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Host Plant Resistance or Tolerance for Control of Yellow Dwarf Viruses in Cereals

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ABSTRACT

Yellow dwarf viruses are the most economically important and devastating viruses affecting cereal crops, resulting in yield and quality losses. Because of recent global climate change, there has been an increase in vector-borne viruses, particularly yellow dwarf viruses transmitted by aphids. YDVs comprise a complex group that includes barley yellow dwarf viruses (BYDVs)/cereal yellow dwarf viruses (CYDVs), as well as newly renamed species. One of the most effective control methods for YDVs is to grow resistant or tolerant cultivars, in addition to late sowing, spraying and covering seeds with insecticides to control aphid vectors, as well as other cultural practices. Resistance to BYDV is complex, and numerous studies have been conducted to date in many efforts to develop resistant cultivars and lines to manage YDVs. Those studies included BYDV resistance derived from wheat-related and wild relatives, as well as resistance attained against aphids. This review will examine breeding studies addressing BYDV resistance in cereals, including wheat, barley, oats, and maize, to date.

Keywords: Cereal, BYDV, resistance, tolerance

Introduction

Yellow dwarf viruses (YDVs) are the most economically important and devastating viruses, causing yield losses in cereal crops worldwide. YDVs infect cereal species, such as wheat, barley, and oats, as well as many annual and perennial monocotyledonous grasses in the Poaceae family (D'Arcy, 1995). YDVs have also been found to infect dicotyledonous grasses, *Geranium dissectum* and *Juncus compressus*, in recent years (İlbağı et al., 2019). They are characterized by yellowing or reddening, depending on the hosts, dwarfing, delayed heading, and reduced cereal grain numbers. Characteristic symptoms include stunted growth of the host, resulting from diminished internode elongation. The discoloration is pervasive on older infected leaves (Oswald and Houston, 1953). Wheat, triticale, and rye leaves are commonly yellow, and sometimes they are red. It has been reported that serration along the leaf margins in wheat and oats, apart from inhibiting root growth, was observed in

plants infected with YDV (Kolb et al., 1991; Hoffman and Kolb, 1997). YDV infection may be confused with symptoms of abiotic stress in plants. Thus, the diagnostic methods should confirm the visual diagnosis of YDV infections. YDVs affect yield by causing sterility, suppressing heading, and reducing the number of tillers and kernels per spike (D'Arcy, 1995). It can cause severe losses, especially in wheat, depending on the YDV species, wheat varieties, weather conditions, and aphid populations. YDVs are phloem-limited and are transmitted in a persistent circulative manner by over 25 aphid vectors. The most common vectors of BYDV are *Rhopalosiphum padi*, *Rhopalosiphum maidis*, *Sitobion avenae*, *Metopolophium dirhodum*, *Schizaphis graminum*, and *Sitobion fragariae* (Parry et al., 2012). Among them, *Rhopalosiphum padi* L. and *Rhopalosiphum maidis* Fitch are the most common and efficient species (Smith and Plumb, 1981). The bird cherry-oat aphid, *Rhopalosiphum padi* L., is a frequent vector of BYDV species (Halbert and Voegtlin, 1995).

The mechanisms associated with YDV infections in the field conditions are complex and influenced by many factors. Given the direct interactions among viruses, aphid vectors, and cereal host plants, it is also crucial to investigate the presence of grass hosts in these agroecosystems (Power and Gray, 1995). After BYDV was named by Oswald and Houston (1953) in California/USA, Rochow (1969) identified five serotypes, classified by their preferred aphid vector species. YDVs comprise a complex virus group, including barley yellow dwarf viruses (BYDVs)/cereal yellow dwarf viruses (CYDVs), as well as newly identified species such as MYDV-RMV and WYDV-GPV (Krueger et al., 2013; Zhang et al., 2009). YDVs cause yield losses of 15-25% in wheat, barley, and oats (Lister and Ranieri, 1995; McKirdy and Jones, 1997). It has been reported that YDVs caused 30% losses in wheat in the UK (Perry et al., 2000) and 80% losses in early-planted winter wheat in Türkiye (İlbağı, 2020). Nancarrow et al. (2021) pointed out that BYDV-PAV caused yield reductions of up to 84% (1358 kg/ha) in wheat and 64% (1456 kg/ha) in barley. Disease control strategies could also be partially achieved by applying insecticides, crop rotation, removing virus reservoirs, avoiding frequent sowing, and using germplasm with tolerance/resistance to the virus or its vectors (Royer et al., 2005; Kennedy and Connery, 2012). Chemical application for controlling aphid populations is an effective and easy method; however, it is not economic. Due to the negative environmental and other organism impacts, the use of pesticides is restricted in certain regions of the world (McNamara et al., 2020). Moreover, specifically, once symptoms become obvious, it would already be too late to control the vector. On the other hand, the sowing of resistance/tolerant varieties adapted to each location (i), late sowing; as of second week of November for the Trakya region, Türkiye (ii), combating of weeds as inoculum sources (iii), rotation; avoiding planting wheat after other cereal or maize crops (iv), avoiding of planting with stubble in the cereal fields (v), and avoiding of frequent sowing were suggested to combat YDVs by İlbağı (2020). In this respect, late sowing is a crucial cultural practice for combating YDVs. Thanks to the late sowing of wheat, YDVs have been successfully managed in the Trakya region of Türkiye (İlbağı, 2020). As shown in Figure 1, the importance of late sowing for controlling YDVs is evident based on late- and early-sowing wheat fields in Trakya/Türkiye. Similarly, the studies worldwide have shown that late sowing is important for controlling YDVs. McKirdy and Jones (1997) noted that delaying sowing reduced BYDV incidence in wheat. Aghnoum

et al. (2017) indicated that late planting plays a crucial role in escaping BYDV infections in the BYDV hot spot region. Foster et al. (2004) noted that virus and aphid incidence may be associated with crop and field characteristics, particularly sowing date. Sowing winter cereals and correctly timing insecticide applications are critical components of BYDV management, as reported by Walsh et al. (2022). On the other hand, breeding resistant or tolerant varieties is the most effective method for controlling YDVs and is a cost-effective approach for controlling BYDV, as reported by Ordon et al. (2004). Arodittir and Crespo-Herrera (2021) noted that challenges and opportunities in resistance to BYDV and its vectors in wheat breeding programs and indicated the importance of identifying resistance sources for Host Plant Resistance (HPR).

BYDV resistance in wheat

Four primary genetic sources of resistance in wheat, three of which are derived from the secondary gene pool (species which are progenitors of the three hexaploid wheat genomes: e.g., *T. dicoccoides*, *T. dicoccum*, *Aegilops tauschii*), though no resistance is known in the primary wheat gene pool. *Bdv1*, *Bdv2*, *Bdv3*, and *Bdv4* resistance genes, which have been reported in wheat; however, their introduction into commercial cultivars has not been effective (Ayala et al., 2001; Kosova et al., 2008). Previous studies have reported that true resistance to BYDV has not been naturally found in wheat; however, BYDV resistance genes have been identified in more than 10 wild relative species belonging to the genera *Thinopyrum*, *Agropyron*, *Elymus*, *Leymus*, *Roegneria*, and *Psathyrostachys* (Zhang et al., 2009). Some *Thinopyrum* species are widely used as sources of combined resistance to BYDV and various rusts in wheat breeding programs (Larkin et al., 1995). Evaluation of resistant sources carrying the *Bdv1* and *Bdv2* genes suggests a polygenic nature for BYDV resistance (Veškrna et al., 2009). The only exception among other genes is the *Bdv1* gene, a semidominant gene, which was detected in the North American bread wheat cultivar Anza. Although *Bdv1* confers tolerance to BYDV-MAV based on field observations, it does not confer resistance to all BYDV serotypes or across all environments. *Bdv1* for a “tolerance” known as “partially effective” and conferring “slow yellowing of infected leaves”. *Bdv1* was reportedly associated with the *Lr34/Yr18* rust resistance gene complex on 7DS, which is also associated with a leaf tip necrosis trait (Singh et al., 1993). Tolerance to BYDV in wheat, which reduces crop losses at high virus concentrations, has been reported to be polygenically controlled (Cisar et al., 1982). A QTL located in the same position as

Bdv1 accounted for approximately 7% of the total variability, like the polygenic nature of BYDV tolerance in wheat (Ayala et al., 2002). Additionally, *Bdv1* was reported to be associated with the *Lr34/Yr18* rust resistance gene complex on 7DS, which is also associated with a leaf tip necrosis trait and powdery mildew resistance (Singh et al., 1993; Spielmeyer et al., 2005). Ayala et al. (2002) indicated that, despite Anza having reduced visual symptoms, especially yellowing, no statistically significant differences were found between genotypes in any of the measures of disease effects. The presence or absence of the *Lr34/Yr18* complex was determined by Lagudah et al. (2006; 2009). Previous studies have shown that *Bdv1*, linked with the *Lr34/Yr18* gene complex, may reduce visible symptoms of BYDV infection; however, there is limited evidence that it is effective in preventing grain or biomass yield losses. The first BYDV resistance gene in *Thinopyrum intermedium* was identified in a disomic chromosome addition line, L1, derived from the wheat-*Th. intermedium* partial amphiploid TAF46 (Cauderon et al., 1973). This gene was located on the long arm of homoeologous group 7 chromosome 7XL (7Ai#IL) of *Th. intermedium* (Brettell et al., 1988; Xin et al., 1991), and was designated as *Bdv2* (Zhang et al., 1999; Stoutjesdijk et al., 2001). Some wheat-*Th. intermedium* translocation lines, such as the Yw series, that show good BYDV resistance, were developed using the CS ph mutant (Xin et al., 2001). Banks and Larkin (1995) transferred the alien chromatin carrying *Bdv2* from L1 to the common wheat background and developed several wheat-*Th. intermedium* translocation lines, including 7D-7Ai#1 recombinants (e.g., TC5-TC6, TC8-TC10, and TC14), and one 7B-7Ai#1 translocation (TC7) (Banks and Larkin 1995; Hohmann et al., 1996; Larkin et al., 2002). These lines were used to produce resistant wheat cultivars with *Bdv2*, such as a winter wheat, Mackellar (with TC14), and a spring wheat, Glover (with TC6) in Australia (Larkin et al., 2002). Some *Th. intermedium* - *Th. ponticum* translocations were recovered, which carry the resistance genes *Lr19* and *Bdv2* through homoeologous pairing in the presence of gene *ph1b* (Ayala-Navarrete et al., 2007). Ayala Navarrete et al. (2007, 2009) developed several EST-based PCR markers for the 7Ai#1L segment, containing *Bdv2*. EST-based PCR markers associated with the *Bdv2*- harbouring segment (Gao et al., 2009). A dominant SCAR marker was also developed for the *Bdv2* resistance gene, which originates on the long arm of chromosome 7A1 of *Thinopyrum intermedium*, by Stoutjesdijk et al. (2001). The BYDV resistance locus in P29 and P107 was named as *Bdv3* (Ohm and Anderson, 2007). Anderson et al. (1998) reported

that P29 is completely resistant to CYDV-RPV and MYDV-RMV, and moderately resistant to BYDV-PAV and BYDV-MAV. Kong et al. (2009) suggested the SSR-*Bdv3* diagnostic marker and investigated the transmission of the *Th. intermedium* 7E segment carrying *Bdv3* in different genetic backgrounds. Another BYDV resistance gene, *Bdv4*, is located on chromosome 2 (2D-2Ai-2) (Larkin et al., 1995; Lin et al., 2006). The BYDV resistance observed in Zhong 5 was determined to be the same as that of L1 to BYDV-GAV and more effective against BYDV-GPV and PAGV (a Chinese wheat yellow dwarf virus strain related to PAV) (Lin et al., 2007). Identifying genome regions associated with BYDV resistance and applying this knowledge to marker-assisted selection (MAS) would enable faster progress in cereal crop breeding (Choudhury et al., 2017). As noted by Shang et al. (2025), comprehensive studies over the past few decades have focused on identifying and characterizing candidate genes associated with resistance to BYDV and its aphid vectors in barley and wheat. Jiang (2013) indicated that very limited information exists on commercial cultivars concerning BYDV resistance genes in wheat. However, current studies have demonstrated promising improvements in BYDV resistance genes in wheat, which can be utilized in breeding programs. A winter wheat variety (G1) was identified as exhibiting significant aphid resistance through antixenosis and antibiosis, and restricted phloem access and salivation by viruliferous *R. padi* in the G1 wheat variety were associated with lower BYDV transmission efficiency (Ilma et al., 2025). Recently, the wheat variety RGT Wolverine, carrying the *Bdv2* gene, was commercially introduced in the United Kingdom. Pichon et al. (2022) indicated that a newly developed wheat variety named RGT Wolverine, carrying the *Bdv2* gene, will allow for observation under natural conditions in terms of the impacts of the *Bdv2* gene on the evolution and adaptation of YDVs, the durability of the resistant phenotype, and the impact of the deployment of a BYDV-resistant material on the epidemiology of YDV diseases. The ensuing study flow for developing resistant cereal cultivars through breeding programs is shown in Figure 2.

BYDV resistance in barley

Four genes and several QTLs in barley have been reported to be associated with resistance/tolerance to BYDV. The first gene, called *Ryd1*, which carries recessive intermediate tolerance, was identified by Suneson (1955) in the cultivar 'Rojo.' It has been rarely used in breeding programs. However, the second resistance gene, *Yd2*, was identified by Schaller et al. (1964) and subsequently introduced into many

barley cultivars, where it was utilized in barley breeding programs. Later, this gene was defined as *Ryd2* by Søgaard and von Wettstein-Knowles (1987). The barley cultivars carrying the *Ryd2* gene exhibit tolerance to BYDV-PAV and BYDV-MAV; however, this gene may be ineffective in inducing resistance to CYDV-RPV (Niks et al., 2004). *Ryd2* has been located on chromosome 3HL (Collins et al., 1996; Paltridge et al., 1998), and markers have been used in breeding programs to incorporate *Ryd2* (Ovesna et al., 2000; Jefferies et al., 2003). The *Ryd2* gene was then transferred to chromosome 3H of the American spring barley cultivar Atlas 68 by crossing Schaller and Chim, (1969). *Ryd2* has been successfully used in breeding tolerant spring and winter barley cultivars (Delogu et al., 1995; Šip et al., 2006). Some QTL for tolerance against BYDV-MAV and BYDV-PAV have been mapped on chromosomes 7H, 4H, and 1H (Tooijinda et al., 2000). Additionally, a new locus, *Ryd3*, derived from an Ethiopian landrace, was identified and located on chromosome 6H (Niks et al., 2004). In barley, no complete resistance to BYDV is known to exist. Through extensive screening, three tolerance genes, including *Ryd1*, *Ryd2*, and *Ryd3*, have been identified. Among these, *Ryd2*, located on chromosome 3HL, has been successfully incorporated into different commercial spring and winter barley cultivars (Ordon et al., 2009). Habekuss et al. (2009) determined that reducing symptom expression and virus extinction in lines combining *Ryd2* and *Ryd3*. Riedel et al. (2011) reported that DH lines carrying the combination of *Ryd2* and *Ryd3* exhibited a significant reduction in virus titre, and a significantly higher relative grain yield was obtained in spring barley DH lines in comparison to lines carrying only *Ryd2* or *Ryd3*. They stated that a combination of *Ryd2* and *Ryd3* confers quantitative resistance to BYDV-PAV rather than tolerance. Additionally, significant levels of resistance to BYDV were obtained by combining the resistance gene *Yd2* with genes detected in moderately resistant cultivars by Ovesna et al. (2000). An additional two QTLs for the relative yield after BYDV infection were detected on chromosomes 2HL and 3HL, accounting for approximately 50% of the phenotypic variance in the relative yield after BYDV infection (Ordon et al., 2009). Collins et al. (1996) determined that the protein product of the gene at the *xylP* locus could provide a convenient assay for the selection of *Yd2* during the breeding of BYDV-resistant barley varieties. Recently, a study reported that *Ryd* genes limit the success of infection (low infection rates) and increase the latency period in infected hosts. These characteristics allow the *Ryd2*- and *Ryd3*-genotypes to be described as

partially resistant to YDVs (Souquet et al., 2025). Jarosova et al. (2020) investigated miRNA profiles in new barley lines and in cultivar Wysor (carrying one resistance gene, *Ryd2*), with and without BYDV infection. They determined that the profile of miRNAs expressed in Vir8:3 and Vir13:8 in response to BYDV was similar and differed from that of Wysor. To identify a novel resistance gene, a study was conducted in 2019. This study demonstrated that the consistently detected new gene on chromosome 5H has the potential to serve as a novel source of tolerance, thereby achieving more sustainable resistance to BYDV in barley. *Ryd4* was identified and localized on chromosome 3HL in barley by Scholz et al. (2009). This resistance was introgressed from *Hordeum bulbosum*, the secondary gene pool of barley. However, it cannot be efficiently used in barley breeding programs, as indicated by Scholz et al. (2009). *Ryd2* and *Ryd3*, when combined, are the most promising approach for barley cultivars expressing quantitative resistance to barley yellow dwarf virus (Riedel et al., 2011). Pidon et al. (2024) reported that high-throughput molecular markers will permit more targeted selection of resistance in breeding for the use of *Ryd4* in barley varieties.

BYDV resistance in oat and maize

Tolerance to BYDV in oat is heritable Mckenzie et al. (1985). Comeau and Burnett (1984) noted that breeding for tolerance to BYDV was greatly accelerated following the severe North American epidemic in 1959. Then, a source of BYDV tolerance was identified in oats, leading to the development of several prominent BYDV-tolerant lines (Brown and Jedlinski, 1973). Endo and Brown (1964) found tolerance in oats to BYDV, which is heritable and easily identified in segregating populations. Jenkins (1966) stated that early BYDV infections caused a decrease in yield in susceptible oat varieties by 93% and 97% in the more tolerant oat varieties. Mckenzie et al. (1985) reported that two to four quantitatively inherited genes could contribute to the tolerance of the four tolerant oats. The highest levels of resistance were found in certain *Avena* species, including *Avena sterilis*, *A. occidentalis*, *A. barbata*, *A. fatua*, *A. hybrida*, *A. macrostachya*, *A. nuda*, and *A. strigosa* (Comeau and Burnett 1984). Landry et al. (1984) developed a model with two to four genes for the segregation of tolerance in hybrids between *A. sativum* and *A. sterilis*. Virus-derived transgenic resistance in oat was investigated, and Koev et al. (1998) proposed a strategy for genetically stable transgenic resistance to BYDVs applicable to all virus hosts. In oat (*Avena sativa*), several QTLs contributing to BYDV tolerance have been detected (Ordon et al., 2009), of which three loci were shown to be of major

importance (Jin et al., 1998). Gray et al. (1993) reported that resistance to BYDV in a spring oat was released as a reduction in the accumulation of viral antigen in the whole plant. In studies on maize, Körber et al. (2013) reported a high potential for breeding BVDY-resistant/tolerant maize. Horn et al. (2014) suggested using SNPs (associated with BYDV resistance) in marker-assisted selection, indicating that this approach can accelerate the breeding process for developing BYDV-resistant maize genotypes. Horn et al. (2015) found that a QTL on chromosome 10 explained 45% of the phenotypic variance, affecting virus extinction traits and infection rates, and suggested that maize resistance is oligogenically inherited; this QTL should be utilized in breeding programs. Recently, Schmidt et al. (2025) reported BYDV-PAV resistance mechanisms in maize that act directly on the virus, rather than on its vector, *R. padi*.

Conclusions

Managing YDVs successfully depends on several factors, including the biology of the aphid vectors, the plant host, and the virus species. The use of insecticides to control aphid vectors is neither environmentally friendly nor economically efficient, making it an unsustainable strategy. However, the use of resistant/tolerant cultivars is an environmentally safe method to control viruses. To this end, breeding programmes have been conducted to find sources of resistance to BYDV/CYDV and its aphid vectors. So far, resistance sources have been found in a primary gene pool and a few species in the secondary gene pool. However, exploring BYDV and aphid-resistant genes in other related species may offer future research. Even so, recent advances in BYDV resistance genes are promising, but further

studies are needed to detect resistance genes against YDVs. On the other hand, in controlling YDVs, it is essential to consider cultural practices, as optimizing planting dates is fairly effective for managing vector aphids of YDVs. Because late sowing reduces virus infections by delaying winter cereal emergence after aphid migrations, early-sowing cereal can significantly increase virus prevalence during the seedling stage. Thus, optimizing sowing dates and combating virus sources, such as grasses, should always be considered strategies for managing YDVs.



Figure 1. Late sown wheat field (A, on the left), early sown wheat field (B, on the right), resistant/tolerant and susceptible wheat cultivars to YDVs in the field (C) (İlbağı, 2017; İlbağı, 2020)

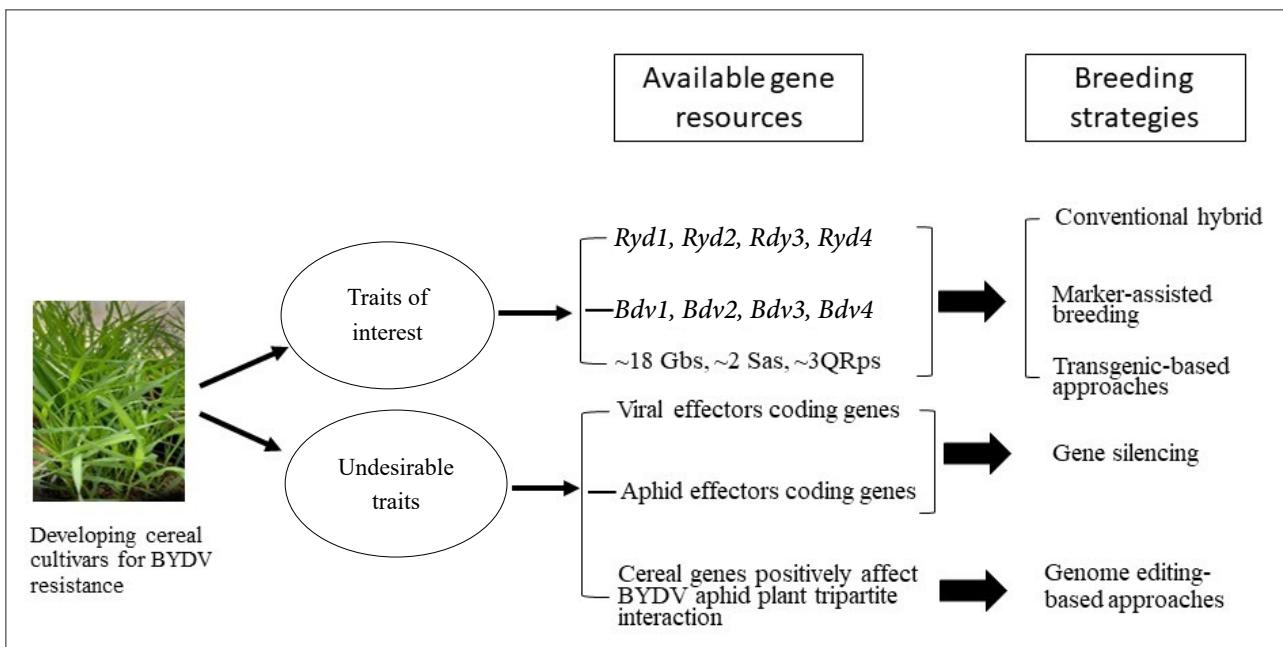


Figure 2. Strategies in breeding programs to develop cereal cultivars against BYDV. Bdvs, BYDV-resistant genes derived from wheat cultivars; Ryds, BYDV-resistant genes derived from barley cultivars; Gbs, greenbug (*Schizaphis graminum*) resistance genes; Sas, English grain aphid (*Sitobion avenae*) resistance genes; QRPs, bird cherry-oat aphid (*Rhopalosiphum padi*) resistance QTLs (Shang et al., 2025).

