847102

Solmaz, I., Sari, N., Gursoy, I., and Kasapoğlu, S. (2011). Comparison of *in vivo* and *in vitro* colchicine application for production of dihaploid 'Kirkagac' and 'Yuva Hasanbey' melons. *African Journal of Biotechnology*, *10*(70), 15717-15724.

Tepe, Ş., Ellialtıoğlu, Ş., Yenice, N., and Tıpırdamaz, R. (2002). *In vitro* kolhisin uygulaması ile poliploid nane (*Mentha longifolia* L.) bitkilerinin elde edilmesi. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi*, *15*(2), 63-69. (In Turkish)

Vaughn, K. C. (2006). The abnormal cell plates formed after microtubule disrupter herbicide treatment are enriched in callose. *Pesticide Biochemistry and Physiology*, *84*(2), 63-71. https://doi.org/10.1016/j.pestbp.2005.03.006

Vural, G. E., Arı, E., Zengin, S., and Ellialtıoğlu, S. S. (2019). Development of androgenesis studies on eggplant (*Solanum melongena* L.) in Turkey from past to present. In *Sustainable Crop Production* (pp. 67-90). IntechOpen. https://doi.org/10.5772/intechopen.88299

Whitaker, T. W., and Davis, G. N. (1962). *Cucurbits: Botany, cultivation, and utilization*. Interscience Publishers. New York, 250p.

Yapıcı, B., Gürsoy, E., Yıldız, B. N., İpek, E., Kavak, S., Kurtar, E. S., Gülşen, O., and Metin, D. (2023). Külleme ve ZYMV dayanımlı nitelikli yazlık kabak (*Cucurbita pepo* L.) hatlarında ışınlanmış polen tekniği ile dihaploidizasyon. *3rd International Antalya Scientific Research and Innovative Studies Congress* (13-14 Şubat 2023, Antalya, Türkiye), s. 773-788. (In Turkish)

Yetişir, H., and Sarı, N. (2003). A new method for haploid muskmelon (*Cucumis melo* L.) dihaploidization. *Scientia Horticulturae*, *98*(3), 277-283. https://doi.org/10.1016/S0304-4238(02)00226-1

Yiğit, M., Tunalı, H., Çardak, T. Z., Altındağ, F. N., Şeker, E., Doğangüzel, E., and Ellialtıoğlu, Ş. Ş. (2023,). Parthenogenetic embryo induction in different zucchini genotypes by irradiated pollen technique [Abstract]. *6th International Agricultural Congress*, p: 55. 31 August-4 September 2023

Yuan, S., Su, Y., Liu, Y., Li, Z., Fang, Z., Yang, L., Zhuang, M., Zhang, Y., Lv, H., and Sun, P. (2015). Chromosome doubling of microspore-derived plants from cabbage (*Brassica oleracea* var. *capitata* L.) and broccoli (*Brassica oleracea* var. *italica* L.). *Frontiers in Plant Science*, *6*, Article 1118. https://doi.org/10.3389/fpls.2015.01118

Research Article

Ekin
Journal of Crop Breeding and Genetics

12(2):153-160, 2026

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Determination of the Agricultural Performance of Soybean (*Glycine max* L. Merr.) Genotypes Under the Ecological Conditions of Konya in Türkiye(§)

İrfan ÖZER[1*] **Seydi Ahmet BAĞCI**[1]

[1] Selcuk University, Vocational School of Sarayönü, Sarayönü/Konya, Türkiye

[*] Corresponding author e-mail: irfanozer@selcuk.edu.tr

Citation:
Özer İ., Bağcı SA., 2026. Determination of the Agricultural Performance of Soybean (*Glycine max* L. Merr.) Genotypes Under the Ecological Conditions of Konya in Türkiye. Ekin J. 12(2):153-160.

Received: 24.06.2026 ***Accepted: 28.06.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

Soybean (*Glycine max* L. Merr.) is an important legume-oilseed crop cultivated worldwide for its higher protein and quality vegetable fat content. In Türkiye, a significant portion of the demand for soybeans is met through imports. This situation necessitates the identification of high-performance genotypes adapted to ecological conditions of Türkiye. This study was carried out to identify soybean genotypes that can be grown under the ecological conditions of Konya, which has high agricultural production potential. Eight soybean varieties (Nova, Defiance, NE 3399, A 3935, ATAEM 7, Çetinbey, Atakişi and Arısoy) and four breeding lines (BDS 07, BDS 11, BDS 21 and BDS 25) were evaluated in a randomized block design with three replications in field trials under irrigated conditions during the 2021 and 2022 growing seasons. Observations were recorded for grain yield, thousand-grain weight, seed oil, ash and protein content, plant height, number of branches and pods per plant, and first pod height. Analysis of variance results showed statistically significant differences between genotypes for most of the examined characteristics ($p < 0.01$). The highest grain yield was produced by BDS 25 (5,881 kg ha^{-1}) followed by BDS 11 (5,859 kg ha^{-1}) while Defiance variety (4,077 kg ha^{-1}) produced the lowest grain yield. The Atakişi variety had the highest protein content (37.48%) followed by Cetinbey (37.34%), while the highest oil content was found in the BDS 21 line (18.6%) closely followed by the Atakişi and NE 3699 varieties (18.4%). The findings indicate that these genotypes (BDS 25, BDS 11, Atakişi and BDS 21) are promising for soybean cultivation under Konya's ecological conditions and could contribute to increasing domestic soybean production in Türkiye.

Keywords: Soybean, genotypes, adaptation, yield, agronomic traits.

