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Adakale Street, No.: 22/12 Kızılay, 06420 Cankaya/Ankara - TURKEY
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Status of Durum Wheat (*T. durum* Desf.) Genetic Resources in the Southeastern Anatolia from Past to Present

İrfan ÖZBERK[1] **Fethiye ÖZBERK**[2*]

[1] Department of Field Crops, Faculty of Agriculture, The University of Harran, Şanlıurfa, Türkiye
[2] Organic Farming Program, Akcakale Vocational Collage, The University of Harran, Şanlıurfa, Türkiye

[*] Corresponding author e-mail: ozberki@harran.edu.tr

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ABSTRACT

Agriculture started to evolve in Anatolia about 10.000 years ago. Genetic diversity of crops plants with their wild relatives and center of domestication of durum wheat were always interested in by scientists. The connection between molecular markers such as AFLP (amplified fragment length polymorphism) and domestication geography showed that the Karacadağ mountain in the Southeast Anatolia was pointed out the origin of domesticated einkorn (*Triticum monococcum*) and emmer (*T. dicoccum*). Durum wheat spread out from the Fertile Crescent and through southern Europe, reaching North Africa around 7000 BC. More recently, 17 *Aegilops* and 11 *Triticum* species or sub species including *T. aestivum* and *T. durum* were defined under both geneses in Türkiye. Twenty-five of them were wild relatives. Wheat landraces are composed of traditional crop varieties developed by farmers through years of natural and human selection. There have been several collection missions for wheat landraces. Durum wheat landraces were mostly grown until 1960 in Türkiye. They used to be called as 'sarı buğday'. The acreage of the landraces grown in Türkiye is about 0.55 million ha. A survey held more recently proved the presence 162 names of wheat landraces in Türkiye. Many beneficial traits such as drought and cold tolerance and high grain quality were detected and tried to be exploited in modern breeding programs. Farmers have access to modern cultivars but keep their landraces. The main reason for maintaining landraces is satisfaction with the landraces' performance under poor input conditions. Tens of landraces are still grown in Türkiye. Unless additional measures are taken, landraces will disappear gradually.

Keywords: Historical evolution, wild relatives, landraces, present and future perspectives.

This review: From Chapter 2 : Özberk F and Özberk I, (2021). Wheat Landraces in Mesopotamia. Wheat Landraces, 13-34, Edts: Zencirci N, Baloch FS, Habyarimana E and Chung G, (Eds.), (2021). Wheat Landraces. Springer International Publishing

Historical background

The information gathered from several excavations suggests that the agriculture started to evolve in Anatolia almost 10.000 years ago. Anatolia hosted many civilizations in the past and was the pathway between Asia and Europe in the history (Harlan 1995; Zeist et al., 1995; Karagöz et al., 2010). Recent excavations in Göbeklitepe of Sanliurfa province have a potential to shed light on the periods prior to known date of agriculture (Killian et al., 2010). For more than two decades, the use of molecular markers has been providing new information on genetic diversity of crop plants in relation to wild relatives, centers of domestication, time frame of the domestication process and specific alleles supporting domesticated traits. The connection between molecular markers and domestication geography took root in the paper by Heun et al., (1997) who found that based on AFLP (amplified fragment length polymorphism) markers, the closest wild relatives of domesticated

einkorn (*Triticum monococcum*, diploid) occur in a very restricted area within the Karacadag mountain range in south-eastern Türkiye (Fig.1). From that they concluded that this represents the site were humans first domesticated einkorn. Important contributions using different molecular markers for other species followed: einkorn (Killian et al., 2007); emmer (Ozkan et al., 2002; Ozkan et al., 2005; Mori et al., 2003; Luo et al., 2007).

Archaeological evidence verifies the occurrence of plant remains at different excavation sites, in different strati graphic layers that were analyzed, and radiocarbon dated (Hillman, 2000) from which a generally consistent picture emerges indicating that western agriculture originated in the Fertile Crescent after the last ice age, in aceramic Pre-Pottery Neolithic (PPN) from about 12,000 to 9,500 years ago (Zohary and Hopf 2000; Nesbitt 2002; Salamini et al., 2002). It is now widely held that Fertile Crescent agriculture originated in a "core area" in south-eastern Türkiye to northern Syria (Fig. 1), where the distribution of wild forms (Fig. 2).

Several issues concerning geography and domestication of wild emmer wheat were recently reviewed by (Ozkan, 2011). The authors considered published molecular and archaeological data and re-analyzed the data of (Ozkan et al., 2005). Wild emmer was probably domesticated in south-eastern Türkiye (Ozkan et al., 2002; Ozkan et al., 2005; Mori et al., 2003; Luo et al., 2007; Jaradat, 2013).

A reconsideration of the domestication geography of tetraploid wheats has been considered by (Ozkan et al., 2005) and (Luo et al., 2007). Phylogenetic analysis indicates that two different races of *T. dicoccoides* exist, the western one, colonizing Israel, Syria, Lebanon, and Jordan, and the central-eastern one, which has been frequently sampled in Türkiye and rarely in Iraq and Iran. It is the central-eastern race that has played the role of the progenitor of the domesticated germplasm. This is supported by the results from the collections of (Ozkan et al., 2002; Mori et al., 2003; Luo et al., 2007). A disagreement is nevertheless appearing at the local geographical scale: the chloroplast DNA data indicate the Kartal mountains at the western border of the "core area" (Abbo et al., 2006), while AFLP finger printing points to the Karacadag range as the putative site of tetraploid wheat domestication. From this area, emmer expanded across Asia, Europe, and Africa (Ozkan et al., 2005). South-western expansion of domesticated emmer generated sympatry with the southern populations of *T. dicoccoides* and the rise of a secondary diversity center (Luo et al., 2007).

Durum wheat (*T. turgidum* spp. *durum*) has been of great historical significance, because it provided a range of sub-species that were cultivated widely across the globe for thousands of years (Feuillet et al., 2007). Durum wheat spread out from the Fertile Crescent and through southern Europe, reaching North Africa around 7000 BC (Feldman, 2001). It came into cultivation originally in the Damascus basin in southern Syria about 9800 BC Zohary and Hopf (2000). A second route of migration occurred through North Africa during the Middle Ages (Moragues et al., 2006). Geographical expansion of durum wheat was intimately associated with human migrations. It is cultivated mainly in the marginal areas of Mediterranean region, Southern Europe, and North Africa, while more recently it has started to expand to Southern Asia (Baloch et al., 2017)

Wild relatives of wheat in Türkiye

Kimber and Feldman (1987) indicated the presence of 25 wide relative species in Türkiye. More recently, 17 *Aegilops* and 11 *Triticum* species or sub species including *T. aestivum* and *T. durum* were defined under both genera (Cabi 2010). Subspecies under *Aegilops* genus Waines and Barnhart (1992) are *Aegilops biuncialis* Vis., *Aegilops markgraffii* (Greuter) Hammer, *Aegilops columnaris* Zhuk, *Aegilops comosa* Sm. in Sibth. &Sm, *Aegilops crassa* Boiss., *Aegilops cylindrica* Host, *Aegilops geniculata* Roth, *Aegilop juvenalis* (Thell.) *Eig, Aegilops kotchyi* Boiss., *Aegilops neglecta* Req. Ex Bertol., *Aegilops peregrina* (Hack. in J. Fraser) Maire&Weiller., *Aegilops speltoides* Tausch., *Aegilops triuncialis* L., *Aegilops umbellulata* Zhuk., *Aegilops uniaristata* Vis., *Aegilops vavilovii* (Zhuk.) Chennav.

Subspecies under *Triticum* genus are; *T. boeticum* Boiss, *T. urartu* Thumanjan ex Gandilyan, *T. monococcum* L., *T. araraticum* Jakupz., *T. dicoccoides* Koern., *T. dicoccon* Schrank, *T. durum* Desf., *T. turgidum* L., *T. polonicum* L., *T. cartlicum* Nevski, *T. aestivum* L., *T. monococcum* in the north, west Anatolia and Marmara region, *T. dicoccon* in the north Anatolia, *T. urartu* and *T. dicoccoides* in the south east Anatolia, *T. boeticum* in the whole country is found extensively (Table 1 and 2).

Wheat landraces in Türkiye

Wheat landraces are composed of traditional crop varieties developed by farmers through years of natural and human selection and are adapted to local environmental conditions and management practices. As distinct plant populations, landraces are named and maintained by traditional farmers to meet their social, economic, cultural, and environmental needs. They are alternately called farmers' varieties or folk varieties to indicate the innovative role of farmer communities in their development and maintenance (Jaradat 2013). The first collection was completed at the first quarter of 20th

century by pioneering Turkish scientist Mirza Gökgöl who collected 2120 wheat landraces from all over Türkiye and evaluated them for basic characteristics. The name of the book is "Türkiye Buğdayları". Gökgöl identified about 18.000 types of wheat and among them he identified 256 new varieties (Gokgol, 1939). In the same period as Gökgöl, well known Russian scientist Zhukovsky conducted 3 collecting missions to Türkiye during 1925-1927. Zhukovsky was encouraged by Vavilov, and his missions were supported by The Botany Society of the Soviet Union. During three years in Türkiye, Zhukovsky collected around 10,000 samples of cereals, forages, and vegetables. The material was an enormous contribution to plant varieties of the Soviet Union (Zhuhovsky, 1951).

Another landrace collection was done by Harlan in 1948 to 1949 with contribution of Agronomy Department of the University of Ankara, the Toprak Ofisi of the Ministry of Trade, and the Plant Breeding Stations of the Office of the Director General of Agriculture. The collection includes in 2121 wheat accession (incl. *T. monococcum*), and 55 wild relatives of wheat. These populations were analyzed for botanical and agronomic composition, providing an unusual opportunity for studies on the behavior of botanical varieties in mixed populations under diverse climatic conditions. The wheat in Türkiye were represented by remarkable diversity and great varietal wealth (Harlan 1950).

Damania et al., (1996) evaluated the collection of 2420 accessions derived from single-spike population samples of durum wheat landraces collected in 1984 from 172 sites in 28 provinces in Türkiye. They found differentiation of these accessions for number of days to heading, maturity, grain filling period as well as for plant height, peduncle length, and number of spikelets per spike, spike length, awn length, and kernel weight. As result of the canonical analysis, significant correlation among province means temperatures, altitude, latitude, and length of growing season. Eight distinct groups of provinces were identified by cluster analysis. They concluded that accessions could be utilized in crop improvement programs targeted at either favorable or stressed environments. Several other regional or local collection missions were fulfilled (Karagoz 1996; Qualset et al.,1997; Tan, 2002; Karagoz and Zencirci, 2005; Akcura and Topal, 2006; Giuliani et al., 2009).

The last survey was carried out in 65 provinces of Türkiye between 2009-2014 (Giuliani et al., 2009; Kan et al., 2015; Morgounov et al., 2016). As a result of the survey, 162 different local wheat landraces' names were detected. The wheat landraces were ranked according from highest frequency to the lowest frequency. In Türkiye, the most common 10 wheat landraces according to the frequency were; 1. Ak Buğday (Durum/bread wheat), 2. Sarı Buğday (Durum/Bread wheat),3. Kırmızı Buğday (Bread Wheat),4. Karakılçık (Durum/ bread wheat), 5. Zerun (Bread wheat), 6. Kırik (Bread wheat), 7. Koca Buğday (Durum/ bread wheat),8. Siyez Buğdayı, 9. Topbaş (Durum/bread wheat), 10. Üveyik Buğdayı (Durum wheat).

Durum wheat landraces mostly grown until 1960 in Türkiye were given in Table 3. In early 20th century, bread and durum wheat landraces grown in Türkiye were so called 'Ak Buğdaylar' and 'Sarı Buğdaylar' respectively. Turkish farmers cultivated their landraces widely until the second half of 20th century. After the World War II, a program was started in Türkiye through an agreement with Rockefeller Foundation. Although it was a modest start in agriculture research, mechanization, use of fertilizers and chemicals, it resulted in unexpected consequences. Among several plant groups involved, wheat program had the greatest impact. It didn't take long for the new varieties to replace the landraces. The heritage begun to be demolished after so called high yielding "Mexican origin wheat varieties" were introduced to the country. The acreage of the landraces grown in Türkiye is about 0,55 mil ha (Karagoz 2014).

Breeding value of durum wheat durum landraces

Although the presence of regional differences, general breeding aims of durum wheat are high yielding, yellow semolina color, gluten quality, resistance to lodging, tolerance to cold, heat and drought, tolerance to rust diseases (Ozberk et al., 2010). In modern era of durum wheat breeding in Türkiye, variety development studies were initiated through the line selection from widely grown landraces. Therefore, Makarnalık Sarı Buğday 710 in 1931, Makarnalık 073/44 and 414/44 in 1944, Fata'S' 185/1 in 1961-63, Kunduru 1149 in 1967 were developed (Ozberk et al., 2016). Apart from molecular genetics studies many morphological, physiological, and quality characterization studies were carried out employing durum wheat landraces. Many beneficial traits were detected and tried to be exploited in modern breeding programs (Genc et al., 1993; Koc, 1993; Barutcular et al., 1993; Alp and Kun, 1999; Sonmez et al., 1999; Altınbas and Tosun, 2002; Ozberk et al., 2005; Alp, 2005; Alp and Akinci, 2005; Alp and Aktas, 2005; Kara and Akman, 2007; Serpen et al., 2008; Koksel et al., 2008; Kutuk et al., 2008; Ozturk et al., 2008; Gumus et al., 2008; Alp and Sagir, 2009; Koyuncu, 2009; Sayaslan et al., 2012; Akcura, 2009). Molecular genetic studies mainly based on characterizations employing some morphological,

physiological, and technological characteristics of landraces (Yıldırım et al., 2011; Baloch, 2017).

Domestic use of durum wheat landraces

Depending on the region, up to 80% of the farmers have tried modern cultivars and most of them kept growing them along with landraces. The proportion of area growing wheat landraces to total wheat area in farmers' fields varied from 45 to 55% in the central Black Sea region and up to 98% in the southern coastal region. Farmers have access to modern cultivars but keep their landraces. The main reason for maintaining landraces is satisfaction with the landraces' performance. While, on average, only 25 and 30% (bread wheat and durum wheat growers, respectively) of the farmers rated yield of the landraces as good; 83% of the respondents for bread wheat and 93% for durum wheat were happy with the grain quality and its suitability for homemade products (Fig. 4). The other highest ranked traits for bread wheat and durum wheat, respectively, were straw yield (74 and 80%) and straw quality (70 and 76%), cold tolerance (78 and 82%), and drought tolerance (71 and 84%). For most of these traits, durum wheat landraces were rated slightly higher than bread wheat landraces (Figure 4) (Morgounov et al., 2016).

Wheat grain in the rural areas is used for two main purposes: bread, including typical loaves and thin types, and bulgur or cracked wheat, which is cooked in water. Respectively, bread and durum wheat are normally used for these two products. Based on the survey of the farmers in the region's growing primarily bread wheat (Aegean, central Anatolia, northeastern Anatolia, and central eastern Anatolia), its grain is mainly used for bread (64.3 to 83% of farmers). Of the four regions dominated by durum wheat, grain in the southern coastal and eastern Mediterranean regions is mainly used for bulgur (55.5 and 87.1%, respectively). The durum grain in the central Black Sea and southeastern Anatolia regions is used for both bulgur and bread (61.1 and 83.3%, respectively). Generally, the farmers were quite flexible in dual use of their grain for bread, bulgur, and other homemade products). Most of the club or compact wheat is used for dual purposes. Hulled einkorn wheat is used for bulgur in Bolu and Kastamonu regions and for animal feed elsewhere. Emmer wheat is consumed by the farmers in Kars and Sinop provinces as well as in north Anatolian region villages in small quantities. It is also used as animal feed. Durum wheat farmers in the central Anatolia region were 100% satisfied with the grain, mostly using it for bulgur. In the southeastern Anatolia and central eastern Anatolia regions, the durum farmers also gave very high ratings to the quality of their landraces, using them for dual purposes (bread and bulgur) (Morgounov et al., 2016).

Conclusion

Some of wheat landraces have so far been conserved in low scale due to their suitability for local dishes. They are not able to compete with the modern cultivars in respect of grain yielding ability and profitability. Unless being profitable none of the landrace can be sustainable. On- farm landrace conservation requires the continuation of the farmer induced selection processes by on how these landraces have been developed and their genetic structure have been shaped. Farmers must keep on seed replacement and renewal. Participatory plant breeding (Fasoula, 2004; Galie, 2013) collaboration with the local self-sufficient farmers can proved farmers to access the improved landrace seed. Sharing of the indigenous knowledge from generation to generation is also vital for sustainable conservation of landraces. Climate change is expected to differentially affect components of complex biological interactions in modern and traditional wheat production systems. Wheat yield and quality will be affected by climate change directly or indirectly through diseases. Wheat landraces and their populations in and outside their centers of diversity might respond to climate change will determine their continued productivity, utility, and survival. Non-breeding approaches to create demand for landrace products to promote on-farm dynamic conservation and sustainable utilization of wheat landraces include; 1. Rising public awareness regarding current and future value of landraces, 2. Diversity fairs to allow for the exchange of landrace materials associated indigenous knowledge, 3. Visits among farmers in various localities to share the seed and experience, 4. Contests for choice of highest diversity holding farmer, 5. Recipe development and niche market creation for landrace products (Jaradat 2013), 6. Growing mixtures for similar phenotypes to meet more local dish demands 7. Amendments in seed certification system allowing landraces to have diversity within the pre-determined ranges, 8. Expand organic farming practices employing more landraces (Karagöz, 2014)

Coordination with the non-breeding approaches to create demand for landrace products to promote on-farm dynamic conservation and sustainable utilization of wheat landraces can be provided by activities generating additional value and profit.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

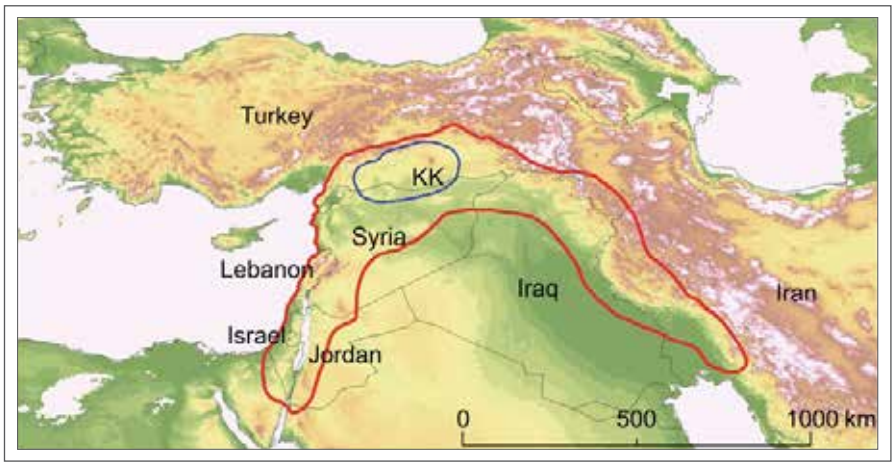

Figure 1. Fertile Crescent and "core area" of plant domestication within the Fertile Crescent. The Fertile Crescent is indicated with a red line and the "core area" is shown with a blue line. KK Karacadag mountain range in south-eastern Türkiye.

Figure 2. Wild einkorn, wild emmer and *Aegilops* species in their natural habitat in Karacadag mountain range. Picture taken by H. Ozkan in early July 2004.

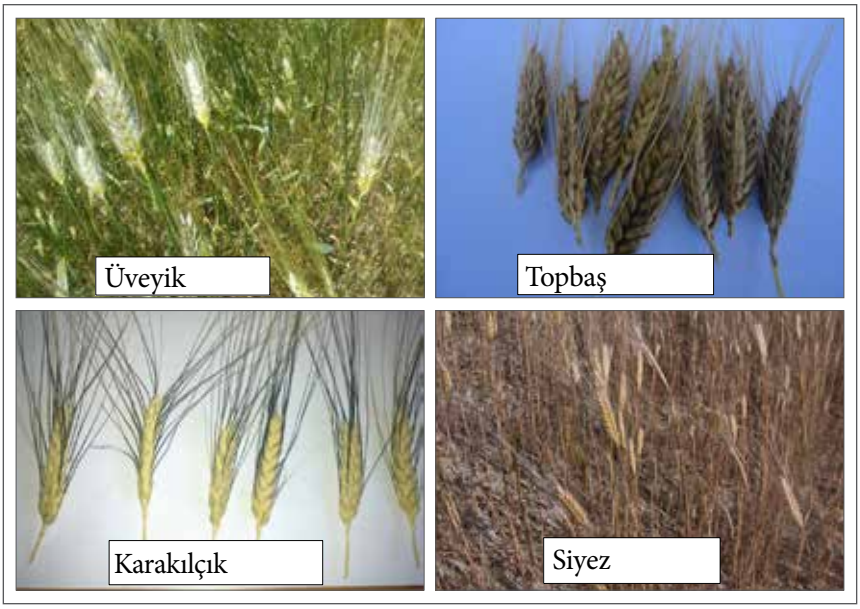

Figure 3. Some of durum wheat landraces still grown in Türkiye.

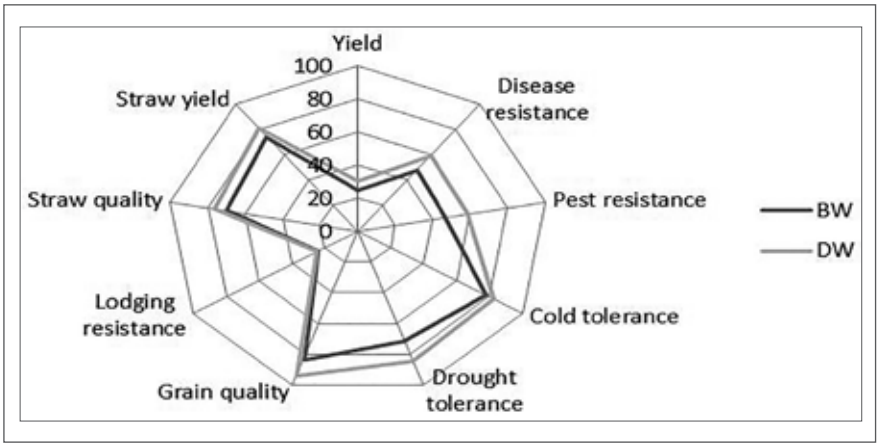

Figure 4. Percentage of farmers' ratings of different traits of bread wheat (BW) and durum wheat (DW) landraces as good based on a survey of 1026 households in Türkiye in 2009 to 2014.