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Molecular Phylogenetic Analyses of Perennial Ryegrass (*Lolium perenne* L.) Populations Selected from the Flora of Türkiye

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ABSTRACT

Perennial ryegrass (*Lolium perenne* L.) is an important forage species widely used in grassland-meadow ecosystems and turfgrass management due to its high forage yield, feed quality, and strong adaptation ability. The effectiveness of breeding programs in this species depends on the accurate determination of genetic differences and phylogenetic relationships among the genetic materials to be used. This study was conducted to reveal, at the molecular level, the phylogenetic relationships among potential *L. perenne* samples collected from the natural distribution areas of Türkiye's Central Anatolia and Mediterranean regions. Phylogenetic analyses indicate that annual species (*L. rigidum* and *L. temulentum*) have made a significant contribution to the evolution of perennial ryegrasses. One of the most important findings of this study is that it confirms the origin of existing perennial ryegrass populations from annual ryegrasses, based on the sampled Anatolian ryegrasses, and provides important insights regarding local populations. In the phylogenetic tree constructed using chloroplast sequences, it is clearly observed from the haplotypes that, except for the Eskil populations, the sampled perennial ryegrasses possess an evolutionary history different from the *L. perenne* taxon and exhibit distinct maternal inheritance patterns. The fact that the sampled from Eskil (LP 16, 17, 18) share a common haplotype with both *L. multiflorum* and *L. perenne* in terms of maternal inheritance suggests a close relationship between biennial and perennial species at the maternal lineage level. Network analyses based on ITS sequences revealed a wide ribotype diversity, while those based on *rpl32* pointed to low haplotype variation and diversity. The presence of different ribotypes, in particular, indicates that perennial ryegrasses have arisen through a more complex natural evolutionary process than previously recognized and perhaps natural hybridisation could have been effective in occurring different lineages by natural crosses and gene flow among *Lolium* and its relatives in Poaceae. These results support the idea that interspecific gene flow plays an important role in the evolutionary history of *Lolium* species and that more taxa or hybrid populations with perennial growth habits are present within Türkiye's natural flora. Furthermore, the study highlights the importance of molecular analyses in determining genetic diversity and guiding parental selection in perennial ryegrass breeding programs.

Keywords: *Lolium perenne*, ITS, rDNA, *rpl32*, cpDNA, network analysis

Introduction

Perennial ryegrass (*Lolium perenne* L.) is a strategic species among cool-season forage crops, distinguished by its high forage quality, strong adaptive capacity, and intensive tillering ability. Thanks to its rapid germination and effective ground-covering characteristics, it is widely used in grassland-pasture establishment and forage

production systems across extensive geographical regions such as Europe, North America, New Zealand, and Australia (Wilkins & Humphreys, 2003). Due to its vigorous vegetative growth, it is preferred in pasture improvement, erosion control, and turf establishment and is considered one of the most extensively bred forage species worldwide (Stewart & Hayes, 2011).

L. perenne is also notable for its high nutritional value, with crude protein content ranging between 14-22% and digestible organic matter between 65-80%. Owing to these properties, it plays a critical role in meeting the roughage requirements of dairy and beef production systems. Its ability to withstand intensive grazing, high regrowth capacity, and dense leaf structure makes it one of the fundamental species in sustainable pasture systems (O'Donovan et al., 2017; O'Donovan et al., 2021).

In Türkiye, *L. perenne* is widely used in pasture and meadow establishment as well as erosion control projects in regions such as Eastern Marmara, the Black Sea, the Mediterranean, and Central Anatolia due to its high adaptability, drought tolerance, and soil-binding capacity (Aygün & Olgun, 2013; Surmen et al., 2013). However, it has been reported that naturally occurring populations within Türkiye's flora have not been sufficiently investigated genetically, and local genetic resources may possess a largely unexplored variation potential (Erdoğan et al., 2018; Özer, 2015).

The success of plant breeding programs depends on the diversity of the genetic material used and the accurate selection of parents. As in many cultivated species, variation among *L. perenne* cultivars is limited, raising concerns regarding its narrow genetic base (Ahmed et al., 2014; Guan et al., 2017; Karn & Jasieniuk, 2017). Furthermore, increasing environmental pressures such as drought, salinity, and heat stress under climate change, have increased the need for new populations with high adaptive capacity. Therefore, accurately determining genetic variation, identifying local genetic resources, and incorporating them into breeding programs constitute essential goals of current research efforts (Sampoux et al., 2011).

Morphology-based variation analyses may be insufficient for determining genetic relationships because they are easily influenced by environmental conditions. Consequently, molecular marker-based characterization studies have become widely used in the evaluation of plant genetic resources in recent years (Cruzan, 1998). DNA markers provide effective tools for analyzing genetic similarity and diversity, determining population structure, identifying phylogenetic relationships, and supporting parental selection in breeding programs (Jones et al., 2002; Dar et al., 2019).

In *Lolium* species, various molecular marker systems such as RAPD, AFLP, SSR, and ISSR have been successfully applied (Warpeha et al., 1998; Nie et al., 2019). However, rDNA ITS and cpDNA-based markers are reported to be more reliable and widely used, especially for uncovering phylogenetic relationships (Hand et al., 2010). The ITS region is

frequently preferred in phylogenetic analyses due to its high evolutionary rate and strong discriminatory power at the species level (White et al., 1990; Baldwin et al., 1995). The cpDNA *rpl32* intron, on the other hand, is accepted as a reliable marker for tracing hybridization and gene flow among species due to its maternal inheritance pattern (Shaw et al., 2007).

The genus *Lolium* is evolutionarily closely related to the genus *Festuca*, and together they form the *Lolium-Festuca* complex (Hand et al., 2010). Molecular studies have demonstrated substantial genomic homology between these two genera and the occurrence of frequent natural hybridization events (Jenkin, 1955; Inda et al., 2008). The integrated genome structures that result from such hybridizations often lead to polytomies in phylogenetic analyses, making taxonomic separation of species more difficult (Torrecilla & Catalán, 2002; Hand et al., 2010; Cheng et al., 2016).

Intense gene flow has been reported particularly between perennial *L. perenne* and annual species such as *L. rigidum*, *L. multiflorum*, and *L. temulentum* (Hu et al., 2011). Moreover, gene exchange between *Festuca arundinacea* and *L. perenne* has also been documented, suggesting that these two species may have evolved from a shared ancestor. This highlights the critical importance of cpDNA- and rDNA-based phylogenetic studies for understanding the evolutionary history of *Lolium* species (Balfourier et al., 2000; Tamura et al., 2011).

Türkiye is among the regions exhibiting high biodiversity and substantial variability and hosts rich genetic diversity within the Poaceae family. It is believed that *Lolium* species have historically been distributed across Anatolia and that local *L. perenne* populations possess high adaptive capacity and genetic differentiation potential. However, studies addressing the molecular characterization of natural *L. perenne* of Türkiye remain limited, and the existing genetic diversity has not yet been fully elucidated scientifically. Therefore, this study was conducted to determine phylogenetic relationships among perennial ryegrass (*Lolium perenne* L.) collected from the natural vegetation of the Central Anatolia and Mediterranean regions of Türkiye. For this purpose, the ITS region of ribosomal DNA (rDNA) and the *rpl32* region of chloroplast DNA (cpDNA) were amplified and sequenced. The obtained sequence data were used to perform phylogenetic analyses (Parsimony and Network), and evolutionary relationships among the wild populations of *Lolium* were revealed. The results are expected to contribute to parental selection in perennial ryegrass breeding programs and shed light on the scientific evaluation of local *Lolium* genetic resources within Türkiye's flora.

Materials and Methods

Material

In this study, wild perennial ryegrass (*Lolium perenne* L.) collected from the natural flora of Türkiye were used as a source of plant material for DNA extractions and PCR sequences works. A total of 18 wild population belonging to naturally distributed *L. perenne* L. populations in the Central Anatolia and Mediterranean regions were included. The studied accessions are preserved at Selçuk University, Faculty of Agriculture, Department of Field Crops. In addition, nuclear and chloroplast sequences of *L. rigidum*, *L. multiflorum*, and *Festuca arundinacea* taxa obtained from the gene bank were incorporated into the data matrix. The provinces, collection number or codes, names, latitude, longitude, elevation data, and morphological characteristics of the sample used in the study are presented in Table 1.

Method

Total genomic DNA was isolated from fresh leaf tissues and silica gel-dried samples using the method of Doyle and Doyle (1987), with modifications by Soltis et al. (1991) and Cullings (1992). Approximately 0.01 g of leaf material was homogenized in CTAB extraction buffer and incubated at 65 °C for 4 hours. DNA was then purified through chloroform/isopropanol extractions, washed with 70% ethanol, and dissolved in 1× TAE buffer. DNA concentration was determined using a NanoDrop 2000 spectrophotometer.

DNA samples were loaded onto a 1.2% agarose gel with bromophenol blue and visualized under a UV transilluminator.

The internal transcribed spacer (ITS) region and the chloroplast *rpl32-trnL* (UAG) region were amplified separately by PCR. For the ITS region, ITS1 and ITS4 primers were used, and the PCR program was initiated at 94°C followed by 30 amplification cycles (White et al., 1990). The amplification of the *rpl32-trnL* (UAG) chloroplast gene region was carried out using the method of Shaw et al. (2007).

The samples used in the molecular analyses are shown in Table 2.

The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, USA) and sequenced. After the obtained sequences were edited in the Chromas Lite 2.1 program. The sequences were aligned using MEGA 6 and BioEdit software, and a data matrix was generated by comparing base pairs for phylogenetic analyses (Swofford, 1990). Phylogenetic networks were analyzed using Network4613 and the beta version of PAUP 4.0 (Swofford, 2003).

Results and Discussion

Molecular Findings

DNA Isolation

Genomic DNA isolated from *L. perenne* accessions collected from their natural distribution areas was determined to be approximately 10-15 kb in size. The DNA purity ratios (A260/A280) ranged between 2.01 and 2.07, indicating low protein contamination and sufficient quality for downstream molecular analyses (Sambrook & Russell, 2001). Nucleic acid concentrations varied between 984 and 2467 ng/μl. Similar levels of DNA purity and concentration obtained from plant tissues have previously been reported as adequate for PCR-based molecular analyses (Doyle & Doyle, 1987; Porebski et al., 1997).

ITS Results

The internal transcribed spacer (ITS) region of the nuclear DNA was amplified at a length of 500-750 bp. Owing to the clarity and distinctness of the amplified bands, the products were purified and sequenced. ITS sequences obtained from 23 samples were aligned using the BioEdit software, and a data matrix was constructed. The final alignment comprised 579 bp, of which 555 characters were constant, 9 were variable, and 15 were parsimony-informative. Parsimony analyses yielded a Consistency Index (CI) of 0.727, a Retention Index (RI) of 0.625, and a Homoplasy Index (HI) of 0.273. These values are consistent with the moderate levels of homoplasy commonly reported in ITS-based phylogenetic studies of grasses (Gaut et al., 2000; Torrecilla & Catalán, 2002).

Phylogenetic analyses indicated that the majority of the collected samples clustered within the same polytomic clade as foreign *L. perenne* taxa (Figure 1; BS 66%; PP 0.91). This finding is in agreement with previous studies reporting close genetic relationships among perennial ryegrass populations across broad geographic regions (Catalán et al., 2004; Cheng et al., 2016). In contrast, sample LP18 was evaluated as a taxon of possible hybrid origin involving *L. perenne* × *L. multiflorum* or *L. rigidum*. Similar ITS-based evidence of hybrid origin within the genus *Lolium* has been reported previously (Gaut et al., 2000; Cheng et al., 2016).

Bayesian analyses further separated the Central Anatolian and Mediterranean populations into two distinct subclades, while certain samples (LP4, LP9, and LP14) exhibited close genetic relationships (Figure 2). Such geographic structuring has frequently been observed in ITS-based phylogenetic analyses of *Lolium* populations (Torrecilla & Catalán, 2002). Network analyses suggested that LP18 may have originated from foreign perennial ryegrass populations (Figure 3), supporting the view that network approaches are more informative than strictly bifurcating trees for

revealing complex evolutionary processes such as gene flow and hybridization (Posada & Crandall, 2001).

***rpl32* Analysis Results**

The chloroplast DNA *rpl32* gene region was amplified at a length of 900-1000 bp (Figure 4). The resulting sequences were aligned using BioEdit, yielding a data matrix with a total length of 866 bp, including 812 constant and 37 variable characters. Parsimony analyses resulted in a Consistency Index (CI) of 0.873, a Retention Index (RI) of 0.867, and a Homoplasy Index (HI) of 0.127, indicating that chloroplast DNA regions provide reliable phylogenetic signals (Shaw et al., 2007).

The *rpl32* phylogenetic trees exhibited lower resolution compared to the ITS results. However, network analyses improved phylogenetic resolution, particularly among closely related taxa (Posada & Crandall, 2001). Haplotype analyses based on the *rpl32* region revealed that a substantial proportion of naturally occurring perennial grass populations in Türkiye's are more closely related to *Festuca arundinacea* in terms of maternal inheritance. Given the predominantly maternal inheritance of chloroplast DNA, this finding is important for understanding hybridization and gene flow processes (McGrath et al., 2006; Diekmann et al., 2012).

In contrast, the Eskil populations (LP16-18) shared the same haplotype with *L. perenne* and *L. multiflorum*, suggesting a common maternal origin. The separation of LP16, LP17, and LP18 from other natural ryegrass populations and their close relationship with the annual species *L. temulentum* var. *arvense* and *L. rigidum* indicate that these populations may have arisen through gene flow between annual and perennial taxa (Figure 4). Network analyses further supported the possible hybrid origin of these populations (Figure 5), a pattern previously reported in *Lolium* species (Catalán et al., 2004; Cheng et al., 2016).

When ITS and *rpl32* analyses were evaluated together, most of the collected natural ryegrass accessions showed a moderate genetic relationship with *L. perenne*. Nevertheless, some populations, particularly LP18, appeared to be of hybrid origin and may have experienced gene flow with different *Lolium* species. The combined use of nuclear and chloroplast DNA data provided robust insights into the phylogenetic structure and evolutionary relationships of the studied populations (Gaut et al., 2000; Torrecilla & Catalán, 2002).

Conclusions

In this study, molecular characterization of perennial *Lolium* species collected from natural

flora of Türkiye revealed important findings about the evolutionary history, gene flow, and speciation dynamics of the genus. Phylogenetic analyses showed that the majority of the studied populations had different ribotypes resulting from natural gene flow and hybridization. In particular, the fact that the LP17 genotype exhibits an intermediate position between annual and perennial groups supports the idea that natural hybridization is an effective mechanism in speciation. In addition, chloroplast region analyses indicate a significant gene flow between annual and perennial *Lolium* populations and suggest that the maternal origin is largely based on annual species. These results reveal that natural hybridization and backcrossing are fundamental processes shaping the genetic structure of perennial ryegrass populations.

The obtained phylogenetic data show that the genus *Lolium* exhibits a monophyletic structure and that its common ancestor is most likely related to the diploid *Festuca pratensis*. Annual taxa were found to have made independent contributions to the evolutionary history of perennial *Lolium* populations in Türkiye. This suggests that *Lolium* populations exhibiting a perennial appearance in nature may not be limited to *L. perenne* alone, and that more perennial grass types exist in natural conditions. In general, it appears that different perennial grasses arose as a result of natural hybridization between annual *Lolium* species and closely related *Festuca* taxa. In this context, mimicking the natural hybridization processes described in this study could significantly contribute to the development of new and superior grass varieties through biotechnological approaches.

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Table 1. The codes and names of the *Lolium perenne* L. samples used in the study, along with their locality, latitude, longitude, elevation information, and morphological characteristics.

Sample No	Sample Code	Genotype Name	Location Name	Latitude (North)	Longitude (East)	Elevation (m)	Plant Height (cm)	Spike Length (cm)	Plant Height/Spike Length Ratio	Last Internode Length (cm)	Leaf Width/Leaf Length Ratio	Leaf Width (cm)	Leaf Length (cm)	Leaf Texture	Density	Leaf Texture	Density	Leaf Texture	Density	Leaf Texture	Density	Leaf Width/Leaf Length Ratio	Leaf Width (cm)	Leaf Length (cm)	Leaf Width	Leaf Length	Leaf Width/Leaf Length Ratio	Leaf Width (cm)	Leaf Length (cm)	Leaf Width	Leaf Length	Seasonal Color Change	Seasonal Formation	Tendency	Very weak, none or strong)
1	LP 1	A-24	Cihanbeyli-Konya	38.42	32.44	989	34.4	15.2	2.26	5.1	6.5	3.7	1.76	2.6	4.8	6.5	6.8	6.5	6.8	6.5	6.8	6.5	6.8	6.5	6.8	6.5	6.8	6.5	6.8	7.0					
2	LP 2	A-43	Between Ankara and Kulu-ANKARA	39.26	32.51	109	33.7	13.6	2.48	8.3	7.7	4.1	1.88	5.2	4.4	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	7.3	8.0			
3	LP 3	A-134	Akşehir-KONYA	38.27	31.19	981	31.2	12.6	2.48	6.7	9.3	4.1	2.27	5.0	5.6	6.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.0				
4	LP 4	A-149	Akşehir-KONYA	38.29	31.21	983	30.8	13.4	2.30	4.6	8.2	3.8	2.16	3.1	4.7	4.5	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	5.4				
5	LP 5	A-155	Akşehir-KONYA	38.30	31.22	984	31.0	12.5	2.48	4.7	9.3	4.5	2.07	4.6	5.6	6.8	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	5.2					
6	LP 6	B-1	Akören-KONYA	37.28	32.14	118	32.4	13.4	2.42	4.5	7.4	3.5	2.11	5.2	3.0	6.4	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	5.9					
7	LP 7	B-35	Kırkpınar Village, Çifteler-ESKİSEHIR	39.12	31.07	914	33.1	13.0	2.55	5.3	8.1	3.4	2.38	4.1	5.1	6.0	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	7.7						
8	LP 8	B-59	Between Karaman and Mut-MERSİN	36.56	33.16	150	30.2	11.8	2.56	5.3	6.0	3.7	1.62	4.9	4.2	6.3	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.9					
9	LP 9	B-110	Seydişehir-Tarasçı-KONYA	37.27	31.40	121	36.4	14.2	2.56	7.2	8.3	3.8	2.18	4.3	4.1	3.9	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	8.0					
10	LP 10	B-117	Between Şarkikaraağaç and Beyşehir	37.45	31.24	113	33.3	11.2	2.97	6.0	6.9	4.0	1.73	5.2	4.3	3.4	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	7.1					
11	LP 11	G-500	Yarpuz-ANTALYA	37.08	31.50	125	32.7	11.9	2.75	7.2	5.1	2.4	2.13	3.2	6.2	3.2	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	8.3				
12	LP 12	G-501	Yarpuz-ANTALYA	37.09	31.51	124	32.8	12.5	2.62	7.1	6.1	3.1	1.97	4.3	6.1	3.2	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	8.1				
13	LP 13	G-504	Akbelenli-ANTALYA	37.35	30.52	966	29.9	11.0	2.72	4.9	5.0	2.3	2.17	5.0	6.2	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	6.8					
14	LP 14	G-506	Eğriğöl-ANTALYA	36.55	32.12	206	34.8	14.0	2.49	6.3	5.6	2.5	2.24	3.2	5.7	2.6	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	8.6				
15	LP 15	234	Eskil (Sicak Plateau)-AKSARAY	38.12	33.23	101	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*					
16	LP 16	601	Eskil (Sicak Plateau)-AKSARAY	38.10	33.24	101	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*					
17	LP 17	602	Eskil (Sicak Plateau)-AKSARAY	38.11	33.24	101	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*					
18	LP 18	603	Eskil (Sicak Plateau)-AKSARAY	38.11	33.23	100	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*						

Source: Obtained from TÜBITAK projects No. 1060159 and 1100312. *: Accessions 234, 601, 602, and 603 used in this study were included later; since data for these Accessions were not available, they were not included in the table.

Table 2. Numerical and molecular samples used in the study.

Collection Code	Samples	ITS	rpl32
LP1_A24	<i>Lolium perenne</i>	+	+
LP2_A43	<i>Lolium perenne</i>		+
LP3_A134	<i>Lolium perenne</i>	+	+
LP4_A149	<i>Lolium perenne</i>	+	+
LP5_A155	<i>Lolium perenne</i>	+	+
LP6_B1	<i>Lolium perenne</i>	+	+
LP7_B35	<i>Lolium perenne</i>	+	+
LP8_B59	<i>Lolium perenne</i>	+	+
LP9_B110	<i>Lolium perenne</i>	+	+
LP10_B117	<i>Lolium perenne</i>	+	+
LP11_G500	<i>Lolium perenne</i>	+	+
LP12_G501	<i>Lolium perenne</i>	+	+
LP13_G504	<i>Lolium perenne</i>	+	+
LP14_G506	<i>Lolium perenne</i>	+	+
LP15_234	<i>Lolium perenne</i>	+	+
LP16_601	<i>Lolium perenne</i>	+	+
LP17_602	<i>Lolium perenne</i>	+	+
LP18_603	<i>Lolium perenne</i>	+	+
KJ599446_rpl32	<i>Lolium multiflorum</i>	+	+
KJ598998_ITS			
KJ599447_rpl32	<i>Lolium perenne</i>	+	+
KJ598999_ITS			
KJ599448_rpl32	<i>Lolium rigidum</i>	+	+
KJ599000_ITS			
KJ599411_rpl32	<i>Lolium temulentum</i> var. <i>arvense</i>	+	+
KJ598964_ITS			
KJ599444_rpl32	<i>Festuca pratensis</i>	+	+
AF171180_ITS			
EF379060_ITS	<i>Festuca gigantea</i>	+	
KJ599440_rpl32	<i>Festuca arundinacea</i>		+

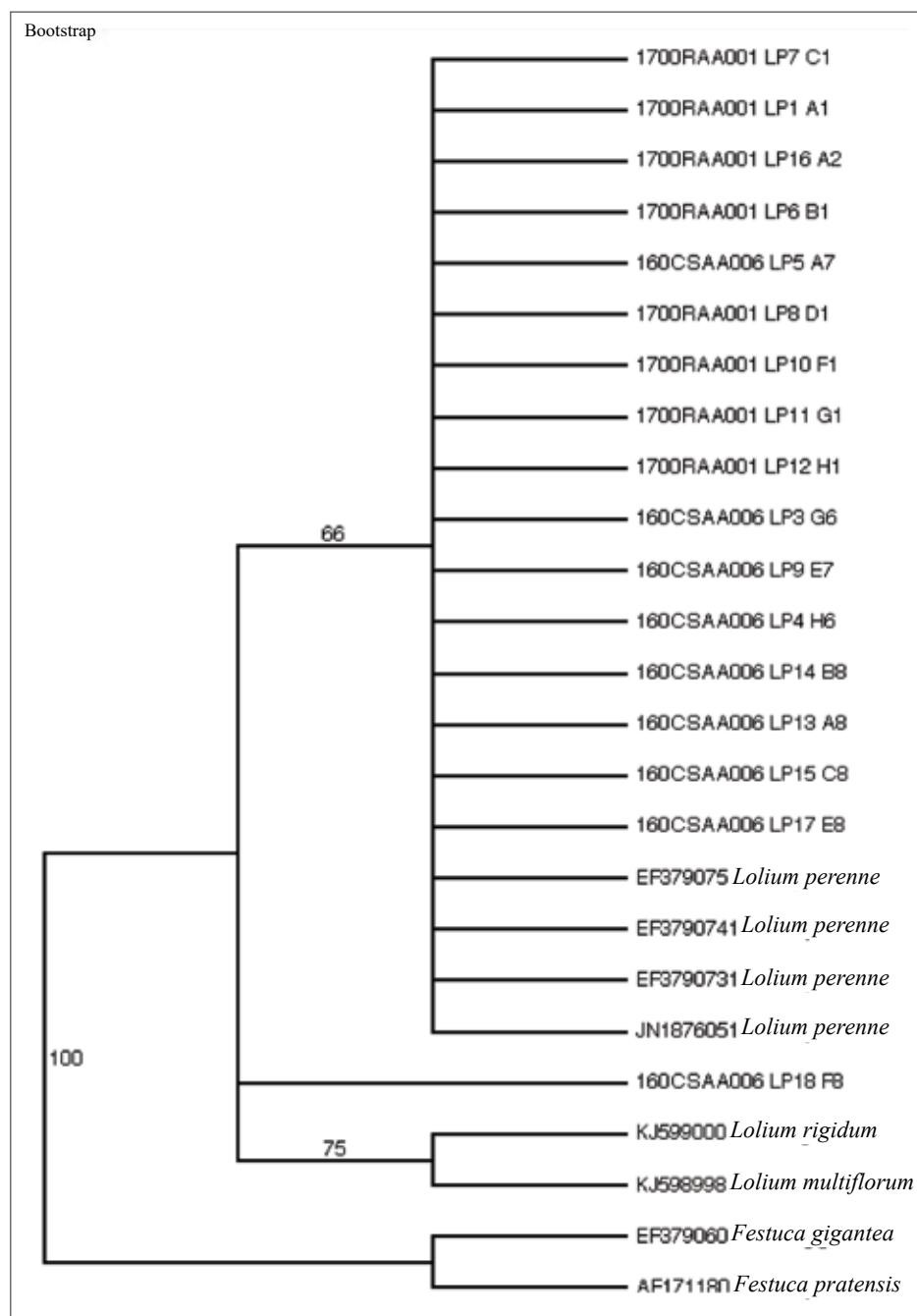


Figure 1. Phylogenetic tree showing the evolutionary relationships of *Lolium* taxa and accessions generated by parsimony analyses of ITS sequences.