Introduction

Soybean (*Glycine max* L. Merr.) is one of the most important legume-oilseed crops cultivated worldwide due to its high protein (35-42%) and oil (18-22%) content (Arıoğlu, 2014). As a major source of plant-based protein and oil, soybean is widely used both as a direct food ingredient in human nutrition and as a high-quality protein feed in animal nutrition. In addition, utilization of soybean in the vegetable oil industry, food products, biodiesel production, and various industrial applications makes it a versatile and high value-added agricultural commodity worldwide (Hartman et al., 2011; Pagano and Miransari, 2016; Wilcox, 2004). Furthermore, the symbiotic nitrogen-fixing capacity of soybean contributes significantly to soil fertility, enhancing sustainability in crop rotation systems (Leggett et al., 2017; Salvagiotti et al., 2008).

The global demand for soybean is increasing markedly due to population growth, rising consumption of animal protein, and the expanding use of vegetable oils. In the year 2023/2024 production season, total soybean production of about 397 million tons was obtained from cultivated area of about 139 million hectares (FAO, 2025; USDA, 2026) making soybean as the world's top oilseed

(§) *This study was presented as an oral presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

crop in terms of both cultivated area and production volume. The global demand of soybean is increasing due to growing demand from the feed industry, as well as from food, biofuel, and industrial sectors (USDA, 2026).

Brazil, United States and Argentina are the leading producer and exporters of soybean contributing approximately 80% of global production (USDA, 2026). China is the world's largest soybean importer and plays a decisive role in global trade flows (Gale et al., 2019; Peng et al., 2026; OECD-FAO, 2023).

As of 2020, national soybean production was approximately 150-160 thousand tons, which is far from meeting the In Türkiye, domestic demand of soybean is more than 3 million tons against the production of only about 150-160 thousand tons (Başalma et al., 2025; TÜİK, 2025). The demand in the country is increasing due to rising demand for plant-based protein and the expansion of the feed industry. This gap is currently being filled through imports of soybean and soybean products.

Soybean production in Türkiye is mainly concentrated in the Çukurova Region, with limited area in the Mediterranean and Black Sea regions. The crop faces stiff competition with maize, cotton and sunflower (Arıoğlu, 2014; Arıoğlu et al., 2016). This situation necessitates the expansion of soybean cultivation into new ecological zones and the identification of high-yielding genotypes well adapted to regional conditions.

The Central Anatolia Region, and particularly the Konya Plain, is considered a potential production basin for soybean cultivation due to availability of agricultural land, irrigation opportunities, and suitability for crop rotation systems. Nevertheless, the semi-arid climate conditions characterized by high summer temperatures and low precipitation may pose limitations for soybean cultivation in Konya Province. Factors associated with climate change such as rising temperatures, irregular precipitation patterns, and water scarcity have intensified genotype X environment interactions in soybean production (Ray et al., 2015). In this context, evaluating genotype performance across different ecologies is of critical importance for the development of sustainable production strategies. These environmental conditions have a significant influence on key agronomic traits such as grain yield, protein content and oil concentration (Acikgoz et al., 2009; Yan et al., 2000).

Numerous studies have demonstrated significant differences among genotypes for yield, yield components, and quality traits under diverse ecological conditions as well as the decisive role of genotype X environment interactions (Gauch, 2013; Yan and Kang, 2002). Research conducted in Türkiye has similarly reported differences in yield and quality characteristics of soybean genotypes under varied environmental conditions across regions (Bakkal et al., 2017; Kocatürk et al., 2019). Sowing time and row spacing, together with genetic potential have been reported to significantly affect yield components (Yılmaz et al., 2022; Barış et al., 2020). However, comprehensive evaluations of genotype performance under continental and semi-arid ecologies such as Konya remain limited. Therefore, identifying genotypes with high yield and quality traits that are well adapted to the Konya ecology will of great importance for developing alternative cropping patterns in the region.

The findings are expected to contribute scientifically to the development of suitable cultivar recommendations for regional producers and to strategies aimed at reducing the national deficit in oilseed production.

Materials and Methods

2.1. Plant Material

Twelve soybean (*Glycine max* (L.) Merr.) genotypes evaluated in the study comprised eight registered cultivars (Defiance, Nova, ATAEM 7, NE 3399, Arısoy, A 3935, Atakişi, and Çetinbey) and four advanced Bahri Dağdaş breeding lines (BDS 11, BDS 07, BDS 25, and BDS 21).

2.2. Experimental Site and Soil Characteristics

Field experiments were conducted over two consecutive growing seasons (2021-2022) at the Selçuk University Sarayönü Vocational School, Sarayönü District of Konya Province, Türkiye. The experimental site is situated at 38.264° K latitude, 32.406° D longitude and an altitude of 1055 m above sea level.

The soil of the experimental field (30 cm depth) was clay loam in texture, slightly alkaline in reaction (pH 8.12), with a medium level of organic matter content (2.33%) and a high $CaCO_3$ (22.07) concentration (Table 1).

2.3. Climatic Conditions

Average daily maximum temperatures in both years were approximately 34-36°C during July and August.

Summer temperatures in 2021 (35,7-35,8°C) were higher as compared with 2022 (34,1-34,4°C).

Precipitation during summer months was lower and in June was relatively higher than long term average in both years. Total rainfall received during the crop season in both years was below long-term averages, and rainfall was unevenly distributed across months.

2.4. Cultural Practices

The experiment was conducted in a randomized complete block design (RCBD) with three replications. Each plot consisted of five rows, each 5 m in length, with a row spacing of 40 cm and an intra-row spacing of 5 cm (gross plot size 10 m^2). Sowing was performed manually. At sowing, diammonium phosphate fertilizer (DAP, 18-

46%) was applied at a rate of 150 kg ha^{-1}. Sowing was carried out on 18 May 2021 and 13 May 2022. Prior to sowing, seeds were inoculated with *Bradyrhizobium japonicum*.