Table 1. *Aegilops, Amblyopyrum* and *Dasypyrum* species, Turkish names, and genome formulas (Cabi and Doğan 2009; Waines and Barhart 1992).

Aegilops species	**Turkish name**	**Genome**
Ae. biuncialis Vis.	İki kılçık	UM
Ae. caudate L.,	Kara ot	C
Ae. columnaris Zhuk.	Kıl buğday	UM
Ae. comosa Sm. In Sibth. & Sm.	Uzun kılçık	M
Ae. crassa Boiss.	Kalın buğday	DM; DDM
Ae. cylindrica Host.	Kirpikli ot	DC
Ae. geniculata Roth.	Konbaş	MU
Ae. juvenalis (Thell.) Eig	Kaba buğday	DMU
Ae. kotchyi Boiss.	Asi buğday	SU
Ae. neglecta Req. Ex Bertol.	Tüylü buğday	UM; UMN
Ae. peregrina (Hack. in J. Fraser) Maire&Weiller	Kum buğdayı	SU
Ae. speltoides Tausch	Akbuğday anası	S
Ae. tauschii Coss.	Tespih buğdayı	D
Ae. triuncialis L.	Üç kılçık	UC; CU
Ae. umbellulata Zhuk.	Hanım buğdayı	U
Ae. uniaristata Vis.	Tek kılçık	N
Ae. vavilovii (Zhuk.) Chennav.	Zarif buğday	DMS
Amblyopyrum muticum (Boiss.) Eig	Narin Buğday	T
Dasypyrum villosum (L.) Candargy	Kızıl ev	V

Table 2. Wild *Triticum* species, Turkish name and genome formulas (Cabi and Doğan 2009).

Triticum species	Turkish name	Genome
T. boeoticum Boiss.	Yabani siyez	A^mA^m
T. dicoccoides (Körn. ex Aschers. et Graebn.) Schweinf	Yabani gernik	AABB
T. timopheevii (Zhuk.) Zhuk. v *araraticum* (Jakubz.) Yen	Deli Rus buğdayı	AAGG
T. urartu Thumanjan ex Gandilyan	Urartu buğdayı	AA
T. monococcum L.	Siyez	A^mA^m
T. turgidum L. ssp. *dicoccon*	Gernik=Çatal kaplıca= Çatal siyez	AABB
T. turgidum L. ssp. *durum*	Makarnalık	AABB
T. turgidum L. ssp. *durum commune*	Asıl makarnalık	AABB
T. turgidum L. ssp. *durum* ssp. *duro-compactum*	Makarnalık topbaş	AABB
T. turgidum L. ssp. *turgidum*	Kaba buğday	AABB
T. turgidum L. ssp. *polonicum*	Turna gagası buğday	AABB
T. turgidum L. ssp. *carthlicum*	Doğu buğdayı	AABB
T. turgidum L. ssp. *turanicum*	Turna dili buğday	AABB

Table 3. Wheat landraces grown in Türkiye before 1960.

Region	Provinces	Durum land races
Central-North Anatolia	Ankara, Çankırı, Uşak, Çorum, Kırşehir, Yozgat, Bolu, Bilecik, Eskişehir, Kütahya	Sarı Buğday, Karakılçık, Kunduru, Şahman, Sarı Bursa, Akbaşak, Üveyik
Central-East Anatolia	Amasya, Malatya, Sivas, Tokat, Tunceli, Elazığ	Üveyik, Menceki, Kunduru
Central-South Anatolia	Afyon, Kayseri, Niğde, Konya, Nevşehir	Bolvadin, Sarı Buğday, Karakılçık
North-Eastern Anatolia	Ağrı, Artvin, Kars, Erzincan, Erzurum	Karakılçık, Hazerik
South-Eastern Anatolia	Bingöl, Bitlis, Van, Hakkâri, Mardin, Muş, Siirt, Şanlıurfa	Bağacak, Sorgül, Sorik, Beyaziye, Menceki, Akbaş, İskenderi, Mısri, Havrani, Karakılçık, Akbaşak, Hamrik
Mediterranean	Antalya, Gaziantep, Hatay, İçel, Maraş, Adana	Akbuğday, Karakılçık, Tığrak Buğdayı, Sarı Buğday ve Kıbrıs Buğdayı
Aegean	İzmir, Aydın, Muğla, Denizli, Burdur, Isparta, Manisa, Balıkesir, Çanakkale	Fata, Gökala, Sarı başak, Kunduru, Menemen, Karakılçık, Sarı Çam, Akbaşak, Akpüsen, Çam Buğdayı, Sarı Buğday, Devedişi, Kırmızı Buğday
Marmara	Bursa, Kocaeli, Sakarya, İstanbul, Edirne, Tekirdağ, Kırklareli	Akbaşak, karakılçık, Tunus Buğdayı, Sarı Başak, Köse Buğday, Arnavut Buğdayı, Kunduz, Koca Buğday, Kokana
Black Sea	Rize, Trabzon, Giresun, Ordu, Samsun, Sinop, Kastamonu, Zonguldak, Gümüşhane	Rumeli (Yunan) Buğdayı, İlik Buğday, Sarı Buğday, Akbuğday, Sarıbaş, Karakılçık, Üveyik, Rumeli, Sarı Hamza, Koçarı, Diş Buğdayı

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Comparison of Agronomic and Physiological Parameters of Durum Wheat Local Landraces and Commercial Cultivars

İrfan ÖZTÜRK

Trakia Agricultural Research Institute, Edirne, Türkiye

* Corresponding author e-mail: ozturkirfan62@yahoo.com

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ABSTRACT

Türkiye has great variation and production experience in terms of both bread and durum wheat landraces and commercial varieties. The study was carried out to evaluate of agronomic and physiological components of durum wheat commercial cultivars and landraces under rainfed conditions. In the experiments, totally 35 durum wheat landraces and commercial varieties were investigated in the 2018-2019 cropping years in the Trakia region, Türkiye. The experiment was laid out in a randomized complete blocks design with three replications. The results of variance analysis showed significant differences ($p<0.01$) among local landraces and commercial varieties for the traits studied except for chlorophyll content. Normalised difference vegetation index (NDVI) was measured in heading stages. Landraces have the highest NDVI compared with commercial varieties. Higher canopy temperature was measured in commercial cultivars (G35, G32, G34 and G33) while lower canopy temperature was detected in landraces (G2, G15). Flag leaf area was measured at heading stages and it was found significant difference among landraces and commercial cultivars. Landraces G3, G4 and G2 had the highest flag leaf area of 42.9, 40.6 and 39.9 cm^2, respectively. Landraces had longer plant height and peduncle length than commercial varieties. The number of grains per spike and number of stomata were higher in commercial varieties and local landraces, respectively. Stomata measurements were made on samples taken from flag leaves during the heading period. Commercial varieties had higher values than landraces in terms of stomata width, height, area and perimeter. Cluster analysis clearly differentiated, commercial cultivars from the landraces based on agro-physiological data.

Keywords: Durum wheat, landraces, commercial cultivars, agro-physiological parameters

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Introduction

Türkiye is considered a diversification centre of durum wheat (*Triticum turgidum* L. var. *durum*). Germplasm presents average diversity showing a large genetic variability. If considerable variation among genotypes could be identified these can be widely used in durum wheat breeding programs (Öztürk, 2019). Durum wheat (*Triticum turgidum* L. ssp. *durum*) expresses approximately 6% of the global wheat production. The Mediterranean Basin is the most extensive durum-producing area, the customer of durum wheat products and the most important import market in the world (Royoa et al., 2020). Durum wheat Mediterranean landraces are essential resources to increase the genetic diversity of modern cultivated varieties and ensure their adaptation to regions affected by biotic and abiotic factors (Soriano et al., 2018). Wheat has been a staple crop in the Anatolian region since prehistoric times. Anatolia has hosted many agricultural cultures from the first waves of Neolithic migrations to modern times. The diversity of wheat in Anatolia is also great, as farmers

have multiplied the crops and preserved them for thousands of years (Brush, 1995). New high-yield varieties of durum wheat that can compete with bread wheat varieties haven't yet been improved. Durum wheat breeding researchers also need to select well-adapted genotypes available in the region (Bilgin et al., 2008). The yield of durum wheat in the Mediterranean regions is frequently restricted by high temperatures and drought stress during grain growth stages (Garcia del Moral et al., 2003). Drought and heat are the most important abiotic stresses limiting wheat cultivation. Local varieties are tolerant to abiotic stresses and are genetic resources that can be used in breeding programs to develop genotypes resistant to stress conditions (Farooq, 2023). While Durum wheat is mainly used in the production of pasta and couscous, it is also used in the production of some other semolina products such as bulgur and unleavened bread. It is known that in the Mediterranean region, Durum wheat is mainly grown in conditions where rainfall is irregular between years and locations and during the plant growth period, thus causing yield differences (Soriano et al., 2018). Some of the morphological and physiological characteristics known to be hereditary and used in breeding programs are early development and early flowering. Early development in genotypes is generally determined by the size of the seed. This feature reduces direct evaporation of soil water by covering the soil after rapid development and increasing plant water use (Richards et al., 2011; Blum, 2011; Elazab et al., 2015). Breeding carried out according to phenological characteristics may ignore genetic characteristics. Scientists recognize that landraces and varieties represent an important group of genetic resources for the development of commercially important traits (Lopes et al., 2015).

Stomatal transpiration is the principal way of water loss in the plant. Stomata features influencing the water-use efficiency of plants are significant factors in assessing genotypes for drought stress. Reducing water loss from the leaf surface during periods of water stress is an important element of maintaining viability in drought (Bilgin et al., 2011). Stomatal characteristics such as density and size of stomata are considered to be the main determinants of the development rate and water balance in plants (Dillen et al., 2008).

Landraces were largely cultivated until the first decades of the twentieth century, being progressively abandoned from the early 1970s and replaced with improved, genetically uniform semi-dwarf cultivars as a consequence of the Green Revolution (Ortiz et al., 2007). Türkiye was one of the genetically diverse countries where landraces and commercial varieties of bread and durum wheat are widely available and produced before green revolution. Still, durum and bread wheat landraces are cultivated in rural areas of highlands where stipend farming systems are common. The experiment was carried out to evaluate agronomic and physiological parameters of durum wheat commercial cultivars and landraces under rainfed conditions.

Materials and Methods

Plant Materials and Studied Traits

During the 2018-2019 growing season, a total of 35 durum wheat landraces and commercial cultivars (Table 1) were tested in the Trakia region, Türkiye. The study was carried out under rainfed conditions at the experimental field of Trakia Agriculture Research Institute in Edirne Türkiye (41° 38′ 52′′ N and 26° 36′ 07′′ N, 40 m elevation), in a randomized complete blocks design (RCBD) with three replications. In the experiment, each plot was 2 m×3 rows, spaced 0.30 meters apart.

Parameters related to yield component and physiological were tested in each genotype using the following criteria. In the study, canopy temperature (CT), chlorophyll content (SPAD) and normalized difference vegetation index (NDVI) were taken at heading stages. Yield components such as the number of spikelet per spike, the number of kernels per spike, spike weight and spike length were determined from each genotype. Flag leaf area (FLA), days of heading (DH), plant height (PH) and peduncle length (PL) were investigated. The stomata area (STA), stomata width (STW), stomata height (STH), stomata perimeter (STP) and number of stomata (STN) were also experienced on flag leaves during the heading (Z55) period.

Normalized difference vegetation index (NDVI): It was scaled at the Z55 (Zadoks et al., 1974) period. Measurements were made using a hand-held Ntech 'Greenseeker' NDVI meter (N Tech Industries (2011) Greenseeker (Pask et al., 2012). NDVI measurements were taken from 11:00h to 14:00h, on a clear, sunny day. Measurements were taken for plant growth at Z55 development stages. Normalised difference vegetative index can be used to estimate biomass accumulation, growth rate, yield estimation, soil cover, early vigour, senescence model predictions, detection of biotic and abiotic stress factors (Araus, 1996; Gutierrez-Rodriguez et al., 2004; Pask et al., 2012).

Chlorophyll content (SPAD): For Chlorophyll content, the SPAD-502 chlorophyll meter (Minolta) was used. Chlorophyll content was measured from ten flag leaves were used to take chlorophyll meter

(SPAD) readings from each plot at the heading stage (Z55) (Adamsen et al., 1999; Babar et al., 2006; Fisher, 2001; Pask et al., 2012; Reynolds et al., 2001).

Canopy temperature (CT): The infrared thermometer was used to measure canopy temperature CT (°C). Canopy temperatures were scaled from each plot at a 1m distance from the edge and approximately 50 cm above the canopy at an angle of 30° to the horizontal. Scaled were made between 13.00 and 15.00 h on sunny and without windy days (Babar et al., 2006; Reynolds et al., 2012; Pask et al., 2012). Measurements were taken for plant growth at the Z55 development stages.

Flag leaf area (cm²): In the research, 10 flag leaves were randomly selected in each subplot and their length (FLL) and width (FLW) were measured by a ruler. Flag leaf area (FLA) was then calculated using the following formula (Dodig et al., 2010).

$FLA\ (cm^2) = (FLL \times FLW) \times 0.75$

Stomata width and height (µm): Stoma measurements were made on samples taken from flag leaves during the heading period. Stoma width and length were determined by taking the average of a total of 10 measurements.

Stomata area (µm²): It was determined by taking the average of the measurements made in 10 samples from the samples taken from the flag leaves.

Stomata perimeter (µm): It was determined by taking the average of the measurements made in 10 samples from the samples taken from the flag leaves.

Statistical Analyses

Data examined in the study were statistically analysed in the method described by Gomez and Gomez (1984). The averages of the parameters examined in the genotypes were determined according to the LSD test (0.05). Relationships between features were determined by Pearson correlation analysis. The cluster analysis was used to see whether the cultivars fell into groups or clusters. The cluster analysis was achieved that adopted squared Euclidian distance as a measure of dissimilarity and Ward's method as the clustering algorithm (Ward, 1963).

Meteorological Data

Total precipitation for the growing cycles from October to June was 523.4 mm. In March (7.6 mm), December (16.8 mm) and February (18.2 mm) rainfall was very low. The mean temperature was 11.6 °C and the mean humidity was 76.2% (Table 2).

Results and Discussion

The analysis of variance in the experiment is listed in Table 3. The combined analysis of variance showed significant differences ($P<0.01$) among durum wheat landraces and commercial cultivars for all traits except chlorophyll content (SPAD) (Table 3).

In the study, it was observed that there were significant differences between local and commercial varieties of durum wheat according to plant height, stem length and spike length. In durum wheat genotypes, plant height and stem structure are significant traits for lodging resistance. Earliness has become the most important feature against drought and heat stress in climate change. Early varieties with low vernalization requirements are less affected by drought. In the research, it has been observed that commercial varieties head earlier than local varieties. In the research, the earliest varieties were G35 with 116.0 days, G27 and G28 with 116.7 days. Long plant height is a preferred trait, especially in arid conditions. Local landraces are taller than commercial varieties. The shortest height was measured at 83.7 cm (G18) in the commercial variety, and the longest height was measured at 158 cm (G29) in the local variety. In the study, local landraces G14 had the longest peduncle (64.5 cm) and the shortest peduncle was measured for commercial cultivar G6 (28.9 cm). Spike length in genotypes may vary depending on genotype and environmental factors. In the experiments, the minimum spike length was 6.09 cm in commercial cultivar (G27). The maximum spike length was 9.10 cm in commercial cultivar (G17) and 9.05 cm in local landraces G1 (Table 4).

The number of spikelet per spike (SNM) and the number of kernels per spike (KNS) are essential yield parameters associated with grain yield. Parameters SNM and KNS may also vary based on genotype and environmental factors. The number spikelet per spike varied from 16.5 to 21.9. Commercial cultivar G26 and local landraces G15 had a higher spikelet number per spike. The number kernel per spike of durum wheat genotypes was examined and it was found a significant difference among genotypes (Table 3). More spikes were counted in commercial varieties. The maximum kernel number per spike was noted in genotype G26 (59.2), followed by G21 (57.3), G28 (52.8) and G22 (52.4). Local landraces G8 produced a minimum kernel number per spike (31.8). Differences in spike weight were determined between commercial and local varieties. The heaviest spike was measured at 3.88 g in the local variety G1. The smallest spike weight was determined in the local variety G7, with 1.99 g (Table 4).

Flag leaf areas in durum wheat landraces and commercial cultivars were tested and it was found differences in genotypes. In the study, the flag leaf area of local varieties was larger than commercial varieties. The largest flag leaf area was measured in G3 (42.9 cm^2),

G4 (40.6 cm^2) and G2 (39.9 cm^2) local varieties. The smallest flag leaf area was measured in G27 (15.9 cm^2) and G28 (18.7 cm^2) commercial varieties (Table 4).

There was significant variation ($p<0.01$) in chlorophyll content (SPAD) of the durum wheat landraces and commercial cultivars. The highest chlorophyll content was 60.9 in G23 and 60.4 in G27 commercial cultivars. Chlorophyll content in durum wheat landraces varied from 53.2 (G8) to 58.8 (G3). Under drought and heat stress conditions canopy temperature is related to yield. The lowest canopy temperature was 22.1°C in G2 and 22.4°C in G15. Lower canopy temperature was measured in local varieties. The normalized difference vegetation index is widely utilised for estimating the rapid ground level of crops, canopy for leaf area index, green area index, biomass and nutrient content (Pask et al., 2012). The high rate of variation in normalised difference vegetation index in durum wheat landraces and commercial cultivars. Normalized difference vegetation index varied from the lowest 0.60 to the highest 0.81 in genotypes. The highest NDVI were determined in genotypes G13, G14, G16, G23 and G26 (Table 5). The results of the study explained differences among durum wheat genotypes according to measured stoma characteristics. It was determined that commercial varieties had wider stomata than local varieties in terms of stoma width, length, area and perimeter. The largest stoma width was measured in G15 and G25, and the longest stoma was measured in G23 and G16. While G23 had the widest stoma, the smallest stoma area was determined in G1. It was determined that local varieties had more stomatal numbers than commercial varieties (Table 5).

Durum wheat local landraces and commercial cultivars were tested for 16 parameters and a wide difference was found for the parameters studied. The cluster analysis was performed and 35 durum wheat genotypes were grouped into 7 clusters based on Ward's method. It has been determined that most of the local landraces are in the first three groups. While Fırat93 (G28) and Harran95 (G29) varieties were the closest to each other according to the examined parameters, G1 and Ankara98 (G25) were the most distant genotypes. The first group of cluster 7 genotypes and the second and third groups of cluster 3 genotypes are located. The fourth group of cluster 9 cultivars, the seventh group of cluster 6 cultivars, and the last group of cluster 5 commercial durum wheat cultivars are located (Figure 1).

Correlation coefficients among studied parameters were established by Pearson's correlation analysis (Table 6). Days to heading were positively correlated with plant height, peduncle length, spike length, spikelet number per spike, normalised difference vegetative index and flag leaf area. A positive correlation was found among plant height with peduncle length, spike length, normalised difference vegetative index, flag leaf area and number of stomata. There was a positive relation between peduncle lengths with spike length, normalised difference vegetative index, flag leaf area and number of stomata. Canopy temperature was negatively correlated with days to heading, peduncle length, plant height, spike length, number of spikelet and normalised difference vegetative index. Flag leaf area positively correlated with days of heading, plant height, peduncle length, spike length, number of spikelet and normalised difference vegetative index. Stomata number in genotypes was positively correlated with days of heading, plant height, peduncle length, spike length and normalised difference vegetative index.

Conclusions

It was observed that there were significant differences between commercial varieties and local landraces in the parameters examined in the study. The higher value of flag leaf area, plant height and normalised difference vegetation index was determined in local landraces. This result showed the importance of using local varieties in breeding studies, especially since flag leaf area and normalised difference vegetation index values are positively related to yield. The fact that local landraces have low canopy temperatures has shown the importance of using breeding studies for drought tolerance. The stomata density in genotypes was higher in local landraces than the commercial cultivars. It was determined that commercial varieties had wider stomata in terms of values such as stoma width, length and area. According to cluster analysis, it was determined that the commercial varieties examined in the research differed from the local varieties. It will be useful to use local varieties in breeding studies due to some of their superior agronomic properties.

Table 1. Local landraces and cultivars durum wheat genotypes investigated in the study.

Genotype No	Landraces	Genotype No	Commercial Cultivars	Genotype No	Commercial Cultivars
G1	Yerli/Bağacak	G16	Ergene	G31	Sarı çanak 98
G2	Sevinç	G17	Tunca 79	G32	Fuatbey 2000
G3	Kızıl Buğday	G18	Gökgöl 79	G33	Balcalı 2000
G4	Cafari	G19	Çakmak 79	G34	Zenit
G5	Gedifla	G20	Kunduru 1149	G35	Svevo
G6	Menceki	G21	Mirzabey 2000		
G7	Hacıhalil	G22	Kızıltan 91		
G8	Sorgül	G23	Eminbey		
G9	Beyaziye	G24	Çeşit-1252		
G10	Devedişi	G25	Ankara 98		
G11	Bağacak	G26	Selçuklu-97		
G12	İskenderi	G27	Ege 88		
G13	Karabaşak	G28	Fırat-93		
G14	Karakılçık	G29	Harran 95		
G15	Akbaşak	G30	G30		

Table 2. Climate data in Edirne location experimental area in 2018-2019 growing year.