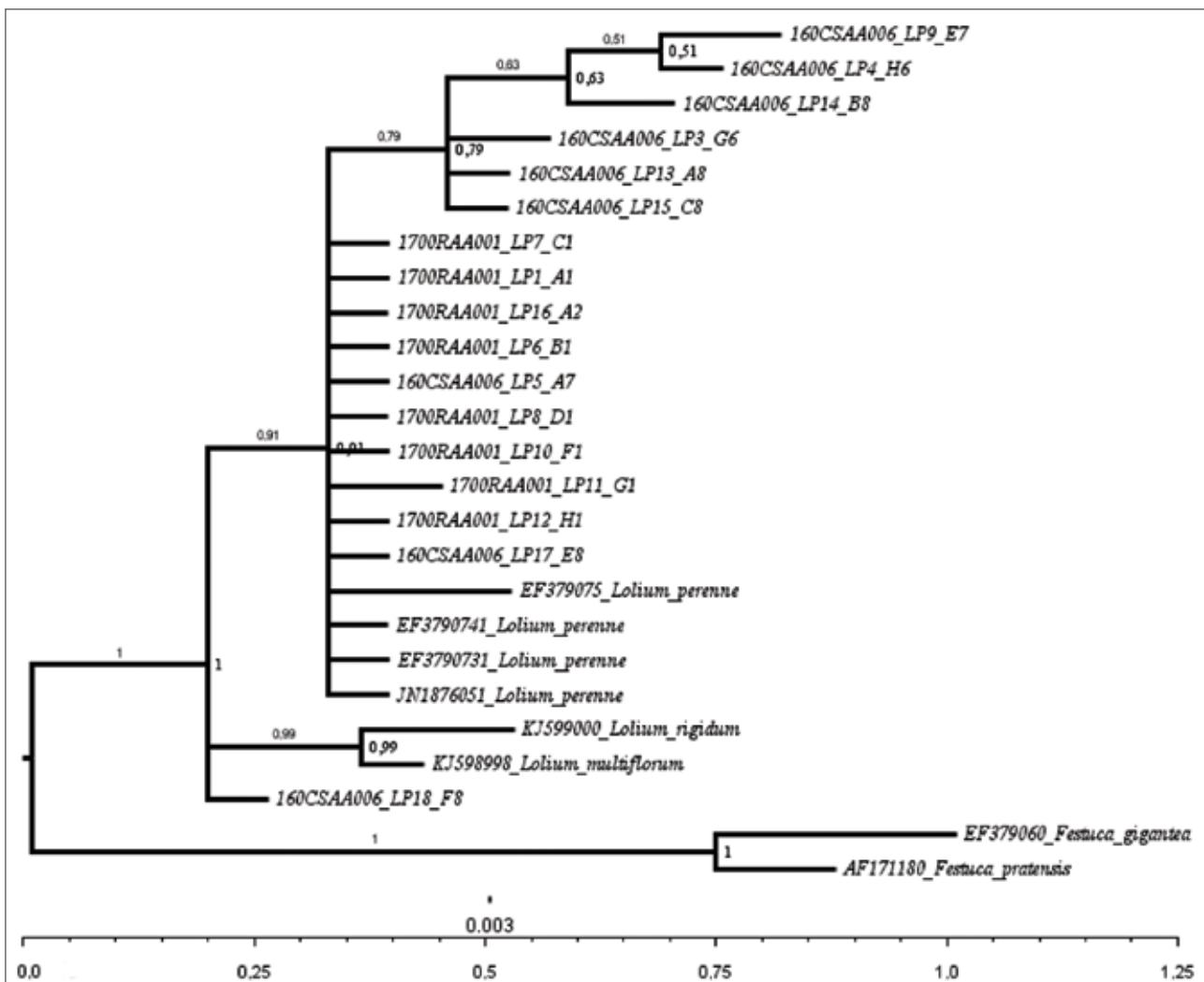


Figure 2. Phylogenetical tree showing the phylogenetic relationships of *Lolium* taxa and populations, constructed by Bayesian analysis of ITS sequences.

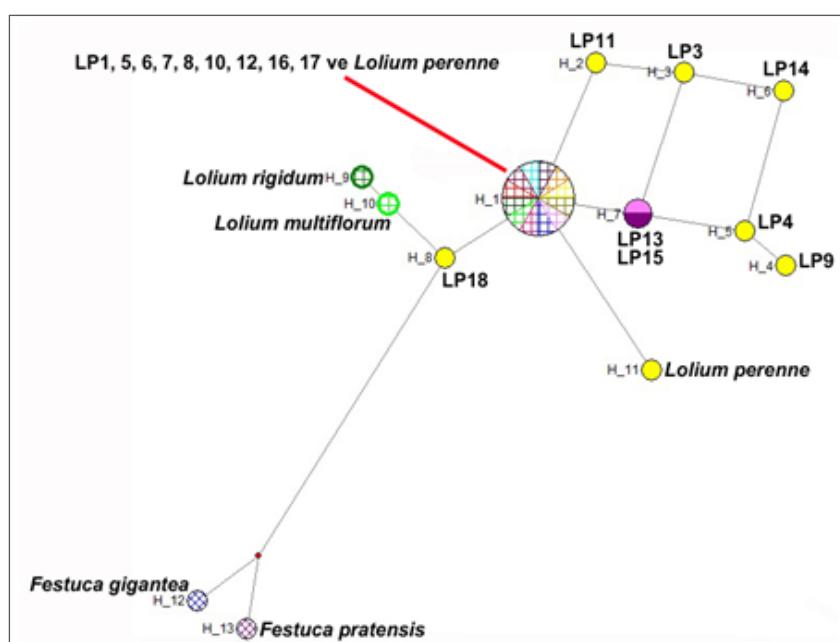


Figure 3. Phylogenetic network of *Lolium perenne* and closely related taxa generated from the aligned ITS gene region sequences.

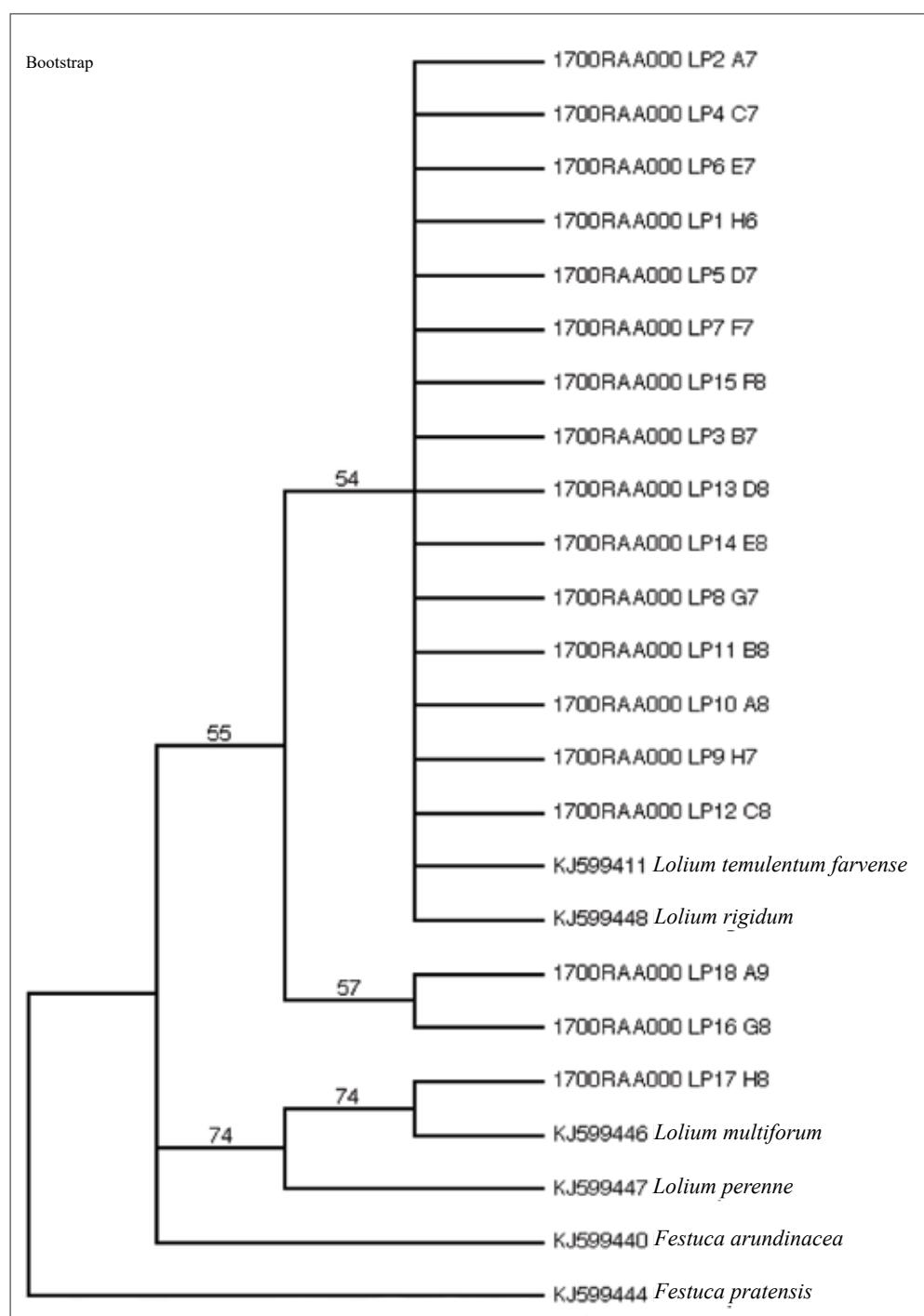


Figure 4. Phylogenetic tree showing the evolutionary relationships of *Lolium perenne* generated by parsimony analyses of *rpl32* sequences.

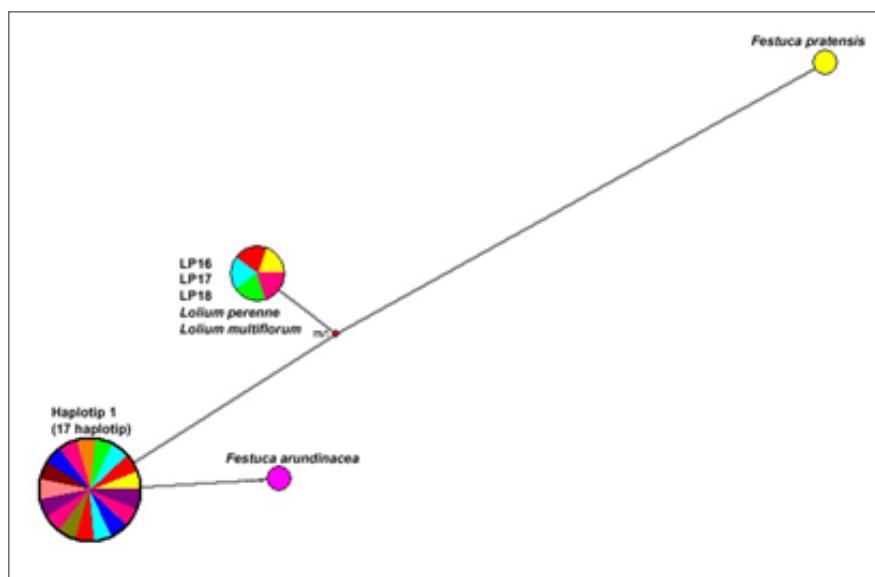


Figure 5. Phylogenetic network of *Lolium* taxa and populations generated by network analyses of *rpl32* gene sequences.

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Morphological and Phenological Variability of Ber Genotypes under Semi-Arid Conditions in Haryana, India

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ABSTRACT

The study on morphological variability in fruiting characteristics of ber genotypes was conducted at the experimental orchard of CCS Haryana Agricultural University Regional Research Station, Bawal. In this study sixteen genotypes were planted in a randomized block design were grown under uniform agronomic practices and evaluated for variability. Different genotypes showed considerable variation in morphological parameters. The shortest time taken from fruit setting to fruit maturity (118.7 days) was observed in Gola, which was statistically at par with Kaithali (121.3 days) and the maximum time taken from fruit setting to fruit maturity (152.0 days) was reported in Bawal Sel-1. The maturity period of Gola, Goma Kriti and Kakrola Gola was observed early, whereas Umran, Bawal Sel-1, Bawal Sel-2 and Katha Phal had late-maturing fruit. Remaining fruit of eight genotypes (Kaithali, Chhuhara, Thar Sevika, Thar Bhubraj, Narendra Ber Sel-1, Narendra Ber Sel-2, Rohtak Safeda, Mudia Murhara and Illaichi) were maturing in mid of season. The longest fruit (40.99 mm) was recorded in Narendra Ber Sel-1, followed by Chhuhara (38.86 mm), Mudia Murhara (38.03 mm) and Umran (37.22 mm), whereas the minimum fruit length (20.34 mm) was reported in Illaichi. The Narendra Ber Sel-1 had the maximum fruit diameter i.e., 38.90 mm, which was followed by Bawal Sel-2 (29.26 mm) and Bawal Sel-1 (28.85 mm). The minimum fruit diameter (17.57 mm) was recorded in Illaichi. Maximum fruit weight of 37.69 g was recorded in Narendra Ber Sel-1, followed by Umran (26.37 g) and Narendra Ber Sel-2 (26.05 g). In contrast, the lowest fruit weight of 6.05 g was observed in Illaichi. Maximum stone length (28.88 mm) and stone diameter (11.44 mm) were recorded in Chhuhara, while stone weight (1.47 g) was recorded maximum in Narendra Ber Sel-1 whereas minimum stone length (11.94 mm), stone diameter (5.01 mm) and stone weight (0.58 g) were recorded in Illaichi. Maximum pulp/stone ratio (26.64) was noted in Narendra Ber Sel-2, followed by Narendra Ber Sel-1 (24.64) and Kaithali (24.53).

Keywords: Genotypes, Indian jujube, pulp stone ratio, fruit size, fruit shape, stone size

Introduction

The Indian ber (*Ziziphus mauritiana* Lamk.) is one of the most ancient and important underutilized fruit crops indigenous to India. It belongs to the family Rhamnaceae and has a chromosome number $2n=48$ (Srinivasan, 1952). Ber is believed to an originated in the Indian subcontinent and extended to Malaya, includes parts of south-western China (Vavilov, 1951;

Hu et al., 2010). The genus *Ziziphus* encompasses about 170 species of spiny shrubs and small trees distributed across warm-temperate and subtropical regions worldwide (Islam and Simmons, 2006). It is commonly known as Indian jujube, Chinese date, Chinese fig, and ‘poor man’s fruit’ as it is easily available among the poor (Kumari et al., 2016). It is also designated as the “King of Arid Fruits” owing to the facts that it can

be successfully grown in barren land or marginal soil in arid and semi-arid regions, it holds considerable economic value.

Nutritionally, the ripe fruit surpasses apples in protein, calcium, phosphorus, carotene and vitamin C content (Godi and Joshi, 2016), providing 20.9 kcal per 100 g pulp. Antioxidants and phenolic compounds such as p-coumaric acid, ferulic acid, caffeic acid and p-hydroxybenzoic acid are also found in its leaves, fruits and seeds (Koley et al., 2011; Krishna and Parashar, 2013; Okala et al., 2014 and Gupta, 2018).

In India, ber occupies an area of about 48000 hectares, with an annual production of nearly 512000 metric tons. Globally, India is the second largest producer of ber after, China (Anonymous, 2024). The breeding programmes of plants need suitable genetic variation. Evaluation of genetic variability is essential for efficient application in breeding. Genetic diversity is investigated using several methods, among which morphological characterization is the most powerful method for breeders to identify genotypes with desired traits (Jannatabadi et al., 2014; Khadivi-Khub et al., 2014).

This crop holds immense potential for improvement, offering ample opportunities to enhance its productivity and adaptability but it remained neglected for a long time. Screening diverse genotypes can facilitate the identification of superior traits, such as higher yield, improved quality, and increased resistance to abiotic and biotic stresses.

Previous studies consistently demonstrate substantial genotypic variability in fruit physical, morphological, and yield traits of *Ziziphus mauritiana* across diverse agro-climatic regions, with wide ranges reported for fruit weight, size, pulp-to-stone ratio, and yield (Abdel-Sattar et al., 2021; Das et al., 2022; Rai et al., 2022; Rajadurai et al., 2022; Nikmatullah et al., 2023; Vikalp et al., 2023). Notably, several cultivars and germplasm lines have been identified for respective growing conditions for selection and genetic improvement in ber.

Although several studies have documented variability in fruit physical traits and yield attributes of *Ziziphus mauritiana* across different agro-climatic regions, systematic evaluations integrating both morphological and phenological traits under the semi-arid conditions of Haryana remain limited. In view of the above, the present study was undertaken to assess the extent of morphological and phenological variability among ber genotypes under semi-arid conditions of Haryana with the aim to identify superior and early-maturing genotypes suitable for cultivation and future improvement programmes in semi-arid regions.

Materials and Methods

The investigation was carried out at the experimental orchard of CCS Haryana Agricultural University, Regional Research Station, Bawal. The location lies in the south-west part of Haryana at an elevation of 266 meters above sea level, with geographic coordinates of 28° 10' N latitude and 76° 50' E longitude. Summers in Bawal are unforgivingly hot, often soaring above 45°C, while winters dip below freezing. May and June are typically the hottest months, while December and January are the coldest. The region receives an average annual rainfall of 456 mm. Of this, around 80-85 per cent is received during the monsoon season, while the remaining rainfall occurs as light showers from December to February.

Plant Material: In total sixteen genotypes viz., Gola, Umran, Kaithali, Chhuhara, Goma Kirti, Thar Sevika, Thar Bhubharaj, Narendra Ber Selection-1, Narendra Ber Selection-2, BS-1, BS-2, Kakrola Gola, Rohtak Safeda, Katha Phal, Mudia Murhara, Illaichi, planted in a randomized block design, were used for the study. All genotypes were maintained under similar agronomic practices during the study period.

Phenological and Morphological Parameters:

Time taken from fruit setting to fruit maturity (days) was calculated by adding up the number of days taken from the date of 50 per cent fruit set to the date of 50 per cent fruit maturity on the tagged branches. Maturity refers to the point at which the fruits attain maximum size and start ripening or turning yellowish with a brownish tinge on the outer skin. The genotypes were classified into three maturity groups based on the maturity period of the fruits: early, mid, and late maturing. The fruits of genotypes that matured before February were classified as early maturing. Fruits that matured between the third week of February and the third week of March were classified as mid-maturing, whereas fruits that matured after the second week of March until April were classified as late-maturing.

Fruit length was measured from the distal to proximal ends, while fruit diameter was measured at its widest point, which is usually the middle or equatorial region of the fruit, using a digital vernier caliper. The average values were calculated for all replications. The weight of twenty fruits from each quarter of the plant was measured with the help of a digital electronic weighing balance (AND EK-6100V) at the ripening stage and the average weight of fruit was calculated and expressed in grams (g). The length and diameter of the stones were measured with the help of digital vernier caliper. The length of the stone was measured as distance from apex to base, and the diameter of the stone was measured at its thickest region.

The extracted stones were also used to determine stone weight. The pulp, which was separated from the fruits during stone weight calculation, was weighed separately. The weight of the pulp was divided by the weight of the stone to estimate the pulp-to-stone ratio.

Statistical Analysis: The statistical analysis of data was done using the software R, MS excel and OPStat. The level of significance between genotypes was estimated with the help of critical difference.

Results and Discussion

Time taken from fruit setting to fruit maturity (days)

The genotypes showed considerable variation in time taken from fruit setting to fruit maturity (Table 1). Time taken from fruit set to fruit maturity ranged from 117.3 days to 151.7 days and 120.0 days to 152.3 days during 2022-23 and 2023-24, respectively. During both years, the minimum duration from fruit setting to fruit maturity (117.3 days and 120.0 days) was recorded in Gola, which was statistically at par with Kaithali (120.3 days and 122.3 days), while the maximum duration was observed in Bawal Sel-1 (151.7 days and 152.3 days).

Mean data analysis revealed that the minimum duration from fruit setting to fruit maturity (118.7 days) was observed in Gola, which was statistically at par with Kaithali (121.3 days), whereas the maximum was observed was in Bawal Sel-1(152.0 days). These results are in agreement with the findings of Tarai and Ghosh (2010), Sharif et al. (2013), Choudhary et al. (2017) and Hardeep et al. (2022) in ber. Kumari et al. (2016) reported that under rainfed conditions of Jammu, Gola took 180 days from fruit setting to fruit maturity and Ranjari Selection-2 took 205 days. Variation in the maturity period among cultivars across regions may be attributed to differences in agro-climatic conditions. Saran (2005) reported that environmental factors such as temperature, humidity and nutritional status along with genetic variability are key determinants responsible for variation in the time taken from fruit setting to fruit maturity among different germplasms.

Fruit length and diameter (mm)

The data presented in Table 2 indicate that fruit length varied from 21.21 mm to 40.03 mm and 19.47 mm to 41.95 mm among selected ber genotypes during the years 2022-23 and 2023-24, respectively. The maximum fruit length (40.03 mm) was recorded in Narendra Ber Sel-1, which was found statistically at par with Mudia Murhara (38.86 mm), while Illaichi (21.21 mm) had the shortest fruit during the year 2022-23. Similarly, during 2023-24, the maximum fruit length (41.95 mm) was recorded in Narendra

Ber Sel-1, followed by Chhuhara (39.74 mm), Bawal Sel-1 (38.42 mm) and Umran (37.25 mm), while the minimum fruit length (19.47 mm) was observed in Illaichi. Mean data of both years revealed that the longest fruit (40.99 mm) was recorded in Narendra Ber Sel-1, followed by Chhuhara (38.86 mm), Mudia Murhara (38.03 mm) and Umran (37.22 mm), whereas the minimum fruit length (20.34 mm) was reported in Illaichi.