Crop was harvested on 26 October 2021 and 1 November 2022. Irrigation was applied using a drip irrigation system in six applications, each lasting five hours. Weed control was performed mechanically. To avoid border effects, the first and last rows of each plot were excluded from measurements and observations. After excluding two rows from each border of the experimental plots and 50 cm from both ends of the plot lengths, the remaining net plot size evaluated was 4,8 m². Measurements and evaluations were performed on 10 randomly selected plants from this net plot area. Agronomic traits such as grain yield, plant height, first pod height, and number of pods per plant were determined according to the methodology of the International Soybean Program (INTSOY) (Bek and Arıoğlu, 2005). Statistical analyses were conducted using JMP software (Altınyüzük and Öztürk, 2017).

Results and Discussion

3.1. Plant Height

The results indicated statistically significant differences among soybean genotypes for plant height ($P < 0.01$). The plants of ATAEM 7, BDS 11, and BDS 25 genotypes attained tallest and statistically similar plant height (85.3-87.4 cm), whereas Defiance exhibited significantly lowest plant height (62.0 cm) and was on par with NE 3399, A 3935, BDS 21 and Çetinbey (Table 4).

Previous studies have also reported that soybean plant height is largely controlled by genetic factors, while environmental conditions play a substantial role in the expression of this trait (Board and Kahlon, 2011; Yang et al., 2021). Shorter genotypes are considered advantageous for mechanical harvesting and contribute to improved production reliability whereas plants with greater height may be more prone to lodging.

3.2. First Pod Height

Significant differences were observed among soybean genotypes for both first branch height and first pod height ($P < 0.05$). The highest first pod height was recorded in BDS 25 and BDS 21 genotypes, while the lowest value was observed in the Defiance cultivar (Table 4). First pod height is a critical agronomic trait for mechanical harvesting of soybean (Board and Kahlon, 2011; Bakal et al., 2020). The non-significant year effect and the absence of a significant year X genotype interaction indicate that these traits are more strongly governed by genotypic factors.

3.3. Number of Branches and Pods

Statistically significant differences were observed for number of branches and number of pods per plant among soybean genotypes. BDS 21, BDS 25 and Atakişi genotypes produced higher number of branches, whereas BDS 11, Atakisi and BDS 25 produced higher numbers of pods per plant (Table 4). ATAEM 7 and Defiance produced the lowest number of branches per plant and pods per plant, respectively. The number of branches and pods is directly related to the photosynthetic efficiency of plants and their capacity to form generative organs (Board and Kahlon, 2011; Board et al., 1992).

3.5. Thousand-Seed Weight (g)

To determine the thousand-seed weight of the genotypes, four separate sets of 100 seeds were weighed individually to calculate the average 100-seed weight, and the resulting value was multiplied by 10.

Genotypes showed significant differences ($P < 0.01$) in thousand-seed weight. Earlier study also reported that seed weight is a trait under strong genetic control and shows considerable variation among cultivars (Ogunbayo et al., 2014).

The highest thousand-seed weight (156 g) was recorded in BDS 25 genotype, followed by BDS 07 and Çetinbey. The lowest thousand-seed weight (approximately 113 g), was observed in the Atakişi cultivar. Thousand-seed weight has a high heritability and represents an important yield component that can be improved through selection (Ramya et al., 2010). Significant effect of year on Thousand-seed indicates that seed weight traits are also influenced by environmental conditions and climatic variability during the growing season. Seed size, which is directly related to thousand-seed weight, is among the key physical quality parameters determining suitability for the feed and food industries. Cultivars with larger seeds are preferred by industry due to higher flour yield, better milling properties, and lower processing losses (Elgün and Ertugay, 2011). In this context, genotypes such as BDS 25, BDS 07, and Çetinbey, which exhibited higher thousand-seed weight, can be considered to have greater potential for use in the feed and food industries.

Multi-environment field trials have demonstrated significant differences among genotypes regarding seed weight and related quality traits ($P<0.01$). These interactions provide valuable guidance for breeding programs and cultivar selection for industrial use (Gökdere et al., 2023).

3.6. Grain Yield

Significant differences were observed among soybean cultivars for grain yield ($P < 0.05$. The year X cultivar interaction was significant, indicating that yield rankings of genotypes varied between years. Mean yield

values ranged from 4077 to 5881 kg ha^{-1}, with the higher yields obtained from the BDS 11, BDS 25, and Nova cultivars.

Similar differences in yield and yield components and positive correlation among number of pods per plant, 100-seed weight, and the number of seeds per pod of soybean genotypes were reported earlier (Nget et al., 2022). Furthermore, genotype X environment interactions have been shown to significantly affect yield performance, with environmental variability altering the yield rankings of genotypes (Addae-Frimpomaah et al., 2025; Pospišil and Pospišil, 2024).

3.7. Quality Parameters

3.7.1. Protein Content (%)

Protein content ranged from 32.30% to 37.48%. The highest protein content was recorded in the Atakişi genotype (37.48%), followed by Çetinbey (37.34%), BDS 25 (37.09%), and Defiance and BDS21 (both 36.97%). The lowest protein content was observed in the Arısoy genotype (32.30%). In general, genotypes with higher protein content are considered more valuable for both human consumption and the feed industry.

The higher protein content observed in some genotypes is an important quality characteristic because soybean protein is a primary determinant of nutritional value for both human food and animal feed. Consequently, the development and selection of high-protein genotypes remain major objectives in soybean breeding programs (Chiluwal, 2025; Liu, et al., 2023; Singer et al., 2023; Shi et al., 2023).