Months	Long Term Rainfall (mm)	Annual Rainfall (mm)	Mean Humidity (%)	Temperature (°C)		
				Min.	Max.	Mean
October 2018	52.9	32.6	74.2	1.6	24.8	15.7
November 2018	72.4	208.8	81.8	-2.9	23.2	9.8
December 2018	61.7	16.8	86.7	-4.5	16.5	3.9
January 2019	48.1	82.4	85.7	-9.4	16.1	4.1
February 2019	46.9	18.2	76.5	-5.5	16.8	5.6
March 2019	52.2	7.6	68.6	-1.9	23.2	9.8
April 2019	51.0	60.4	72.8	-0.3	25.8	12.4
May 2019	56.0	63.4	75.1	3.8	32.2	18.2
June 2019	41.5	33.2	64.8	19.8	36.2	24.5
Total/Mean	482.7	523.4	76.2	0.1	23.9	11.6

Table 3. Mean square and F ratio for parameters investigated in durum wheat genotypes.

Parameters	Genotypes (G)	
	Mean square (MS)	F Ratio
Days of heading (DH)	77.7171**	70.472
Plant height (PH)	2063.745**	66.001
Peduncle length (PL)	269.215**	15.708
Spike length (SL)	2.382**	9.790
Spikelet number per spike (SNS)	5.366**	5.256
Number of kernel per spike (KNS)	90.402**	3.393
Spike weight (SW)	0.6763**	4.604
Normalized difference vegetation index (NDVI)	0.013**	2.654
Chlorophyll content (SPAD)	12.186	1.406
Canopy temperature (CT)	4.148**	2.061
Flag leaf area (FLA)	108.437**	12.533
Stomata width (STW)	25.720**	7.726
Stomata height (STH)	12.420**	4.869
Stomata area (STA)	36799.867**	7.836
Stomata perimeter (STP)	139.877**	8.546

Table 4. Mean value of the durum wheat landraces and commercial cultivars for parameters.

Genotype	DH	PH	PL	SL	SNS	KNS	SW	FLA
G1	127.7fg	155.7ab	60.3ab	9.05a	19.8b-e	48.9c-h	3.88a	35.2c-f
G2	127.3fg	158.0a	55.7b-e	8.62a-d	19.3b-f	46.9c-l	3.45abc	39.9abc
G3	130.0b-e	110.7h	48.6f-ı	6.84k-n	18.3e-ı	41.1g-m	2.85c-ı	42.9a
G4	128.3ef	115.7gh	49.5e-h	6.84k-n	19.5b-f	41.6f-m	2.64d-j	40.6ab
G5	119.7ı	88.3j-n	33.1nop	6.37mn	17.8f-j	45.3d-l	2.47g-k	24.2p-s
G6	130.7a-d	136.7c	54.9b-f	7.91d-ı	19.1b-f	42.9e-m	3.07b-g	35.2c-f
G7	129.7cde	132.0cde	53.5c-f	6.69k-n	17.4g-j	39.5j-m	1.99k	29.4h-n
G8	126.3gh	124.7efg	46.7h-j	6.59lmn	17.0ıj	35.1m	2.10jk	28.6j-p
G9	130.3bcd	127.0def	53.8b-f	7.71e-j	17.9f-j	44.8d-l	2.86c-ı	31.1f-l
G10	131.7ab	146.7b	58.0a-d	8.19b-g	18.6e-ı	40.9h-m	3.13b-f	33.0d-j
G11	131.7ab	119.7fgh	56.1b-e	7.04j-m	16.5j	40.4ı-m	2.28h-k	31.4f-k
G12	131.0abc	135.0cd	51.5d-g	8.33a-e	19.7b-e	47.3c-l	2.87c-ı	34.0d-h
G13	130.7a-d	153.3ab	49.5e-h	8.03c-h	18.4e-ı	39.0lm	2.34h-k	34.2d-g
G14	131.0abc	154.7ab	64.5a	8.63a-d	20.8ab	48.4c-ı	3.38abc	36.4b-e
G15	132.3a	150.0ab	59.2abc	8.33a-e	21.7a	46.5c-l	3.24bcd	37.1bcd
G16	127.3fg	92.0ı-l	35.8l-o	8.00c-h	19.6b-e	41.8f-m	2.40h-k	24.1p-s

Continuing Table 4

Genotype	DH	PH	PL	SL	SNS	KNS	SW	FLA
G17	128.3ef	88.7j-n	36.8l-o	9.10a	20.4abc	47.9c-j	2.60e-k	32.3e-k
G18	125.3h	83.7lmn	35.2m-p	8.23b-f	18.7d-h	41.1g-m	2.26ıjk	32.2e-k
G19	128.3ef	90.0ı-n	42.5ı-k	7.41g-k	18.4e-ı	49.7b-f	2.31h-k	26.4l-s
G20	131.3abc	136.3c	56.2bcd	7.81e-j	18.7d-h	45.5c-l	3.08b-g	33.7d-ı
G21	131.0abc	91.0ı-m	41.7j-m	8.91ab	20.4abc	57.3ab	3.11b-f	30.1g-m
G22	130.3bcd	98.7ı	43.6h-k	8.05c-h	19.0c-g	49.0b-h	2.52f-k	22.6st
G23	127.7fg	90.0ı-n	41.0j-m	8.01c-h	20.3a-d	53.8abc	3.14b-f	25.3m-s
G24	126.0gh	93.3ıjk	38.7k-n	8.74abc	18.9c-g	48.2c-ı	2.60e-k	23.7qrs
G25	125.3h	95.3ıj	41.5j-m	7.46f-k	19.2b-f	50.9a-e	3.12b-f	29.0ı-o
G26	130.3bcd	90.0ı-n	37.7k-o	8.32a-e	21.9a	59.2a	2.68d-j	30.5f-l
G27	116.7k	85.7k-n	35.4m-p	6.09n	17.8f-j	52.4a-d	2.89c-h	15.9u
G28	116.7k	91.7ı-m	40.1j-m	7.13ı-m	19.3b-f	52.8a-d	3.47abc	18.7tu
G29	119.0ıj	88.0j-n	37.9k-o	6.75k-n	18.4e-ı	49.9b-f	3.55ab	25.3n-s
G30	129.0def	86.3j-n	38.6k-n	7.28h-l	19.5b-f	49.3b-g	2.21jk	23.2rst
G31	126.3gh	87.3j-n	28.9p	6.57lmn	17.4g-j	48.4c-ı	3.17b-e	31.4f-k
G32	120.3ı	88.7j-n	38.1k-o	6.72k-n	18.8c-g	51.9a-d	3.31abc	27.7k-r
G33	119.0ıj	89.3j-n	38.4k-n	6.18n	17.0ıj	40.9h-m	2.57e-k	28.2k-q
G34	117.3jk	81.7n	31.6op	6.66k-n	17.2hıj	39.2klm	2.10jk	24.5o-s
G35	116.0k	82.7mn	35.3m-p	6.43mn	16.5j	47.5c-k	2.59e-k	28.5j-q
Average	126.6	109.7	44.8	7.57	18.8	46.4	2.81	29.9
$LSD_{(0.05)}$	1.69	9.07	6.72	0.8	1.63	8.38	0.62	4.77

DH: Days of heading, PH: Plant height (cm), PL: Peduncle length (cm), SL: Spike length (cm), SNS: Spikelet number per spike, KNS: Kernel number per spike, SW: Spike weight (g), FLA: Flag leaf area (cm^2)

Table 5. Agro-physiological parameters of the durum wheat landraces and commercial cultivars.

Genotype	SPAD	CT	NDVI	STW	STH	STA	STP	STN
G1	55.7b-g	23.7e-j	0.77a-e	42.1lm	24.0mno	803.1q	110.4ll	16.7a
G2	55.1c-g	22.1j	0.78a-d	44.8ı-l	25.7h-o	894.3m-q	116.3jkl	13.1c-h
G3	58.8a-d	24.5b-ı	0.76a-e	41.1m	24.4l-o	809.4pq	109.9l	14.7b
G4	54.6d-g	24.2c-j	0.78a-d	43.6j-m	23.4o	807.1pq	112.6kl	13.0c-ı
G5	54.0efg	24.7b-ı	0.73a-f	45.4h-k	24.5l-o	864.3opq	116.3jkl	12.6e-j
G6	58.2a-f	24.6b-ı	0.79abc	41.9lm	24.5l-o	803.0q	110.0l	16.7a
G7	56.2a-g	25.2a-h	0.76a-f	43.2klm	27.7b-j	917.3l-p	116.3jkl	12.6e-j
G8	53.2g	25.7a-f	0.79abc	50.7a-d	26.6e-m	1052.9c-ı	129.7a-e	11.6h-l
G9	55.4c-g	24.5b-ı	0.81ab	49.6b-e	25.2j-o	967.9g-o	124.0e-ı	14.7b
G10	54.2d-g	23.4f-j	0.79ab	46.2f-j	24.5l-o	889.5m-q	117.7ıjk	16.6a
G11	55.6c-g	24.8b-h	0.80ab	45.8g-k	26.9d-l	944.9ı-o	119.6hıj	14.7b
G12	57.9a-g	24.0d-j	0.79abc	45.9g-k	24.8k-o	882.7n-q	117.9ıjk	14.3bc
G13	58.6a-e	24.1d-j	0.81a	48.6d-g	23.9no	917.2l-p	122.3g-j	11.1jkl
G14	55.6c-g	23.2hıj	0.81a	46.1f-k	25.3ı-o	915.4l-p	119.2h-k	12.8c-ı
G15	56.9a-g	22.4ıj	0.78a-d	52.5a	28.8b-f	1160.6abc	134.8ab	11.9g-l
G16	57.5a-g	23.3g-j	0.81a	45.7h-k	30.1ab	1037.3d-k	122.9f-j	12.3f-k
G17	53.5fg	25.5a-h	0.77a-e	46.3f-j	28.5b-g	994.2e-n	121.9g-j	13.0c-h
G18	55.1c-g	23.4f-j	0.77a-e	48.1d-h	27.4c-k	1022.6e-l	124.5d-h	12.6e-j
G19	58.2a-f	24.1d-j	0.80ab	47.8d-h	29.3bcd	1103.8b-e	127.0c-g	14.2bcd
G20	59.8abc	23.2hıj	0.80ab	51.9ac	26.8d-l	1078.6b-g	130.8a-d	13.7b-f
G21	57.3a-g	24.8b-h	0.80ab	49.2b-e	28.1b-h	1065.1c-h	127.7c-g	13.5b-g
G22	59.5abc	24.0d-j	0.79abc	46.2f-j	26.6e-m	939.6j-o	119.3hıj	14.1b-e
G23	60.9a	25.4a-h	0.81ab	51.6abc	32.3a	1243.1a	136.3a	12.8c-ı
G24	56.6a-g	25.7a-f	0.79ab	48.9c-f	29.1b-e	1090.0b-f	128.9b-f	12.6e-j
G25	56.6a-g	25.3a-h	0.78a-d	52.2ab	27.8b-ı	1141.6a-d	132.4abc	11.8h-l
G26	58.1a-f	24.4b-ı	0.81ab	50.5a-d	26.1g-n	1040.7d-j	127.7c-g	13.4b-g
G27	60.4ab	25.9a-e	0.71a-g	47.2e-ı	27.7b-j	1023.3e-l	124.1e-ı	12.7d-ı
G28	58.2a-f	25.6a-g	0.65fgh	47.0e-ı	27.6b-j	985.4f-n	122.9f-ı	10.6l
G29	57.3a-g	25.2a-h	0.67d-h	46.1f-k	26.3f-n	943.4ı-o	119.3hıj	11.5h-l
G30	58.6a-e	25.0a-h	0.70b-h	46.2f-j	28.3b-h	996.0e-m	121.4g-j	13.1c-h
G31	53.2g	25.0a-h	0.68c-h	48.1d-h	24.9k-o	928.8k-o	121.9g-j	10.8kl
G32	56.4a-g	26.7ab	0.60h	47.8d-ı	25.9h-o	944.7ı-o	122.3g-j	11.5h-l
G33	58.3a-f	26.3a-d	0.62gh	46.0f-k	26.6e-l	960.0h-o	120.1hıj	11.4ı-l
G34	56.3a-g	26.5abc	0.60h	50.5a-d	29.5bc	1177.3ab	133.2abc	11.6h-l
G35	56.6a-g	27.2a	0.66e-h	46.1f-k	25.4ı-o	886.0m-q	118.2h-k	12.3f-k
Average	56.8	24.7	0.75	47.2	26.7	978.0	122.3	13
$LSD_{(0.05)}$	4.77	2.29	0.11	2.96	2.58	111.2	6.56	3.12

SPAD: Chlorophyll content, CT: Canopy temperature (°C), NDVI: Normalized difference vegetation index (NDVI), STW: Stomata width (µm), STH: Stomata height (µm), STA: Stomata area (µm²), STN: Stomata number, STP: Stomata perimeter (µm)

Table 6. Coefficients of correlation among parameters investigated in durum wheat genotypes.

Traits	DH	PH	PL	SL	SNS	KNS	SW	NDVI	SPAD	CT	FLA	STA
PH	0.594**											
PL	0.651**	0.927**										
SL	0.621**	0.411*	0.410*									
SNS	0.443**	0.188	0.228	0.726**								
KNS	-0.102	-0.346*	-0.250	0.272	0.559**							
SW	-0.019	0.302	0.315	0.308	0.422*	0.507**						
NDVI	0.818**	0.498**	0.542**	0.639**	0.421*	-0.035	-0.047					
SPAD	-0.019	-0.146	-0.042	-0.021	0.181	0.354*	0.058	0.018				
CT	-0.673**	-0.647**	-0.608**	-0.589**	-0.492**	0.091	-0.242	-0.659**	0.056			
FLA	0.589**	0.657**	0.630**	0.346*	0.232	-0.310	0.224	0.390*	-0.313	-0.537**		
STA	-0.072	-0.366*	-0.313	0.098	0.197	0.263	-0.166	0.040	0.276	0.133	-0.432**	
STN	0.541**	0.429**	0.561**	0.408*	0.134	-0.047	0.156	0.496**	0.040	-0.382	0.365*	-0.393*

*, ** Significant at P<0.05 and P<0.01 respectively. DH: Days of heading, PH: Plant height (cm), PL: Peduncle length (cm), SL: Spike length (cm), SNS: Spikelet number per spike, KNS: Kernel number per spike, SW: Spike weight (g), NDVI: Normalised difference vegetative index, SPAD: Chlorophyll content, CT: Canopy temperature (°C), FLA: Flag leaf area (cm^{-2}), STA: Stomata area, STN: stomata number.

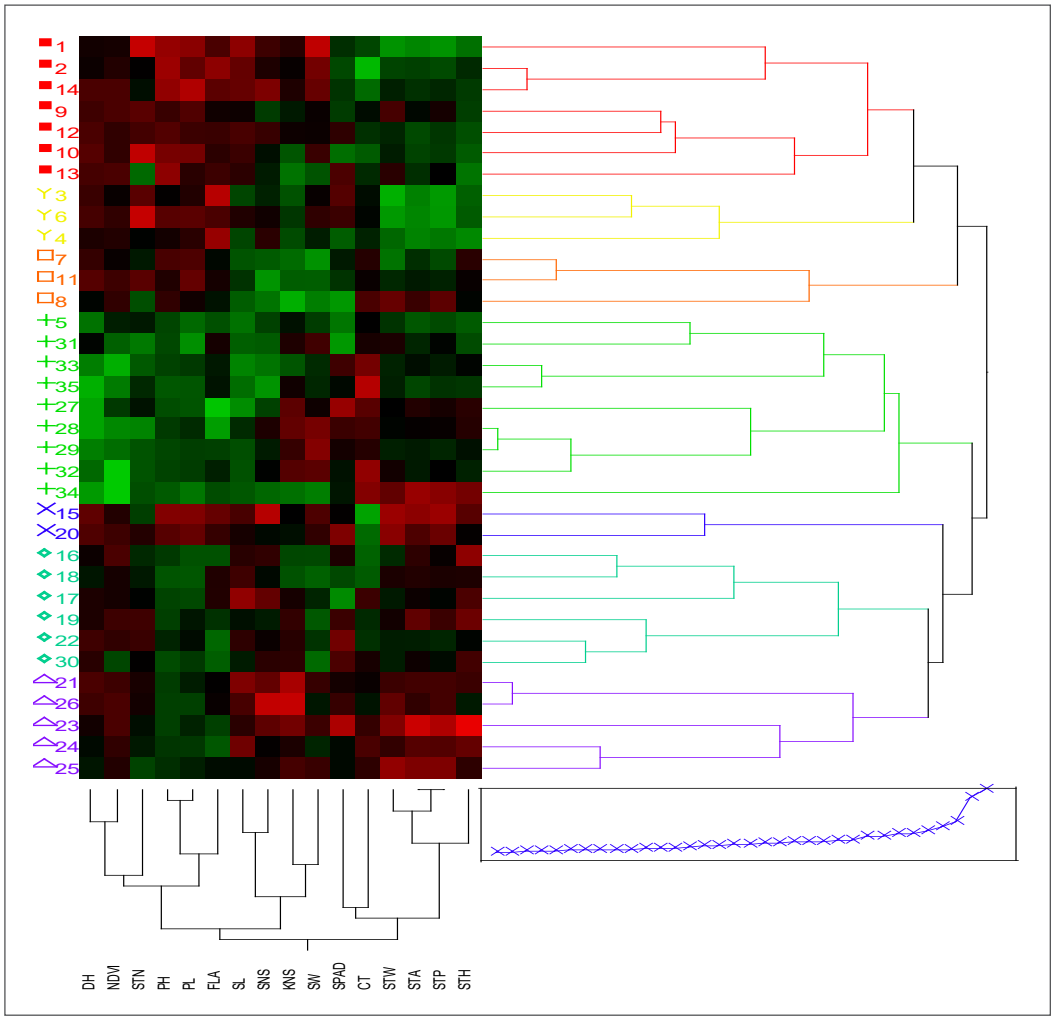

Figure 1. Cluster diagram of 35 durum wheat genotypes for parameters.

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Studies on Identification of Stable Genotypes of Lemongrass for Semi-Arid Regions

Ravinder SINGH Pritam Kumar VERMA Rishi Kumar BEHL Rajesh Kumar ARYA*

MAP Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar-125004 (India)

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

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ABSTRACT

The present research work was carried out for identification of stable genotypes of lemongrass for different characters over different spacing environments (60x60, 60x45 and 45x45) at Research area of MAP Section (GPB), CCS HAU, Hisar in RBD. The ANOVA for the stability revealed presence of both linear and non - linear G X E interactions. The results on oil content % (FWB) revealed that, out of all the thirty three genotypes / varieties, only twelve genotypes exhibited stable performance with high mean. NLG-4, Krishna, NLG-5, NLG-118, OD-58, NLG-84, HL-11, RRL-16 and CKP-25 were found best genotypes for oil content % (FWB) viewing high mean performance with above average (b_i>1) responsiveness; and genotype, OD-19, OD-23, and OD-388 were suitable for favourable environments, none were suitable for poor environments.

Keywords: *Cymbopogon* spp., stability, G X E, herbage yield, oil content

Introduction

The Indian subcontinent prospers in many aromatic plants. Chiefly three kinds of Lemongrasses are in cultivation, i.e. (i) East Indian / Malabar or Cochin Lemongrass (*Cymbopogon flexuosus*), (ii) West Indian Lemongrass (*C. citratus*) and (iii) North Indian Lemongrass (*C. pendulus*). East Indian Lemongrass is mainly cultivated in Kerala, A.P., Karnataa, T.N., Maharastra and U.P. In addition to this, Java citronella (*C. winterianus*) and Ceylon-citronella (*C. nardus*) mainly cultivated in Ceylon, Indonesia, India, and Sumatra, respectively. Lemongrass oil is used for making perfumes, cosmetics, creams and soaps. The bioactive compound, 'Citral' extracted from the oil, is a flavouring agent for soft drinks, scenting soaps and detergents, which has germicidal properties (Arya et al., 2021). After oil extraction, spent lemongrass may be utilized as raw material for paper making, or manure/ compositing and also as a fuel. Being a medicinal herb, lemongrass is found as a good carminative and antimicrobial. *C. nardus* is considered as an excellent source of citronella oil. This oil is an insect repellent and useful in ridding off dogs and cats parasites. Moreover, its oil also found helpful to clear the mind with a general toning as it has a very good tonic effect on human body. It also seems helpful to relieve cold and flu, and has antiseptic and deodorizing properties (Arya et al., 2021).

The genotypes - environments interaction, significantly contributed to the non realization of expected gain in relation to selection (Comstock and Moll, 1963). This condition imparts a serious hitch to the crop breeder in appropriate evaluation of genotype/ variety under different growing environments. Therefore, such a situation is complicated by the relationship of several environmental factors which vitiate the expression of the genotype/variety, when same are assessed over different environments. To overcome this difficulty, two types of schemes, statistical (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966) and the other genetical (Perkins and Jinks, 1968 a, b and Breeze, 1969) are utilized by

different research workers, which could be useful to give reliable estimation of these g x e interactions. From the research of these scientists, the most valuable finding which has came out is that the bulk of g x e interaction is often a linear function played a major part in building of total genotype-environment interaction. The range of genotypes/varieties could give a capable tool to measure and grade a progression of environments. Eberhart and Russell (1966) has been pointed out that in order to get unbiased estimates of stability parameters, the genotypes/varieties must be grown in an adequate number of environments.

Good stability and wider adaptability is a significant criterion to improve the herbage yield, oil yield, quality of oil and active compounds over a wide range of environments. It is always pleasing that a good yielding clone/genotype must be stable over different locations. Keeping in view the above discussion and increasing demand o essential oils produced from lemongrass, present investigation was carried out with the objective to identify the stable genotypes for different characters.

Materials and Methods

The present research work was carried out during *spring-summer-rainy* seasons for identification of stable genotypes of *Cymbopogon flexuosus* for different characters under three different environments created by planting the genotypes in different spacing, i.e. environment E_1 (60x60 cm), E_2 (60x45 cm) and E_3 (45x45 cm) at Research area of MAP Section (GPB), CCS Haryana Agricultural University, Hisar in randomized block design. Each genotype was accommodated in two rows of three meter length in each environment. For present study 33 genotypes of lemongrass viz., GRL-1, Krishna, NLG-1, Chirharit, NLG-2, NLG-3, NLG-5, NLG-4, NLG-6,NLG-7, NLG-9, NLG-8, NLG-118, NLG-10, NLG-84, OD-388, OD-23, OD-58, OD-19, RRL-16, HL-1, HL-2, HL-4, HL-3, HL-5, HL-7, HL-6, HL-8, HL-9, CKP-25, HL-10, HL-11 and HL-12. The observations were recorded on ten randomly selected planted in each genotype in each replication in each environment. Data recorded on plant height (cm), tillers per plant, fresh herb yield per plant (g) and oil content (%) FWB was subjected to analysis of variance as per standard procedure. The stability parameters were estimated as per procedure suggested by Eberhart and Russell, 1966.