Fruit diameter among different genotypes varied from 18.03 mm to 38.73 mm and 17.13 mm to 39.07 mm during the years 2022-23 and 2023-24, respectively (Table 2). The maximum fruit diameter (38.73 mm and 39.07 mm) was recorded in Narendra Ber Sel-1, followed by Bawal Sel-1 (29.45 mm and 28.24 mm) and Narendra Ber Sel-2 (28.53 mm and 29.00 mm), whereas the minimum fruit diameter (18.03 mm and 17.13 mm) was recorded in Illaichi in both years. Mean data of both years revealed represented that Narendra Ber Sel-1 had maximum fruit diameter (38.90 mm), followed by Bawal Sel-2 (29.26 mm) and Bawal Sel-1 (28.85 mm), while the minimum fruit diameter (17.57 mm) was recorded in Illaichi.

Flora et al. (2015) also reported maximum fruit length in Narendra Ber Sel-1 (48 mm) under Rahuri conditions. Similarly, Singh et al. (2015) in eastern Uttar Pradesh, Kumar et al. (2017) in West Bengal conditions and Gupta (2018) in Punjab conditions also reported minimum fruit length in Illaichi. Overall, the maximum fruit diameter (38.90 mm) was observed in Narendra Ber Sel-1, followed by Bawal Sel-2 (29.26 mm) and Bawal Sel-1 (28.85 mm). The minimum fruit diameter (17.58 mm) was recorded in Illaichi. The variation in fruit length and diameter among different genotypes may primarily result from the inherent genetic traits of each genotype. However, these traits can also be influenced to some extent by environmental factors, such as climate, which may alter growth conditions (Saran, 2005). The variation in fruit size can be attributed to the accumulation of food materials within the fruit during its growth (Kumari et al., 2016). The length and width of the fruit were important traits for breeders, as these parameters directly influence the fruit's marketability and suitability for fresh consumption. Additionally, fruit size-related traits are important for logistical considerations such as packaging and shipping. Larger and more uniform fruits are easier to pack efficiently, reducing the risk of damage during transport and improving overall shipping efficiency. These characteristics are essential in the commercial production of fruits like ber, where uniformity in size can also enhance consumer appeal (Liu et al., 2009).

Fruit weight (g)

The data presented in Table 2 indicates that the fruit weight among different genotypes ranged from 6.11 g to 36.31 g during the year 2022-23 and 5.99 g to 39.06 g during 2023-24. During both years, the highest fruit weight (36.31 g and 39.06 g) was recorded in Narendra Ber Sel-1, followed by Narendra Ber Sel-2 (26.36 g and 25.73 g) and Umran (25.47 g and 27.27 g). Conversely, the lowest fruit weight (6.11 g and 5.99 g) was consistently observed in Illaichi during both years. The mean data across both years revealed that Narendra Ber Sel-1 exhibited the maximum fruit weight of 37.69 g, followed by Umran (26.37 g) and Narendra Ber Sel-2 (26.05 g). In contrast, the lowest fruit weight of 6.05 g was observed in Illaichi.

Tarai and Ghosh (2010) also reported the minimum fruit weight in Illaichi under West Bengal conditions. Similar variations in ber fruit characteristics were also recorded by Singh et al. (2015), Godi et al. (2016), Sharif et al. (2019), Singh et al. (2019), Yadav et al. (2020), Das et al. (2022), Rai et al. (2022), Rajadurai et al. (2022) and Singh and Deen (2022). Fruit weight is a crucial parameter in the evaluation and selection of promising cultivars, as it directly influences yield and quality. The variation in fruit weight may be attributed to a longer fruit retention period on the plant, which allows extended time for growth and ripening. Additionally, the increased uptake of nutrients and water, coupled with the efficient translocation of photosynthates from the source (leaves) to the sink (fruits), likely contributed to the enhanced development and weight gain of the fruits (Patel et al., 1977). These factors collectively enhance the accumulation of dry matter and other essential compounds in the fruits, promoting their growth and quality. Umbreen et al. (2018) reported that variation in fruit weight might be due to agro-climatic conditions of the growing region, the genetic makeup of the genotype, and the availability of nutrients to the plant. These factors collectively impact fruit development, particularly in terms of its length and width. Climatic conditions like temperature, humidity, and light directly affect physiological processes, while genetic traits determine the inherent potential for fruit size. Nutrient supply further enhances growth by providing essential elements needed for cell expansion and overall fruit development. Genotypes with larger fruit sizes and higher weights are ideal for breeding programs focused on fresh fruit production, as they offer the potential for higher yields and better market appeal.

Fruit maturity

Fruits of genotypes Gola, Goma Kriti and Kakrola Gola matured early, while late maturity was observed

in Umran, Rohtak Safeda, Bawal Sel-1, Bawal Sel-2 and Katha Phal. Remaining eight genotypes (Chhuhara, Kaithali, Thar Sevika, Thar Bhubraj, Narendra Ber Sel-1, Narendra Ber Sel-2, Mudia Murhara and Illaichi) matured in mid-season. Similar observations with respect to fruit maturity in ber were reported by Saran et al. (2006), Godi et al. (2016), Krishna et al. (2016), Adhikary et al. (2019) and Kumari et al. (2024). These variations in fruit maturity may be attributed to climatic factors such as temperature and rainfall, as well as the genetic constitution of the germplasm (Godi et al., 2016).

Stone characteristics and pulp-to-stone ratio

The data on various stone parameters revealed significant variation among the genotypes. The minimum stone length (11.94 mm) was observed in Illaichi, succeeded by Kakrola Gola (18.94 mm) and Gola (19.55 mm) and the maximum stone length (28.88 mm) was found in Chhuhara. The genotype Illaichi had the minimum stone diameter (5.01 mm), succeeded by Goma Kriti (7.07 mm), Kaithali (7.14 mm) and Mudia Murhara (7.49 mm) and the maximum stone diameter (11.44 mm) was found in Narendra Ber Sel-1. Stone weight was recorded as the minimum (0.58 g) in Illaichi, succeeded by Goma Kriti (0.81 g) and Kaithali (0.86 g) whereas the maximum stone weight (1.47 g) was reported in Narendra Ber Sel-1. Further, maximum pulp/stone ratio (26.64) was noted in Narendra Ber Sel-2, followed by Narendra Ber Sel-1 (24.64) and Kaithali (24.53). The minimum pulp/stone ratio (9.53) was recorded in Illaichi. Similar results regarding minimum stone length in ber were reported by Gupta (2018). The findings of the present study align with those of Singh et al. (2019), who reported that the genotypes displayed a broad range of diversity in various morphological traits. Similar variations in stone characteristics among different ber germplasm were reported by Sathyanarayana et al. (2010), Godi et al. (2016), Gupta (2018), Abdel-Sattar et al. (2021) and Rai et al. (2022). This variability in stone adherence across different ber genotypes may be attributed to a combination of factors, including the genetic makeup of the genotypes, environmental conditions, cultivation practices and positioning of the fruit. These factors collectively impact the size, shape, and weight of the stones. Such variabilities are critical for selecting superior genotypes with desirable traits for breeding and improvement programs (Gupta, 2018).

There is mix correlation between different parameters some parameters have weak while other have moderate and strong correlation. The colour in the correlogram indicate that the greenish colour has positive correlation, greener more positive correlation,

as the colour become lighter the correlation becomes weaker. None of the correlation is showed saffron colour correlogram means no negative correlation between parameters. Strong positive correlation were observed between stone length with fruit length, fruit diameter with fruit length and stone diameter, stone diameter with stone weight and fruit length, and stone weight with fruit weight. As per Fig. 1 none of the correlation is negatively correlated with the other studied parameter. In this figure, the values above 0.80 has very strong correlation, and values 0.60 to 0.79 has strong correlation.

Conclusions

This study was planned to identify suitable genotypes with higher consumers acceptability and potential for inclusion in breeding programme. Variability in the measured parameters was observed among the different genotypes. However, this variability showed varying degree of correlation with other traits. The physical or visual variation is one of the most important criteria for breeders when selecting genotypes for a breeding programme. Therefore, greater emphasis was placed on physical parameters in the present study.

Table 1. Time taken from fruit set to maturity (days), fruit maturity group and pulp to stone ratio of different ber genotypes under semi-arid conditions of Haryana.

Genotypes	Time taken from fruit set to maturity (days)			Fruit maturity group	Pulp to stone ratio		
	2022-23	2023-24	Mean		2022-23	2023-24	Mean
Gola	117.3	120.0	118.7	Early	21.27	20.97	21.12
Umran	143.7	145.3	144.5	Late	18.60	20.30	19.45
Kaithali	120.3	122.3	121.3	Mid	24.09	24.97	24.53
Chuhhara	132.7	130.7	131.7	Mid	17.77	15.96	16.87
Goma Kriti	122.3	124.7	123.5	Early	21.99	21.19	21.59
Thar Sevika	130.7	129.7	130.2	Mid	19.06	18.86	18.96
Thar Bhubraj	131.7	132.7	132.2	Mid	23.56	23.08	23.32
Narendra Ber Sel-1	136.0	134.3	135.2	Mid	24.11	25.16	24.64
Narendra Ber Sel-2	132.7	132.0	132.3	Mid	27.48	25.80	26.64
Rohtak Safeda	131.0	130.0	130.5	Late	17.31	15.61	16.46
Bawal Sel-1	151.7	152.3	152.0	Late	16.75	15.32	16.04
Bawal Sel-2	146.3	145.3	145.8	Late	18.26	16.60	17.43
Kakrola Gola	126.3	127.7	127.0	Early	17.94	18.44	18.19
Mudia Murhara	135.0	134.3	134.7	Mid	22.34	22.64	22.49
Katha Phal	148.7	151.7	150.2	Late	16.98	15.35	16.16
Illaichi	135.7	133.0	134.3	Mid	10.18	8.89	9.53
Range	118.7				10.18-	8.89-	9.53-
	117.3-	120.0-	-		27.48	25.80	26.64
C.D (p = 0.05)	3.1	3.6	2.9		1.75	1.39	1.12

Table 2. Length, diameter and weight of fruit of different ber genotypes under semi-arid conditions of Haryana.

Genotypes	Length of fruit (mm)			Diameter of fruit (mm)			Weight of fruit (g)		
	2022-23	2023-24	Mean	2022-23	2023-24	Mean	2022-23	2023-24	Mean
Gola	32.70	30.65	31.67	28.16	27.45	27.80	23.73	24.75	24.24
Umran	37.18	37.25	37.21	27.17	26.04	26.60	25.47	27.27	26.37
Kaithali	33.87	31.22	32.54	24.24	23.52	23.88	21.25	22.49	21.87
Chhuhara	37.98	39.74	38.86	23.08	22.63	22.86	18.77	17.78	18.27
Goma Kriti	31.58	32.90	32.24	22.49	23.28	22.88	17.94	18.63	18.29
Thar Sevika	35.82	36.99	36.40	24.67	24.34	24.50	23.11	23.97	23.54
Thar Bhubraj	35.06	34.31	34.68	23.89	24.21	24.05	22.43	21.83	22.13
Narendra Ber Sel-1	40.03	41.95	40.99	38.73	39.07	38.90	36.31	39.06	37.69
Narendra Ber Sel-2	36.24	34.90	35.57	28.53	29.00	28.77	26.36	25.73	26.04
Rohtak Safeda	29.30	29.20	29.25	26.96	25.51	26.24	24.66	23.36	24.01
Bawal Sel-1	35.44	38.42	36.93	29.45	28.24	28.84	19.42	17.50	18.46
Bawal Sel-2	34.73	36.67	35.70	28.40	30.12	29.26	20.29	19.36	19.82
Kakrola Gola	30.61	27.97	29.29	26.55	25.03	25.79	24.94	25.49	25.21
Mudia Murhara	38.86	37.19	38.03	25.33	24.70	25.02	22.71	24.12	23.41
Katha Phal	29.72	31.42	30.57	27.51	28.06	27.78	21.58	20.37	20.97
Illaichi	21.21	19.47	20.34	18.03	17.13	17.57	6.11	5.99	6.05
Range	21.21	19.47	20.34	18.03	17.13	17.58	6.11	5.99	6.05
-	-	-	-	-	-	-	-	-	-
	40.03	41.95	40.99	38.73	39.07	38.90	36.31	39.06	37.69
C.D (p = 0.05)	1.92	1.52	1.29	1.28	1.48	0.97	1.62	1.35	1.07

Table 3. Stone parameters of different ber genotypes under semi-arid conditions of Haryana.

Genotypes	Length of stone (mm)			Diameter of stone (mm)			Weight of stone (g)		
	2022-23	2023-24	Mean	2022-23	2023-24	Mean	2022-23	2023-24	Mean
Gola	19.24	19.85	19.54	9.45	9.90	9.67	1.12	1.16	1.14
Umran	24.19	25.48	24.84	8.40	8.20	8.30	1.30	1.28	1.29
Kaithali	23.59	24.30	23.95	7.21	7.07	7.14	0.85	0.87	0.86
Chhuhara	28.47	29.28	28.88	7.92	7.72	7.82	1.00	1.05	1.02
Goma Kriti	22.90	23.43	23.16	7.11	7.02	7.06	0.78	0.84	0.81
Thar Sevika	27.54	26.73	27.13	8.78	8.48	8.63	1.15	1.21	1.18
Thar Bhubraj	23.82	22.65	23.24	8.08	7.82	7.95	0.91	0.91	0.91
Narendra Ber Sel-1	23.20	23.87	23.54	11.33	11.55	11.44	1.45	1.49	1.47
Narendra Ber Sel-2	20.17	20.25	20.21	8.48	8.86	8.67	0.93	0.96	0.94
Rohtak Safeda	19.54	20.87	20.21	10.71	10.58	10.65	1.35	1.41	1.38
Bawal Sel-1	26.47	25.79	26.13	9.80	9.61	9.70	1.09	1.07	1.08
Bawal Sel-2	20.48	21.72	21.10	9.02	9.29	9.16	1.05	1.10	1.08
Kakrola Gola	18.62	19.25	18.94	10.13	10.34	10.24	1.25	1.27	1.26
Mudia Murhara	27.76	28.66	28.21	7.64	7.33	7.48	0.97	1.02	1.00
Katha Phal	19.97	20.19	20.08	11.06	10.84	10.95	1.20	1.25	1.22
Illaichi	11.27	12.60	11.94	5.05	4.97	5.01	0.55	0.61	0.58
Range	11.27	12.60					0.55	0.61	0.58
-	-	-	11.94-	5.05-	4.97-	5.01-	-	-	-
	28.47	29.28					1.45	1.49	1.47
C.D (p = 0.05)	1.27	1.48	1.07	0.89	0.93	0.62	0.04	0.04	0.03

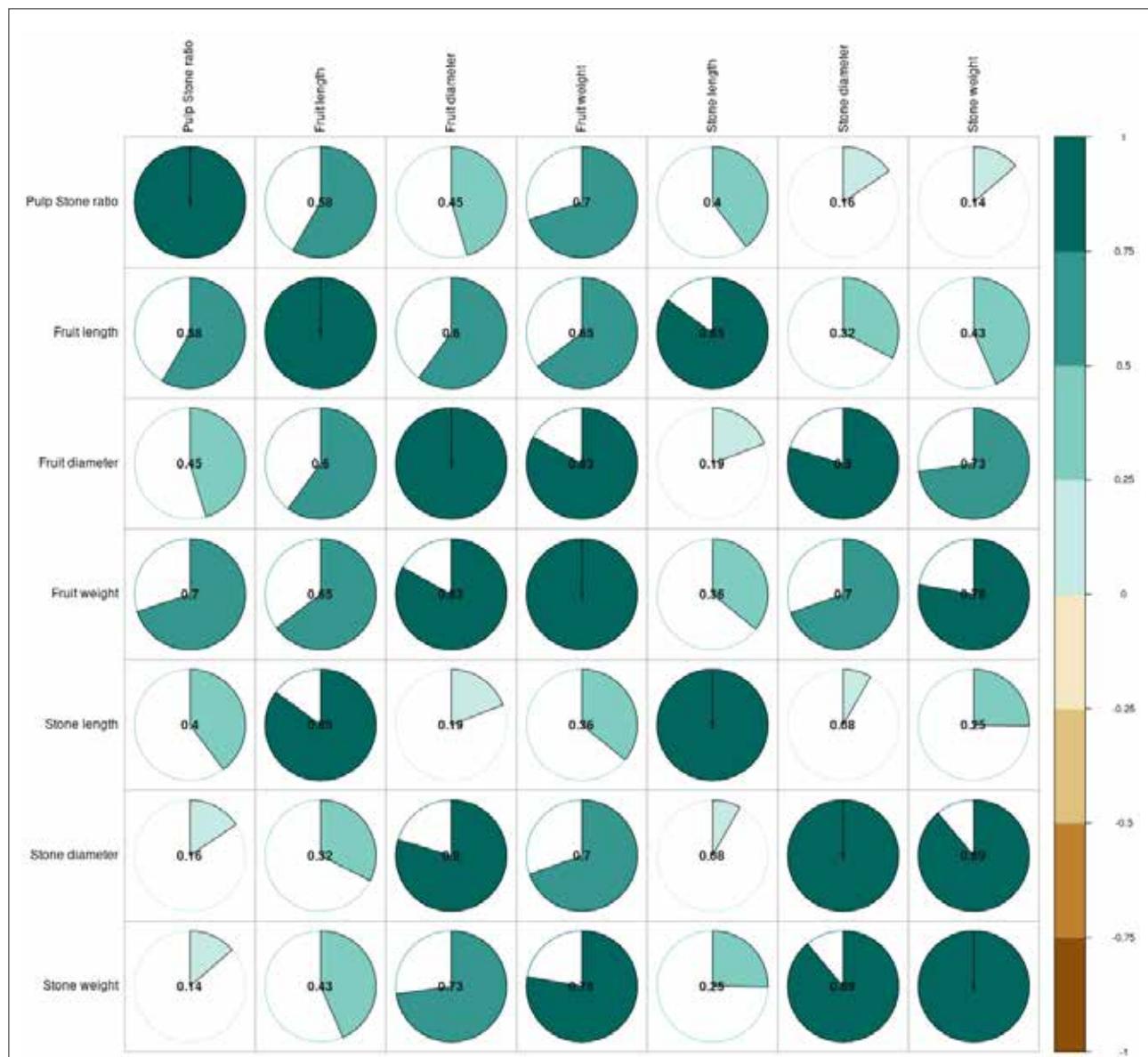


Figure 1. Correlogram between different parameters.

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Yield Performances of Advanced Bread Wheat Mutant Lines

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ABSTRACT

The aim of this study is to identify the advanced mutant lines created by mutagen application to Sagittario, Flamura 85, NKÜ Lider, NKÜ Asiya and Tekirdağ varieties that are superior in terms of yield compared to their parents and commercial varieties. Thirty-five mutant lines developed by through gamma rays parent varieties and nine bread wheat commercial check varieties were used material. Forty-nine wheat genotypes were tested using a partially balanced lattice design. According to the variance analysis, there were statistically significant differences in grain yield among the parent varieties, mutant lines and commercial varieties. The NZFE 285 mutant line was the highest grain yield with 5961.9 kg ha⁻¹. The mutant lines of NZFE 285, NZFE 289, NZFE 249, NZFE 256, NZFE 242, NZFE 260, NZFE 284, NZFE 288, NZFE 287, NZFE 292, NZFE 239, NZFE 267, NZFE 245, NZFE 274, NZFE 269, NZFE 262 and NZFE 255 were the other highest grain yielding lines. The lowest grain yield was in NKÜ Asiya variety with 4804.6 kg ha⁻¹, followed by NZFE 271 with 4814.6 kg ha⁻¹, NZFE 277 with 5006.6 kg ha⁻¹. The three mutant lines from Sagittario variety, one mutant line from the NKÜ Lider variety, two mutant lines from Tekirdağ variety, and two of mutant lines from the NKÜ Asiya variety were the higher grain yield compared to parent variety means. The average grain yield of the nine commercial bread wheat varieties was 5693.8 kg ha⁻¹. The mutant lines NZFE 285, NZFE 289, NZFE 249, NZFE 256, NZFE 242, NZFE 260, NZFE 284 and NZFE 288 were higher grain yield than the average of commercial varieties and their parents.

Keywords: Bread wheat, mutant line, grain yield, control, standard variety

Introduction

Cereals have been one of the most widely produced and consumed agricultural commodities since humanity adopted a settled lifestyle and adopted traditional agriculture. Wheat is the largest contributor, accounting for approximately 30% of global grain production and 50% of global grain trade (Akter and Rafiqul Islam, 2017). It is the second-largest cultivated cereal crop after rice, with global wheat production of approximately 761 million tons annually. The largest world producers of wheat are China (with an estimated output of 137.7 million tons), EU (134.2 million tons), India (104.0 million tons), Russia (92.0 million tons), and the USA (44.9 million tons), and Türkiye ranked 9th with a production of 22.3 million tons (FAOSTAT, 2023). It is

estimated that developing countries will need to increase their wheat production by 77% to meet the nutritional demands of the growing population, and the world will need an additional 198 million tons of wheat by 2050 to meet future demands (Sharma et al., 2015). Among the various ways and methods of increasing production, the most realistic one is to increase productivity per unit area. Among the various ways and methods of increasing production, the most realistic one is the increase in yield per unit area that can be achieved through new variety breeding and improvements in agronomic practices. Breeding of wheat varieties that combine high grain yield and stability under drought stress conditions is crucial to boost yield gains to ensure food security and enhance climate resilience in wheat production systems.

For this purpose, the most commonly used breeding method for breeders to obtain new genotypes is cross breeding. Mutation breeding is one of the most popular breeding methods among breeders in many countries around the world. Mutagenesis has played a key role in generating new genetic stocks for the improvement of economic traits, including grain yield and quality, phenological traits, disease resistance, and heat and drought tolerance (Kumar et al., 2024, Wang et al., 2024). Today, a total of 3,401 mutant cultivars have been developed directly or indirectly through mutations in 233 plant species across 75 countries worldwide. The largest number of mutant cultivars has been registered in China (835), Japan (505), India (348), Russia (216), the Netherlands (176), and Germany (171), respectively. In our country, a total of 15 mutant varieties have been registered in different plant species (IAEA, 2022). Considering the advances in mutation breeding globally, unfortunately, there are no commercial wheat varieties developed through mutation in our country yet. Mutagenesis has significant potential in the development of novel wheat varieties to enhance genetic gains for key traits, which are vital for ensuring food security (OlaOlorun et al., 2021). Mutagenesis shows as an easy and effective mean of inducing genetic variation. Several researchers have used mutation breeding to improve grains yield of bread wheat (Balkan, 2018; Nazarenko, et al., 2018). Physical mutagens were the most commonly used method in developing mutant varieties, with a rate of 78% compared to chemical mutagens (11%). Of the mutant varieties obtained with physical mutagens, 69% were treated with gamma rays and 22% with X-rays (IAEA, 2022). Mutation breeding has some advantages compared to crossbreeding breeding; Homozygosity occurs at F_6 or F_7 in crossbreeding breeding, whereas M_2 or M_3 occurs in mutation breeding (Chakraborty and Paul, 2013). Mutational breeding is used to improve plant traits when conventional breeding has failed, when desired traits are recessive, or to improve one or two other traits in a commercial variety (Van Harten, 1998; Ahloowalia and Maluszynski, 2001). It is also possible that a new character will be discovered that is not present in the parent genotype. Given that mutation is a viable, sustainable, flexible, unregulated, non-hazardous, environmentally acceptable, highly effective and cost-effective plant breeding method (Kainthura and Srivastava, 2015), mutation techniques need to be used more effectively in wheat breeding programs in our country. As a result of the mutations induced by mutagens, plants can exhibit a wide range of variations in morphological and yield-related characteristics compared to normal plants. Scientists have demonstrated the role of induced mutations in increasing the genetic

variability for agronomic traits in various crop plants (Chen et al., 2019)

The aim of the study is to determine candidate elite variety lines by examining the yield performances of advanced bread wheat mutant lines developed from populations generated by gamma irradiation to commercial bread wheat varieties with different characteristics.