3.7. 2. Ash Content (%)

Ash content ranged from 4.89% to 7.23%. The highest ash content was recorded in the NE 3399 genotype (7.23%), followed by Çetinbey (6.73%), Defiance (6.71%), and BDS 25 (6.53%). The lowest ash content was observed in the BDS 11 genotype (4.89%). Since ash content reflects the total mineral content of the seed, genotypes with higher ash content can be considered richer in mineral nutrients.

Ash content is commonly regarded as an indicator of the total mineral content of seeds. Therefore, genotypes with higher ash content are generally considered to possess greater concentrations of essential mineral nutrients, which contributes positively to their nutritional quality (Sonia et al., 2024; Singer et al., 2023).

3.7.3. Oil Content (%)

Oil content ranged from 16.40% to 18.60%. The highest oil content was recorded in the BDS 21 genotype (18.60%), followed by NE 3399 and Atakişi, both with 18.40%. The lowest oil content was observed in the BDS 11 genotype (16.40%). Genotypes with higher oil content are considered more advantageous for vegetable oil production.

Genotypes with higher seed oil content are generally considered more desirable for vegetable oil production because oil concentration is one of the major determinants of the economic value of soybean. Consequently, improving seed oil content remains a key objective of soybean breeding programs aimed at food, feed, and industrial applications (Li et al., 2026; Kim et al., 2024; Vargas-Almendra et al. 2024).

Conclusions

The results of this study demonstrated significant differences among cultivars for plant height, first pod height, number of branches, number of pods, and thousand-seed weight. The significant cultivar X year interaction for yield indicates that cultivars respond differently under varying environmental conditions. Overall, the BDS 11 and BDS 25 genotypes, along with the Nova cultivar, stood out in terms of high yield and favorable morphological traits, indicating good adaptation to the region. Consequently, ecological conditions similar to Konya Plain, Nova, BDS 11 and BDS 25 genotypes can be recommended for grain yield, while Atakişi cultivar and BDS 21 genotype can be recommended for higher oil and protein content.

When the quality parameters, namely protein, oil, and ash contents, were evaluated together, considerable differences were observed among the soybean genotypes. Atakişi stood out for its superior nutritional quality, exhibiting both the highest protein content (37.48%) and a high oil content (18.40%). BDS 21 was distinguished by the highest oil content (18.60%), together with high protein (36.97%) and relatively high ash (6.05%) contents. BDS 25 can also be considered a promising genotype due to its high protein (37.09%) and ash (6.53%) contents, as well as its highest 1000-seed weight. Although NE 3399 exhibited the highest ash content (7.23%) and high oil content (18.40%), its protein content (33.28%) was lower than that of the other outstanding genotypes. In contrast, BDS 11 had the lowest ash (4.89%) and oil (16.40%) contents but produced one of the highest seed yields (5,859 kg ha^{-1}), indicating a different balance between seed quality and productivity. These findings demonstrate that the genotypes possess distinct advantages depending on their intended use whether for high protein, high oil or high mineral content.

Acknowledgments

A preliminary version of this work was presented as an abstract at the 5th International Plant Breeding Congress held in Antalya, Türkiye, from December 1 to 5, 2025. This article provides a significantly expanded and fully developed analysis of that initial work.

Table 1. Soil properties of the experimental area.

Texture	pH	E.C.(mS/cm)	Lime(%)	Organic Matter (%)	Phosphorus (kg/da)	Potassium (kg/da)
Clay Loam	8.12	2.77	22.07	2.33	3.42	153.75

Table 2. Mean monthly temperatures and cumulative rainfall of the experimental site for the crop years 2021-2022.

Meteorological Parameters	Years	Months											
		Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec
Mean Daily Maximum Temperature (°C)	2021	18.8	19.9	21.5	28.1	31.2	30.6	35.7	35.8	30.5	26.2	21.9	16.3
	2022	13.5	12.5	18.8	28.5	30.9	30.2	34.1	34.4	33.3	31.9	19.9	16.3
	Long-term averages	20.6	21.5	26.8	31.5	35.0	37.2	40.1	40.5	38.8	33.3	24.9	20.4
Mean Air Temperature (°C)	2021	2.7	2.6	3.9	11	18.3	17.4	23.1	22.7	16.4	11.1	8	1.8
	2022	-2.4	0	-0.7	12.9	14.1	19.1	20.7	23.7	18.7	11.6	7.9	4.4
	Long-term averages	1.6	3.7	7.9	13.0	17.4	21.8	25.3	25.7	20.6	14.4	8.0	3.6
Mean Daily Minimum Temperature (°C)	2021	-11.7	-15.7	-6.7	-3.5	5.4	4.1	10.7	9	3.6	-3.6	-8.6	-20.2
	2022	-22.5	-15.6	-15.3	-4.9	1.6	10.3	7.3	11.8	1	-2.8	-3.5	-4.8
	Long-term averages	-18.0	-14.7	-7.7	-3.8	2.5	9.2	13.1	12.9	5.1	0.1	-7.7	-16.2
Rainfall (mm)	2021	20.3	0.4	60.7	12.9	2.9	71.4	14.4	12	38.5	0.7	22.1	25.6
	2022	18.2	19.8	10.3	3	20	27.9	20.4	9.7	4.4	26.2	6.9	21.9
	Long-term averages	45.32	24.24	33.88	25.80	35.69	35.80	6.44	6.69	17.93	29.85	34.01	43.42

Source: TİGEM (Gözlü Agricultural Enterprise Directorate) meteorological station - DMGM data

Table 3. Analysis of Variance for the effects of treatments on agronomic traits.