Results and Discussion

The results on mean sum of squares due to g x e interaction revealed that genotypes/varieties have differential response to the change in environmental conditions. The performance of genotypes/varieties was found different under different environmental conditions. This pointed about the presence of g x e interaction for oil yield per plant and its related characters. Similar finding were also reported by Arya et al., (2022). It was also observed that both linear and non linear parameters extensively contributed to the total G x E interaction for all the traits but their magnitude varied (Table 1). There was preponderance of linear components for all the traits. This revealed that there is no association or complex relationship between the genotypes/varieties and environmental effects and in such a situation prediction is not possible. The results on the basis of the present investigation in relation to the stability parameters of individual genotype/variety are given in Table 2. The proportion of genotypes/varieties indicating predictable performance was high for all the traits. Linear components exhibited preponderance for yield per plant in analysis of variance here escaped in this analysis of stability parameters for individual genotypes/varieties, and oil content (FWB) came into notice in present study. This incongruity might be due to the discrepancy testing procedures in the two analyses.

As per stability model of Eberhart and Russell (1966), regression coefficient b_1, represents the linear component of G x E interaction and is a suitable measure of response of a variety/genotype to the alteration in the environment. A genotype / variety which reflect above average response ($b_1 \geq 1$) has b_1 value significantly greater than unity; such a genotype/ variety suitable for the better environment because improvement in the environment could only enhance the yield of such genotypes/varieties. Opposite to this, genotype with below average response ($b_1 \leq 1$) has b_1 value significantly less than unity; such a genotype/variety does not exhibit significant decrease with the decline of the environment. A genotype, which is relatively indifferent toward the change in the environment is believed to be average responsive ($b_1 = 1$) and will have regression coefficient value do not differ significantly from unity. Such genotypes/varieties are valuable for all the environments (Abhay et al., 2013, Arya et al., 2022).

In stability study, s main question comes in the mind of breeder that which type of linear regression is to be selected. The selection in crop plants for the type of response would differ with the alteration in the environmental conditions. The required level of interaction should be as low as possible to give maximum uniformity of presentation. But, according to Allard and Bradshaw, (1964) for inhibited factors like date of sowing, the desired level of interaction could

be as high as possible to increase the yield. It is always looked-for to select genotype/variety with high mean performance and above unity response because only such genotypes are going to make the use of superior environmental conditions. The difficulties arise in evaluating the required level of responsiveness when the two types of environment variables i.e. controllable and uncontrollable, are functioning at the same time. In such situation, it will be desirable to have a universal level of interaction, so that genotypes/varieties can be selected which combine low level of interaction with controllable variables. For such a condition, the genotypes/varieties could be chosen having, high average yield, regression of unity one (b_1=1) and least deviation from regression (S^2_{di}=0). Such genotypes designated as ideal genotypes.

Stability analysis in present investigation identified based on 33 genotypes/varieties which could be suitable for different kinds of environments are presented in Table 3. None of the genotype /variety conferred stable for all the traits under investigation. Out of 33 genotypes/varieties, six genotypes for plant height, four for number of tillers per plant, and nine for oil content % (FWB) were found stable. Out of 33 genotypes, tall genotypes were 19, of which six genotypes/ varieties viz., OD-19, OD-23, NLG-3, GRL-1, NLG-5, and NLG-6 were stable in performance (S^{-2}_{di}=0) and found suitable for wide range of environmental conditions (b_1=1). Fourteen genotypes/varieties revealed above average mean performance for number of tillers per plant, out of which only six genotypes/varieties exhibited stable performance. Most of them were fit for general adaptability (b_1=1) viz., NLG-118, NLG-8, NLG-3 and NLG-10. Only NLG-1 and NLG-9 revealed suitability for favourable environmental conditions and no genotypes was suitable for unfavourable conditions.

In the present investigation fresh herbage yield per plant is very important trait for which only 16 genotypes/varieties exhibited above average herbage yield per plant and remaining 17 genotypes/varieties revealed below average herbage yield per plant but none of the genotype/variety was found stable for this trait. More or less similar findings were also observed by Lal (2002) in citronella grass stability studies, where clones/ varieties were extremely unstable for elemol content (SFi=28.67) followed by herbage yield per plant (SFi=14.67). In present study environments were created by spacing, first environment E_1(60x60 cm) was most favourable due to availability of more nutrition and less compaction among plants, second environment E_2(60x45 cm) was moderate and environment E_3(45x45 cm) was least favourable due to more competition among plants.

Out of 33 genotypes/varieties, the findings on oil content % (FWB), showed only 12 genotypes with high mean and stability performance i.e. HL-11, NLG-4, Krishna, NLG-5, RRL-16, CKP-25, OD-58, NLG-84, and NLG-118 were found ideal genotypes/varieties. However, the genotypes, OD-19, OD-23, and OD-388 were found suitable for favourable conditions for oil content % (FWB) having high mean with above average (b_i>1) responsiveness. None of genotype was found suitable for poor environmental conditions. These results indicated that there was sufficient difference for mean performance among the genotypes / varieties under different environmental conditions. This revealed the incidence of high g x e interactions for oil yield in lemongrass genotypes / varieties. Above results were supported by Sharma et al., (1988), Lal (2012 and 2023), Kumar et al., (2022, 2023a,b).

Table 1. Magnitude of linear and non-linear components (%) of G x E in lemongrass.

Characters	Lemongrass	
	Linear (%)	Non linear (%)
Average Plant height (cm)	62.80	37.20
Tillers per plant	68.62	31.38
Fresh herbage yield per plant (g)	73.7	26.22
Oil content (%) FWB	50.00	50.00

Table 2. Distribution of different genotypes on the basis of different stability parameters in lemongrass.

Characters	Predictable		Unpredictable	
	Both bi and S^2_{di} Non-significant	Only bi significant	Both bi and S^2_{di} significant	Only S^2_{di} significant
Average Plant height (cm)	11	07	00	15
Tillers per plant	16	03	02	12
Fresh herbage yield per plant (g)	00	00	10	23
Oil content (%) FWB	27	05	00	01

Table 3. Stability parameters' estimates for different characters in lemongrass.

Sr. No.	Genotypes	Plant height (cm)			Tillers per plant			Fresh herb yield per plant (g)			Oil content (%) EWB		
		Mean	b_1	S^{-2}_{di}	Mean	b_1	S^{-2}_{di}	Mean	b_1	S^{-2}_{di}	Mean	b_1	S^{-2}_{di}
1.	Krishna	139.59	1.49	0.87*	82.15	2.00	9.93**	804.67	0.81	0.37**	0.72	0.34	1.00
2.	Chirharit	136.96	1.56	2.51**	77.56	2.36	5.18**	907.11	0.94	0.60**	0.35	-0.15*	1.77
3.	GRL-1	123.89	1.24	0.24	79.41	2.25	4.39**	868.56	0.63*	0.20**	0.47	0.07	0.83**
4.	NLG-1	139.19	1.67	3.65**	77.74	2.24**	1.06	1230.56	1.63*	0.57**	0.34	0.14**	0.67
5.	NLG-2	123.04	0.48**	0.09	59.96	-0.65*	3.56**	575.41	0.38**	0.09**	0.35	2.33**	0.67
6.	NLG-3	126.67	0.67	0.33	69.07	0.88	0.63	807.82	0.79	0.18**	0.37	1.27	0.37
7.	NLG-4	113.89	1.20	0.25	31.30	0.93	0.54	692.22	0.75	0.43**	0.48	0.58	4.46
8.	NLG-5	134.78	1.35	0.32	66.19	1.12	0.49	936.48	0.84	0.09**	0.40	0.63	2.88
9.	NLG-6	129.96	0.87	0.22	60.48	1.65	1.88*	907.59	0.84	0.32**	0.28	0.74	0.98
10.	NLG-7	142.33	1.64**	0.33	53.44	1.30	1.41	857.70	0.35	0.73**	0.37	0.77	0.14
11.	NLG-8	133.04	1.57**	0.15	69.26	1.41	0.65	956.85	1.36	0.77**	0.29	1.04	2.15
12.	NLG-9	128.41	1.47**	0.16	71.11	1.61	0.88	990.70	0.75	0.37**	0.28	0.90	1.39
13.	NLG-10	136.26	1.55	0.91*	72.85	-0.77**	0.22	721.22	0.89	0.09**	0.33	1.03	0.30
14.	NLG-118	117.44	0.94	0.12	68.48	1.09	1.32	662.96	0.77	0.26**	0.43	1.66	1.56
15.	NLG-84	116.15	0.81	0.10	55.37	0.72	3.00*	646.78	0.47**	0.05**	0.46	1.18	1.40
16.	OD-23	126.89	0.98	0.61*	64.04	1.34	0.58	736.96	1.05	0.33**	0.43	1.99**	0.35
17.	OD-388	122.07	1.41	0.92*	57.78	1.07	0.92	811.56	0.98	0.37**	0.44	2.45*	2.27
18.	OD-19	123.74	1.12	0.32	65.33	1.22	0.97	733.30	1.08	0.44**	0.43	2.10*	1.84
19.	OD-58	130.07	1.78	2.49**	73.78	0.60	1.90*	837.89	1.67*	0.57**	0.40	0.66	1.52
20.	RRL-16	121.78	1.55	2.83**	67.37	1.06	3.67**	667.67	1.23	0.16**	0.43	0.97	2.40
21.	HL-1	125.52	0.07**	0.43	65.19	0.15	2.36*	864.04	0.57	1.18**	0.29	0.45	0.67
22.	HL-2	116.41	0.89	0.06	46.56	1.08	0.71	687.63	1.06	0.47**	0.33	1.06	1.33
23.	HL-3	120.22	0.89	0.61	54.41	1.51	1.03	724.82	0.79	0.40**	0.33	1.86	1.74
24.	HL-4	94.04	0.64	2.78**	52.89	1.28	0.35	664.82	1.20	0.19**	0.31	0.96	1.15
25.	HL-5	91.67	0.57	2.37**	60.07	0.99	1.13	689.33	1.22	0.20**	0.29	1.01	0.70
26.	HL-6	94.44	0.49	1.36**	57.78	0.93	0.73	686.59	1.53**	0.23**	0.32	0.55	1.05
27.	HL-7	110.59	0.53*	0.19	64.44	1.18	1.14	866.22	1.47**	0.06**	0.35	1.02	2.47
28.	HL-8	102.44	0.74	1.38**	81.44	-0.67**	1.93*	873.96	1.11	0.44**	0.34	0.22	2.32
29.	HL-9	125.48	0.36**	0.10	78.82	1.81	1.90*	994.33	1.49*	0.34**	0.31	0.69	0.58
30.	HL-10	101.56	0.22	2.17**	51.96	-0.08	2.03*	821.93	1.65	0.89**	0.31	1.18	2.25
31.	HL-11	130.67	1.19	1.04*	57.52	-0.02**	0.96	688.67	0.41*	0.39**	0.40	1.54	3.44
32.	HL-12	139.00	0.34	1.11**	88.82	0.45	3.12**	1244.96	1.59*	0.48**	0.30	0.27*	0.64
33.	CKP-25	111.00	0.70	0.52	136.15	0.98	6.62**	836.67	0.71	0.22**	0.63	1.50	2.14
Pooled mean		122.09	1.00		67.23	1.00		818.12	1.00		0.38	1.00	
SE_m+ (mean)		0.25	0.36		0.50	0.53		0.53	0.23		0.29	0.78	

*,**= Significant at 5% & 1%, respectively.

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Genetic Diversity Analysis for Morphological Traits in Sorghum [*Sorghum bicolor* (L.) Moench]

Mohammad SHAFIQURRAHAMAN **Gajraj Singh DAHIYA** **Ashok Kumar DEHINWAL** **Vinay KUMAR***

Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar-125004, Haryana, India

* Corresponding author e-mail: kumar.vinay51012@gmail.com

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ABSTRACT

In present investigation 150 sorghum germplasm lines were studied for two years. The findings exhibited high heritability in association with high genetic advance. During 2015-16,82 genotypes (maximum) were grouped in cluster I, followed by cluster IV and cluster II with 22 and 19 genotypes, respectively, and cluster III having 11 genotypes, cluster VII having nine genotypes only, cluster X consisted of three genotypes, while V, VI, VIII and IX clusters remain confined to single genotype. The cluster distances ranged from 16.98 to 84.52 (within the clusters) and 40.65 to 73.37 (between clusters). Similarly, for 2016-17 are grouped into different clusters revealed that the highest number of genotypes (96) were confined to cluster I, followed by cluster IV, cluster II, cluster V, cluster III and cluster VI with 18, 17, 10, 8 and 1 genotype(s), respectively. The cluster distances ranged from 29.30 to 76,38 (within the clusters) and 0.00 to 71.11 (between clusters). Further for pooled data sorghum genotypes are grouped in to different clusters indicated that the 82genotypes (maximum) were associated with cluster I, followed by cluster IV, cluster III, cluster II, cluster VIII and cluster V with 19, 18, 15, 8 and 4 genotypes, respectively, cluster VI, cluster VII and cluster IX had only one genotype. The cluster distances ranged from 26.85 to 117.88 (within the clusters) and 65.87 to 117.88 (between clusters). The inter-cluster distances were more than intra-cluster distances, which pointed towards wide genetic diversity among the genotypes of various clusters than those of same cluster.

Keywords*:* Sorghum, clustering, polymorphisms, genetic divergence

Introduction

Among the forage crops, forage sorghum (*Sorghum bicolor* (L.) Moench) could be a deliberate choice because of the crop's xerophilic physiognomies, quick growing habit, adaptation potential, high palatability, rationality, digestibility, and widespread range of uses as fresh green fodder, roughage, and silage fodder. Moreover, it also has adaptability over a wide range of soils and climates (Borad et al., 2007). It is a well-known *kharif* crop for animal fodder, further genetic modification in its agronomic traits for forage will certainly benefit to reduce the gap between fodder demand and supply for the maximizing livestock production. In order to start enterprising with sorghum as a fodder and remunerative crop, there is an instant need to develop new cultivars/hybrids having high forage yield and quality (Shafiqurrahaman et al., 2022). To develop such forage varieties or hybrids, information and knowledge on genetic make-up is most important for the devising of an efficient breeding strategy for genetic improvement of sorghum as a forage crop. The genetic studies of quantitative and qualitative characters is needed before to start any breeding program for improvement of forage sorghum germplasm for these traits.

Possibility of attaining required genetic improvement in a crop depends mainly on the magnitude of genetic variability (Kaushik et al., 2020). The morphological variability uttered by a plant genotype or a group of genotypes in any plant species can be

divided into genotypic and environmental parameters (Raiger et al., 2021). The genotypic parameters being the heritable portion of the total variability in study material, its magnitude for fodder yield and its attributes, influences the selection approaches to be implemented by the plant breeder (Vu et al., 2019). The realization of any hybridization generally relays upon the selection of best suited diverse parents in genetic characters (Nguyen et al., 2017). Mahalanobis D^2 statistics founded on multivariate studies of quantitative characters is a commanding tool for the measurement of genetic divergence among different populations based on statistical distances for multivariate analysis (Mahalanobis, 1936). A complete awareness of the genetic relationship with diversity among the genotypes of sorghum will be helpful to development of new cultivars that can avoid drought stress, stand with low soil fertility, and resist against pests and diseases and also increase crop productivity under low input environments (Yuvaraja et al., 2019). Diversity study can also be a helpful device for mining germplasm collections for provinces associated with adaptive or agronomic desirable characters. Therefore, keeping said points in view, present investigation on forage sorghum was done.

Materials and Methods

Experimentation and data recording: The field trial was sown in a randomized block design (RBD) with three replications during 2015-16 and 2016-17 to examine the morphological genetic divergence among the genotypes of sorghum (*Sorghum bicolor* (L.) Moench.). All the150 sorghum genotypes (Table 1) were collected from NBPGR, New Delhi and planted at, Forage Section Research Area, Department of Genetics and Plant Breeding (CCSHAU Hisar, India). Hisar is located in the semi-arid subtropics and the experimental site in Hisar was situated at 29° 10' N latitude, and 75° 46' E longitude at an altitude of 215.2 meters above mean sea water level. Each genotype was accommodated in 3m row length with spacing 45x15 cm. The data was recorded on plant height (cm), stem diameter (cm), number of leaves/ plant, effective tillers/plant, leaf length of blade (cm), leaf width of blade (cm), panicle length without peduncle (cm), 1000 seed weight (g), green fodder yield/plant (g) and dry fodder yield/plant (g).

Statistical analysis: The analysis of variance (ANOVA)according to RBD was done on the basis of the model described in Panse and Sukhatme (1967). Phenotypic coefficient of variance (PCV) and genotypic coefficient of variability (GCV) were estimated according to Singh and Chaudhary (1982). Heritability in broad sense and Genetic advance were estimated as suggested by Burton and Devane (1953). Genetic divergence estimated as per Mahalanobis (1936). All the germplasm accessions were clustered into various groups according to Tocher's method (Rao, 1952). The intra- and inter-distances were also estimated as per the criterion used in clustering to the same cluster should at least on the average, display a lesser D^2 values, than those belonging to diverse clusters.

Results and Discussion

Heritability, PCV, GCV and Genetic Advance

For initiating any crop breeding, evidence on the nature and magnitude of genetic variability is of immense importance because occurrence of significant variability in the base germplasm confirms better probabilities of evolving desired outcome. During 2015-16, PCV, GCV, heritability and genetic advance (Table 2) are found valuable in defining the method of selection to make genetic improvement in a specific population for a definite character. It was constantly not essential for high heritability to be related with desirable genetic advance. The high heritability joined with desirable genetic advance specifies that additive genetic effects are dominant and simple will be useful for desirable improvement. High heritability was perceived for studied traits, except leaf length and leaf width. High heritability was found associated with high genetic advance for the characters viz., plant height, stem diameter, number of leaves/plant, effective tillers/ plant, panicle length excluding peduncle, 1000-seed weight, green fodder yield/plant (g) and dry fodder yield/plant (g). It may be because of the occurrence of additive gene action for above traits and selection for their genetic improvement is recommended. Moderate heritability coupled with moderate genetic advance was observed for leaf length of blade (cm) and leaf width of blade (cm). The high GCV and PCV were detected for plant height, stem diameter, number of tillers/plant, and number of leaves/plant, panicle length excluding peduncle, 1000-seed weight, green fodder yield/plant and dry fodder yield/plant. Moderate GCV and moderate PCV was observed for leaf length. Whereas, moderate GCV and high PCV for leaf width of blade (cm) was observed. The differences in GCV and PCV is low for these traits indicating less environmental effect for these traits.

Likewise, during 2016-17, PCV, GCV, heritability and genetic advance are convenient in decisive the technique of selection desired genetic improve in a particular population for a particular character. High heritability is not necessary to be found accompanying with high genetic advance for the required trait. High heritability and high genetic advance linkage specify

the presence of additive genetic effects therefore simple selection method is suggested for desirable improvement. In the present study except leaf length and leaf width high heritability was detected for the characters studied. High heritability and high genetic advance, both were associated with each other for plant height, stem diameter, number of leaves/plant, number of tillers per plant, panicle length except peduncle, 1000-seed weight, green fodder yield/plant and dry fodder yield/plant. It may be due to the presence of additive gene action for these characters and selection may be effective for their improvement. Moderate heritability coupled with moderate genetic advance was observed for leaf length of blade (cm) and leaf width of blade (cm). High GCV and high PCV were observed for traits like plant height (cm), stem diameter (cm), number of tillers per plant, number of leaves per plant, panicle length without peduncle, 1000 seed weight, green fodder yield per plant (g) and dry fodder yield per plant (g). Moderate GCV and moderate PCV was observed for leaf length of blade (cm) and leaf width of blade (cm). The differences between GCV and PCV is fewer for these traits indicating less environmental effect for these characters.

The differences among GCV and PCV are less for these traits indicating less environmental effect for these traits. Similar findings were described by Vinodhana et al., (2009) for PCV and GCV for plant height, and 1000- seed weight. Bello et al., (2007) reported high PCV, high GCV and high heritability for leaf length, leaf width, number of leaves per plant, plant height and 1000 seed weight. Likewise, high heritability and high genetic advance for plant height and fodder yield per plant was reported by Wadikar et al., (2018). More or less similar research findings were stated for high PCV, GCV, high heritability associated with high genetic advance for plant height, number of tillers/plant, green fodder yield/plant, dry fodder yield/plant, 1000-seed weight and panicle length excluding peduncle, and also moderate GCV, PCV, heritability associated with moderate genetic advance for leaf length and leaf width by Singh et al., (2010) and Deepak et al., (2017).

Genetic divergence

Development of high yielding varieties is documented as a definite area since population explosion with expansion and decreasing crop cultivation areas are the serious aspects causing fodder uncertainty for animals in emerging countries Most of the varieties available with us were developed by selection so new varieties have reduced genetic variability and selection in these genotypes further reduced the genotypic variability. As the genotypic variability for the desirable traits has exhausted from the genotypes there is need to identify new genes contributing to desirable traits. Diversity in germplasm offers chance for breeders to create new and genetically superior variety with required traits as germplasm has broad genetic base. That's why, deification of genotypes for crossing should be relay on genetic divergence among genotypes and not on geographic background. Therefore, genotypes grouping based on different ecogeographic areas into single group could be credited to the regular exchange of germplasm among different locations and its further selection of different geographic areas, may consequence in genetic drift.