Materials and Methods

Seeds of Sagittario, Flamura 85, NKÜ Lider, NKÜ Asiya and Tekirdağ bread wheat varieties were irradiated with gamma rays from ^{60}Co source at the Ankara Nuclear Research and Training Center of the Turkish Atomic Energy before sowing the 2017. No selection was made until 2021, and the single ears of plants that were superior in terms of agronomic characteristics in the same year, that is, the M_4 generation, were sown as single ear rows in 2022. In 2023, seeds taken from single spike rows were sown in separate plots, and 35 mutant lines with high agricultural value (agronomically) were identified from them. These 35 promising mutant lines, were included in the experiment, along with non-mutagen treated parents and nine bread wheat varieties commonly sown in the region. The study was carried out with 7x7 partially balanced lattice design with three replications. The study was conducted in Süleymanpaşa (Tekirdağ), Hayrabolu (Tekirdağ), Edirne and Silivri locations in 2024-2025 growing season. The 50 kg ha^{-1} of pure nitrogen and phosphorus (20.20.0 fertilizer) at sowing, 69 kg ha^{-1} of pure nitrogen at the tillering stage, 46 kg ha^{-1} of pure nitrogen at the beginning of stem formation, and 39 kg ha^{-1} of pure nitrogen before heading was applied. Herbicides were used to control weeds in the trial. The plots were harvested with a HEGE-160 plot combine harvester, and the obtained grain yield values were converted to yield per hectare.

The test of significance of the differences between the means for mutant lines, control varieties and commercial varieties was determined using the TARPOP-GEN statistical analysis program, using a partially balanced lattice design for variance analysis. Because the differences between the blocks were statistically insignificant, analyses were conducted using a randomized complete block design. Differences between the means were determined using Tukey's significance test.

Results and Discussion

The results of variance analysis performed on grain yield data obtained from experiments conducted in 4 different locations of Thrace region with 35 advanced mutant lines developed by gamma irradiation of five

bread wheat varieties, their parent varieties and check varieties, showed that the differences between the means of genotypes and locations were statistically significant. The average grain yields and significance of the genotypes obtained at four different locations are presented in Table 1.

In the study, the average grain yields of bread wheat mutant lines and their parent varieties ranged from 6755.0 to 4855.7 kg ha⁻¹ for Edirne location, ranged from 6210.0 to 4670.0 kg ha⁻¹ for Hayrabolu location, ranged from 5892.5 to 3632.5 kg ha⁻¹ for Silivri location and ranged from 7363.4 to 4553.3 kg ha⁻¹ for Tekirdağ location. The general average grain yield of genotypes for the four locations ranged from 5962.7 to 4864.6 kg ha⁻¹.

The average grain yield in the parent variety Sagittario changed between 5152.7 and 6198.4 kg ha⁻¹ for the locations and the general mean was 5693.0 kg ha⁻¹. While 8 mutant lines for the Edirne location, 7 mutant lines for the Hayrabolu location and 7 lines for the Tekirdağ location yielded above the average of the parent variety, it was observed that no mutant line showed such a feature in the Silivri location. The average grain yield across locations was 5693.0 kg ha⁻¹, exceeded by the yields of the NZFE 265, NZFE 260 and NZFE 284 mutant lines.

The four mutant lines was higher the grain yield mean than that of parent variety of Flamura 85 of 6155.0 kg ha⁻¹ for Edirne location. The five mutant lines for Hayrabolu location, all mutant lines for Silivri location and Seven mutant lines for Tekirdağ location gave the more yield than the parent variety. All mutant lines yielded higher grain yields than the parent variety mean across locations. Of the two mutant lines obtained from the NKÜ Lider variety, 1 mutant line in each of the 4 locations and 1 mutant line on average yielded higher grain yields overall. Of the three mutant lines obtained from the NKÜ Ergene variety, two in the Edirne and Hayrabolu locations, and one in the Silivri location, yielded grain yields higher than the non-mutagen treated parent, while three mutant lines failed to meet the non-mutagen treated parent in the Tekirdağ location. The average grain yield of the Tekirdağ bread wheat variety in the Edirne location was 5236.7 kg ha⁻¹, the four mutant lines gave higher grain yield over the mean. Three mutant lines in the Hayrabolu location, three mutant lines in the Silivri location, and one mutant line in the Tekirdağ location were higher grain yield than the parent variety. The average across locations was 5218.1 kg ha⁻¹ and all mutant lines yielded higher yields.

The out of 35 mutant lines NZFE 285, NZFE 289, NZFE 249, NZFE 284, NZFE 269 and NZFE 275

gave higher grain yield than the average grain yield of nine commercial cultivars was 6028.3 kg ha⁻¹ for Edirne location. The mutant lines NZFE 249, NZFE 256, NZFE 288, NZFE 287, NZFE 239, NZFE 245, NZFE 269, NZFE 274 and NZFE 247 gave higher grain yield for Hayrabolu location compare to the average grain yield of nine commercial bread wheat cultivars of 5387.3 kg ha⁻¹. The average grain yield of 8 commercial varieties (4997.2 kg ha⁻¹) was lower than that of NZFE 285, NZFE 289, NZFE 249, NZFE 242, NZFE 260, NZFE 284, NZFE 288, NZFE 287, NZFE 267, NZFE 274, NZFE 269, NZFE 273, NZFE 281 and NZFE 246 mutant lines grain yield means for Silivri location. Regarding grain yield for Tekirdağ location, NZFE 285, NZFE 289, NZFE 249, NZFE 256, NZFE 242, NZFE 260, NZFE 284, NZFE 292, NZFE 267, NZFE 245, NZFE 278, NZFE 255 and NZFE 286 mutant lines gave higher values compared to check means. Comparing the average grain yield of 5600.1 kg ha⁻¹ of commercial varieties at four locations, it is understood that the mutant lines NZFE 285, NZFE 289, NZFE 249, NZFE 256, NZFE 242, NZFE 260 and NZFE 284 gave higher grain yield. The study showed that the average grain yields of the mutant lines obtained from 4 different locations ranged from 4877.1 to 5960.0 kg ha⁻¹, indicating promising yield potential. These yields are similar to those reported by other researchers (Aydöğan and Soylu, 2017; Öztürk and Korkut, 2018; Kahraman et al., 2021; Ersöz and Başçiftci, 2024).

Conclusions

The results obtained showed that gamma irradiation of five bread wheat varieties resulted in a wide variation, and genotypes with different characteristics were obtained successfully. While the yields of the mutant lines varied across locations, 16 of the mutant lines showed higher yields than their parent varieties. Of the 35 mutant lines obtained, 13 mutant lines (NZFE 285, NZFE 289, NZFE 249, NZFE 256, NZFE 242, NZFE 260, NZFE 284, NZFE 292, NZFE 267, NZFE 245, NZFE 278, NZFE 255 and NZFE 286) surpassed the commercial check and parent varieties, demonstrating that these are promising elite lines and can be variety candidates for both the region and the wheat production regions of our country. In conclusion, it is understood that the mutant lines have promising results and could be considered as potential variant candidates.

Author Contribution Statement

All authors contributed equally to the preparation of this paper.

Conflicting Interest Statement

All authors declare that they have no conflict of interest regarding this article.

Table 1. Mean grain yield per hectare and significance groups for advanced mutant lines and their parent varieties.

Genotypes	Edirne	Hayrabolu	Silivri	Süleymanpaşa	Mean
Sagittario	5528.3 b-i	5152.7 abc	5892.5 a	6198.4 a-g	5693.0 a-f
NZFE 256	5483.3 c-i	5583.4 abc	5042.5 a-g	7363.4 a	5868.1 ab
NZFE 260	6024.3 a-h	4669.0 c	5817.5 ab	6688.4 a-d	5799.8 a-d
NZFE 284	6533.3 a-d	4928.3 bc	5220.0 a-f	6418.3 a-e	5775.0 a-e
NZFE 267	5790.0 a-i	4817.1 c	5387.5 a-d	6616.7 a-d	5652.8 a-g
NZFE 245	5175.0 ghi	5575.0 abc	5065.0 a-g	6706.7 a-d	5630.4 a-g
NZFE 274	5756.7 a-i	5422.7 abc	5355.0 a-e	5926.7 b-g	5615.3 a-g
NZFE 262	5743.3 a-i	5170.0 abc	5115.0 a-g	5976.7 b-g	5501.3 a-h
NZFE 273	5150.0 hi	5200.0 abc	5600.0 abc	6021.7 b-g	5492.9 a-h
NZFE 278	5270.0 f-i	4988.3 bc	4982.5 a-g	6623.3 a-d	5466.0 a-h
NZFE 281	5833.3 a-i	4775.7 c	5220.0 a-f	5900.0 b-g	5432.3 a-i
NZFE 286	5413.3 e-i	4985.7 bc	4825.0 a-i	6450.0 a-e	5418.5 a-i
NZFE 251	5753.3 a-i	5416.7 abc	4660.0 a-i	5530.0 d-h	5340.0 b-j
NZFE 263	5653.0 b-i	5107.7 abc	4638.5 b-i	5860.0 b-g	5314.8 b-j
NZFE 246	5345.0 e-i	4695.00 c	5582.5 abc	5611.7 c-h	5308.6 b-j
NZFE 265	5515.0 b-i	4957.7 bc	4760.0 a-i	5825.0 b-g	5264.4 c-j
NZFE 247	4855.7 i	5626.7 abc	4713.0 a-i	5585.6 c-h	5195.2 f-j
NZFE 266	5385.0 e-i	4873.4 bc	4597.5 b-i	5876.7 b-g	5183.1 f-j
NZFE 241	5148.4 hi	4645.0 c	5045.0 a-g	5671.7 b-h	5127.5 g-j
Flamura 85	6155.0 a-h	5180.3 abc	4645.0 b-i	5920.0 b-g	5475.1 a-h
NZFE 285	6211.7 a-h	5366.7 abc	5547.5 abc	6725.0 abc	5962.7 a
NZFE 289	6535.0 abc	5155.0 abc	5300.0 a-e	6723.3 abc	5928.3 a
NZFE 249	6755.0 a	5568.4 abc	5122.5 a-g	6200.0 a-g	5911.5 a
NZFE 242	5771.7 a-i	5280.0 abc	5462.5 a-d	6791.7 ab	5826.5 abc
NZFE 288	5981.7 a-h	5480.8 abc	5517.5 abc	5986.7 b-g	5741.7 a-f
NZFE 287	5735.0 a-i	5701.8 abc	5182.5 a-f	6042.4 b-g	5665.4 a-g
NZFE 292	5985.0 a-h	5038.4 bc	4952.5 a-g	6683.4 a-d	5664.8 a-g
NZFE 275	6406.7 a-e	5148.3 abc	4700.0 a-i	5903.4 b-g	5539.6 a-h
NKÜ Lider	5881.7 a-i	5191.3 abc	4562.5 c-i	5593.1 c-h	5307.1 b-j
NZFE 279	5466.7 c-i	5689.0 abc	4790.0 a-i	5743.3 b-g	5422.3 a-i
NZFE 283	6311.7 a-f	5096.7 abc	4436.5 c-i	5061.7 gh	5226.6 e-j
NKÜ Ergene	6226.7 a-h	4982.7 bc	4270.0 d-i	5426.7 e-h	5226.5 e-j
NZFE 243	6113.4 a-h	5035.7 bc	4605.0 b-i	5126.7 fgh	5220.2 e-j
NZFE 277	5836.7 a-i	5201.7 abc	3690.0 hi	5278.4 e-h	5001.7 hij
NZFE 271	6044.3 a-h	4670.0 c	3632.5 i	5161.7 fgh	4877.1 ij
Tekirdağ	5236.7 f-i	5168.4 abc	4262.5 d-i	6205.0 a-g	5218.1 e-j
NZFE 239	5726.7 a-i	6210.0 a	4950.0 a-g	5737.1 b-g	5656.0 a-g
NZFE 269	6240.0 a-g	4940.2 bc	5183.0 a-f	6071.7 b-g	5687.0 a-g
NZFE 255	5253.3 f-i	5204.4 abc	4925.0 a-h	6341.7 a-e	5431.1 a-i
NZFE 264	5453.0 c-i	5609.0 abc	4145.0 e-i	5754.7 b-g	5240.4 d-j

Table 2. Mean grain yield per hectare and significance groups for advanced mutant lines and commercial check varieties.

Genotypes	Edirne	Hayrabolu	Silivri	Süleymanpaşa	Mean
NZFE 285	6211.7 a-h	5366.7 abc	5547.5 abc	6725.0 abc	5962.7 a
NZFE 289	6535.0 abc	5155.0 abc	5300.0 a-e	6723.3 abc	5928.3 a
NZFE 249	6755.0 a	5568.4 abc	5122.5 a-g	6200.0 a-g	5911.5 a
NZFE 256	5483.3 c-i	5583.4 abc	5042.5 a-g	7363.4 a	5868.1 ab
NZFE 242	5771.7 a-i	5280.0 abc	5462.5 a-d	6791.7 ab	5826.5 abc
NZFE 260	6024.3 a-h	4669.0 c	5817.5 ab	6688.4 a-d	5799.8 a-d
NZFE 284	6533.3 a-d	4928.3 bc	5220.0 a-f	6418.3 a-e	5775.0 a-e
NZFE 288	5981.7 a-h	5480.8 abc	5517.5 abc	5986.7 b-g	5741.7 a-f
NZFE 287	5735.0 a-i	5701.8 abc	5182.5 a-f	6042.4 b-g	5665.4 a-g
NZFE 292	5985.0 a-h	5038.4 bc	4952.5 a-g	6683.4 a-d	5664.8 a-g
NZFE 239	5726.7 a-i	6210.0 a	4950.0 a-g	5737.1 b-g	5656.0 a-g
NZFE 267	5790.0 a-i	4817.1 c	5387.5 a-d	6616.7 a-d	5652.8 a-g
NZFE 245	5175.0 ghi	5575.0 abc	5065.0 a-g	6706.7 a-d	5630.4 a-g
NZFE 274	5756.7 a-i	5422.7 abc	5355.0 a-e	5926.7 b-g	5615.3 a-g
NZFE 269	6240.0 a-g	4940.2 bc	5183.0 a-f	6071.7 b-g	5608.7 a-g
NZFE 275	6406.7 a-e	5148.3 abc	4700.0 a-i	5903.4 b-g	5539.6 a-h
NZFE 262	5743.3 a-i	5170.0 abc	5115.0 a-g	5976.7 b-g	5501.3 a-h
NZFE 273	5150.0 hi	5200.0 abc	5600.0 abc	6021.7 b-g	5492.9 a-h
NZFE 278	5270.0 f-i	4988.3 bc	4982.5 a-g	6623.3 a-d	5466.0 a-h
NZFE 281	5833.0 a-i	4775.7 c	5220.0 a-f	5900.0 b-g	5432.3 a-i
NZFE 255	5253.3 f-i	5204.4 abc	4925.0 a-h	6341.7 a-e	5431.1 a-i
NZFE 279	5466.7 c-i	5689.0 abc	4790.0 a-i	5743.3 b-g	5422.3 a-i
NZFE 286	5413.3 e-i	4985.7 bc	4825.0 a-i	6450.0 a-e	5418.5 a-i
NZFE 251	5753.3 a-i	5416.7 abc	4660.0 a-i	5530.0 d-h	5340.0 b-j
NZFE 263	5653.0 b-i	5107.7 abc	4638.5 b-i	5860.0 b-g	5314.8 b-j
NZFE 246	5345.0 e-i	4695.0 c	5582.5 abc	5611.7 c-h	5308.6 b-j
NZFE 265	5515.0 b-i	4957.7 bc	4760.0 a-i	5825.0 b-g	5264.4 c-j
NZFE 264	5453.0 c-i	5609.0 abc	4145.0 e-i	5754.7 b-g	5240.4 d-j
NZFE 283	6311.7 a-f	5096.7 abc	4436.5 c-i	5061.7 gh	5226.6 e-j
NZFE 243	6113.4 a-h	5035.7 bc	4605.0 b-i	5126.7 fgh	5220.2 e-j
NZFE 247	4855.7 i	5626.7 abc	4713.0 a-i	5585.6 c-h	5195.2 f-j
NZFE 266	5385.0 e-i	4873.4 bc	4597.5 b-i	5876.7 b-g	5183.1 f-j
NZFE 241	5148.4 hi	4645.0 c	5045.0 a-g	5671.7 b-h	5127.5 g-j
NZFE 277	5836.7 a-i	5201.7 abc	3690.0 hi	5278.4 e-h	5001.7 hij
NZFE 271	6044.3 a-h	4670.0 c	3632.5 i	5161.7 fgh	4877.1 ij
<i>NKÜ Asiya</i>	5626.7 b-i	4991.0 bc	4047.5 f-i	4553.3 h	4804.6 j
<i>Rumeli</i>	5450.0 d-i	5306.0 abc	4787.5 a-i	6349.3 a-e	5473.2 a-h
<i>Maden</i>	5940.0 a-i	5406.7 abc	5570.0 abc	6280.0 a-f	5799.2 a-d
<i>LG Albufera</i>	6239.7 a-g	5640.0 abc	3932.5 ghi	6081.7 b-g	5473.5 a-h
<i>Oğalıs</i>	6143.3 a-h	5335.0 abc	5837.5 ab	6056.7 b-g	5843.1 ab
<i>Axum</i>	6573.3 ab	5348.3 abc	5092.5 a-g	5975.0 b-g	5747.3 a-f
<i>Saban</i>	6044.7 a-h	5040.0 bc	4957.5 a-g	6060.0 b-g	5525.5 a-h
<i>Gelibolu</i>	6180.0 a-h	5401.7 abc	5342.5 a-e	6196.7 a-g	5780.2 a-e
<i>Glosa</i>	6056.7 a-h	6006.7 ab	5407.5 a-d	6345.2 a-e	5954.0 a
Commercial Varieties Mean	6028.3	5387.3	4997.2	5988.7	5600.1

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Determination of Resistance to Broomrape and Yield Performances of IMI Type Sunflower Hybrids^(*)

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is the most widely cultivated oilseed crop in Türkiye. However, sunflower production is severely constrained by broomrape (*Orobanche cumana* Wallr.). Herbicide-tolerant sunflower cultivars resistant to imazamox (IMI) play a crucial role in controlling broomrape and other weeds. In Türkiye, newly emerging broomrape races are currently present in almost all sunflower-growing areas. This study aimed to evaluate the yield performance and broomrape resistance of IMI-tolerant hybrid sunflower cultivars developed within the scope of TARI's National Sunflower Project. Field experiments were conducted in four locations in 2024. Experimental design was a randomized complete block design with four replications. Weed control was achieved by applying imazamox (40 g L⁻¹) at a rate of 1.25 L ha⁻¹ at the 6-8 leaf stage. Statistical analyses were performed using the JMP software. Broomrape resistance was evaluated under both field conditions in Keşan and artificial inoculation tests in pots. In field trial, plots with two replicates. Each plots consisted 32 plants. Infection frequency, infection intensity, and aggressiveness levels were assessed, and genotypes were classified as susceptible, tolerant, or resistant. The results showed that TTAE IMI 23-130 and TTAE IMI 23-135 exhibited superior seed yield, oil yield, and high tolerance to new broomrape races, and they were identified as the most promising hybrids for variety registration. The susceptibility of OR7 gene-carrying genotypes further indicates the emergence of new broomrape races in the region.

Keywords: Sunflower, broomrape, imazamox, inoculation, yield

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Introduction

Sunflower is one of the most extensively grown oilseed crops in Türkiye and exhibits a principal source of digestible vegetable oil. Due to its wide adaptability, sunflower can be successfully grown under both irrigated and rainfed conditions across many regions of the country. The growing global population has led to an increasing demand for food. Consequently, the need for vegetable oils has risen both globally and nationally. In Türkiye, the high consumer preference for sunflower oil

magnifies this demand, emphasizing the importance of maximizing yield per decare. Despite its strong adaptive capacity, sunflower production often can not reach to desired levels by cause of various limiting factors.

Sunflower (*Helianthus* spp.), which known as native American, covers 51 species, including 14 annual and 37 perennial species. Sunflower was initially cultivated as an ornamental plant. Its use as an oil crop began in the 17th century, after which it rapidly spread throughout Europe (Fick and Miller, 1997).

Following World War II, sunflower cultivation was introduced to Thrace region, Türkiye by migrants from the Balkans and its cultivation has been expanded across the country (Kaya, 2021).

Oilseed sunflower is among the most widely cultivated oil crops worldwide due to its high oil content and exceptional adaptability. The interaction between genotype, environmental conditions and cultivation methods form yield, yield-related traits, and quality characteristics. Average seed yield generally ranges between 2000 and 3500 kg ha⁻¹, and appropriate irrigation, sowing time, and fertilization can significantly enhance productivity (Evci et al., 2012). Seed oil content mostly varies between 40% and 50%, depending on genetic structure. Adequate water and nutrient during the seed-filling period improve oil biosynthesis (Flagella et al., 2002). Plant height ranges from 120 to 180 cm, depending on nitrogen fertilization, planting density, and genotype (Killi, 2004). Head diameter usually varies between 15 and 25 cm and shows a positive correlation with seed number and yield (Gholinezhad et al., 2009). Thousand-seed weight, which is affected by environmental factors, ranges from 50 to 80 g in oilseed sunflower (Hassan et al., 2013).

Sunflower production is inhibited by several abiotic and biotic factors such as diseases, weeds, and broomrape (*Orobanche cumana* Wallr.) in Türkiye. Although breeding programs have strengthened genetic resistance to these stresses, resistance is getting weaker over time has caused new challenges for farmers. Downy mildew (*Plasmopara halstedii* (Farlow) Berlese et de Toni), one of the most important sunflower diseases, is spreaded throughout sunflower-growing regions in Türkiye and has caused severe yield losses during epidemic years.

Weed management in sunflower starts prior to sowing and continues after emergence. The best practice is achieved through an integrated approach combining cultural, mechanical, and chemical methods. Chemical control practices include pre-plant integrated, pre-emergence, and post-emergence applications, performed when sunflower plants reach the 4-6 leaf stage. Major weed species such as cocklebur (*Xanthium strumarium*), wild oat (*Avena sterilis*), goosefoot (*Chenopodium album*), barnyard grass (*Echinochloa crus-galli*), wild mustard (*Sinapis arvensis*), tumbleweed (*Amaranthus albus*, *A. retroflexus*), black nightshade (*Solanum nigrum*), and thornapple (*Datura stramonium*) are suppressed by these strategies efficiently (Beres et al., 2005).

In both Türkiye and globally, the cultivation of sunflower varieties resistant to IMI (Imazamox) and

SU (75% tribenuron-methyl) herbicides has become increased. These technologies enable effective weed management and also providing efficient control of broomrape through imazamox applications.

Broomrape is an obligate parasitic plant belonging to the Orobanchaceae family and roots a serious threat to sunflower production in many regions. *Orobanche cumana* weakens photosynthetic ability and parasitizes sunflower roots. It represents a major constraint to sunflower cultivation, especially in the Black Sea basin and Spain (Molinero-Ruiz et al., 2013).

Each broomrape flower patterns a capsule containing approximately 600 to 5,000 seeds, with a single plant capable of producing up to 500,000 seeds (Habimana et al., 2014). These seeds can remain viable in the soil for up to 20 years. Optimal soil temperatures for seed germination range from 20 to 25°C, and around 30-60 days after germination, flowering takes place (Pathak and Kannan, 2014).

Recent studies have reported the emergence of a new broomrape race (race H) in Romania (Pacureanu-Joita et al., 2009), Russia (Gontcharov, 2009; Antonova et al., 2011), northeastern Ukraine (Maklyak et al., 2018), and Türkiye (Kaya et al., 2009). Currently, broomrape races F, G, and H are known to exist in Türkiye, although the races were not clearly identified (Kaya et al., 2004; Molinero-Ruiz et al., 2015; Bilgen et al., 2019; Uludağ et al., 2021). A new race has emerged in sunflower fields in Adana and has also begun to infect known resistant sunflower lines. Additionally, a distinct infection pattern observed in the Şahinköy region of Thrace has led to the identification of a new race, designated as race I (Yonet et al., 2018).