Source of Variation (SV)	df	Plant Height (cm)	First Pod Height (cm)	Number of Branches per Plant	Number of Pods per Plant	Thousand-Seed Weight (g)	Grain Yield (kg ha^{-1})
Replication	2	55.04	33.39	1.14	87.29	317.38	46700.1
Year	1	6952.17*	249.57	25.92	369.46	8383.51*	26692.8
Error 1 (Rep X Year)	2	125.23	111.17**	4.22**	356.89**	357.70	41379.4*
Genotype	11	378.19**	35.83*	2.20**	97.93*	949.91**	19927.6*
Year X Genotype	11	45.83	9.61	0.49	42.30	144.97	18487.2*
Error 2	44	114.96	13.65	0.51	40.93	112.77	8575.4
Coefficient of Variation (CV, %)	-	14.08	19.42	25.01	23.79	8.17	19.02

**Significant at P <0.05; **Significant at P < 0.01.*

Table 4. Effects of treatments on the performance of soybean genotypes.

Genotype	Plant Height (cm)	First Pod Height (cm)	Number of Branches per Plant	Number of Pods per Plant	Thousand-Seed Weight (g)	Grain Yield (kg ha^{-1})	Protein (%)	Ash (%)	Oil (%)
Defiance	62.0 e	14.5 d	2.75 b-d	21.6 c	129.3 b-e	4077 c	36.97	6.71	17.15
Nova	74.7 b-d	17.6 b-d	2.68 cd	25.9 bc	118.2 ef	5198 ab	34.47	5.27	16.50
ATAEM 7	87.4 a	20.0 a-c	1.60 e	24.8 c	116.5 f	5058 a-c	34.64	5.04	16.95
NE 3399	68.7 de	17.6 b-d	3.05 a-d	26.2 bc	129.1 c-e	4447 bc	33.28	7.23	18.40
Arısoy	78.9 a-c	18.9 bc	3.13 a-d	27.5 bc	119.7 ef	5040 a-c	32.30	5.11	17.10
A 3935	68.6 de	19.3 bc	3.03 a-d	27.2 bc	123.3 d-f	4253 bc	33.32	5.46	17.05
Atakişi	74.1 b-e	16.1 cd	3.43 a-c	32.3 ab	113.3 f	4435 bc	37.48	4.92	18.40
BDS 07	82.8 a-c	20.1 a-c	2.43 de	27.1 bc	141.6 b	4740 bc	35.93	5.71	17.05
BDS 11	85.3 ab	19.4 bc	2.38 de	36.0 a	134.8 b-d	5859 a	34.88	4.89	16.40
BDS 21	72.5 c-e	21.2 ab	3.83 a	24.1 c	136.7 bc	4580 bc	36.97	6.05	18.60
BDS 25	85.7 ab	24.0 a	3.57 ab	28.2 bc	155.7 a	5881 a	37.09	6.53	16.70
Çetinbey	73.2 b-e	19.4 bc	2.57 d	22.0 c	141.4 bc	4855 a-c	37.34	6.73	16.50

References

Acikgoz, E., Sincik, M., Karasu, A., Tongel, O., Wietgrefe, G., Bilgili, U., Oz, M., Albayrak, S., Turan, Z. M., and Goksoy, A. T. (2009). Forage soybean production for seed in mediterranean environments. *Field Crops Research*, *110*(3), 213-218. https://doi.org/10.1016/j.fcr.2008.08.006

Addae-Frimpomaah, F., Amenorpe, G., Sossah, F. L., Adazebra, G. A., Amiteye, S., Adjebeng-Danquah, J., Nelimor, C., Yirzagla, J., Amoako, O. A., and Amoatey, H. (2025). Genetic variability, heritability, and genetic gain for yield and associated traits in recently introduced soybean genotypes in the guinea savannah zone of Ghana. *Australian Journal of Crop Science*, *19*(5), 489-500. https://doi.org/10.3316/informit.T2025070300000100514249057

Altınyüzük, H., and Öztürk, Ö. (2017). Soya çeşitlerinin Çukurova koşullarında II. ürün olarak verim ve kalite özelliklerinin incelenmesi. *Selcuk Journal of Agriculture and Food Sciences*, *31*(3), 101-110. https://doi.org/10.15316/SJAFS.2017.41 (In Turkish).

Arıoğlu, H. H. (2014). *Yağ bitkileri yetiştirme ve ıslahı*. Çukurova Üniversitesi Ziraat Fakültesi Yayınları (No. 220, Ders Kitapları No. A-70). (In Turkish).

Arıoğlu, H., Bakal, H., Güllüoğlu, L., Kurt, C., and Onat, B. (2016). Ana ürün koşullarında yetiştirilen bazı yerfıstığı çeşitlerinin önemli agronomik ve kalite özelliklerinin belirlenmesi. *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi*, *25*(Özel Sayı-2), 24-29. https://doi.org/10.21566/tarbitderg.281656 (In Turkish).

Bakal, H., Gulluoglu, L., Onat, B., and Arıoglu, H. (2017). The effect of growing seasons on some agronomic and quality characteristics of soybean varieties in Mediterranean Region in Turkey. *Turkish Journal of Field Crops*, *22*(2), 187-196. https://doi.org/10.17557/tjfc.356213

Bakal, H., Kenetli, A., and Arıoglu, H. (2020). The effect of plant density on pod yield and some agronomic characteristics of different growthtype peanut varieties (*Arachis hypogaea* L.) grown as a main crop. *Turkish Journal of Field Crops*, *25*(1), 92-99. https://doi.org/10.17557/tjfc.748671

Barış, M., Tunçtürk, M. ve Söğüt, T. (2020). Ekim zamanı uygulamalarının bazı soya fasulyesi (Glycine max (L.) Merrill) çeşitlerinde verim ve verim özelliklerine etkisi. ISPEC Tarım Bilimleri Dergisi, 4(4), 717-731. https://doi.org/10.46291/ISPECJASvol4iss4pp717-731 (In Turkish).