In the present study150 genotypes of sorghum were categorized into different clusters using Tocher's method (Rao 1952) based on the D^2 values (Table 3-4). Grouping of sorghum genotypes into ten clusters showed that the 82 genotypes were grouped in cluster I, followed by the cluster IV and cluster II with 22 and 19 genotypes respectively, cluster III having 11 genotypes, cluster VII having nine genotypes, cluster X having three genotypes, while V, VI, VIII and IX clusters having single genotype. The cluster distances ranged from 16.98 to 84.52 (within the clusters) and 40.65 to 73.37 (between clusters) for year 2015-16. Similarly, for year 2016-17 genotypes were placed in different groups indicating that the 96 genotypes were involved in cluster I, followed by cluster IV, cluster II, cluster V, cluster III and cluster VI with 18, 17, 10, 8 and 1 genotype, respectively. The cluster distances ranged from 29.30 to 76.38 (within the clusters) and 0.00 to 71.11 (between clusters). Further for pooled data, genotypes were assembled in to different clusters indicating that the highest number of genotypes were involved in cluster I, followed by cluster IV, cluster III, cluster II, cluster VIII and cluster V with 19, 18, 15, 8 and 4 genotypes, respectively. However, cluster VI, cluster VII and cluster IX had single one only.

The results on intra- and inter- cluster distances are accessible in Table 5-6. The data range revealed the cluster distances from 26.85 to 117.88 (within the clusters) and 65.87 to 117.88 (between clusters). The higher inter-clusters distances than the intra-cluster, revealed the extensive diversity among the genotypes of different clusters rather than the same one. This advocated that genotypes occurring in same cluster had very less diversity and selection of parents for hybridization within the cluster is not found promising for the development of noble segregants. The greater distances among the cluster, further demonstrating substantial volume of diversity amongst the genotypes used in present studied. Based on D^2 analysis, inter-cluster distance is the chief selection criterion for genotypes for hybridization.

The data on cluster means are presented in Table 7 for 2015-16, Table 8 for 2016-17 in compasses the presence of huge genetic diversity in sorghum study material. Genetic diversity available in the germplasm was also advocated by the considerable volume of difference among cluster means for diverse traits. Similar findings were noticed by Prasad and Biradar (2017) in which the different genotypes were classified into 22 groups, whereas cluster-I had maximum of 115 genotypes, followed by cluster-II having 45 genotypes only. Highest inter-cluster distance was found among clusters-III and XXI, followed by among cluster-XIII and XXI. Damor et al., (2017) reported five clusters of sorghum genotypes. According them, Cluster I had maximum of 40 genotypes but, cluster II had 16 genotypes, cluster IV only two genotypes, while cluster III & V with single genotype. Meena et al., (2016) also observed the genotypes were grouped into ten clusters. Maximum distance among clusters was observed in clusters II & IX, whereas minimum was in VI & VIII. Maximum distance within the cluster was found in cluster-IX followed by cluster-VII. Likewise, Kumar et al., (2010) also grouped accessions into eight clusters. The cluster-I comprised of 15 genotypes and cluster-V of 10 genotypes, cluster IV of 9 ones. The inter cluster distances were higher among cluster-VII & VIII followed by cluster-III and VII and cluster V and VIII. In sorghum, such findings were also observed by Yuvaraja et al., 2019.

Character contribution in genetic divergence

The data of present study depicted that each trait had performed at number one rank and its respective contribution (%) towards genetic divergence (Table 9). For 2015-16, relative contribution of characters such as panicle length without peduncle was highest towards genetic divergence (31%), followed by 1000 seed weight (29.03%), green fodder yield (20.48%), followed by total tillers/plant, plant height, leaves per plant, dry fodder yield/plant, stem diameter and leaf length of blade, respectively, to the genetic divergence in decreasing order. Similarly, for 2016-17 share of panicle length without peduncle was highest in total genetic divergence (33.44%), followed by 1000 seed weight (27.45%), green fodder yield (17.66%), followed by total tillers per plant, plant height, leaves per plant, dry fodder yield and stem diameter respectively to the genetic divergence in decreasing order. Similar results were reported by Singh et al., (2008) for number of leaves/plant found greatest involvement towards plant divergence followed by green fodder yield and leaf breadth. Khadakabhavi et al., (2014) for yield/plant reported maximum contribution in genetic divergence followed by 1000-seed weight, length of panicle, height and days to 50% flowering, these characters can be exploited for further genetic enhancement.

To develop new varieties or hybrids of forage sorghum, information and knowledge on genetic make-up is most important for the devising of an efficient breeding strategy for genetic improvement of forage sorghum. In present study, information on genetic variability, divergence, inheritance and genetic advance of important quantitative and qualitative characters seems to very important to draft a new breeding program for genetic improvement of forage sorghum germplasm for these traits.

Table 1. List of forage sorghum germplasm lines.

S. No	Accession No	S. No	Accession No	S. No	Accession No	S. No	Accession No	S. No	Accession No
1	IC-485180	31	IC-240855	61	IC-485003	91	IC-485233	121	IC-585202
2	EC-486333	32	IC-240856	62	IC-485009	92	EC-464430	122	IC-585203
3	IC-484860	33	IC-240859	63	IC-485011	93	IC-298598	123	IC-585204
4	IC-546929	34	IC-240860	64	IC-485244	94	IC-298601	124	IC-585205
5	IC-121559	35	IC-240861	65	IC-484515	95	IC-298605	125	IC-585209
6	IC-484320	36	IC-240862	66	IC-484583	96	IC-309905	126	IC-585218
7	IC-484895	37	IC-240864	67	IC-484628	97	IC-309906	127	IC-585219
8	IC-484962	38	IC-240865	68	IC-484696	98	IC-309907	128	IC-585225
9	IC-484968	39	IC-240866	69	IC-484714	99	IC-309914	129	IC-585233
10	IC-485002	40	IC-240871	70	IC-485145	100	IC-309944	130	IC-585234
11	IC-485024	41	IC-240872	71	IC-485177	101	IC-353607	131	IC-585239
12	IC-240831	42	IC-240876	72	IC-484591	102	IC-585143	132	IC-585240
13	IC-240832	43	IC-240877	73	IC-484729	103	IC-585174	133	IC-296496
14	IC-240833	44	IC-240879	74	IC-484750	104	IC-585176	134	IC-395722
15	IC-240835	45	IC-240880	75	IC-484767	105	IC-585177	135	IC-395816
16	IC-240837	46	IC-240881	76	IC-484826	106	IC-585180	136	IC-436867
17	IC-240838	47	IC-436857	77	IC-484855	107	IC-585184	137	IC-413297
18	IC-240839	48	IC-240883	78	IC-484351	108	IC-585185	138	IC-413299
19	IC-240840	49	IC-240884	79	IC-484418	109	IC-585189	139	IC-436523
20	IC-240841	50	IC-484974	80	IC-484430	110	IC-585190	140	IC-436527
21	IC-240842	51	IC-485023	81	IC-484444	111	IC-585191	141	IC-436572
22	IC-240843	52	IC-485028	82	IC-484445	112	IC-585192	142	IC-436577
23	IC-240845	53	IC-485030	83	IC-484489	113	IC-585193	143	IC-527019
24	IC-240846	54	IC-485039	84	IC-484491	114	IC-585194	144	IC-527022
25	IC-240848	55	IC-484819	85	IC-484510	115	IC-585195	145	IC-397246
26	IC-240849	56	IC-484869	86	IC-484637	116	IC-585196	146	IC-436682
27	IC-240850	57	IC-484870	87	IC-484658	117	IC-585197	147	IC-436752
28	IC-240851	58	IC-484911	88	IC-485143	118	IC-585198	148	IC-436791
29	IC-240852	59	IC-484989	89	IC-485188	119	IC-585200	149	IC-436916
30	IC-240853	60	IC-484997	90	IC-485202	120	IC-585201	150	IC-436796

Table 2. Heritability, GCV, PCV and Genetic advance value % of sorghum genotypes in 2015-16 and 2016-17.

S. No	Year	Heritability (%)	GCV (%)	PCV (%)	Genetic Advance Value % of Mean
Plant height	2015-16	78.28	23.15	26.17	42.20
	2016-17	79.18	21.80	24.50	39.96
Stem diameter (cm)	2015-16	64.05	21.49	26.86	35.44
	2016-17	68.27	22.20	26.87	37.79
Number of tillers per plant	2015-16	85.39	29.62	32.06	56.39
	2016-17	86.51	29.54	31.76	56.60
Number of leaves per plant	2015-16	63.33	23.20	29.16	38.04
	2016-17	69.75	23.71	28.38	40.78
Leaf Length of blade (cm)	2015-16	45.29	11.88	17.65	27.98
	2016-17	49.15	12.19	17.39	17.60
Leaf width of blade (cm)	2015-16	40.56	13.87	21.78	18.20
	2016-17	41.07	12.74	19.87	16.81
Panicle length without peduncle (cm)	2015-16	92.17	32.26	33.60	63.80
	2016-17	92.38	32.55	33.87	64.45
1000 seed weight (g)	2015-16	93.41	38.89	40.24	77.44
	2016-17	93.11	38.31	39.70	76.15
Green fodder yield (g)	2015-16	86.16	42.58	45.87	81.42
	2016-17	84.91	42.31	45.91	80.31
Dry fodder yield (g)	2015-16	81.94	42.53	46.99	79.31
	2016-17	81.65	42.27	46.78	78.69

Table 3. Number of genotypes in each cluster for 2015-16.

Cluster	Genotypes
Cluster1	80, 87, 126, 43, 2, 81, 88, 150, 105, 37, 149, 94, 147, 97, 29, 92, 63, 70, 106, 74, 89, 95, 28, 144, 112, 107, 114, 102, 64, 134, 135, 123, 26, 122, 6, 121, 13, 130, 60, 27, 104, 73, 133, 90, 3, 31, 52, 139, 48, 91, 8, 131, 100, 68, 38, 88, 115, 47, 128, 148, 33, 103, 111, 14, 101, 145, 54, 146, 7, 50, 59, 120, 160, 124, 98, 96, 99, 136, 49, 110,1 25, 108
Cluster 2	84,85,83,143,66,32,44,24,86,41,77,61,45,127,9,53,75,76,71
Cluster 3	132,138,129,118,141,140,117,119,12,72,10
Cluster 4	57,109,55,56,142,51,137,67,20,62,36,25,93,46,21,11,22,58,34,40,69,16
Cluster 5	15
Cluster 6	35
Cluster 7	4,5,18,42,17,19,1,113,30
Cluster 8	78
Cluster 9	79
Cluster 10	23,39,65

Table 4. Number of genotypes in each cluster for 2016-17.

Cluster	Genotypes
Cluster1	111,121,122,27,6,8,106,134,135,28,147,13,64,14,115,130,123,94,105,29,92,126,26,139,60,73,80,112,114,89,95,74,63,70,87,43,81,2,107,150,104,3,97,91,37,88,133,48,52,31,90,131,82,38,68,47,144,146,128,100,103,148,149,7,50,145,102,75,9,54,33,59,62,110,124,101,49,119,72,142,116,98,67,12,120,96,99,136,141,108,32,44,10,20,85,84
Cluster 2	57,109,55,56,137,51,25,36,93,46,11,22,21,58,45,61,127
Cluster 3	132,138,129,125,118,140,117,65
Cluster 4	71,76,77,78,79,41,83,143,66,53,24,86,69,1,23,40,34,39
Cluster 5	15,16,113,19,17,4,5,42,18,30
Cluster 6	35

Table 5. Intra and inter-cluster distances for 2015-16.

0	1	2	3	4	5	6	7	8	9	10
1	25.73	38.62	34.64	40.11	52.02	38.01	68.21	50.43	50.03	49.61
2		30.22	52.18	48.01	57.73	46.81	72.64	35.02	35.70	54.45
3			28.56	49.88	47.56	45.54	64.73	69.68	69.39	43.01
4				35.13	47.30	45.31	58.30	52.66	47.21	58.81
5					0.00	56.39	29.35	73.47	66.97	40.65
6						0.00	69.66	57.39	51.91	52.21
7							35.74	84.52	77.29	57.24
8								0.00	16.98	73.37
9									0.00	71.23
10										45.20

Table 6. Intra and inter-cluster distances for 2016-17.

Group	1	2	3	4	5	6
1	29.30	43.92	41.32	44.98	69.52	44.33
2		35.20	64.11	49.01	59.58	51.86
3			29.55	62.16	76.38	56.63
4				41.69	69.98	50.54
5					36.92	71.11
6						0.00

Table 7. Cluster means for 2015-16.

	Char.1	Char.2	Char.3	Char.4	Char.5	Char.6	Char.7	Char.8	Char.9	Char.10
Group.1	132.40	8.69	1.08	9.80	47.04	4.75	14.67	20.04	83.09	38.21
Group.2	117.46	9.48	1.21	9.59	49.14	4.89	24.17	17.64	84.05	38.49
Group.3	151.85	9.75	1.15	11.43	49.45	5.00	9.76	13.11	111.42	51.64
Group.4	120.06	10.64	1.40	10.20	49.17	5.00	16.07	32.80	113.12	52.28
Group.5	164.00	10.47	2.00	10.10	53.00	5.47	15.53	21.42	197.00	84.67
Group.6	50.33	10.13	2.00	12.07	46.33	4.37	12.70	20.88	53.67	25.03
Group.7	145.74	10.89	2.14	12.70	48.70	5.63	15.91	27.07	216.00	99.30
Group.8	105.33	9.53	1.00	13.60	57.00	4.20	29.80	28.32	65.33	27.33
Group.9	92.67	8.63	1.43	9.93	35.00	3.70	28.47	31.54	73.67	30.00
Group.10	133.11	10.94	1.70	11.99	50.00	5.01	15.48	6.78	143.89	67.54

Table 8. Cluster means for 2016-17.

	Char.1	Char.2	Char.3	Char.4	Char.5	Char.6	Char.7	Char.8	Char.9	Char.10
Group.1	132.47	8.95	1.10	9.66	47.28	4.96	15.14	20.50	87.00	40.26
Group.2	118.21	11.35	1.33	9.23	51.25	5.37	17.71	34.14	114.12	53.57
Group.3	164.37	9.50	1.06	11.82	49.25	5.11	8.70	9.26	108.75	50.88
Group.4	106.20	9.89	1.44	10.76	46.94	4.89	23.59	18.18	92.07	43.22
Group.5	148.57	10.99	2.16	12.61	51.03	5.53	15.24	26.75	215.53	98.30
Group.6	53.67	10.13	2.00	12.53	45.67	4.10	12.63	21.20	56.67	27.00

Table 9. Contribution (%) of different traits to diversity of fodder sorghum.

Sr. No.	**Source**	**Times Ranked 1st**		**Contribution %**	
		2015-16	**2016-17**	**2015-16**	**2016-17**
1.	Plant height(cm)	655	555	5.86%	4.97%
2.	Stem diameter (cm)	31	14	0.28%	0.13%
3.	Number of tillers/plants	796	1366	7.12%	12.22%
4.	Number of leaves/plants	222	262	1.99%	2.34%
5.	Leaf Length of blade (cm)	2	0	0.02%	0.00%
6.	Leaf width of blade (cm)	0	0	0.00%	0.00%
7.	Panicle length without peduncle (cm)	3722	3737	31.00%	33.44%
8.	1000 seed weight (g)	3244	3067	29.03%	27.45%
9.	Green fodder yield (g)	2289	1967	20.48%	17.66%
10.	Dry fodder yield (g)	214	207	1.91%	1.85%

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Diversity Analysis for Drought Tolerance in Pearl Millet Inbred Lines using SSR Markers

Jagdeep SINGH[1*] Ashok K CHHABRA[2] Rishi K Behl[3] Akshay K VATS[4] Pooja MALIK[5]

[1,3,4,5] Department of Agriculture Maharishi Markandeshwar University Mullana-Ambala, Haryana, India
[2] Department of Genetics and Plant Breeding, CCSHAU Hisar, Haryana, India

[*] Corresponding author e-mail: jagdeep.singh@mmumullana.org

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ABSTRACT

50 pearl millet genotypes (inbred lines) were grown in RBD design at two contrasting locations in Haryana, India including one at CCSHAU Hisar and another at RRS, Bawal. Genomic DNA was isolated from leaves of 2-3 weeks old plants using CTAB extraction method. The SSR diversity data was used to determine the genetic relationship among the fifty genotypes. A similarity matrix was first made using SIMQUAL subprogram of software. The dendrogram was then constructed based on the simple matching coefficient using SAHN sub-program. The SAHN sub-program uses UPGMA algorithm to perform cluster analysis. Out of 50 SSRs used for identification of single marker analysis 9 SSRs were found polymorphic for further checking their behaviour on known drought tolerant (HTP 93-37, HTP 03/13-901-1) and drought-sensitive inbreeds (HM S33B, HMS 42B) and were used for amplification of DNA. SSRs showed amplification for all genotypes and thus confirmed in other genotypes for the study of drought in pearl millet genotypes. Primers amplified a total of 237 alleles which varied from 2 to 8 with a mean of 4.54 alleles per locus. The overall size of PCR-amplified products ranged from 140 bp (*PSMP 2271*) to 810 bp (*ICMP 10*). Polymorphic information content (PIC) value ranged from 0.326 (*PSMP2201*) to 0.89 (*XCUMP 009*) with an average of 0.579. 50 Inbred lines based on SSR marker polymorphism data were resolved into 11 diverse clusters two genotypes HTP 9337 and HMS 43B were failed to fall into any cluster. Based on SSR markers and morphophysiological data two inbred lines (HTP 93-37, HTP 03/13-901-1) appeared drought tolerant which may be used for hybrid development program.

Keywords: Inbred, drought tolerant, polymorphism, genotypes, SSR markers

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the fourth most important Nutri cereal crop in India, after rice, wheat and sorghum. It is grown in the arid ecology of Indian states like Rajasthan, Maharashtra, Gujarat and Haryana as well as many other sub-Saharan African countries. It occupies an area of 6.93 million ha with an average production of 8.61 million tons and productivity of 1,243 kg ha^{-1} (Directorate of Millets Development, 2020). The productivity of pearl millet is influenced by the genotypes of plants, its growing environment and genotype x environment interaction (Arya and Yadav, 2009). There is considerable variability was found in pearl millet for adaptation to different environments including water-stress environments leading to drought. This genetic variability can be accessed at morphophysiological and molecular levels. Assessment of genetic variability at morphophysiological is confronted with an unknown degree of G X E interaction influencing character expressions, hence it is less reliable. On the other hand, genetic variability at the molecular level is precise as the DNA is independent of environmental conditions and its genes by itself, thus genetic variability at the molecular level represents true variability that can be transmitted (Satyavathi et al., 2013; Bairwa et al., 2023). Several molecular markers have been used to characterize different crops and their varieties for their molecular diversity including RAPD,

ISSR, SSR SNPS (Jaiswal et al., 2007; Priya et al., 2022; Singh et al., 2023; Bairwa et al., 2023). SSR method is found to be the most appropriate method to study the molecular diversity among the pearl millet genotypes (Colagar et al., 2016).

Pearl millet is multi useful crop as food feed and biofuel which has high protein and minerals. Being gluten-free is an important crop for the wellness of human health particularly for challenging people with diabetes and cardiovascular diseases (Gagan et al. 2023). Although pearl millet is adapted to water stress conditions there are considerable variability for genotype-dependent drought tolerances is there among its genotypes. Therefore, development of pearl millet hybrids possessing high production potential coupled with high tolerance is the most important concern of pearl millet breeders for food security in arid and semi-arid regions (Abhay Bikash 2013; Arya et al., 2014). Screening of pearl millet inbreds plants for drought tolerance plants and their molecular characterization is their fore imperative to identify pearl millet inbred lines possessing desirable traits and diversity at the molecular level. Current study deals with the determination of molecular diversity among pearl millet inbred lines using molecular markers.

Materials and Methods

Materials

The experimental materials comprised fifty pearl millet inbred lines procured from Chaudhary Charan Singh Haryana Agricultural University (CCSHAU) Hisar listed in Table 1. The experimental material was raised at two contrasting locations in Haryana the first location was at CCSHAU, Hisar is situated in the semi-arid climate at 29° 17′ N latitude and 75° 47′E longitude at an altitude of 215.2 meters above mean sea level in the subtropical climatic zone of India. The second location at Regional Research Station (RRS), Bawal, CCSHAU, Hisar is situated at a latitude of 28^{0}08'N, longitude of 76^{0}58'E and altitude of 266 m above sea level in the semi-tropical region of the western zone of India. Chemicals used for preparing DNA extraction buffer, PCR amplification and gel electrophoresis were obtained from G-Biosciences, USA and Sigma Chemicals Co. USA. All other chemicals used were of molecular biology grade or analytical grade and procured from Sigma Chemicals Co., USA, G-Biosciences, USA, Sisco Research Laboratories Pvt. Ltd., India and Affymetrix Inc., USA. Glassware of Borosilicate quality and plastic-ware used throughout the investigation were obtained from Borosil India Ltd. and Tarsons Products Pvt. Ltd. respectively. Specifically, design 50 SSR primers were selected for studying molecular diversity among genotypes. These were synthesized on order from Imperial Life Sciences, USA, the primer pairs included 10 PSMP, 6 ICMP, 5 UMP, 5 CT M and 4 PGIRD series of markers (Allouis et al., 2001; Qi, 2004; Budak et al., 2003; Mariac et al., 2006). The sequence information of forward and reverse primers used for genotyping pearl millet SSR loci is given in Table 2.

Methods

Fifty pearl millet genotypes (inbred lines) were obtained from diverse sources and were grown at Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar in Randomized Block Design (RBD) at CCSHAU Hisar (normal environment) and at Bawal (drought stress environment). Leaves samples were drawn from each of the 50 inbred lines to extract DNA using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method given by Murray and Thompson (1980) and modified by Saghai-Maroof et al. (1984). The extracted DNA was purified by removing RNA through the RNase enzyme. DNA samples were treated with 2 µl of RNase A solution (5 mg/ml) per 50 µl DNA sample to remove RNA contamination. The samples were incubated in water bath at 37°C for 4-5 h. After incubation samples were again checked for any RNA left. The purified DNA was analyzed for qualitatively and quantitatively. Quality and Quantity of the isolated genomic DNA was estimated by UV spectrophotometer and agarose gel electrophoresis. Absorbance at 260 nm and 280 nm wavelength was noted using UV Spectrophotometer, the ratio of two wavelengths was calculated and samples with a ratio of 1.7 to 1.8 was considered to be of good quality.