At present, new broomrape races are spotted in nearly all sunflower-growing areas of the Thrace–Marmara region equals to almost half of the national sunflower production areas. However, the development of genetically resistant and IMI-tolerant sunflower varieties has significantly reduced the impact of broomrape in recent years (Kilic et al., 2016; Kaya, 2020).

This study investigated the yield performance of candidate hybrid sunflower varieties developed within an institutional breeding program, alongside commonly cultivated registered varieties, across multiple locations. Resistance to broomrape was estimated under both natural field conditions and artificial inoculation.

Materials and Methods

Materials

The materials used in this study were IMI-resistant hybrid sunflower varieties developed within the scope of the National Sunflower Project of the Thrace

Agricultural Research Institute. Twelve IMI-tolerant oilseed sunflower candidate varieties (TTAE IMI 23-22, TTAE IMI 23-54, TTAE IMI 23-90, TTAE IMI 23-123, TTAE IMI 23-124, TTAE IMI 23-130, TTAE IMI 23-132, TTAE IMI 23-135, TTAE IMI 23-142, TTAE IMI 23-150, TTAE IMI 23-154, TTAE IMI 23-155) were tested. Some of the most commonly grown commercial varieties (LG 50550 CLP, P64 LP130, P64 LC108, SUN 2259 CL) in the region were used as control varieties.

Field Trials

Field trials were conducted at four locations (Edirne, Çorlu, Keşan, and Kırklareli) using a randomized complete block design with four replications and four-row plots. Rows were 7.5 m in length, with 70 × 30 cm plant spacing (Figure 1a and Figure 1b). Four widely cultivated commercial hybrids were included as check varieties (Table 1). Weed control was achieved by applying imazamox (40 g L⁻¹) at a rate of 1.25 L ha⁻¹ at the 4–6 or 6–8 leaf stage (Figure 1a). Phytotoxicity observations were recorded at 7 and 14 days after application. Statistical analyses were performed using the JMP software package.

Broomrape Field Tests

Field trial of broomrape test was located in Kesan (Figure 1c). The experimental design was Randomized Complete Block Desing with 2 replicates. Plots with two rows were 4-m long and plant spacing was 70 x 25 cm. Each plots consisted 32 plants. The location was selected according to the observations on high broomrape intensity of the field between the seasons of 2020-2023. There was no herbicide (imazamox) applications to have a better understanding of variety's resistance to broomrape. Frequency of infection (F), intensity of infection (I), levels of aggression (A) were examined for each genotype. Frequency of infection was obtained by calculating the percentage of infected plants. The data of Intensity of infection was gathered by counting the broomrapes per infected plants. Levels of aggression was calculated with this formula: (Frequency of Infection x Intensity of Infection) / 100. Hybrids, which have 0-10% F score and 0-1 A score, were considered as resistant-tolerant hybrid (Pustovoit, 1975).

Broomrape Inoculation Tests

The resistance of material to broomrape was tested in pots with full of artificially infected soil by broomrape. Broomrape seeds were obtained from different locations in the Thrace region. In the climate chamber, 1-2 g broomrape seeds were mixed into the soil in each plastic cup. 35 days after planting, the plants in cups were removed, the roots were washed, the tubers of the rootstock were counted and the degree of resistance was determined (Figure 1d). It is evaluated

as susceptible, tolerant and resistant according to the tubers on the roots (TARI, 2012).

Results and Discussion

An analysis of variance was performed using the data obtained from the field experiments. Seed yield per decare varied significantly among locations. Edirne recorded the lowest yield values, whereas Keşan and Kırklareli were identified as the highest-yielding locations. A similar trend was observed for oil content, with the Edirne location showing lower average oil percentages compared to the other test environments (Table 1). These findings are in agreement with the study conducted by Skoric (2009) in Serbia, who reported that prolonged drought conditions affect sunflower growth and development negatively, and it leads to yield reductions and causes serious challenges for sunflower production under dry environments.

When seed yield performance was evaluated by location, the candidate varieties TTAE IMI 23-54 (1018 kg ha⁻¹) and TTAE IMI 23-150 (1021 kg ha⁻¹) exhibited remarkable performance in Edirne. In Çorlu, TTAE IMI 23-155 achieved the highest yield (1522 kg ha⁻¹), followed by TTAE IMI 23-154 (1415 kg ha⁻¹), TTAE IMI 23-123 (1354 kg ha⁻¹), TTAE IMI 23-135 (1275 kg ha⁻¹), TTAE IMI 23-124 (1269 kg ha⁻¹), TTAE IMI 23-150 (1234 kg ha⁻¹), TTAE IMI 23-130 (1226 kg ha⁻¹), and TTAE IMI 23-54 (1205 kg ha⁻¹), all of which exceeded the yield levels of the standard varieties. In Keşan, no statistically significant differences were detected among varieties; however, TTAE IMI 23-90 (2149 kg ha⁻¹), TTAE IMI 23-54 (2134 kg ha⁻¹), TTAE IMI 23-130 (2109 kg ha⁻¹), TTAE IMI 23-142 (2103 kg ha⁻¹), and TTAE IMI 23-154 (2101 kg ha⁻¹) emerged as the most promising genotypes. In Kırklareli, the highest yields were recorded for TTAE IMI 23-54 (2347 kg ha⁻¹), TTAE IMI 23-22 (2307 kg ha⁻¹), and TTAE IMI 23-123 (2278 kg ha⁻¹) (Table 1).

Evaluation of oil content revealed that in Edirne, the candidate varieties TTAE IMI 23-130 (39.9%), TTAE IMI 23-142 (39.2%), and TTAE IMI 23-132 (38.7%) surpassed the average oil content of the standard varieties (38.4%). In the Çorlu location, TTAE IMI 23-142 (43.8%), TTAE IMI 23-130 (43.3%), and TTAE IMI 23-135 (42.6%) were identified as the highest oil-yielding genotypes. Results from Keşan indicated that a considerable number of candidate varieties exceeded the mean oil content of the standard cultivars (41.9%). Among all tested genotypes, including the standards, TTAE IMI 23-155 (44.1%), TTAE IMI 23-130 (44.0%), TTAE IMI 23-142 (44.0%), and TTAE IMI 23-154 (43.7%) exhibited the highest oil content values. In Kırklareli, TTAE IMI 23-142 (43.0%), TTAE IMI 23-

154 (42.5%), TTAE IMI 23-130 (42.3%), and TTAE IMI 23-155 (42.3%) exceeded the average oil content of the standard varieties (42.1%) and were ranked among the leading genotypes (Table 1).

Overall evaluation of the experimental results demonstrated yield differences among all locations, showing strong agreement with the findings reported by Cetin and Ozturk (2018) in their study conducted in the Altınekin, Çumra, and Obruk locations of Konya Province.

Based on field-based broomrape resistance assessments, the candidate varieties TTAE IMI 23-90, TTAE IMI 23-130, TTAE IMI 23-135, TTAE IMI 23-142, TTAE IMI 23-150, TTAE IMI 23-154, and TTAE IMI 23-155 were identified as tolerant to the broomrape parasite. Results obtained from artificial inoculation trials further confirmed that TTAE IMI 23-90, TTAE IMI 23-130, TTAE IMI 23-135, TTAE IMI 23-142, TTAE IMI 23-154, and TTAE IMI 23-155 exhibited high levels of tolerance, while TTAE IMI 23-22, TTAE IMI 23-132, and TTAE IMI 23-150 were classified as tolerant genotypes. Combined evaluation of both field and inoculation results revealed the resistance of genotypes, although certain genotypes carrying the OR7 resistance gene displayed susceptibility. These observations are coherent with previous reports indicating the emergence of new broomrape races (H race) in Türkiye (Kaya et al., 2009) and the identification of a distinct infection pattern in the Şahinköy region of Thrace, which has been designated as race I (Yonet et al., 2018).

Conclusions

As a result of this study, it is seen that genotypes coded TTAE IMI 23-130, TTAE IMI 23-135, TTAE IMI 23-142, and TTAE IMI 23-155 exhibit remarkable performance in terms of grain yield and oil content in all 4 locations. Tolerance to broomrape races of genotypes is also determined as an outcome of tests. Although TTAE IMI 23-54 produced the highest yield values, it was susceptible to broomrape. Herbicide applications are not the main option especially for the fields without severe weed pressure. In order to prevent yield losses caused by broomrape, herbicide-tolerant varieties that have strong genetic tolerance, is essential in sunflower cultivation. Resistance evaluation results indicated that genotypes carrying the OR7 resistance gene were susceptible, and these results are proof of the presence of new broomrape races in the region. Based on a combined assessment of yield, oil content, broomrape resistance, and stability analysis (Figure 2), TTAE IMI 23-130 and TTAE IMI 23-135 were identified as the most suitable candidates for variety registration.

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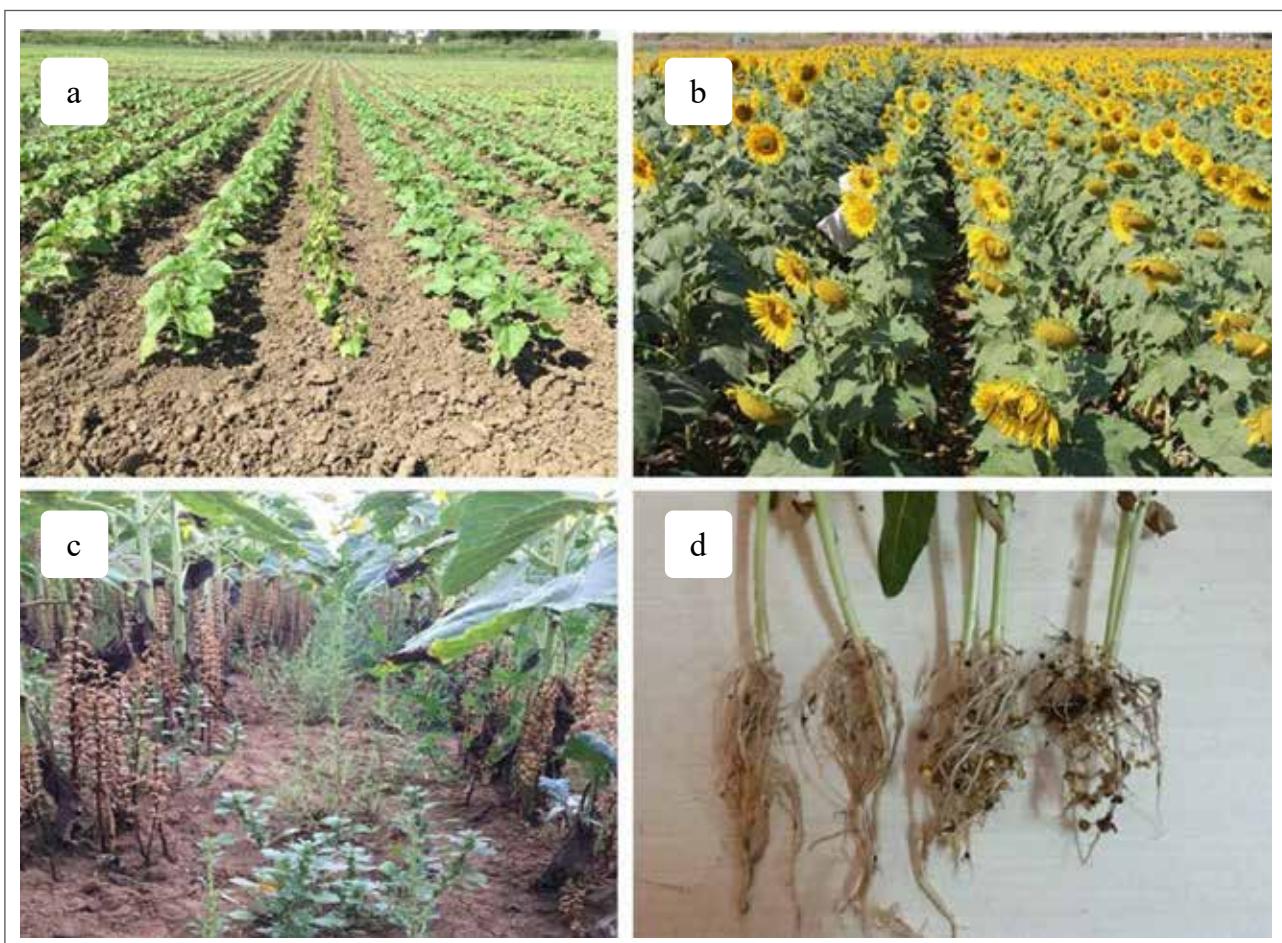


Figure 1. Sunflower yield trial, Edirne location (a-b), broomrape field tests, Keşan location (c) and (d) broomrape inoculation tests (Original).

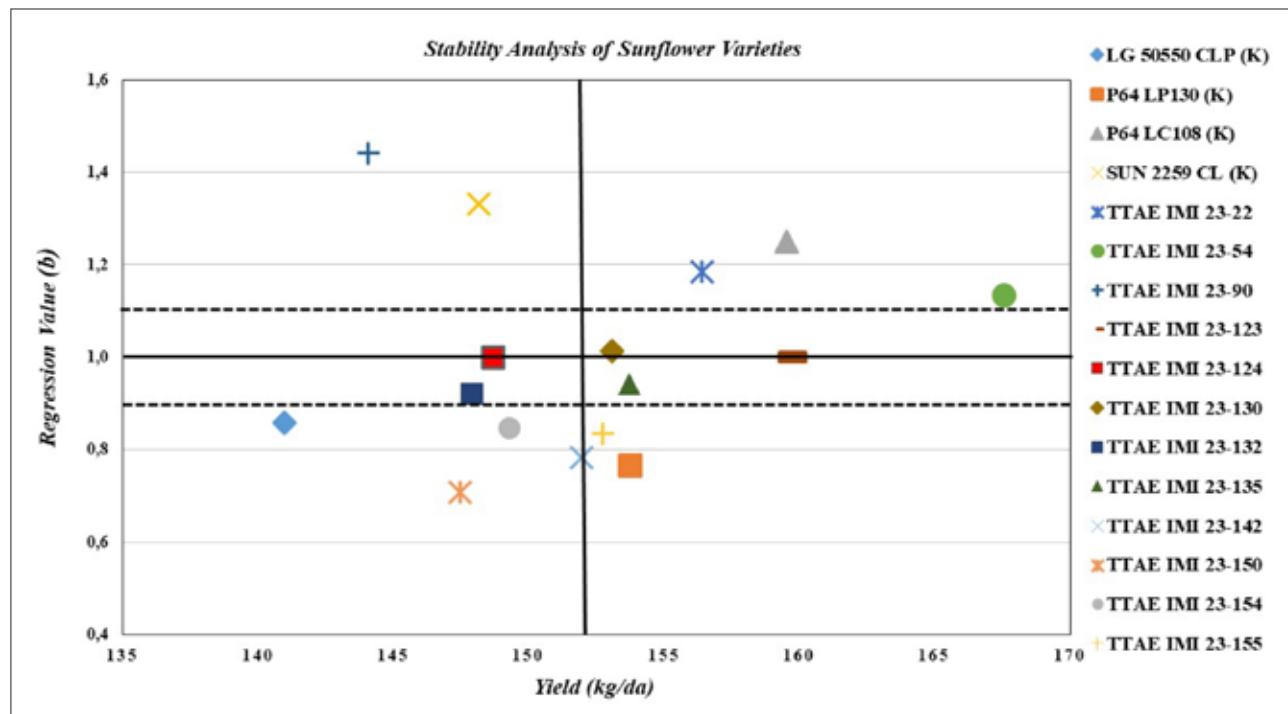


Figure 2. Stability analysis of sunflower varieties.

Table 1. Yield Trial, Field Broomrape Tests and Inoculation Broomrape Tests Observations and Results.

No	Varieties	Edirne		Çorlu		Keşan		Kırklareli		Yield		SOC		Yield		FBT		
		Yield (kg/ha)	SOC (%)	Yield (kg/ha)	SOC (%)	Yield (kg/ha)	SOC (%)	Yield (kg/ha)	SOC (%)	Avg. (kg/ha)	Avg. (%)	Rank	F	I	A	F	I	A
1	LG 50550 CLP(C)	859 ^{bc}	38,9	1132 ^b	42,7	1688 ^{ab}	42,6	1959 ^{ef}	41,7	1410 ^b	41,5	16	0	0	0	0	0	R
2	P64 LP130 (C)	1194 ^a	39,1	1083 ^b	43,5	1578 ^b	43,7	2296 ^{ab}	44,0	1538 ^{ab}	42,6	5	0	0	0	0	0	R
3	P64 LC108 (C)	846 ^{bc}	38,2	1095 ^b	42,1	2364 ^a	41,2	2076 ^{ad}	41,0	1595 ^{ab}	40,6	2	3,1	1	0	0	0	R
4	SUN 2259 CL (C)	562 ^{de}	37,5	1143 ^b	40,0	2027 ^{ab}	40,4	2194 ^{ad}	41,8	1481 ^{ab}	40	12	6,2	1	0,1	0,1	R	
5	TTAE IMI 23-22	803 ^{bc}	37,8	1184 ^b	41,9	1961 ^{ab}	42,3	2307 ^{ab}	41,5	1564 ^{ab}	40,9	4	6,2	1	0,1	0,1	T	
6	TTAE IMI 23-54	1018 ^{ab}	35,1	1205 ^{ab}	38,3	2134 ^{ab}	41,3	2347 ^a	40,0	1676 ^a	38,6	1	66	3,1	2	2	S	
7	TTAE IMI 23-90	379 ^c	36,2	1167 ^b	38,0	2149 ^{ab}	39,7	2067 ^{ad}	39,8	1440 ^b	38,4	15	0	0	0	0	R	
8	TTAE IMI 23-123	899 ^{bc}	36,9	1354 ^{ab}	37,9	1840 ^{ab}	42,6	2278 ^{ac}	41,3	1593 ^{ab}	39,6	3	100	3,9	3,9	3,9	S	
9	TTAE IMI 23-124	774 ^{cd}	36,7	1269 ^{ab}	37,8	1987 ^{ab}	42,1	1919 ^{df}	39,4	1487 ^{ab}	39	11	53	3,3	1,8	1,8	S	
10	TTAE IMI 23-130	859 ^{bc}	39,9	1226 ^{ab}	43,3	2109 ^{ab}	44,0	1930 ^{df}	42,3	1531 ^{ab}	42,4	7	0	0	0	0	R	
11	TTAE IMI 23-132	900 ^{bc}	38,7	1166 ^b	41,0	1792 ^{ab}	42,5	2058 ^{ad}	40,1	1479 ^{ab}	40,6	13	31	2,3	0,7	0,7	T	
12	TTAE IMI 23-135	901 ^{bc}	37,7	1275 ^{ab}	42,6	1979 ^{ab}	41,4	1992 ^{be}	39,2	1537 ^{ab}	40,2	6	0	0	0	0	R	
13	TTAE IMI 23-142	957 ^{bc}	39,2	1337 ^{ab}	43,8	2103 ^{ab}	44,0	1679 ^{ef}	43,0	1519 ^{ab}	42,5	9	0	0	0	0	R	
14	TTAE IMI 23-150	1021 ^{ab}	34,3	1234 ^{ab}	37,9	1957 ^{ab}	40,4	1686 ^{ef}	38,5	1475 ^{ab}	37,8	14	0	0	0	0	R	
15	TTAE IMI 23-154	812 ^{bc}	37,0	1415 ^{ab}	41,8	2101 ^{ab}	43,7	1646 ^f	42,5	1493 ^{ab}	41,2	10	0	0	0	0	R	
16	TTAE IMI 23-155	811 ^{bc}	36,1	1522 ^a	41,7	1900 ^{ab}	44,1	1876 ^{df}	42,3	1527 ^{ab}	41,1	8	0	0	0	0	R	

T: Tolerance

F (%)=Frequency of Infection

FBT=Field Broomrape Tests

IBT=Inoculation Broomrape Tests

Edirne CV (%): 16,6 LSD: 235

Çorlu CV (%): 19,1 LSD: 337

Keşan CV (%): 25,6 LSD: 723

Kırklareli CV (%): 11,3 LSD: 325

Average CV (%): 20,5 LSD: 234

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Predictive Genetic Profiling of High Molecular Weight Glutenin Subunits in Indian Wheat Varieties for Bread-Making Quality

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ABSTRACT

This study aimed at providing a predictive assessment of bread-making quality of 25 Indian bread wheat genotypes and the German variety Bussard based on the characterization of their established high-molecular-weight (HMW) glutenin subunits quality scores using SDS-PAGE, without direct rheological or baking validation. HMW glutenin subunits at the Glu-A1, Glu-B1, and Glu-D1 loci were identified and classified, and Glu-1 quality scores (1-10 scale) were calculated based on SDS-sedimentation associations. High-molecular-weight glutenin profiles were used to compute similarity indices and construct a dendrogram using Genstat. The genotypes Bussard, C-591, C-306, and WH-533 exhibited the highest Glu-1 quality scores, associated with favourable alleles such as Glu-D1 (5+10) and Glu-A1 (1 or 2*) in combination with Glu-B1 (7+8 or 7+9), conferring association with superior dough strength and bread-making quality. Genetic similarity coefficients among the 26 genotypes ranged from 0.53 to 1.00, and the dendrogram separated them into two major clusters, each with two sub-clusters; C-591 and C-306 grouped in SG2b, whereas WH-533 and Bussard clustered in SG1a. The genetic similarity in glutenin composition, based on the clustering, indicates the quality potential of bread wheat cultivars without measuring the functional performance.

Keywords: Glutenin subunits, Glu-1 quality score, bread-making quality, wheat genotypes, SDS-PAGE, genetic similarity

Introduction

One of the important breeding goals in bread wheat is its end-use quality as it determines the quality of products. The end use quality in wheat is determined by gluten as it confers specific viscoelastic characteristics to wheat dough (Islam et al., 2019). Glutenins in general and high molecular weight glutenin subunits (HMW-GS) are considered important to determine processing quality in wheat (Sharma et al., 2020). High-molecular-weight (HMW) subunits of glutenin are encoded by genes at major three homologous loci viz. Glu-A1, Glu-B1,

and Glu-D1 positioned on the stretched arms of chromosomes 1A, 1B and 1D, respectively (Nimbal et al., 2017). Since, the glutenins are major polymeric component of gluten, therefore, the differences in glutenins determine physicochemical (elasticity) and rheological (extensibility) properties of the dough (Abedi and Pourmohammadi, 2021). Two major seed storage protein groups namely Glutenins and gliadins are present in *Triticum aestivum* L. (hexaploid wheat) whose biochemistry as well as the genetics have been broadly studied, revealing both as major determinants of bread-making quality (BMQ) (Li et al., 2021;

Shewry, 2023). Well-characterized inheritance and high polymorphism have made them invaluable for wheat breeding and genetic research.

Quality scores assigned to individual or paired HMW glutenin subunits enable evaluation of bread-making quality (BMQ) potential based on Glu-1 patterns (Nimbal et al., 2017). Studies in European and Indian wheat varieties indicate that HMW glutenin subunit composition accounts for 33–50% of BMQ variation (Wang et al., 2022), with Glu-1 scores positively correlating with bread-making and negatively with biscuit-making qualities. Thus, Glu-1 scores serve as a valuable selection criterion in wheat breeding as a predictive and prescreening mechanism (Jain et al., 2002), while end-use quality (e.g., chapati, bread) classification relies on direct rheological and baking tests such as dough strength, SDS-sedimentation values, solvent retention capacity and gluten index (Coventry et al., 2011). The present study utilizes the glutenin subunit quality scoring in 26 wheat genotypes and their clustering via genetic similarity matrices, to evaluate the genetic diversity and their quality potential, which can be used as a predictive and prescreening tool in wheat improvement programs by the breeders. The selected wheat genotypes in present study were chosen as they thrive well in different agronomic management like Bussard in intensive input conditions, C-591 and C-306 in medium input conditions and WH-533 in water deficit conditions. These genotypes may be involved in recombination breeding to develop high yielding high quality wheat cultivars, based on their association with superior bread-making quality interpreted through Glu-1 quality score.