Başalma, D., İşler, N., Arslan, M., Göksoy, A. T., Sincik, M., Arslanoğlu, F., Önemli, F., Öztürk, Ö., Arslan, H., Day, S., and Kayaçetin, F. (2025). Yağlı tohumlar üretiminde mevcut durum ve sürdürülebilirlik. *Türkiye Ziraat Mühendisliği X. Teknik Kongresi Bildiriler Kitabı*, *1*, 430-455. (In Turkish).

Bek, D., and Arıoğlu, H. (2005). Çukurova koşullarında farklı soya genotiplerinin adaptasyon ve verim potansiyellerinin saptanması. *Türkiye VI. Tarla Bitkileri Kongresi*, *2*, 1101-1105. (In Turkish).

Board, J. E., Kamal, M., and Harville, B. G. (1992). Temporal importance of greater light interception to increased yield in narrow-row soybean. Agronomy Journal, 84(4), 575-579. https://doi.org/10.2134/agronj1992.00021962008400040006x

Board, J. E., and Kahlon, C. S. (2011). Soybean yield formation: What controls it and how it can be improved. In H. A. El-Shemy (Ed.), *Soybean physiology and biochemistry* (pp. 1-36). InTechOpen.

Chiluwal, A. (2025). US soybean seed protein concentrations-Current status, challenges, and some potential crop management solutions. *Agronomy Journal*, *117*(1), e21731. https://doi.org/10.1002/agj2.21731

Elgün, A., Ertugay, Z., 2011. Tahıl işleme teknolojisi, Atatürk Ünv. Ziraat Fakültesi Yayınları, No:718, Erzurum, 411 s.

FAO. 2025. Agricultural production statistics- 2010-2024. FAOSTAT Analytical Briefs, No. 121. Rome. https://doi.org/10.4060/cd8035en

Gale, F., Valdes, C., and Ash, M. (2019). *Interdependence of China, United States, and Brazil in soybean trade* (OCS-19F-01). USDA, Economic Research Service. https://www.ers.usda.gov/media/10166/ocs-19f-01.pdf?v=57181

Gauch, H. G., Jr. (2013). A simple protocol for AMMI analysis of yield trials. *Crop Science*, *53*(5), 1860-1869. https://doi.org/10.2135/cropsci2013.04.0241.

Gökdere, H. İ., Yılmaz, A. B., Tekin, M., Yeken, M. Z., and Çiftçi, V. (2023). Ekmeklik buğday (*Triticum aestivum* L.) genotiplerinin dane verimi ve bazı önemli kalite özellikleri için Trakya Bölgesinde bulunan farklı çevrelerde testlenmesi. *Journal of the Institute of Science and Technology*, *13*(4), 3040-3052. https://doi.org/10.21597/jist.1358515 (In Turkish).

Hartman, G. L., West, E. D., and Herman, T. K. (2011). Crops that feed the world 2. Soybean-worldwide production, use, and constraints caused by pathogens and pests. *Food Security*, *3*(1), 5-17. https://doi.org/10.1007/s12571-010-0108-x.

Kim, J., Scaboo, A., Rainey, K. M., Fritschi, F. B., and Bilyeu, K. (2024). Redesigning soybean with improved oil and meal traits. *Theoretical and Applied Genetics*, *137*(9), Article 218. https://doi.org/10.1007/s00122-024-04732-8

Kocaturk, M., Cubukcu, P., Goksoy, A. T., Sıncık, M., Ilker, E., Kadıroglu, A., Vurarak, Y., Sahın, Y., Karakus, M., and Akgun Yıldırım, U. (2019). GGE biplot analysis of genotype X environment interaction in soybean grown as a second crop. *Turkish Journal of Field Crops*, *24*(2), 145-154. https://doi.org/10.17557/tjfc.615175.

Leggett, M., Diaz-Zorita, M., Koivunen, M., Bowman, R., Pesek, R., Stevenson, C., and Leister, T. (2017). Soybean response to inoculation with *Bradyrhizobium japonicum* in the United States and Argentina. *Agronomy Journal*, *109*(3), 1031-1038. https://doi.org/10.2134/agronj2016.04.0214.

Li, H., Sun, J., Zhang, Y., Wang, N., Li, T., Dong, H., Yang, M., Xu, C., Hu, L., Liu, C., Chen, Q., Foyer, C. H., and Qi, Z. (2026). Soybean oil and protein: Biosynthesis, regulation and strategies for genetic improvement. *Plant, Cell & Environment*, *49*(7), 3730-3746. https://doi.org/10.1111/pce.15272

Liu, S., Liu, Z., Hou, X., and Li, X. (2023). Genetic mapping and functional genomics of soybean seed protein. *Molecular Breeding*, *43*(4), Article 29. https://doi.org/10.1007/s11032-023-01373-5

Nget, R., Aguilar, E. A., Cruz, P. C. S., Reaño, C. E., Sanchez, P. B., Reyes, M. R., and Prasad, P. V. V. (2022). Responses of soybean genotypes to different nitrogen and phosphorus sources: Impacts on yield components, seed yield, and seed protein. *Plants*, *11*(3), 298. https://doi.org/10.3390/plants11030298.

OECD/FAO. (2023). *OECD-FAO agricultural outlook 2023-2032*. OECD Publishing. https://doi.org/10.1787/08801ab7-en.

Ogunbayo, S. A., Sié, M., Ojo, D. K., Sanni, K. A., Akinwale, M. G., Toulou, B., Shittu, A., Idehen, E. O., Popoola, A. R., Daniel, I. O., and Gregorio, G. B. (2014). Genetic variation and heritability of yield and related traits in promising rice genotypes (*Oryza sativa* L.). *Journal of Plant Breeding and Crop Science*, *6*(11), 153-159. https://doi.org/10.5897/JPBCS2014.0457.