A260 / A280 = 1.8 (pure DNA)

Quality of DNA was also checked by submerged horizontal electrophoresis. A 0.8% (w/v) agarose gel was prepared for this (Sambrook et al., 1989). Gel casting plate was washed air-dried and its ends were sealed with tape. Agarose was melted in 0.5 X TBE buffer and ethidium bromide (10 mg/ml) was added, 1 µl per 50 ml of the gel. Gel solution was then poured into gel casting plate inserted with an appropriate comb to get a 0.4-0.6 cm thick gel. After setting of gel, sealing tapes were removed from both the ends. Gel plate was placed in the electrophoresis chamber and submerged using 0.5 X TBE buffer, combs were removed gently. Samples were prepared by adding 1 µl 6X loading dye along with 8 µl sterile distilled water and pulse centrifuged for proper mixing. Samples were loaded in the wells and electrophoresis was carried out at constant voltage (3 V/cm of gel) until dye migrated to other end of the gel. Gel was then viewed under UV transilluminator and photographed using UV Gel

documentation system. For estimation of quantity of the DNA by UV Spectrophotometer, aliquot of each DNA sample was diluted to the appropriate concentration and absorbance was measured at 260 nm as well as at 280 nm wavelengths. Using the relationship of 1.0 O.D. at 260 nm equivalent to 50 µg DNA per ml, the quantity of DNA was estimated by using the following formula:

DNA (µg⁄ml)=A260×Dilution factor × 50 (µg⁄ml)

For estimation of quantity by 0.8% agarose gel electrophoresis, a lambda DNA of known concentration (50 ng/ µl) was run along with DNA samples.

Fifty specifically designed SSR markers were used to characterize diversity at the DNA level and to identify qualitative genes conferring drought tolerance. The PCR amplification reaction was carried out in G-Storm and Bio-Rad thermocyclers. The PCR reaction contained;

DNA template (50ng)	: 1.0 µl
DMSO	: 1.0 µl
PCR buffer (10 X)	: 2.0 µl
dNTPs mix (10 mM)	: 0.5 µl
F. primer (2.5 µM)	: 1.0 µl
R. primer (2.5 µM)	: 1.0 µl
Taq DNA Polymerase (5 U/µl)	: 0.5 µl
Sterile distilled water	: 12.5 µl
Total volume	: 20 µl

The PCR reaction (20 µl) was set up in 0.2 ml thin-walled PCR tubes with the following reaction conditions:

i. 94°C for 3 min (initial denaturation)
ii. 94°C for 45 s (denaturation)
iii 46-61°C for 1 min (primer annealing)
iv. 72°C for 45 s (primer extension)
Step ii to iv for 5 cycles
v. 94°C for 45 s (denaturation)
vi 44-59°C for 1 min (primer annealing)
vii. 72°C for 45 s (primer extension)
Step v to vii for 30 cycles
v. 72°C for 10 min (final primer extension)
The product was kept at 4°C till further use.

PCR-amplified products were first checked for amplification on 2.5% agarose gel electrophoresis. For this 2-3 randomly selected PCR amplified products for a particular SSR were resolved and viewed using UV transilluminator. The marker-positive samples were then finally resolved using Polyacrylamide Gel Electrophoresis. Bands for SSR analysis were scored based on the presence (taken as 1) or absence (taken as 0) of bands. The size (in nucleotides base pairs) of the most intensely amplified bands for each microsatellite marker was determined based on its migration relative to the standard DNA marker (20 or 100 bp DNA ladder). Multiple alleles were inferred whenever a given marker produced more than one cluster of bands. The polymorphism information content (PIC) for each SSR marker was calculated according to the formula given by Anderson et al. (1993).

Only 0/1 matrix of allele scoring was used to calculate the similarity genetic distance using 'SIMQUAL' sub-programme of NTSYS-pc (version 2.02e) software (Numerical Taxonomy and Multivariate Analysis System Programme, Rohlf, 2000). The dendrogram was constructed by using the distance matrix in SAHN sub-programme of NTSYS-pc by the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) algorithm. Principal Component Analysis (PCA) was done to construct 2 and 3-dimensional diagrams**.** The PAGE was not run due to technical reasons; therefore, no information is available with regard to genetic diversity parameters.

Results

The quantity of DNA obtained from different plants ranged from 200-1000 µg/ml. A260/A280 ratio ranged from 1.75 to 1.85, indicating that the DNA was free from contaminants like polyphenols, polysaccharides, proteins and RNA, etc. A single band of high molecular weight, obtained on 0.8% agarose gel electrophoresis, confirmed that genomic DNA was intact and free from any mechanical or enzymatic degradation.

Variation in allelic profile for SSR markers

To check polymorphism among fifty genotypes, enlisted SSR markers were screened using 2.5% agarose gel electrophoresis for the resolution of bands. Agarose gels displaying allelic polymorphism among genotypes for SSR markers are shown in Fig. 1 to 8. Salient features of microsatellite marker analysis are as follows:

Primers amplified a total of 237 alleles which varied from 2 to 8 with a mean of 4.54 alleles per locus. The overall size of PCR-amplified products ranged from 140 bp (*PSMP 2271*) to 810 bp (*ICMP 10*). Polymorphic information content (PIC) value ranged from 0.326 (*PSMP2201*) to 0.84 (*ICMP 3056*) with an average of 0.579. The amplification range (bp), number of alleles per locus and polymorphic information content (PIC) value of PCR amplified product for individual primer is shown in Table 3.

Molecular marker-based genetic diversity analysis

The SSR diversity data was used to determine the genetic relationship among the fifty genotypes using NTSYS-pc software version 2.02e. A similarity matrix was first made using SIMQUAL subprogram of software. The dendrogram was then constructed based on the simple matching coefficient using the

SAHN sub-program. The SAHN sub-program uses UPGMA algorithm to perform cluster analysis. In this dendrogram (Fig. 9) fifty genotypes formed 11 clusters at a similarity coefficient value of 0.54 whereas two genotypes HTP 9337 and HMS 43B were failed to fall into any cluster. The cluster IV (HMS33B, HMS7B, H77/833-2-202, G73-107 and HBL0538), cluster VII(99HS-24, ARS 07114, MP 293/4, EMRT 11-112, RAJ 3 and HTP 92/80) and cluster IX (HMS 42B, HMS 39B, HMS 45B, H 77/833-2, HTP 93/4 and EMRT 11-116) consisted 6 lines flowed by cluster II (HMS 37B, H 90/4-5, H 77/29-2, MSS 833-22B and TCH 26-1) and cluster X (HMS 32B, HMS 41B, HFEL 10-163, HMS 38B and 78/11) consisted 5 lines each, cluster I(HMS 6B, HMS 22B, HMS 34B and HMS 49B), cluster III (HMS 50B, VCF 6862/98-1, AC O4/13 and EMRT 11-133) and cluster XI (S 97/120, H 94/46R and HTP 03/13-901-1) consisted 4 lines each, cluster V (HMS 40B, 1600 MT and EMRT 11-104), IV (HPT 94/54, H 96/4-5 x H 77/29 and EMRT 11-115), cluster VIII (HBL 11, HBL 056 and A5R10-119) consisted 3 lines each. Furthermore, the simple matching matrix was subjected to Principal component analysis (PCA) for the three principal components. The groupings of fifty genotypes using PCA analysis in 2-D (Fig. 10) and 3-D scaling (Fig. 11) followed the same pattern as depicted in the dendrogram with minor differences.

Discussion

The analysis of genetic variation in breeding materials is of fundamental interest to plant breeders, as it contributes to selection, monitoring of germplasm and prediction of potential genetic gain (Chakravarthi and Naravaneni, 2006). Traditionally, breeders have relied on visible traits to select for improvement of varieties which is less reliable. With the advent of molecular markers, diversity analysis is being conducted using various markers including SSR markers (Koli and Arya, 2022). SSRs markers show polymorphism between species and within species in wheat (Plaschke and Röder 1995) and can help breeders to assess genetic diversity and select genotypes carrying gene(s) of interest. The high reproducibility of SSRs makes them ideal for genome mapping and landmarks for map-based cloning of genes, therefore, molecular maps based on these markers provide the breeders powerful tools for MAS that may optimize time and resources (Plaschke et al., 1995, Korzun et al., 1998, Song et al., 2005). SSRs associated with QTLs have been reported for many important traits. After a linkage between a QTL and a molecular marker has been determined, the QTL can be transferred into any genetic background by marker-assisted selection.

In the present investigation, out of 50 SSRs used for identification of single marker analysis 9 SSRs were found polymorphic for further checking their behaviour on known drought tolerant (HTP 93-37, HTP 03/13-901-1) and drought-sensitive inbreeds (HMS 33B, HMS 42B) and were used for amplification of DNA. SSRs showed amplification for all genotypes and thus confirmed in other genotypes for the study of drought in pearl millet genotypes. Primers amplified a total of 237 alleles which varied from 2 to 8 with a mean of 4.54 alleles per locus. The overall size of PCR-amplified products ranged from 140 bp (*PSMP 2271*) to 810 bp (*ICMP 10*). The molecular size difference between the smallest and the largest allele at an SSR locus varied from 47 bp (*XCUMP 001*) to 880 bp (*ICMP 10*). Polymorphic information content (PIC) value ranged from 0.326 (*PSMP2201*) to 0.89 (*XCUMP 009*) with an average of 0.579 which is near to 0.582 (Kapila et al., 2008), 0.58 (Nepolean et al., 2012) and higher than 0.44 (Singh et al., 2013). The molecular analysis was conducted from pre-selected fifty inbred lines for drought-tolerant traits the current study therefore focused on discerning differences among inbred lines for diversity and not on drought-tolerance traits. It would have been useful to use GenAlEx Software for determining differences among various diversity groups however non-availability of software precluded its use.

Thus, in the study, it could be concluded drought tolerance caused due to rainfed leads to a reduction in the mean performance of the varieties for almost all economic traits. However, this reduction can be avoided to some extent by using drought-tolerant varieties. Breeding for such genotypes/varieties can be eased by identifying markers using molecular marker-assisted selection.

SSR markers exhibited significant variability and divergence among the pearl millet genotypes. Further considering the importance of these molecular tools in the present study drought tolerant genotypes of pearl millet i.e. HTP 93-37, HTP03/13-901-1 were identified. The genetic relationship presented among these genotypes is quite more useful for further hybridization as both these genotypes belong to genetically diverse clusters. Therefore, the study can be helpful in marker-assisted breeding for genetic enhancement of pearl millet genotypes for drought tolerance.

Acknowledgements

The authors wish to acknowledge CCSHAU, Hisar pearl millet section and Regional Research Station (RRS), Bawal, for their support for conducting this experiment.

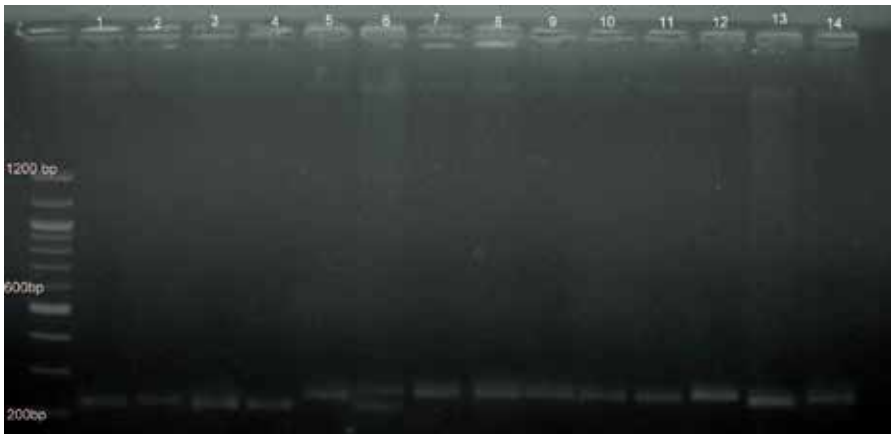

Figure 1. Agarose gel showing allelic polymorphism among pearl millet genotypes at PSMP 2263 locus. Lane L1=100bp ladder, 1 to 14 represents HMS 6B(1), HMS 22B(2), HMS 34(3), BHMS 37B(4), HMS 32B(5), HMS 33B(6), HMS 7B(7), HMS 38B(8), HMS 40B(9), HMS 41B(10), HMS 42B(11), HMS 39B(12), HMS 50B(13), and HMS 49B(14).

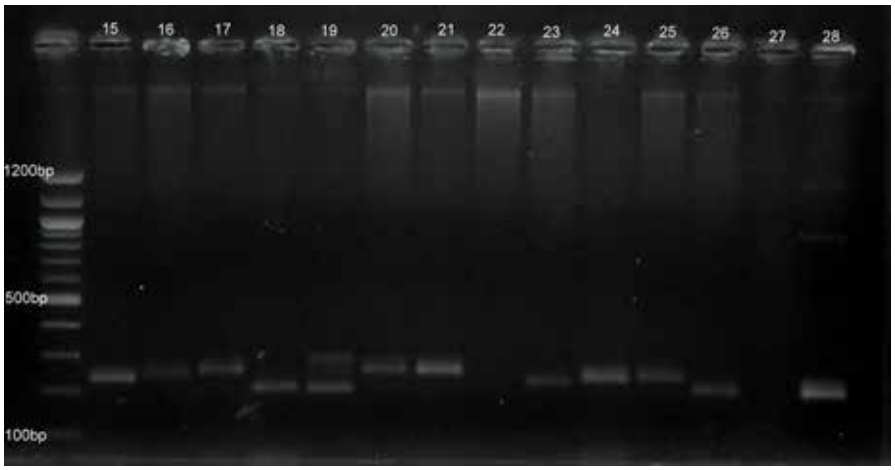

Figure 2. Agarose gel showing allelic polymorphism among pearl millet genotypes at PSMP 2263 locus. Lane L1=100 bp ladder, 15 to 28 represents HMS 45B(15), HMS 43B(16), MS 833-22B(17), HTP 94/54(18), H 77/29-2(19), H 77/833-2(20), H 77/833-2-202(21), G 73-107(22), H 90/4-5(23), HBL 11(24), TCH-26-1(25), HBL 0565(26), VCF6 862/98-1(27), and HBL 0538(28).

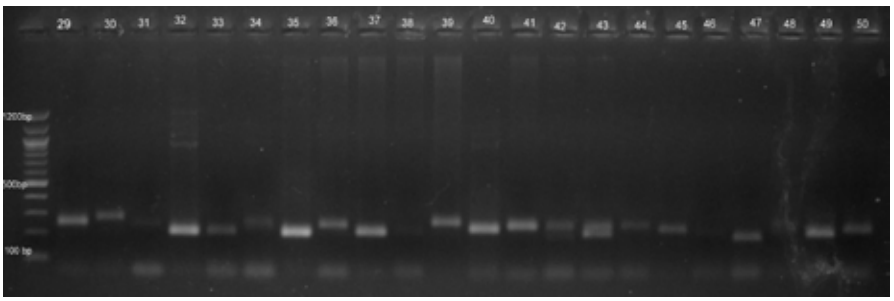

Figure 3. Agarose gel showing allelic polymorphism among pearl millet genotypes at PSMP 2263 locus. Lane L1=100 bp ladder, 29 to 50 represents HTP 93/4(29), AC O4/13(30), EMRT 11-104(31), 78/711(32), (H 96/4-5 x H 77/29-2)(33), A5R 10-119(34), 1660 (MT)(35), EMRT 11-115(36),99 HS-24(37), RAJ 3(38), S 97/120(39), ARS 07114(40), MP 293-4(41), EMRT 11-133(42), HTP 92/80(43), EMRT 11-112(44), HTP 93-37(45), EMRT 11-137(46), EMRT 11-116(47), HTP 03/13-901-1(48), HFeL 10-163(49), and H 94/46R(50).

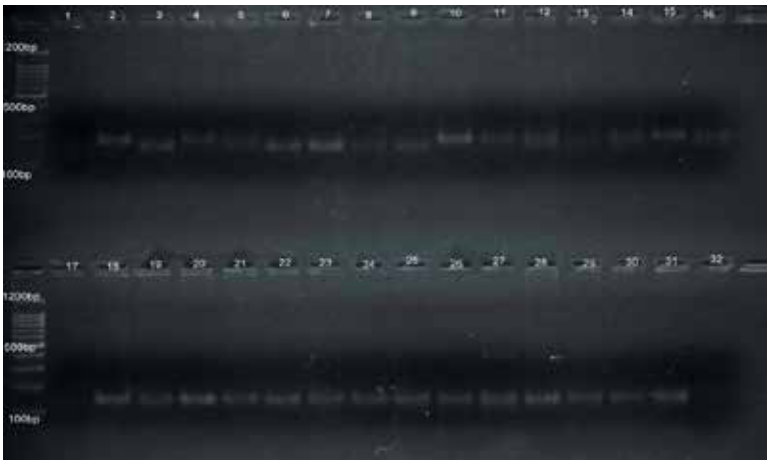

Figure 4. Agarose gel showing allelic polymorphism among pearl millet genotypes at ICMP 3050 locus. Lane L1=100 bp ladder, 1 to 32 represents HMS 6B(1), HMS 22B(2), HMS 34(3), BHMS 37B(4), HMS 32B(5), HMS 33B(6), HMS 7B(7), HMS 38B(8), HMS 40B(9), HMS 41B(10), HMS 42B(11), HMS 39B(12), HMS 50B(13), HMS 49B(14), HMS 45B(15), HMS 43B(16), MS 833-22B(17), HTP 94/54(18), H 77/29-2(19), H 77/833-2(20), H 77/833-2-202(21), G 73-107(22), H 90/4-5(23), HBL 11(24), TCH-26-1(25), HBL 0565(26), VCF6 862/98-1(27), HBL 0538(28), HTP 93/4(29), AC O4/13(30), EMRT 11-104(31), and 78/711(32).

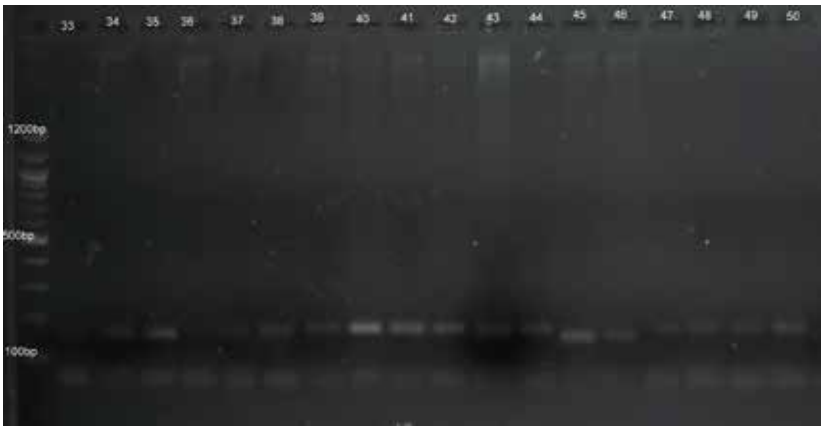

Figure 5. Agarose gel showing allelic polymorphism among pearl millet genotypes at ICMP 3050 locus. Lane L1=100 bp ladder, 33 to 50 represents (H 96/4-5 x H 77/29-2)(33), A5R 10-119(34), 1660 (MT)(35), EMRT 11-115(36), 99 HS-24(37), RAJ 3(38), S 97/120(39), ARS 07114(40), MP 293-4(41), EMRT 11-133(42), HTP 92/80(43), EMRT 11-112(44), HTP 93-37(45), EMRT 11-137(46), EMRT 11-116(47), HTP 03/13-901-1(48), HFeL 10-163(49), and H 94/46R(50).