Materials and Methods

For conducting the present study on profiling of glutenin subunits using SDS-PAGE to predict bread-making quality potential based on established Glu-1 scoring systems, seed samples of twenty-six wheat varieties were used in this study (table 1). These varieties were Bussard (German Wheat variety) and 25 Indian hexaploid wheat genotypes namely C-306, C-591, CS, HD 2009, HD 2204, HD 2285, HIG 17, HUW 134, K 68, KS, Norin 10, Raj 3077, Sonalika, UP 262, UP 368, WH 147, WH 147M, WH 157, WH 283, WH 291, WH 331, WH 416, WH 533, WH 542, and WH 553. SDS-PAGE analysis for gluten proteins of these samples was conducted by extracting wheat flour (30mg) in 400 μ L of buffer (1M Tris-HCl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol and 1% bromophenol blue), heating the extract at 80°C for 30 min, cooling and mixing it with 30 μ L tracking dye

and then centrifuging it at 10000 rpm at 10 mA for 15 minutes to obtain the supernatant. The electrophoresis was carried out by loading 35 μ L of the obtained sample on a 12% running gel and 5% stacking gel (1.0 mm thick). The gel was run at 10-20 mA for the dye to reach the gel's bottom (Nimbal et al., 2010). Following electrophoresis, gel was separated and stained with Coomassie Brilliant Blue R-250 and destained for identification of the HMW glutenin subunits. The methods of Payne and Lawrence (1983) were used for identification and nomenclature of the high molecular weight glutenin subunits at the Glu-A1, Glu-B1 and Glu-D1 loci and for classification of each subunit or subunit pair according to their standard allele designations obtained on banding patterns in SDS-PAGE. Samples of these varieties were scored for High and Low molecular weight profiles of glutenin subunit patterns following (Rogers et al., 1989), and Glu-1 quality scores were calculated according to Payne et al. (1987) and Wang et al. (2022) by assigning a numerical quality value to each observed HMW glutenin subunit allele at Glu-A1, Glu-B1 and Glu-D1 based on their SDS-sedimentation associations and then adding the three locus specific values to obtain a single Glu-1 score for each variety. In the applied scoring system, the quality score values range from 1 to 10, where a score of 10 denotes the highest gluten and better break making quality, and 1 denotes the lowest quality associated with weak dough and lower bread-making quality. Similarity index analysis was performed on the Glu-1 score-based HMW-GS compositions and dendrogram were prepared using Genstat computer programme. The dendrogram was inferred as a representation of genetic similarity of glutenin composition and not the indicator of functional quality performance. Moreover, the low-molecular weight (LMW) glutenin bands were recorded for comprehensiveness of the protein profiles and were not used in quality scoring or clustering as it is exclusively based on HMW-GS composition due to their established role in Glu-1 quality assessment.

Results and Discussion

The documentation of bread-making quality of wheat is generally very intricate, but is mainly controlled by its protein quality and protein content (Khalid et al., 2023). Based on HMW glutenin subunit composition and SDS- sedimentation value known for each genotype, Glu-1 quality score was assigned to each genotype by method described previously (Payne et al., 1987; Omogbolahan et al., 2025) except for the genotype Raj-3077, having subunit 11+18.

A conservative provisional score of 2 was assigned based on its reported association with moderate dough strength in earlier studies, therefore it was excluded from main comparative readings to avoid bias in estimating Glu-1 quality score. Clustering strength was also not driven by this genotype (Table 1 & Table 2).

Genotypes Bussard, C-591, C-306 and WH-533 were found to have the highest Glu-1 quality score of 9. So, these genotypes are referred as genetically favorable for bread-making characters, followed by HD-2009, HD-2204, WH-147, WH-283 which depicted Glu-1 quality score of 8, while the Glu-1 quality scores of other varieties fluctuated from 3-6 (Table 3).

To provide contextual validation for the Glu-1 quality scores and clustering patterns observed in the present study, previously reported functional quality associations of major HMW-GS combinations were compiled from the literature (Table 4). This comparison helps interpret the predictive relevance of glutenin subunit compositions identified herein in the absence of direct rheological measurements.

The consistency between literature-reported functional performance and the Glu-1 quality scores observed in the present study supports the use of HMW-GS profiling as a reliable predictive and prescreening tool for bread-making quality in wheat breeding programs.

Previous studies identified subunits 5+10 (Glu-D1), 1 and 2* (Glu-A1), and 7+8 (Glu-B1) to be linked with superior quality attributes (Bhagwat & Bhatia, 1993; Ivanov et al., 1998). 13 allelic variations were reported by Zhang et al. (2001) at Glu-D1, having 1.5+10 and 5+12 subunits showing quality potential comparable to 5+10. In this study (Table 3), 1 and 2* subunits of Glu-A1, 7+8 and 7+9 subunits of Glu-B1, and 5+10 subunits of Glu-D1 were linked to excellent bread-making quality.

The missing bands for the Glu-D1 could be due to two main reasons i.e. either they are 4x or 2x, having in their pedigree like Nap Hal, where there is a null allele at Glu-D1. However, the impact of non-null Glu-A1 alleles on durum wheat quality is unclear, with some studies suggesting no significant effect, while others indicate improved gluten strength and extensibility. Previous studies reported recurrent presence of 7+8 and 7+9 HMW glutenin subunits at Glu-B1 in European groups (Sontag-Strohm, 1996; Igrejas et al., 1999) and spring wheat cultivars (Tohver et al., 2001). Moreover, a strong positive effect of the 5+10 allele at Glu-D1 on wheat quality was demonstrated by Lukow et al. in 1989, with

optimal combinations including 1 or 2* subunits in Glu-A1, 7+8 or 7+9 subunits in Glu-B1, and 5+10 subunits in Glu-D1 (Ivanov et al., 1998). Bread-making quality is principally determined by Glu-D1 (5+10), followed by Glu-A1 (1, 2*) alleles, while combinations like 5+10/2+12 are valuable in variable environments (Bedó et al., 1995).

Shitre et al. (2016) identified 10 alleles across loci (Glu-A1: null [48%], 1 [30%], 2* [22%]; Glu-B1: 17+18 [33%], 7+9 [27%], etc.; Glu-D1: 2+12 [60%], 5+10 [40%]), with quality scores ranging 4–10 (mean 6.95). Jang et al. (2021) found 22 HMW-GS alleles, with Glu-1 scores of 10 in 15.79% of genotypes featuring combinations like 2*/7+8/5+10. These subunit-HMW correlations enable SDS-PAGE-based screening for bread-making quality (Galova et al., 2002; Siddiqi et al., 2020).

Genetic similarity coefficients among the 26 genotypes ranged from 0.53 to 1.00. Cluster analysis (Fig. 1) revealed two major clusters: Cluster I (20 genotypes) with sub-clusters SG1a (4 genotypes) and SG1b (16 genotypes), and Cluster II (6 genotypes) with sub-clusters SG2a (4 genotypes) and SG2b (2 genotypes: C-306, C-591), indicating substantial genetic diversity.

This suggested that these two genotypes are closely related with each other. Variety C 591 developed in 1935 at Layalpur now in Pakistan (Pal, 1966) and C 306 developed in 1966 at Hisar, Haryana, India (Yunus and Srivastava, 1994) are suitable for low input conditions. They are still considered to be the premium wheats in view of being best quality wheats for chapati making. The genotypes Bussard (high input variety) and WH-533 (suitable for water deficit condition) both have a quality score of 9 that falls in sub-cluster 2a and show high similarity index and hence resemblance for HMWs, while the genotypes C-591 (Quality score 9) and Bussard had low resemblance and clustered separately. All these four genotypes (Bussard, C 306, C 591 and WH 533) possessed desirable combination of Glu-1D (5+10), as well as Glu-1A (1) and Glu-1B (7+9, 20). Keeping in view the genetic polymorphism for HMW and quality scores (Goel et al., 2015, Nuttall et al., 2017), it would be possible to realize improvement in wheat quality through recombination breeding *vis-à-vis* sustainable wheat production in target environments (high/low input, water deficit) to support export-oriented agriculture.

While Glu-1 quality scores are not substitutes for direct rheological measurements, they have been shown to explain 33–50% of variation in bread-making quality and remain essential for early-generation screening (Michel et al., 2018).

The present clustering reflects genetic similarity in glutenin composition rather than absolute functional performance. Therefore, the identified superior genotypes represent promising candidates for further phenotypic validation under controlled baking and rheological assays.

Conclusions

In the present study, the electrophoretic patterns of glutenin protein profiles and Glu-1 quality scores of 26 wheat genotypes revealed that genotypes Bussard, C-591, C-306, and WH-533, with the highest Glu-1 quality score of 9, associated with superior bread-making potential due to the presence of favorable HMW glutenin subunits such as 5+10, 1, and 2*. Other genotypes, including HD-2009, HD-2204, WH-147, and WH-283, with quality scores of 8, also associated with good bread-making quality. This study emphasizes the importance of HMW glutenin subunit composition in predicting the bread-making quality. The results emphasize the utility of Glu-1

quality scores as a reliable selection criterion for prescreening wheat cultivars in breeding programs aimed at improving bread-making quality. The observed variation in glutenin subunit composition and corresponding quality scores highlights the genetic diversity among wheat varieties, which can be leveraged for targeted breeding to enhance wheat processing quality and end-use performance. However, future studies integrating SDS-sedimentation, solvent retention capacity, and gluten index measurements will be essential to fully validate the quality potential indicated by glutenin subunit composition.

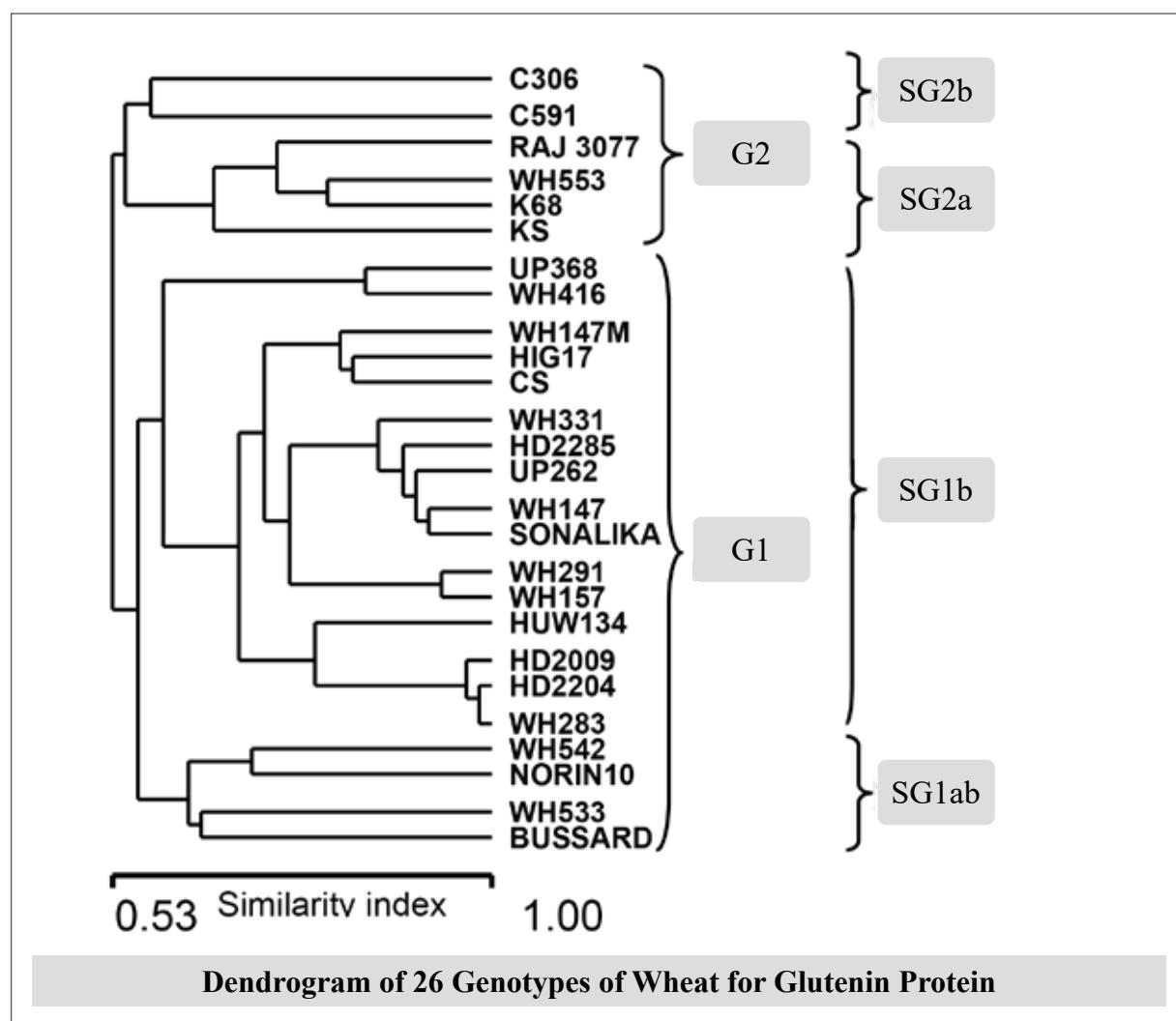


Figure 1. Dendrogram depicting genetic similarity among 26 wheat genotypes based on high molecular weight (HMW) glutenin subunit composition (Glu-1 loci).

Table 1. Profiles of high and low molecular weight glutenin protein subunits in bread wheat.

No.	Genotypes	Glu-A1	Glu-B1	Glu-D1
1	Bussard	1	7+9	5+10
2	C306	1	20	5+10
3	C591	1	20	5+10
4	CS	N	7+8	2+12
5	HD 2009	2*	7+8	2+12
6	HD 2204	2*	7+8	2+12
7	HD 2285	2*	7+8	
8	HIG 17	N	7+8	
9	HUW 134	N	7+8	2+12
10	K 68	N	17+18	
11	KS	N	17+18	2+12
12	Norin 10	N	7+9	2+12
13	Raj 3077	N	11+18	
14	Sonalika	2*	7+9	
15	UP 262	2*	7+8	
16	UP 368	2*	13+16	
17	WH 147	2*	7+8	2+12
18	WH 147M	N	7+8	
19	WH 157	2*	7+9	
20	WH 283	2*	7+8	
21	WH 291	2*	7	
22	WH 331	2*	7+8	
23	WH 416	2*	13+16	
24	WH 533	1	7+9	5+10
25	WH 542	N	7+9	
26	WH 553	N	17+18	

Table 3. HMW glutenin subunit composition and Glu-1 quality score.

No.	Genotype	Subunits	Total
1	Bussard	3 + 2 + 4	9
2	C-306	3 + 2 + 4	9
3	C-591	3 + 2 + 4	9
4	CS	1 + 3 + 2	6
5	HD 2009	3 + 3 + 2	8
6	HD 2204	3 + 3 + 2	8
7	HD 2285	3 + 3 + 0	6
8	HIG 17	1 + 3 + 0	4
9	HUW 134	1 + 3 + 2	6
10	K 68	1 + 3 + 0	4
11	KS	1 + 3 + 2	6
12	Norin 10	1 + 2 + 2	5
13	Raj 3077	1 + 2 + 0	3
14	Sonalika	3 + 2 + 0	5
15	UP 262	3 + 3 + 0	6
16	UP 368	3 + 3 + 0	6
17	WH 147	3 + 3 + 2	8
18	WH 147M	1 + 3 + 0	4
19	WH 157	3 + 2 + 0	5
20	WH 283	3 + 3 + 2	8
21	WH 291	3 + 1 + 0	4
22	WH 331	3 + 3 + 0	6
23	WH 416	3 + 3 + 0	6
24	WH 533	3 + 2 + 4	9
25	WH 542	1 + 2 + 0	3
26	WH 553	1 + 3 + 0	4

Table 2. SDS-sedimentation test-based bread-making quality scores allocated to HMW glutenin subunits (single and pairs).

Score	Glu-A1	Glu-B1	Glu-D1
4 (good)	-	-	5+10
3	1	17+18	-
3	2*	7+8	-
3	-	13+16	-
2	-	7+9	2+12
2	-	-	3+12
1 (poor)	Null	7	4+12
1	-	6+8	2 + 10
1	-	20	-

Table 4. Literature-reported functional quality associations of major HMW-GS combinations identified in this study

HMW-GS Combination	Reported SDS / Dough Strength	Literature Source
1 / 7+9 / 5+10	High	Payne et al., 1987; Lukow et al., 1989
2* / 7+8 / 2+12	Moderate	Bedó et al., 1995
Null / 7 / 2+12	Low	Jain et al., 2002

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Edible Chickpea (*Cicer arietinum* L.) Variety “TOLGA 01”

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ABSTRACT

Tolga 01 was developed and submitted for registration as a result of chickpea breeding studies carried out at the Eastern Mediterranean Agricultural Research Institute Directorate, Adana location; it was registered in 2024 with the name “Tolga 01” as a result of yield, Ascochyta blight tolerance and quality values in registration trials. As a result of chickpea registration yield trials established in different regions of Türkiye, the average yield of Tolga 01 chickpea variety was 247.9 kg/da, while the highest yield value was 395.2 kg/da grain yield. According to the results of the experiment, flowering period of the varieties was 61-154 days, plant height was 38-67 cm and hundred grain weight was 29.3-44.0 g. In terms of technological characteristics, protein ratio was determined in the range of 23.7-27.1%. The gradual seed production of our Tolga 01 edible chickpea variety, which was registered in 2024, will be planted as of 2025 and will be offered to the service of our farmers.

Keywords: Edible chickpea, yield, quality

Introduction

Among edible grain legumes, chickpea is the second most resistant to drought and low temperature after lentil. It is not very selective in terms of soil requirements. It is drought resistant thanks to its small vegetative parts, short development period and taproot system. The importance of chickpea plant in crop rotation increases the importance of its ability to utilize the free nitrogen of the air with *Rhizobium* bacteria in its roots. At the same time, in addition to these, the contribution of protein richness in eliminating the nutritional deficit makes the chickpea plant indispensable. Chickpea has an important place in Türkiye as a human food with its high protein content. It is inevitable to supply the food deficit in the world and in our country from different sources. Chickpea is a protein and vitamin-rich edible grain legume plant that contains 18-31% protein in its grain, as well as important essential amino acids such as leucine, alanine, lysine, isoleucine, methionine,

tryptophan, valine, elements such as K, P, Ca, Mg, S, Fe, Mn and vitamins such as A, B and C, which are the basic building blocks of the human body.

The data for chickpea in Türkiye for 2022 show a cultivation area of 456.480 ha, a production of 580,000 tons, and a grain yield of 1270.0 kg/ha per unit area (FAO, 2024). Chickpea is grown as a winter crop in the Mediterranean and Southeastern Anatolia regions. Chickpea plants to be grown as winter crops should be tolerant/resistant to Ascochyta blight. The most important biotic factor limiting the winter cultivation and yield of chickpea is *Ascochyta rabiei* (Pass) Labr, which causes Ascochyta blight. Ascochyta blight is a fungal disease. The development and rate of the disease varies according to climatic conditions; it occurs mostly in rainy, hot weather with high relative humidity. Especially rain is an important factor in the spread of the disease. For this reason, it is very important that the varieties are tolerant/resistant to diseases and pests in breeding. Our aim in

breeding studies is to identify chickpea varieties with high yield, high market value, good quality values and tolerant/resistant to Ascochyta blight. Tolga 01 Chickpea variety is an edible grain legume chickpea variety registered for this purpose.

Materials and Methods

Our material sources in our edible grain legume breeding studies; We provide our materials from material sharing within the scope of the national project, The International Center for Agricultural Research in the Dry Areas (ICARDA) material exchange programs, new variations created from our own hybridization programs or local varieties.

Tolga 01 chickpea variety is a variety developed by selection method. Tolga 01 Chickpea (*Cicer arietinum* L.) variety was registered by the Eastern Mediterranean Agricultural Research Institute in 2024, suitable for winter cultivation in the Mediterranean, Aegean and Southeastern Regions and summer cultivation in other regions. Tolga 01 edible chickpea variety was bred from ICARDA origin (FLIP 09 186C) materials by using Introduction breeding method from breeding methods; in 2021 and was registered in 2024 with the variety name "Tolga 01" and offered to the service of farmers.

Results and Discussion

Grain yield is the most important breeding objective in edible grain legumes as in other cultivated plants; in addition, grain size is also a highly demanded trait in chickpea breeding. However, due to the negative correlation between grain yield and grain size and between grain size and Ascochyta blight, the optimum grain size should be determined very carefully according to the regional conditions.

As a result of the two-year multi-location registration trials carried out, the findings obtained with the "Tolga 01" chickpea variety were determined by the Seed Registration Office (Anonymous, 2024). Biological characteristics of Tolga 01 chickpea variety vary between 61-154 days for flowering and 107-196 days for physiological maturity. The cultivation method is suitable for winter cultivation. Morphological characteristics; plant height 38-67 cm, first pod height 19-42 cm, plant growth form is semi-erect; it is a variety suitable for machine harvesting. Plant grain characteristics 100 grain weight is 29.3-44.0 g, grain color is beige, grain shape is angular round (Figure 1). Technological characteristics of Tolga 01 chickpea variety were determined as water absorption capacity 0.39-0.47 g/grain; swelling capacity 0.36-0.46 ml/grain; water absorption index

1.12-1.25%; swelling index 2.44-2.57%; sieve values 1.6-24.6% for 9 mm sieve; 14.9-58.2% for 8 mm sieve; protein rate 23.7-27.1%.

Grain yield value of Tolga 01 chickpea variety was determined to be 247.9 kg/da on average, the highest yield value was 395.2 kg/da and it was determined to be tolerant for Ascochyta blight. Cooking time for cooking showed a cooking value between 37-43 minutes.

Conclusions

Improving chickpea agriculture in our country through chickpea breeding studies, increasing cultivation areas, reducing fallow areas by taking chickpea into crop rotation in fallow areas and supporting sustainable agriculture are important for the country's agriculture and our future.

The introduction of new registered varieties such as "Tolga 01" chickpea variety and chickpea varieties that are suitable for winter and summer cultivation, high yielding, suitable for machine harvesting, high quality, tolerant/resistant to diseases and pests, high market value, will carry chickpea agriculture forward.

Tolga 01
Chickpea (*Cicer arietinum* L.)



Registration year	2024	
Place and year of breeding	Adana - 2021	
The organization that owns the variety	The Eastern Mediterranean Agricultural Research Institute Directorate-Adana/Türkiye	
Breeding organization	Eastern Mediterranean Agricultural Research Institute Directorate	
Breeding method	Introduction	
Biological properties	Number of days to flowering	61-154 days
	Number of days to Physiological maturity	107-196 days
Morphological features	Plant height(cm)	38-67
	First pod height(cm)	19-42
	Plant growth form	Semi erect
	Cultivation method	Winter sowing
Grain properties	Hundred seed weight(g)	29.3-44.0
	Grain color	Beige
	Grain shape	Round to angular
Technological features	Water absorption capacity (g/grain)	0.39-0.47
	Swelling capacity (ml/grain)	0.36-0.46
	Water absorption index (%)	1.12-1.25
	Swelling index (%)	2.44-2.57
	Cooking time (min.)	37-43
	Protein rate (%)	23.7-27.1
	Sieve values(%)	9 mm----1.6-24.6 8 mm----14.9-58.2
Agricultural properties	In registration trials; Average yield (kg/da)	
	247.9 kg/da Highest yield (kg/da)	
Places where registration trials are carried out	Diyarbakır, Adana, Manisa, Şanlıurfa, Kahramanmaraş	

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Oil Sunflower (*Helianthus annuus* L.) Variety “1931 CL”

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ABSTRACT

As a result of the sunflower breeding studies carried out at the Trakya Agricultural Research Institute Directorate, Edirne location, the sunflower variety was developed and submitted for registration. In the registration trials, yield, oil rate, oil yield, imazamox (40g/l) resistant and morphological observations as a result, it qualified to be a variety and was registered in 2024 with the name “1931 CL”. As a result of sunflower registration yield trials established in different regions of Türkiye, the average yield of “1931 CL” variety was 2306 kg/ha, while the highest yield value was 3486 kg/ha grain yield. According to the results of the experiment, flowering period of the varieties was 54-74 days, physiological maturity 88-106 days, plant height was 143-186 cm, and head diameter was determined in the range of 13-20,3 cm. In terms of technological characteristics, oil ratio was determined in the range of 36-42,4%. In phytotoxicity observations, although the plants were light green (2) and yellow green (3) when the first application was done one week after the first application, it was observed that the harmful effect of imazamox (40 g/l) completely disappeared in the second week. Certified seed production of our “1931 CL” sunflower variety; which was registered in 2024, was produced as of 2025 and offered to our farmers.