Pagano, M. C., and Miransari, M. (2016). The importance of soybean production worldwide. In M. Miransari (Ed.), *Abiotic and biotic stresses in soybean production* (pp. 1-26). Academic Press. https://doi.org/10.1016/B978-0-12-801536-0.00001-3

Peng, D., Zhang, H., Zhang, Y., Yu, L., Chen, M., Chen, J. M., You, L., Li, P., Liu, J., Zhang, X., Arvor, D., Kuchler, P., Huang, J., Zhang, H., Hao, P., Huang, J., Shi, Z., Wang, F., Song, K., Zhang, B. (2026). Global soybean trade dynamics: Drivers, impacts, and sustainability. *The Innovation*, *7*(2), Article 101124. https://doi.org/10.1016/j.xinn.2025.101124

Pospišil, A., and Pospišil, M. (2024). Soybean yield and yield components depending on a sowing rate and sowing date. *Poljoprivreda*, *30*(2), 10-16. https://doi.org/10.18047/poljo.30.2.2

Ramya, P., Chaubal, A., Kulkarni, K., Gupta, L., Kadoo, N., Dhaliwal, H. S., Chhuneja, P., Lagu, M., and Gupta, V. (2010). QTL mapping of 1000-kernel weight, kernel length, and kernel width in bread wheat (*Triticum aestivum* L.). *Journal of Applied Genetics*, *51*(4), 421-429. https://doi.org/10.1007/BF03208872.

Ray, D. K., Gerber, J. S., MacDonald, G. K., and West, P. C. (2015). Climate variation explains a third of global crop yield variability. *Nature Communications*, *6*(1), Article 5989. https://doi.org/10.1038/ncomms6989

Salvagiotti, F., Cassman, K. G., Specht, J. E., Walters, D. T., Weiss, A., and Dobermann, A. (2008). Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Research*, *108*(1), 1-13. https://doi.org/10.1016/j.fcr.2008.03.001

Shi, D., Hang, J., Neufeld, J., Zhao, S., and House, J. D. (2023). Effects of genotype, environment and their interaction on protein and amino acid contents in soybeans. *Plant Science*, *337*, Article 111891. https://doi.org/10.1016/j.plantsci.2023.111891

Singer, W. M., Lee, Y.-C., Shea, Z., Vieira, C. C., Lee, D., Li, X., Cunicelli, M., Kadam, S. S., Khan, M. A. W., Shannon, G., Mian, M. A. R., Nguyen, H. T., and Zhang, B. (2023). Soybean genetics, genomics, and breeding for improving nutritional value and reducing antinutritional traits in food and feed. *The Plant Genome*, *16*(4), Article e20415. https://doi.org/10.1002/tpg2.20415

Soni, K., Frew, R., and Kebede, B. (2024). A review of conventional and rapid analytical techniques coupled with multivariate analysis for origin traceability of soybean. Critical reviews in food science and nutrition, 64(19), 6616-6635.

Soni, K., Frew, R., and Kebede, B. (2024). A review of conventional and rapid analytical techniques coupled with multivariate analysis for origin traceability of soybean. *Critical Reviews in Food Science and Nutrition*, *64*(19), 6616-6635. https://doi.org/10.1080/10408398.2023.2171961.

TÜİK. (2025). *Bitkisel üretim istatistikleri*. Türkiye İstatistik Kurumu. https://veriportali.tuik.gov.tr/tr/statistical-themes

USDA. (2026). *World agricultural supply and demand estimates* (WASDE-673). U.S. Department of Agriculture. https://www.fas.usda.gov/data/wasde

Vargas-Almendra, A., Ruiz-Medrano, R., Núñez-Muñoz, L. A., Ramírez-Pool, J. A., Calderón-Pérez, B., and Xoconostle-Cázares, B. (2024). Advances in soybean genetic improvement. *Plants*, *13*(21), Article 3073. https://doi.org/10.3390/plants13213073

Wilcox, J. R. (2004). World distribution and trade of soybean. In R. M. Shibles, J. E. Harper, R. F. Wilson, and R. C. Shoemaker (Eds.), *Soybeans: Improvement, production, and uses* (3rd ed., Vol. 16, pp. 1-14). American Society of Agronomy. https://doi.org/10.2134/agronmonogr16.3ed.c1

Yan, W., Hunt, L. A., Sheng, Q., and Szlavnics, Z. (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science*, *40*(3), 597-605. https://doi.org/10.2135/cropsci2000.403597x

Yan, W., and Kang, M. S. (2002). *GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists*. CRC Press.

Yang, Q., Lin, G., Lv, H., Wang, C., Yang, Y., and Liao, H. (2021). Environmental and genetic regulation of plant height in soybean. *BMC Plant Biology*, *21*(1), Article 63. https://doi.org/10.1186/s12870-021-02836-8.

Yılmaz, A., Yılmaz, H., Soydemir, H. E., and Çiftçi, V. (2022). Soya (Glycine max L.)'da PGPR ve AMF uygulamalarının verim özellikleri ve protein içeriğine etkisi. *International Journal of Agriculture and Wildlife Science*, *8*(1), 108-118. https://doi.org/10.24180/ijaws.1077704 (in Turkish).