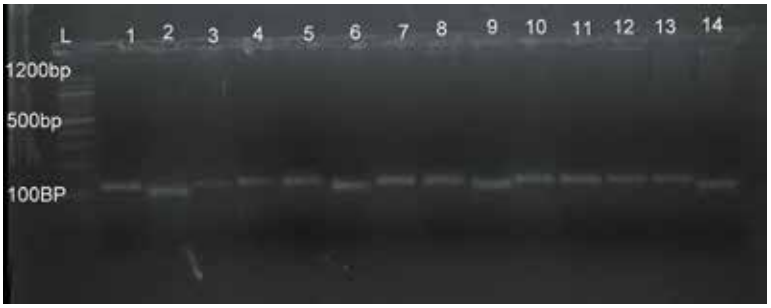

Figure 6. Agarose gel showing allelic polymorphism among pearl millet genotypes at ICMP 3088 locus. Lane L1=100 bp ladder, 1 to 14 HMS 6B(1), HMS 22B(2), HMS 34(3), BHMS 37B(4), HMS 32B(5), HMS 33B(6), HMS 7B(7), HMS 38B(8), HMS 40B(9), HMS 41B(10), HMS 42B(11), HMS 39B(12), HMS 50B(13), and HMS 49B(14)

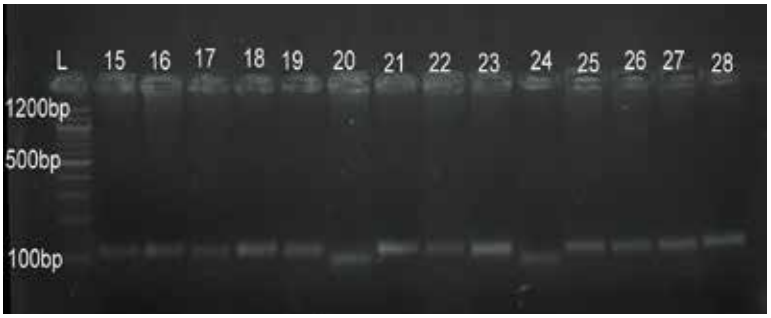

Figure 7. Agarose gel showing allelic polymorphism among pearl millet genotypes at ICMP 3088 locus. Lane L1=100 bp ladder, 15 to 28 represents HMS 45B(15), HMS 43B(16), MS 833-22B(17), HTP 94/54(18), H 77/29-2(19), H 77/833-2(20), H 77/833-2-202(21), G 73-107(22), H 90/4-5(23), HBL 11(24), TCH-26-1(25), HBL 0565(26), VCF6 862/98-1(27), and HBL 0538(28)

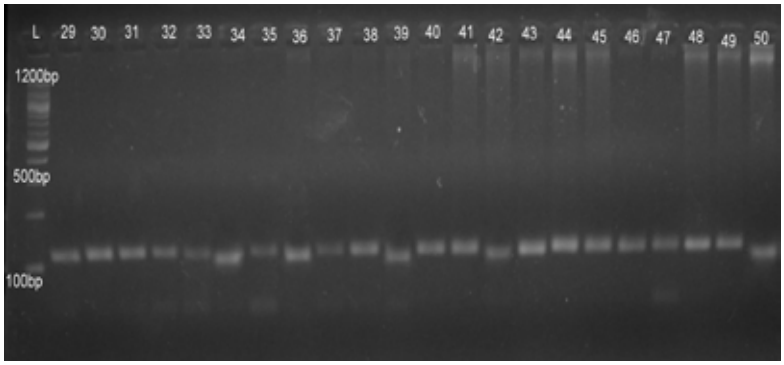

Figure 8. Agarose gel showing allelic polymorphism among pearl millet genotypes at ICMP 3088 locus. Lane L1=100 bp ladder, 29 to 50 represents HTP 93/4(29), AC O4/13(30), EMRT 11-104(31), 78/711(32), (H 96/4-5 x H 77/29-2)(33), A5R 10-119(34), 1660 (MT)(35), EMRT 11-115(36), 99 HS-24(37), RAJ 3(38), S 97/120(39), ARS 07114(40), MP 293-4(41), EMRT 11-133(42), HTP 92/80(43), EMRT 11-112(44), HTP 93-37(45), EMRT 11-137(46), EMRT 11-116(47), HTP 03/13-901-1(48), HFeL 10-163(49), and H 94/46R(50)

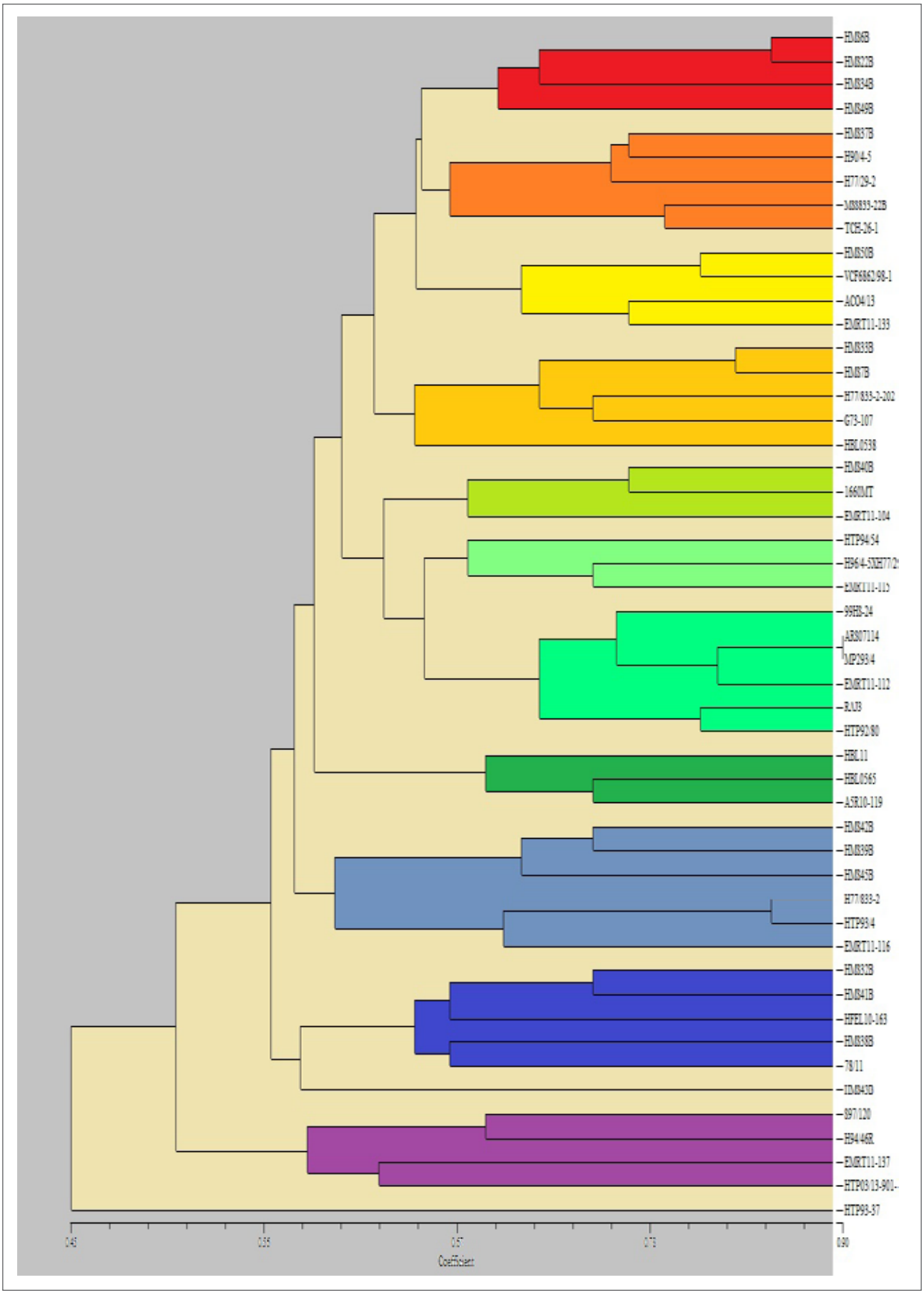

Figure 9. Dendrogram showing relationship among fifty pearl millet genotypes based on similarity matrix data using 50 SSR markers

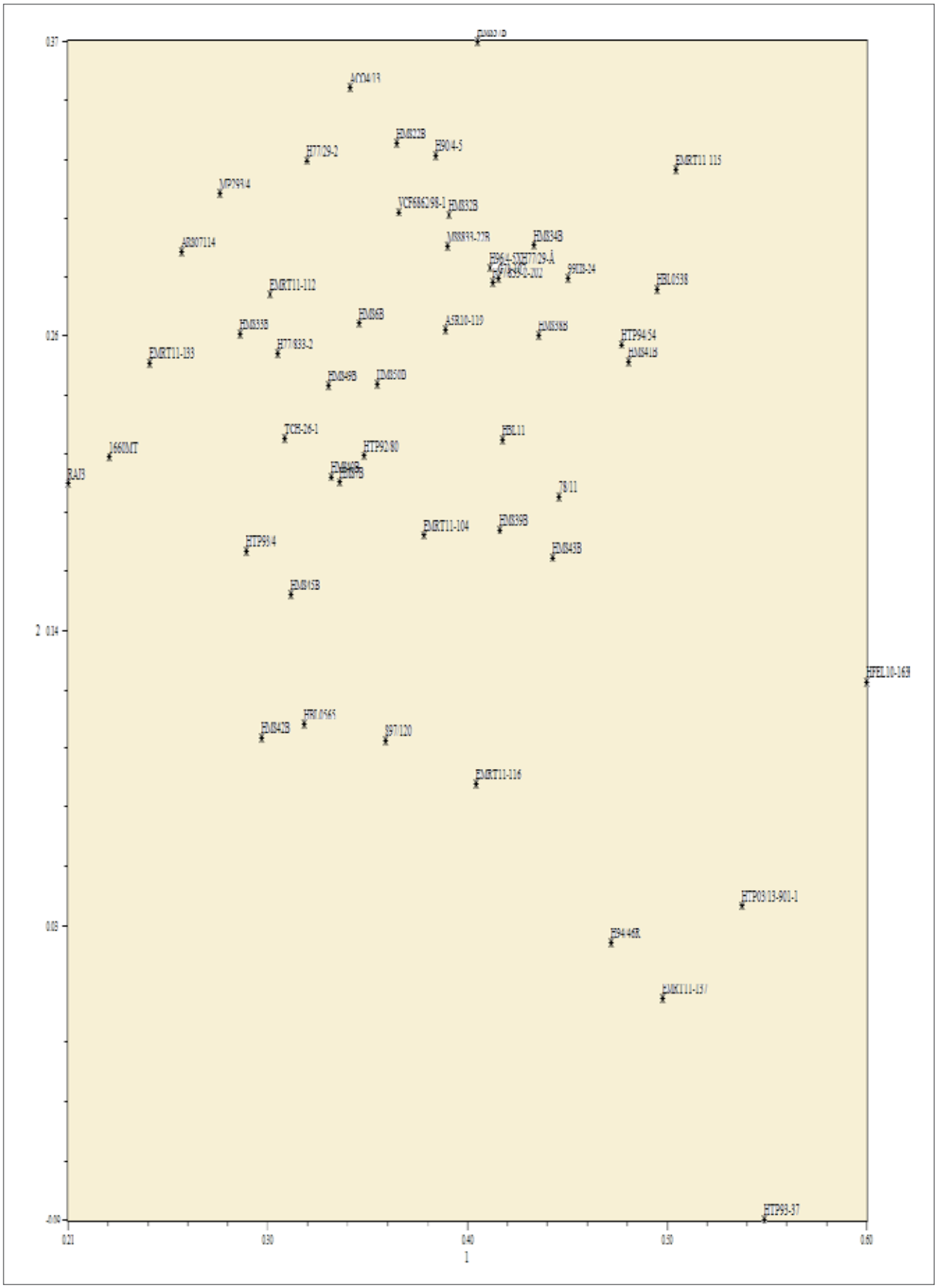

Figure 10. Two dimensional PCA (Principal component analysis) scaling of fifty pearl millet genotypes using similarity matrix data of 50 SSR markers

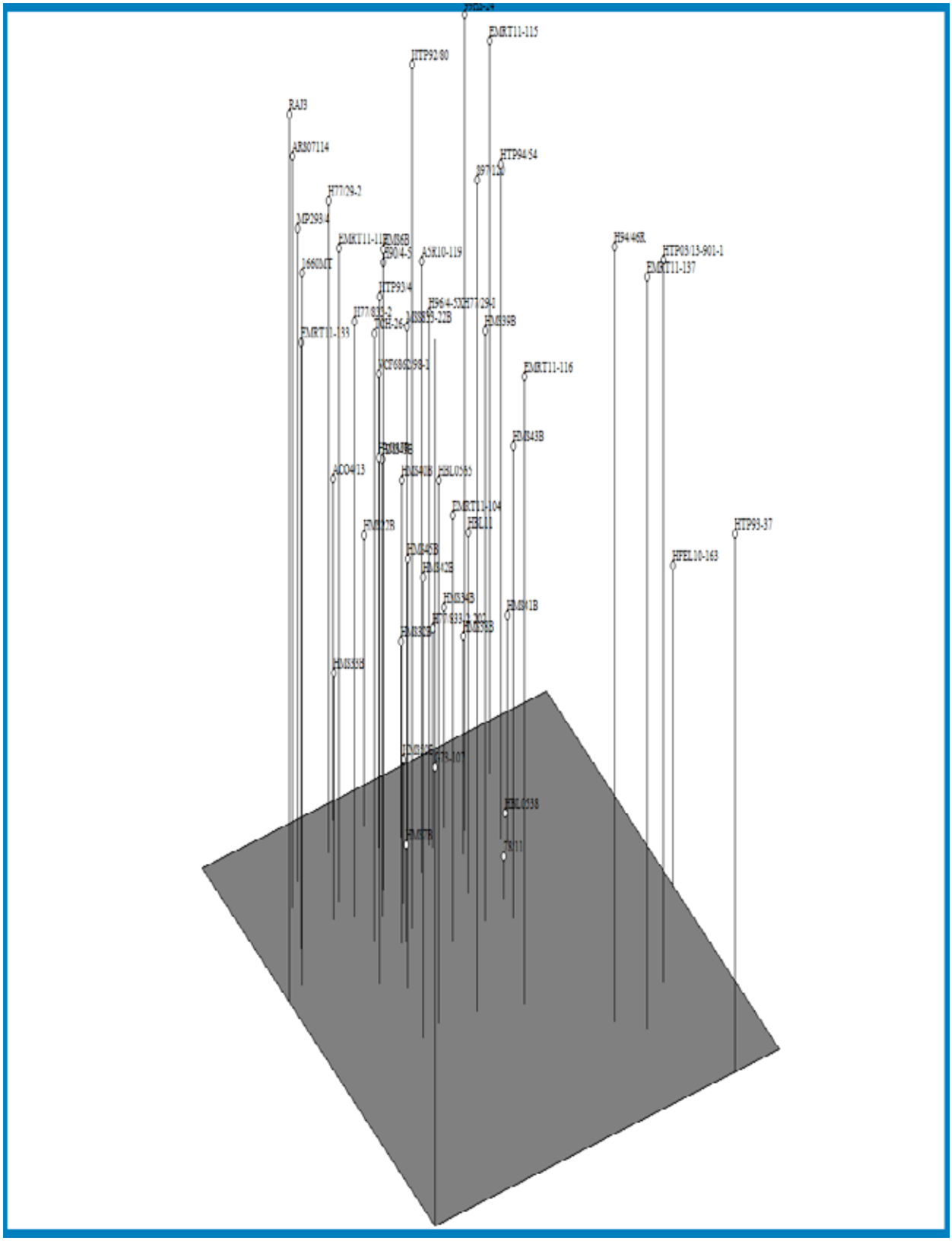

Figure 11. Three dimensional PCA (Principal component analysis) scaling of fifty pearl millet genotypes based on 50 SSR markers

Table 1. List of pearl millet genotypes used in the present study.

S. No.	Genotype	Source	S. No.	Genotype	Source
1	HMS 6B	CCSHAU, Hisar	26	HBL 0565	CCSHAU, Hisar
2	HMS 22B	CCSHAU, Hisar	27	VCF6 862/98-1	CCSHAU, Hisar
3	HMS 34B	CCSHAU, Hisar	28	HBL 0538	CCSHAU, Hisar
4	HMS 37B	CCSHAU, Hisar	29	HTP 93/4	CCSHAU, Hisar
5	HMS 32B	CCSHAU, Hisar	30	AC O4/13	CCSHAU, Hisar
6	HMS 33B	CCSHAU, Hisar	31	EMRT 11-104	CCSHAU, Hisar
7	HMS 7B	CCSHAU, Hisar	32	78/711	CCSHAU, Hisar
8	HMS 38B	CCSHAU, Hisar	33	(H96/4-5xH 77/29-2)	CCSHAU, Hisar
9	HMS 40B	CCSHAU, Hisar	34	A5R 10-119	CCSHAU, Hisar
10	HMS 41B	CCSHAU, Hisar	35	1660 (MT)	CCSHAU, Hisar
11	HMS 42B	CCSHAU, Hisar	36	EMRT 11-115	CCSHAU, Hisar
12	HMS 39B	CCSHAU, Hisar	37	99 HS-24	CCSHAU, Hisar
13	HMS 50B	CCSHAU, Hisar	38	RAJ 3	CCSHAU, Hisar
14	HMS 49B	CCSHAU, Hisar	39	S 97/120	CCSHAU, Hisar
15	HMS 45B	CCSHAU, Hisar	40	ARS 07114	CCSHAU, Hisar
16	HMS 43B	CCSHAU, Hisar	41	MP 293-4	CCSHAU, Hisar
17	MS 833-22B	CCSHAU, Hisar	42	EMRT 11-133	CCSHAU, Hisar
18	HTP 94/54	CCSHAU, Hisar	43	HTP 92/80	CCSHAU, Hisar
19	H 77/29-2	CCSHAU, Hisar	44	EMRT 11-112	CCSHAU, Hisar
20	H 77/833-2	CCSHAU, Hisar	45	HTP 93-37	CCSHAU, Hisar
21	H 77/833-2-202	CCSHAU, Hisar	46	EMRT 11-137	CCSHAU, Hisar
22	G 73-107	CCSHAU, Hisar	47	EMRT 11-116	CCSHAU, Hisar
23	H 90/4-5	CCSHAU, Hisar	48	HTP 03/13-901-1	CCSHAU, Hisar
24	HBL 11	CCSHAU, Hisar	49	HFeL 10-163	CCSHAU, Hisar
25	TCH-26-1	CCSHAU, Hisar	50	H 94/46R	CCSHAU, Hisar

Table 2. List of 50 SSR markers used for studying polymorphism in fifty pearl millet genotypes.

Sr. No.	Primer	Forward Sequence	Reverse Sequence	Temp (°C)
1	PSMP 2008	GATCATGTTGTCATGAATCACC	ACACTACACCTACATACGCTCC	55
2	PSMP 2013	GTAACCCACTAACCCTTACC	GTAACCCACTAACCCTTACC	54
3	PSMP 2027	AGCAATCCGATAACAAGGAC	AGCTTTGGAAAAGGTGATCC	50
4	PSMP 0020	CATTACACGTTTCTTCAAACGC	TCTTCGGCCTAATAGCTCTAAC	53
5	PSMP 2059	GGGGAGATGAGAAAACACAATCAC	TCGAGAGAGGAACCTGATCCTAA	56
6	PSMP 2084	AATCTAGTGATCTAGTGTGCTTCC	GGTTAGTTTGTTTGAGGCAAATGC	54
7	PSMP 2087	GGAACAGACTCCATACCTGAAA	TACCTGCCTGTGCTGTTAGT	53
8	PSMP 2090	AGCAGCCCAGTAATACCTCAGCTC	AGCCCTAGCGCACAACACAAACTC	59
9	PSMP 2201	CCC GAC GTT ATG CGT TAA GTT	TCCATCCATCCATTAATCCACA	52
10	PSMP 2224	GGCGAAATTGGAATTCAGATTG	CGTAATCGTAGCGTCTCGTCTAA	55
11	PSMP 2227	ACACCAAACACCAACCATAAA	TCGTCAGCAATCACTAATGACC	53
12	PSMP 2229	CCACTACCTTCGTCTTCCTCCATTC	GTCCGTTCCGTTAGTTGTTGCC	59
13	PSMP 2232	TGTTGTTGGGAGAGGGTATGAG	CTCTCGCCATTCTTCAAGTTCA	55
14	PSMP 2233	TGTTTTCTCCTCTTAGGCTTCGTTC	ACCTTCTCCGCCACTAAACAACT	56
15	PSMP 2237	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTATCCACACCCCA	61
16	PSMP 2246	CGGATGCTAAATTAACCGAAGC	CCAGCTTGCTTCTGTTCGGTTC	57
17	PSMP 0022	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC	60
18	PSMP 2263	AAAGTGAATACGATACAGGAGCTGAG	CATTTCAGCCGTTAAGTGAGACAA	56
19	PSMP 2270	AACCAGAGAAGTACATGGCCCG	CGACGAACAAATTAAGGCTCTC	57
20	PSMP 2271	CCTTATATTGGACCGACTGCTGAC	CTCCCCCATACACGAGCGAGAA	59
21	PSMP 2273	AACCCCACCAGTAAGTTGTGCTGC	GATGACGACCAAGACTTCTCTCC	59
22	PSMP 2274	CACCTAGACTCTACACAATGCAAC	AATATCAAGTGATCCACCTCCCAA	56
23	ICMP 3016	GTCAACCATTTGGGCTCACT	GGGAGAAATGTGGGGAGAGA	52
24	ICMP 3017	CACCAAACAGCATCAAGCAG	AGGTAGCCGAGGAAGGTGAG	56
25	ICMP 3018	ACGAGGACAAGCTCTTGGAA	ACGGCGCATACTCGATCATA	52

Continuing Table 2

Sr. No.	Primer	Forward Sequence	Reverse Sequence	Temp (°C)
26	ICMP 3019	GCGCACCACCTGTGTCTAT	CATGCAGAGAAAAATCAAGCA	53
27	ICMP 3020	GTTCCATGGAGCTGGAAGC	GCTAGAACAGGGCCGTTACA	54
28	ICMP 3029	ATCGATCTGTTCCACCCAGT	GGACTGGTACTGCTGCTGCT	56
29	ICMP 3050	ATGTCCAGTGTTGACGGTGA	CGGGGAAGAGACAGGCTACT	56
30	ICMP 3056	ACGGAGCTACGGTTGGAATA	CACAAGGGACCCCACGATA	53
31	ICMP 3088	TCAGGTGGAGATCGATGTTG	TTACGGGAGGATGAGGATG	54
32	ICMP 10	ATCCCCTACAGCATCAGCAC	CGGCGGAGAGATCTTATTCA	54
33	XCUMP 001	GCACGAGGCTTATCTGTGTTTC	CAACTCTTGCCTTTCTTGGCCT	55
34	XCUMP 005	GCACGAGGGCCAGATTCTAGAA	CACGGTGATGACACGACATGGT	57
35	XCUMP 006	GAAATCGGCAGAGGGCAT	CAATGAGTATGTGCACGCTGCA	55
36	XCUMP 009	ATCTGATCGTGAGGCCTCAAC	GCCGACCAAGAACTTCATACAAT	54
37	XCUMP 0011	TGATGGGAACCGAGAGCATGA	TAGCACAGCAATAACATGGCATC	54
38	XCUMP 0012	TGTGATCTGTGGTCTCAGGC	CGTGAAAGCTCTCCAGGACT	54
39	XCUMP 0016	CATTTCTCTCGCCAGTGCTC	ATCTCCAGAACCGAGCGCA	54
40	XCUMP 0017	TGCTTTCTTCCCAACCAGTGG	TGCTGAGTGGGGTGCTGCT	54
41	XCUMP 0018	TGCTTTCTTCCCAACCAGTGG	TGCTGAGTGGGGTGCTGCT	55
42	XCUMP 0019	GGCCTAACTCTCTGTTCTTCTTC	GAGAAGCTAACATTTGGGGCCTA	55
43.	CTM 8	GCTGCATCGGAGATAGGGAA	CTCAGCAAGCACGCTGCTCT	56
44	CTM 10	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA	52
45.	CTM 21	ATGCCTCCCACCCCACGTCG	CGTCGCACTAGCCACAGTCA	60
46.	CTM 25	GCGAAGTAGAACACCGCGCT	GCACTTCCTCCTCGCCGTCA	58
47	CTM26	GCAAGTGATCCATGACATTACGA	ACTTGCTAGCTGCTGCTCTTG	54
48	CTM 27	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT	55
49	CTM 55	CGTCTTCTACCACGTCCT	CATAATCCCACTCAACAATCC	50
50	CTM 56	GCGTTGTTTCGGTGACCAC	GCGTATCTTTAAATTGCCTTTGTT	53

a 2°C lesser T_m was used for step wise of PCR amplification

Table 3. Amplification results of 50 SSR markers.