Keywords: Sunflower, yield, oil rate, imazamox

Introduction

Sunflower (*Helianthus annuus* L.) belongs to the *Helianthus* genus of the Asteraceae family, which includes 51 species and 19 subspecies. A large portion of *Helianthus* species are ornamental plants. The agriculturally important varieties are *Helianthus annuus* L. (sunflower) and *Helianthus tuberosus* (Jerusalem artichoke) (Meral, 2019). Sunflower species have a basic chromosome number of n=17, and are found in diploid, tetraploid, and hexaploid structures and those containing more than two ploidy levels (Arioglu, 2007).

Sunflower, the most widely cultivated oilseed crop in our country, is one of the most important oilseed plants. Due to its wide adaptability, it is grown in many parts of our country under both irrigated and dryland conditions. However, there are many biotic and abiotic factors that affect yield and quality characteristics in

its cultivation. The most important biotic factors are disease, weeds, and the parasitic plant *Orobanche* (*Orobanche cumana*).

Our aim in breeding studies is to determine high yielding, high market value, good quality, IMI group herbicides tolerant/resistant varieties or variety candidates of sunflower. 1931 CL sunflower variety is an hybrid IMI group herbicides tolerance variety registered for this purpose.

Materials and Methods

1931 CL sunflower (*Helianthus annuus*) variety is a hybrid which developed by hybridization method and it is an outcome of the national sunflower breeding projects conducted by Trakya Agricultural Research Institute (TARI). 1931 CL sunflower variety was registered by TARI and due to its high adaptations

to the different conditions across Türkiye. 1931 CL sunflower variety was bred from IMI 1091 A (CMS line) and IMI 540 R (Restorer line) materials in 2019 by using hybridization breeding method. It was registered as the variety name "1931 CL" in 2024 and offered to farmers. IMI 1091 A is a high oleic type, downy mildew-tolerant, cytoplasmic male sterile line (CMS). IMI 540 R (Restorer line) is linoleic type and tolerant to broomrape and downy mildew. Both lines were developed within the national plant breeding project of TARI.

Field trials were conducted in eight different locations in 2022 and 2023. Experimental design and agricultural practices were applied according to the instructions created by Variety Registration and Seed Certification Center Directorate (TTSMS, 2001). Randomized complete block design was used with four replications and four rows. Rows were 7.5 m long, and plant spacing was 70 × 30 cm. Total planting area of each plots was 21 m². Two rows of each plot located in the middle were harvested and total harvesting area was 9,66 m². There were no irrigations in all locations, 8 kg/da N and 5 kg/da P₂O₅ was used for fertilization. In the trials, imazamox (40g/l) application dose was 1,25 L/ha and it applied at the 4-6 or 6-8 leaves stage of sunflower. After the application, first phytotoxicity observations were taken one week later, and the second took place two weeks later. A 1-9 scale was used for observations. 1-9 scale: 1 = no damage, 2 = light green, 3 = yellow-green, 4 = yellow, 5 = reduced growth, 6 = some plants with deformities, 7 = many plants with deformities, 8 = some dead plants, 9 = all plants dead.

Resistance to broomrape and downy mildew were evaluated at TARI. The resistance to broomrape was evaluated in pots artificially infected soil. Broomrape seeds were collected from several locations in the Thrace region. In the climate chamber, each plastic cups contained different sunflower genotypes and mixed soil with broomrape seeds (1-2 g). 35 days after planting, the plants in cups were removed, the roots were washed, the tubers of the rootstock were counted and the degree of resistance was determined by measuring frequency of infection, intensity of infection and levels of aggression. Genotypes were evaluated as susceptible, tolerant and resistant according to results (Evci et al., 2011a). In terms of downy mildew, inoculation method was also used. Seeds were germinated in climate chamber for 2 days at 26°C. Germinated seeds (with 0,5-1 cm rootlets) were infected by the bulk races of downy mildew collected from the region in climate chamber (15°C, 60 % moisture, 12h/12h and 1 week). Infected plants were observed and scored according to the sporulation on the plants (Evci et al., 2011b).

Analysis of variance for each locations and combined analysis of variance across locations were done by using SAS 9.0. software. Least Significant Difference (LSD) test at 5% probability level was used for the comparison of hybrids performances.

Results and Discussion

In the registration trials, due to the data of yield, oil rate, oil yield, imazamox (40g/l) resistance and morphological observations, 1931 CL was qualified to be a variety and was registered in 2024 with the name "1931 CL". The average yield of "1931 CL" variety was 2306 kg/ha, while the highest yield value was 3486 kg/ha grain yield. Average yield across the locations (Table 4) shows that 1931 CL is ranked 4th and 2.5% behind the average of standard varieties (2365 kg/ha). It is also not significantly different than two of the check varieties. Grain yield was reported as 639-4247 kg/ha by Kaya et al. (2009), 325 kg/ha-1352 kg/ha by Kılıç (2010), 438 kg/ha-3569 kg/ha by Öz et al. (2011), and 1438-1938 kg/ha by Cabar (2024). Yılmaz and Kınay (2015) explained that the different values found in grain yield vary depending on the variety characteristics, environmental conditions, and cultivation technique. Our findings are similar to those obtained in different studies.

When the Table 5 is examined in terms of oil content, the 1931 CL variety was found to have the same oil content as the LG 5542 CL standard variety in the Tekirdağ-Muratlı location. It had a higher oil content than the same standard variety in the Edirne-Sarayakpınar location and the TR 2242 CL standard variety in the Edirne-Havsa location. When the overall averages are examined, it is in the same statistical group as the LG 5542 CL standard variety. In terms of oil yield during the 2022 (Table 5) production season, the 1931 CL variety was found to have a higher oil yield than the TR 2242 CL standard variety in the Tekirdağ-Muratlı and Tekirdağ-Ergene locations, as well as in the Edirne-Sarayakpınar and Edirne-Havsa locations. Furthermore, it had a higher oil yield than the LG 5542 CL standard variety in the Edirne-Havsa and Edirne-Center locations. Considering the overall average, it was in the same statistical group as the LG 5542 CL and TR 2242 CL standard varieties. In the 2023 production season (Table 6), the 1931 CL variety was found to have a higher oil content than the LG 5542 CL standard variety in the Edirne-Central location. When the overall averages were examined, it was in the same statistical group as the LG 5542 CL standard variety. 1931 CL variety has a higher oil yield than the Başaran CL and LG 5542 CL standard varieties in the Edirne-Central location (Table 6). Considering

the overall averages, the 1931 CL variety was in the same statistical group as the standard varieties P64LC108, LG 5542 CL, and TR 2242 CL. Kaya et al. (2009) reported oil content as 38.1%-53.4%, Kılıç (2010) as 41.2%-48.3%, Öz et al. (2011) as 36.3%-37.6%, Poyraz (2012) as 40.36%-45.05%, and Cabar (2024) as 33.0%-43.9%. The varying values found in oil content measurements can be attributed to the fact that oil content is a quantitative characteristic. In sunflowers, oil content varies depending on climate and soil structure, variety/line characteristics, harvest maturity time, and cultural practices (Kılıç, 2010).

According to the results of the experiment, flowering period of the varieties was 54-74 days, physiological maturity 88-106 days, plant height was 143-186 cm, and head diameter was determined in the range of 13-20,3 cm. In terms of technological characteristics, oil ratio was determined in the range of 36-42,4%. In different studies, the flowering period of the varieties was 59-86 days according to Kaya et al. (2009), 59.2-70.0 Kılıç (2010) and 55.8-64.3 Cabar (2024); the number of days to physiological maturity was days according to 94.0-107.7 Kılıç (2010), 87.8-89.9 Poyraz (2012) and 95.5-106.8 days according to Cabar (2024). The differing values observed in the number of days to flowering period and physiological maturity are thought to be due to the climate during the plant's growing period, the genetic characteristics of the varieties, and ecological differences. Plant height was reported as 108.7-177.5 cm Kılıç (2010), 145.0-158.3 cm Poyraz (2012), and 110.0-152.2 cm by Cabar (2024). Kaya et al. (2009) reported that they found the head diameter to be Kaya et al. (2009) 10-24 cm, Kılıç (2010) 12.2-20.7 cm, Poyraz (2012) 11.6-19.7 cm, and Cabar (2024) 12.5-14.7 cm. Cabar (2024) reported that the different values found in head diameter measurements plant height may be due to climatic characteristics during the plant's growing period, planting density, soil structure, cultural practices, genetic characteristics of varieties, and ecological differences.

In phytotoxicity observations (Table 7, Table 8), although the plants were light green (2) and yellow green (3) when the first application was done one week after the first application, it was observed that the harmful effect of imazamox (40 g/l) completely disappeared in the second week.

Conclusions

Despite sunflower is the most widely cultivated, produced, and consumed oilseed crop in Türkiye, domestic seed companies have not yet achieved a comparable level of capacity in seed production and cultivar breeding. Trakya Agricultural Research Institute, operating under the Ministry of Agriculture and Forestry, serves as the national coordinating institute for sunflower breeding and cultivation activities in Türkiye. Owing to its long-established hybrid breeding program, variety development and registration processes have progressed efficiently over the years. The registered sunflower variety 1931 CL is one of the outcomes of this breeding program. Through this variety, the Institute has reached not only sunflower growers in the Trakya region but also farmers in various sunflower-producing areas across Türkiye. In recent years, the cultivated area of sunflower varieties developed by the Institute has shown a consistent increase.



Figure 1. 1931 CL Sunflower (*Helianthus annuus* L.) (Original).

Table 1. 1931 CL some biological, morphological and technological characters.

Registration year	2024
Place and year of breeding	Edirne-2019
The organization that owns the variety	The Trakya Agricultural Research Institute Directorate Edirne, Türkiye
Breeding organization	Trakya Agricultural Research Institute Directorate
Breeding method	Pedigree
Biological properties	Number of days to flowering 65-74 Number of days to physiological maturity 88-106 Mildew resistant High tolerance Broomrape resistant High tolerance Plant height (cm) 145-186 Head diameter (cm) 16-20 Self pollination (1-5)* 4-5 Head center seed filling (1-5)** 4-5 Uniformity (1-5)*** 1-2 Thousand seed weight(g) 32,8-52,8 Hectoliter weight (g/lt) 363-424,5 Oil rate (%) 40-42
Morphological features	In registration trials; Average yield (kg/ha) 2300 Highest yield (kg/ha) 3490
Technological features	Yes Imazamox (40 g/l)
Agricultural properties	Tekirdağ (Muratlı, Ergene) 2 location Edirne (Center, Hasköy, Havsa, Saraykapınar) 4 location Kırklareli (Babaeski, Ahmetbey) 2 location
Herbicide resistance	
Places where registration trials are carried out	

(*) 1... very weak 2. weak 3. medium 4. good 5... very good.

(**) 1... wide space 5... narrow space.

(***) 1 = very uniform 2 = uniform 3 = medium 4 = heterogeneous 5 = very heterogeneous.

Table 2. Yield Results of 2022 IMI Group Sunflower Agricultural Values Measurement Trials (kg/ha).

Varieties	Tekirdağ (Muratlı)	Tekirdağ (Ergene)	Edirne (Hasköy)	Edirne (S.akpınar)	Edirne (Havsa)	Edirne (Merkez)	Average
1- P64LC108 (c)	3152 ab	2573 a	2572 ab	3977 a	2196 c	2526	2833 a
2- LG 5542 CL (c)	3131 ab	2243 b	2566 ab	3948 ab	2376 abc	2375	2773 ab
3- Başaran CL (c)	2709 c	2031 cd	2758 a	3731 abc	2333 bc	2713	2713 ab
4-Sy Paladium	2807 abc	2190 bc	2309 b	3464 bc	2387 abc	2704	2643 bc
5- 1931 CL	2753 bc	1886 de	2510 ab	3486 abc	2609 ab	2602	2641 bc
6- 1916 CL	2685 c	1839 de	2276 b	3325 c	2702 a	2375	2534 cd
7- SUN 2259 CL	2734 c	1945 d	2318 b	3532 abc	2136 c	2540	2534 cd
8- TR 2242 CL	2504 c	1721 e	2381 ab	3304 c	2401 abc	2609	2487 d
F	*	**	*	*	*	ns	**
CV (%)	9.4	6.5	10.8	9.5	10.0	8.4	9.5
LSD (kg)	389	197	389	501	352	-	143

*: p<0.05 level, **: p<0.01 level, ns: not significant

Note 1: Values are taken from Variety Registration and Seed Certification Center Directorate, 2024 IMI type sunflower variety registration report (TTSM, 2024). LSD value of Edirne Merkez is absent because of F test were found not significant.

Table 3. Yield Results of 2023 IMI Group Sunflower Agricultural Values Measurement Trials (kg/ha).

Varieties	Kırklareli (Babaeski)	Edirne (Merkez)	Kırklareli (Ahmetbey)	Average
1- LG 5542 CL (c)	2631	1395	1194	1740
2- P64LC108 (c)	2490	1432	1285	1736
3- TR 2242 CL (c)	2459	1384	1123	1656
4- Başaran CL (c)	2580	1093	1244	1639
5- 1931 CL	2275	1443	1192	1636
6- Hysun 180 IT	2525	1089	1040	1551
7- 1916 CL	2222	1297	1054	1524
8- Acsun	2416	1077	1050	1514
F	ns	ns	ns	ns
CV (%)	11,1	17,8	16,8	14,3
LSD (kg)	-	-	-	-

ns: not significant

Note 1: Values are taken from Variety Registration and Seed Certification Center Directorate, 2024 IMI type sunflower variety registration report (TTSM, 2024). LSD values are absent because of F tests of each location were found not significant.

Table 4. Yield Results of 2022-2023 IMI Group Sunflower Agricultural Values Measurement Trials (kg/ha).

Varieties	Tekirdağ		Kırklareli				Edirne			Average
	Ergene	Muratlı	Babaeski	Ahmetbey	Merkez	Havsa	S.akpınar	Hasköy		
	2022	2023	2023	2023	2022	2023	2022	2022	2022	
1-P64LC108 (c)	2573	3152	2490	1285	2526	1432	2196	3977	2572	2467 a
2-LG 5542 CL (c)	2243	3131	2631	1194	2375	1395	2376	3948	2566	2429 a
3-Başaran CL (c)	2031	2709	2580	1244	2713	1093	2333	3731	2758	2355 ab
4-TR 2242 CL (c)	1721	2504	2459	1123	2609	1384	2401	3304	2381	2210 c
5-1931 CL	1886	2753	2275	1192	2602	1443	2609	3486	2510	2306 bc
6-1916 CL	1839	2685	2222	1054	2375	1297	2702	3325	2276	2197 c
F										**
CV (%)										10.7
LSD (kg)										116

**: p<0.01 level

Note 1: Values are taken from Variety Registration and Seed Certification Center Directorate, 2024 IMI type sunflower variety registration report (TTSM, 2024).

Table 5. Oil Rate (%) and Oil Yield (kg/ha) Results of 2022 IMI Group Sunflower Agricultural Values Measurement Trials.

Varieties	Tekirdağ (Muratlı, Kırkkepenekli)	Tekirdağ (Ergene, Vakıflar)	Edirne (Hasköy)	Edirne (Sarayakpi- nar)	Edirne (Havsa, Habiller)	Edirne (Merkez)	Average	Oil Rank							
	Oil Rate (%)	Oil Yield (kg/ha)	Oil Rate (%)	Oil Yield (kg/ha)	Oil Rate (%)	Oil Yield (kg/ha)	Oil Rate (%)								
Başaran CL (st)	44.3	1200	41.4	841	48.7	1343	49.4	1843	49.4	1153	46.5	1262	46.6 a	1274 a	1
P64LC108 (st)	41.1	1295	41.4	1065	45.2	1163	41.2	1639	47.3	1039	44.5	1124	43.5 b	1221 a	2
LG 5542 CL (st)	37.8	1184	38.4	860	42.9	1101	40.2	1587	41.8	993	40.1	952	40.2 cd	1113 b	3
TR 2242 CL (st)	40.8	1022	37.4	643	45.6	1086	42.3	1398	39.4	946	42.9	1119	41.4 c	1036 b	8
SY PALADIUM	37.9	1064	37.0	809	43.4	1002	41.1	1424	43.1	1029	38.9	1052	40.2 cd	1063 b	4
SUN 2259 CL	39.8	1088	37.4	726	43.0	997	43.0	1519	44.5	951	42.7	1085	41.7 c	1061 b	5
1931 CL	37.8	1041	36.0	679	42.4	1064	40.6	1415	40.7	1062	40.0	1041	39.6 d	1050 b	6
1916 CL	39.4	1058	39.7	729	42.8	974	42.1	1400	40.4	1092	41.3	981	40.9 cd	1039 b	7
F									**	**					
CV %													3.4	7.1	
LSD													1.7	92	

**: p<0.01 level

Note 1: Values are taken from Variety Registration and Seed Certification Center Directorate, 2024 IMI type sunflower variety registration report (TTSM, 2024).

Table 6. Oil Rate (%) and Oil Yield (kg/ha) Results of 2023 IMI Group Sunflower Agricultural Values Measurement Trials.

Varieties	Kırklareli (Babaeski)		Edirne (Merkez)		Kırklareli (Ahmetbey)		Average		Oil Rank
	Oil Rate (%)	Oil Yield (kg/ha)	Oil Rate (%)	Oil Rate (%)	Oil Rate (%)	Oil Rate (%)	Oil Rate (%)	Oil Yield (kg/ha)	
Başaran CL (st)	45.3	1169	47.0	51.3	45.5	56.6	45.9 a	749 a	1
P64LC108 (st)	43.8	1091	41.6	59.6	41.5	53.3	42.3 bc	740 ab	2
LG 5542 CL (st)	41.6	1094	38.1	53.2	43.5	51.9	41.1 bcd	715 ab	3
TR 2242CL (st)	42.6	1048	40.4	55.9	44.8	50.3	42.6 b	703 abc	4
Acsun	44.4	1073	47.5	51.2	45.6	47.9	45.8 a	688 abc	5
1931 CL	38.6	878	38.3	55.2	41.1	49.0	39.3 d	640 bc	6
Hysun 180 IT	40.7	1028	39.3	42.8	43.4	45.1	41.1 bcd	636 bc	7
1916 CL	39.1	869	39.2	50.9	40.8	43.0	39.7 cd	603 c	8
F							**	ns	
CV %							3.8	8.7	
LSD							2.8	105	

**: p<0.01 level, ns: not significant

Note 1: Values are taken from Variety Registration and Seed Certification Center Directorate, 2024 IMI type sunflower variety registration report (TTSM, 2024).

Table 7. Phytotoxicity Effects of Imidazolinone Group Herbicide-Resistant Sunflower Varieties: 2022 Observation Results (4-8 Leaf Stage).

	Plant Date: 12.05.2022		Herbicide Application: 14.06.2022						
	Varieties 1250 cc/ha								
Tekirdağ (Murath - Kırkkepenekli)		1 st Week Observation: 21.06.2022							
		Varieties 1250 cc/ha							
		LG 5542 CL (st)	SUN 2259 CL	P64LC108 (st)	TR 2242 CL (st)	Başaran CL (st)	SY PALADIUM	1931 CL	1916 CL
	A	3	3	4	3	3	2	3	3
	B	3	3	4	3	3	2	3	3
	C	3	3	4	3	3	2	3	3
	D	3	3	4	3	3	2	3	3
2 nd Week Observation: 28.06.2022		Varieties 1250 cc/ha							
	A	1	1	2	1	1	1	1	1
	B	1	1	2	1	1	1	1	1
	C	1	1	2	1	1	1	1	1
	D	1	1	2	1	1	1	1	1
Edirne (Sarayakpınar)		Plant Date: 31.03.2022		Herbicide Application: 06.05.2022					
		Varieties 1250 cc/ha							
		1 st Week Observation: 13.05.2022							
		Varieties 1250 cc/ha							
	A	2	3	3	2	2	3	2	2
	B	3	2	2	2	3	2	2	3
	C	2	2	2	2	2	2	3	3
	D	3	2	2	3	3	2	3	2
2 nd Week Observation: 20.05.2022		Varieties 1250 cc/ha							
	A	1	2	2	2	1	2	1	1
	B	2	1	1	1	2	1	1	2
	C	1	1	1	1	1	1	2	2
	D	1	1	1	2	2	2	2	1

1-9 scale: 1 = no damage, 2 = light green, 3 = yellow-green, 4 = yellow, 5 = reduced growth, 6 = some plants with deformities, 7 = many plants with deformities, 8 = some dead plants, 9 = all plants dead

Note 1: Values are taken from Variety Registration and Seed Certification Center Directorate, 2024 IMI type sunflower variety registration report (TTSM, 2024).

Table 8. Phytotoxicity Effects of Imidazolinone Group Herbicide-Resistant Sunflower Varieties: 2023 Observation Results (4-8 Leaf Stage).

Kırklareli (Babaeski)	Plant Date: 16.05.2023		Herbicide Application: 09.06.2023						
	Varieties 1250 cc/ha							Hysun 180 IT	TR 2242CL (st)
1 st Week Observation: 16.06.2023		Başaran CL (st)	1931 CL	1916 CL	P64LC108 (st)	Acsun	LG 5542 CL (st)		
A	3	3	3	3	3	3	3	3	2
B	3	2	2	2	2	2	2	2	2
C	2	3	2	2	2	3	2	2	3
D	2	2	3	2	3	2	3	3	2
2 nd Week Observation: 23.06.2023		Varieties 1250 cc/ha							
A	1	2	2	1	1	1	1	2	1
B	2	1	1	1	1	1	1	1	1
C	1	1	1	1	1	2	1	1	1
D	1	1	1	1	1	1	1	1	1
Kırklareli (Ahmetbey)	Plant Date: 16.05.2023	Herbicide Application: 17.06.2023							
1 st Week Observation: 24.06.2023		Varieties 1250 cc/ha							
A	1	1	1	1	1	2	2	2	1
B	1	2	1	1	1	2	2	2	1
C	1	1	2	1	1	2	2	2	1
D	1	1	2	1	2	2	2	2	1
2 nd Week Observation: 31.06.2023		Varieties 1250 cc/ha							
A	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1

1-9 scale: 1 = no damage, 2 = light green, 3 = yellow-green, 4 = yellow, 5 = reduced growth, 6 = some plants with deformities, 7 = many plants with deformities, 8 = some dead plants, 9 = all plants dead

Note 1: Values are taken from Variety Registration and Seed Certification Center Directorate, 2024 IMI type sunflower variety registration report (TTSM, 2024).

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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 rabiei (Pass.) Labr.], yield and yield criteria. Turk J Agric For 27: 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.). Euphytica doi: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.

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Dissertation (Thesis):

Yasar M (2012). Penetrance and expressivity of double podding characteristic in chickpea (*Cicer arietinum* L.). Dissertation, Akdeniz University, Antalya.

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Abbreviations

Abbreviations should be defined at first mention and used consistently.



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