About the Journal

Ekin, Journal of Crop Breeding and Genetics, is an international journal owned and edited by the Plant Breeders Sub-Union of Turkey (BISAB). Ekin is aimed at keeping information among plant breeders about new advances in the plant breeding and genetics as well as genetic diversity of plant species. Ekin publishes research papers and critical reviews on all aspects of plant breeding, genetics and plant registrations cover; old and new cultivars, local populations and introduction materials, germplasm, resistance sources for biotic and abiotic stresses, parental lines, genetic stocks, breeding materials, mapping populations. All manuscripts submitted for publication are reviewed by at least two referees and accepted for publication by editors based on advice from referees.

The journal is peer reviewed journal and published twice a year by BISAB.
Full contents of EKIN are freely available for download http: http://www.ekinjournal.com. To receive Table of Contents alerts of each new issue via e-mail registration is needed.

Manuscript Submission

Author(s) have to imply: that the submitted manuscript has not been published before or it is not under consideration for publication in other journals. For the mentioned issue, the submitted manuscript should be approved by the corresponding author on behalf of co-authors or all co-authors. BISAB will not be held legally responsible when there be any claims for compensation.

The copyright permission is under responsibility of authors if they wish to include figures, tables, or text passages that have already been published elsewhere. Any material received without such permission will be assumed as original from the authors.

Online Submission

Author(s)have to submit their manuscripts online using http: http://www.ekinjournal.com. Figures and Tables should be presented at the end of text (after list of references). The submitted manuscript should include the following sections:

Manuscript

Manuscripts should be written in double space and submitted in Word with Times New Roman font as 12 point. The submitted manuscript includes the automatic page number.

Title

The title should be concise and informative, and fallowed by the name(s) of the author(s), the affiliation(s) and address(es) of the author(s), the e-mail address, telephone and fax numbers of the corresponding author

Abstract

Abstract is one of the most important parts of the articles since it is the most published and cited in international databases. Abstract should be started with a sentence that provides an importance and aim of the study. It also contains materials and methods, main findings or results and a conclusion sentence for readers. It is not more than 250 words. Abbreviations or references cannot be written in the abstract.

Keywords

Keywords should be provided between 4 to 6 words for indexing purposes.

Introduction

Introduction should be reviewed of available literature and the similar nature works published before. It should be outlined the reason why the study was performed or the originality of study.

Materials and Methods

Materials and methods could be divided into sub-headings but headings could be used up to three levels. All materials, experiments conducted, conditions and course should be described in details. The whole methodology in the submitted manuscript should be detailed if it is original; in other cases, it is sufficient to cite the relevant reference(s) published before. Statistical methods processed in the submitted manuscript should also be described with the software used.

Results

The results obtained from the materials, experiments, and analyses should be given as figure and tables. The important findings from the results should be outlined but the irrelevant findings should not be given. Statistical evaluation and commentary should also be given without ANOVA table.

Discussion

Results section can be merged with Discussion section. Author(s) should confront own findings and results with data published by the other authors. For different results, scientific questions should be answered and discussed. The surname of the first author(s) and year of publication must be cited in Discussion section directly or indirectly. Some examples;

In Turkey, wheat was produced 10 million tons in 1923 (Gokgol 1939).

This result was in agreement with result of Sahin and Yildirim (2004).

Similar effect has been widely studied prior to this study (Eser 1991; Bagci et al. 1995; Uzun and Yol 2013).

At the end of Discussion section, the conclusion sentence(s) should be presented for readers.

References

The list of references should include cited works in the text. Personal communications (Personal com. with Prof./Dr./Mr./Ms. Ucar, Ankara, Turkey, 2012) should only be mentioned in the text. The works under consideration, submitted and unpublished works should not be listed in the References section. References should be chronologically alphabetized by the surnames of the first author of each work. Some examples;

Journal article:
Toker C (1998). Adaptation of kabuli chickpeas (*Cicer arietinum* L.) to the low and high lands in the West Mediterranean region of Turkey.Turk J Field Crop 3:10-15.

Toker C and Canci H (2003). Selection of chickpea (*Cicer arietinum* L.) genotypes for resistance to ascochyta blight [*Ascochyta rabie*i (Pass.) Labr.], yield and yield criteria. Turk J Agric For27: 277-283.

Toker C, Canci H and Ceylan FO (2006). Estimation of outcrossing rate in chickpea (*Cicer arietinum* L.) sown in autumn.Euphytica 151: 201-205.

Article by Digital Object Identifier (DOI) number:
Yasar M, Ceylan FO, Ikten C and Toker C (2013). Comparison of expressivity and penetrance of the double podding trait and yield components based on reciprocal crosses of kabuli and desi chickpeas (*Cicer arietinum* L.). Euphyticadoi:10.1007/s001090000086

Book:
Toker C (2014). Yemeklik Baklagiller. BISAB, Ankara.

Book chapter:
Toker C, Lluch C, Tejera NA, Serraj R and Siddique KHM (2007). Abiotic stresses. In: Chickpea Breeding and Management,Yadav SS, Redden B, Chen W and Sharma B (eds.),CAB Int. Wallingford, pp: 474-496.

Online document:
FAOSTAT J (2013) http://faostat.fao.org/site/567/default.aspx# anchor. Accessed 15 May 2013.

Dissertation (Thesis):

Yasar M (2012). Penetrance and expressivity of double podding characteristic in chickpea (*Cicer arietinum* L.). Dissertation, Akdeniz University, Antalya.

Acknowledgments

Acknowledgments of people, grants, funds, etc. could be placed before the reference list. The names of funding organizations should be written.

Abbreviations

Abbreviations should be defined at first mention and used consistently.

bisab
Plant Breeders Union of Turkey
www.bisab.org.tr

ISSN 2149-1275

Adakale Street, No: 22/12 Kızılay, 06420 Çankaya / Ankara - TÜRKİYE
Phone: +90 312 433 30 65-66 Fax: +90 312 433 30 06
Email: bisab@bisab.org.tr