Sr. No.	Primer	Amp. Range (bp)	Allele No.	PIC	Sr. No.	Primer	Amp. Range (bp)	Allele No.	PIC
1	PSMP 2008	170 - 500	5	0.49	26	ICMP 3019	250 - 700	4	0.57
2	PSMP 2013	250 - 600	3	0.50	27	ICMP 3020	220 - 310	6	0.62
3	PSMP 2027	210 - 550	4	0.50	28	ICMP 3029	170 - 450	2	0.45
4	PSMP 20	300 - 810	2	0.51	29	ICMP 3050	310 - 700	5	0.57
5	PSMP 2059	180 - 600	4	0.32	30	ICMP 3056	250 - 600	8	0.84
6	PSMP 2084	250 - 700	4	0.60	31	ICMP 3088	210 - 550	3	0.45
7	PSMP 2087	190 - 650	7	0.64	32	ICMP 10	300 - 810	5	0.65
8	PSMP 2090	200 - 650	2	0.10	33	XCUMP 001	148 - 195	4	0.72
9	PSMP 2201	145 - 570	3	0.09	34	XCUMP 005	145 - 570	2	0.62
10	PSMP 2224	260 - 710	5	0.47	35	XCUMP 006	180 - 500	3	0.41
11	PSMP 2227	200 - 580	4	0.66	36	XCUMP 009	216 - 250	8	0.89
12	PSMP 2229	210 - 225	8	0.74	37	XCUMP 0011	170 - 500	3	0.51
13	PSMP 2232	190 - 400	4	0.56	38	XCUMP 0012	250 - 700	4	0.56
14	PSMP 2233	216 - 250	3	0.52	39	XCUMP 0016	190 - 260	7	0.84
15	PSMP 2237	148 - 195	7	0.75	40	XCUMP 0017	170 - 500	2	0.45
16	PSMP 2246	145 - 570	5	0.64	41	XCUMP 0018	250 -700	5	0.65
17	PSMP 22	180 - 500	2	0.56	42	XCUMP 0019	170 - 500	8	0.82
18	PSMP 2263	210 - 320	8	0.75	43.	CTM 8	180 -500	6	0.61
19	PSMP 2270	200 - 580	5	0.54	44	CTM 10	210 - 320	7	0.84
20	PSMP 2271	140 - 410	4	0.65	45.	CTM 21	200 -580	2	0.49
21	PSMP 2273	200 - 620	5	0.54	46.	CTM 25	140 - 410	3	0.54
22	PSMP 2274	250 - 450	4	0.62	47	CTM26	200 - 650	8	0.85
23	ICMP 3016	170 - 500	2	0.32	48	CTM 27	145 -570	5	0.62
24	ICMP 3017	250 -700	3	0.58	49	CTM 55	260 – 710	4	0.55
25	ICMP 3018	190 - 260	5	0.62	50	CTM 56	190 - 650	5	0.56

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Determination of Resistance to Tomato Yellow Leaf Curl Virus by Molecular Methods in Pink Beef Tomatoes

Mine BULUT* **Sevinç TEKİN** **Elif ÜRÜN KÖKSALAN** **Veysel ARAS**

Alata Horticultural Research Institute, Erdemli, Mersin, TÜRKİYE

* Corresponding author e-mail: minebulut33@gmail.com

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ABSTRACT

In both public and private tomato breeding projects, marker assisted selection (MAS) for disease resistance is frequently used. In tomato molecular breeding programs, the development and application of molecular markers have been extensively pursued, particularly for disease resistance to enable the selection of a single resistance gene or a combination of multiple resistance genes. Tomato Yellow Leaf Curl Virus (TYLCV) is one of the most dangerous viruses affecting tomato production and growth worldwide. Using resistant cultivars is the most effective and eco-friendly way to combat TYLCV. In this study, the TYLCV was tested against 155 genotypes of pink beef tomatoes utilizing the MAS (Marker-Assisted Selection) technique. Resistance against TYLCV was determined with the SCAR (P6-25) primer developed in connection with the *Ty-3* gene. 42 pink tomato genotypes were determined to be susceptible (rr), 99 to be heterozygous resistant (Rr), and 8 to be homozygous resistant (RR) to TYLCV as a consequence of MAS testing. Furthermore, no molecular marker was found in any of the six pink beef tomato genotypes. These findings suggested that P6-25 (SCAR) primers could be used successfully in breeding studies to identify disease resistance.

Keywords: Tomato, MAS, TYLCV, resistance breeding

Introduction

The tomato is a member of the nightshade family Solanaceae, which is classified in the following orders: Solanales, suborder Solanineae, division Magnoliophyta, class Magnoliopsida, and subclass Asteridae. It is estimated that the 96 genera and over 2800 species that make up the incredibly diverse and huge Solanaceae family are divided into three sub-families: Solanoideae (which Lycopersicon belongs to), Cestroideae, and Solanineae (Knapp et al., 1992; Knapp et al., 2004). The tomato (*Solanum lycopersicum* L.) is the most necessary after potatoes. Unquestionably, it is the most widely grown vegetable crop worldwide (Bhandari et al., 2017). The crop is grown almost anywhere in the world, despite being a tropical plant (Robertson and Labate, 2007). The tomato is a crop with significant global economic importance (Foolad, 2007). It is estimated that 4.9 million hectares of tomatoes are farmed annually, yielding over 186 million tons of tomatoes (FAO, 2022). Abiotic and biotic stress are the main factors limiting tomato cultivation. Approximately 200 distinct pathogens have been identified for the tomato plant, making it vulnerable to numerous fungus, bacteria, viruses, and microorganisms (Jones et al., 1991). Globally, a number of biotic stress, including as viral infections, are to blame for large losses in tomato output. Whitefly-transmitted geminiviruses (genus: Begomovirus) are among the viral illnesses that significantly limit tomato output in tropical and subtropical areas of the world. Tomato yellow leaf curl disease (TYLCD) and tomato leaf curl disease (ToLCD), which are harmful diseases with a variety of symptoms, are caused by these viruses (Cohen and Lapidot, 2007). One of the most dangerous viruses in the world is the TYLCV. This disease, which is spread by whiteflies, is caused by single-spinning DNA

from the geminivirus genus (Laterrot,1995). TYLCV can result in yield losses of up to 100% in tomato disease-affected areas. Early in the 1960s, it began to spread from the Middle East and is currently found over much of Africa, America, and Asia. Turkey was affected by the illness in the early 1980s (Polston and Anderson,1997; Moriones and Navas-Castillo, 2000; Agnihotri et al. 2013). The disease, which was initially found in the Middle East and later spread to many other nations, is now a significant problem restricting the output of tomatoes. There are few methods for controlling TYLCV in tomatoes and they are expensive. The most effective approach to combating nematodes and diseases is to create varieties that are resistant to pests and diseases (Glick et al., 2009; Melomey et al., 2019; Ogunsola and Ogunsina, 2021). In wild species, such as *S. chilense* (*Ty-1, Ty-3, Ty-4*, and *Ty-6*), *S. habrochaites* (*Ty-2*), and *S. peruvianum* (*Ty-5*), many resistance genes against TYLCV have been found. New tomato cultivars have been successfully bred using the genes *Ty-1/Ty-3* and *Ty-2* (Gill et al., 2019; Ji et al., 2007; Ji et al., 2009a; Ji et al., 2009b). The identification, mapping, and transfer of several disease resistance genes and quantitative trait loci (QTLs) in tomatoes have been made easier by the application of MAS approaches and genetic markers. In both public and private tomato breeding projects, marker assisted selection (MAS) for disease resistance is frequently used (Foolad, 2007; Jung et al., 2015). Tomatoes are a major product for both domestic and export in Türkiye, so it is crucial that the information and techniques developed on the topic be applied in Türkiye as well as the rest of the globe to increase tomato competitiveness through the development of new varieties. Therefore, the aim of this research is to determine the resistance against TYLCV of tomato genotypes propagated from a commercially resistant hybrid using molecular methods.

Materials and Methods

The material of this study consists of 155 pink tomato genotypes in the gene pool of Alata Horticultural Research Institute. Seeds were sown in peat-perlite medium at a ratio of 1:1 and DNA was isolated from these seedlings. The plants were employed for DNA analysis when they had three or four true leaves.

DNA isolation was performed by modifying the CTAB method developed by Doyle and Doyle (1990). While Chloroform:Isoamyl alcohol was used in the ratio of 24:1 in the CTAB method developed by Doyle and Doyle (1990), in our study, Chloroform:Octanol was used in the ratio of 24:1. Resistance against TYLCV was determined with the SCAR (P6-25) primer developed in connection with the *Ty-3* gene (Ji et al. 2007). The DNA primers used in the research are given in Table 1. PCR reactions for TYLCV were performed in a total volume of 15 µl; 2 µl master mix, 1 µl each of forward and reverse primers, 1.5 µl DNA and 9.5 µl ddH_2O were added to a total volume of 15 µl.

In the reactions of PCR the first denaturation was started at 94°C for 4 minutes and the cycle was performed 35 times, including denaturation at 94°C for 30 seconds, annealing at 53.7°C for 1 minute and 1 minute at 72°C, and this cycle was performed for 10 minutes at 72°C. The PCR products obtained as a result of the study were conditioned on a 1.5-2% agarose gel and the results were evaluated.

Results

In this study, 155 pink beef tomato genotypes were screened with the SCARP6-25 primer providing resistance against TYLCV. PCR findings (Figure 1) were analyzed genotypically: The homozygous (RR) resistant samples yielded a single 630 bp band, but the heterozygous (Rr) genotype samples showed two bands, one at 630 bp and the other at 320 bp. Lastly, 320 bp was found in a single band in samples with homozygous recessive (rr) genotypes (Table 2). 42 pink tomato genotypes were determined to be susceptible (rr), 99 to be heterozygous resistant (Rr), and 8 to be homozygous resistant (RR) to TYLCV as a consequence of MAS. Furthermore, no molecular marker was found in any of the six tomato genotypes-pink beef.

Discussion

A significant disease that severely reduces tomato yield is TYLCV, a begomovirus belonging to the Geminiviruidae family. Treatment for viral illnesses can be very difficult. Cultivars that are resistant to various diseases and pests during growth are therefore among the most important strategies. Numerous attempts have been attempted to introduce resistance into elite cultivars since host resistance is an economical and environmentally beneficial approach of controlling this virus. Through molecular-assisted selection, TYLCV-resistant genotypes can be generated quickly by screening a large number of plant materials. There have been several reported gene-linked markers for the six TYLCV-resistant genes (*Ty-1* to *Ty-6*) (Ji et al. 2007; Yang et al., 2014; Caro et al., 2015; Jung et al., 2015; Lapidot et al., 2015; Gill et al., 2019). For tomato breeding initiatives to improve MAS, gene-based or functional indicators still need to be established. Several researchers have accepted *Ty-1* and *Ty-3* as the markers that indicate tomato resistance to the TYLCV virus, and these findings have been published

in MAS (Zamir et al. 1994; Agrama and Scott, 2006; Ji et al., 2007). Kim et al., (2020) investigated non-synonymous sequence variations between resistant and susceptible varieties for the *Ty-2* and *Ty-3* genes, and the resulting resistance-associated SNPs and InDels were subsequently used to develop molecular markers for MAS. In their study, Aktaş and Aydın (2022) identified 22 homozygous resistant, 4 heterozygous resistant and 128 susceptible individuals in tomatoes (*S. lycopersicum*) at the F_5-F_8 stage. Using molecular DNA markers, the study assessed the TYLCV resistance of various cherry and cocktail tomato varieties. Additionally, 409 different cherry and cocktail tomato varieties had their TYLCV resistance determined by polymerase chain reaction (PCR) with the Ty3P6-25 primer. Of these, 291 were found to be TYLCV susceptible (rr), 66 to be heterozygous resistant (Rr), and 45 to be homozygous resistant (RR). Furthermore, in seven tomato varieties-cherry and cocktail-no molecular marker was found (Basım et al., 2023). Pınar et al., (2013) found that 24 out of 92 tomato genotypes had both bands, but only 50 had homozygous resistant and susceptible bands following testing of the P6-25 marker for the *Ty-3* resistance gene. Similar results were obtained in our study. In their research, Prasanna et al. (2014) shown that Indian breeding studies can make good use of molecular markers created for the tomato leaf curl virus.

Conclusions

Positive results were found from testing 155 pink tomato genotypes with the SCAR P6-25 marker, which was designed for the tomato leaf curl virus and reported in the literature and it was successfully identified that the pink beef tomato genotype is resistant to the TYLCV disease. The molecular DNA marker that was employed was found to be helpful in identifying pink beef tomato resistance responses to TYLCV and could yield fast, accurate, and repeatable findings. It has been determined that the primers can be used in future breeding experiments due to the availability of this information and the fact that some tomatoes exhibit disease resistance.

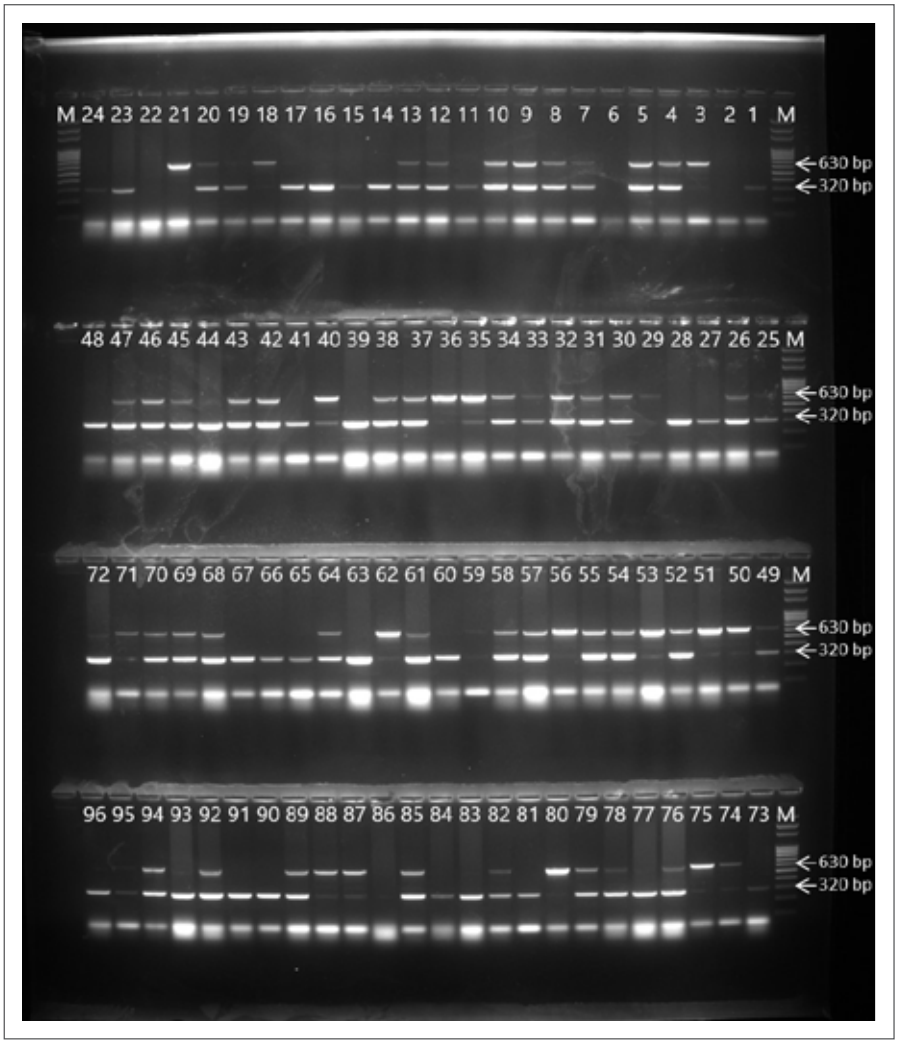

Figure 1. PCR results of tomato genotypes for P6-25. M, Marker 100 bp; Tomato cultivars, 1-96

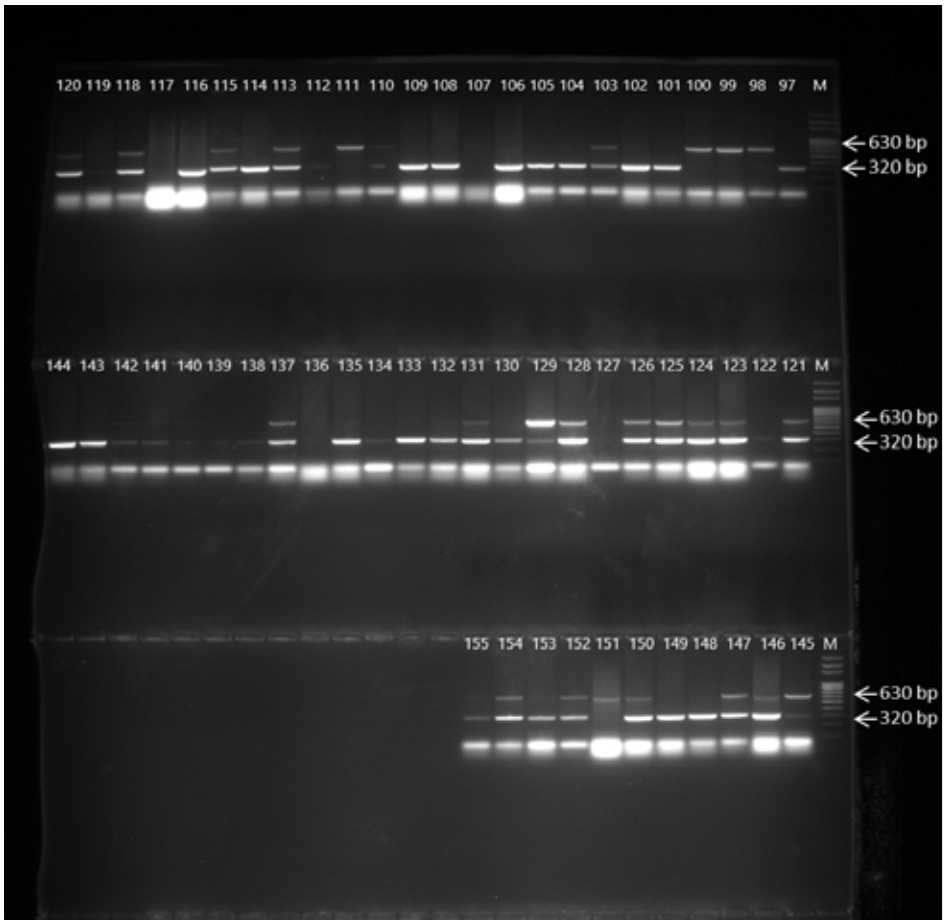

Figure 1 continued, 97-155

Table 1. Used Primer Names and Sequences.

Gene	Primer Name	Primer Sequences	Amplified Product (bp)	
			R***	S*
Ty-3	P6-25-F2 P6-25-R5	5' GGTAGTGGAAATGATGCTGCTC-3' 5' GCTCTGCCTATTGTCCCATATATAACC-3'	450(*Ty3*) 630(*Ty3a*)	320

Table 2. Genotypic characteristics of tomato genotypes (1-155) analyzed by PCR.

Genotype No	P6-25	Genotype No	P6-25	Genotype No	P6-25	Genotype No	P6-25
1	rr	40	Rr	111	RR	118	Rr
2	-	41	rr	112	rr	119	rr
3	Rr	42	Rr	113	Rr	120	Rr
4	Rr	43	Rr	114	Rr	121	Rr
5	Rr	44	Rr	115	Rr	122	rr
6	-	45	Rr	116	rr	123	Rr
7	Rr	46	Rr	65	rr	124	Rr
8	Rr	47	Rr	66	rr	125	Rr
9	Rr	48	rr	67	rr	126	Rr
10	Rr	49	Rr	68	Rr	127	-
11	rr	50	Rr	69	Rr	128	Rr
12	Rr	51	Rr	70	Rr	129	Rr
13	Rr	52	Rr	71	Rr	130	rr
14	rr	53	Rr	72	Rr	131	Rr
15	rr	54	Rr	73	rr	132	Rr
16	rr	55	Rr	74	Rr	133	Rr
17	Rr	56	Rr	75	Rr	134	rr
18	Rr	57	Rr	76	Rr	135	Rr
19	Rr	58	Rr	77	rr	136	-
20	Rr	59	Rr	78	Rr	137	Rr
21	RR	60	rr	79	Rr	138	rr
22	-	61	Rr	80	Rr	139	rr
23	rr	62	Rr	81	rr	140	Rr
24	rr	63	rr	82	Rr	141	Rr
25	Rr	64	Rr	83	rr	142	Rr
26	Rr	97	Rr	84	rr	143	rr
27	rr	98	RR	85	Rr	144	rr
28	rr	99	RR	86	rr	145	Rr
29	Rr	100	RR	87	Rr	146	Rr
30	Rr	101	rr	88	Rr	147	Rr
31	Rr	102	rr	89	Rr	148	rr
32	Rr	103	Rr	90	rr	149	rr
33	Rr	104	Rr	91	rr	150	Rr
34	Rr	105	Rr	92	Rr	151	RR
35	Rr	106	Rr	93	Rr	152	Rr
36	RR	107	RR	94	Rr	153	rr
37	Rr	108	rr	95	Rr	154	Rr
38	Rr	109	rr	96	Rr	155	Rr
39	rr	110	Rr	117	-		

RR: Homozygous Resistant, Rr: Heterozygous, rr:Susceptible, -;Non detected

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Research Article

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New Single Hybrid Popcorn Variety "ATASAM"

Erkan ÖZATA*[1] Ahmet ÖZTÜRK[2] Şekip ERDAL[3] Mehmet PAMUKÇU[3]

[1] Black Sea Agricultural Research Institute, Samsun, Türkiye
[2] Provincial Directorate of Agriculture and Forestry, Karaman, Türkiye
[3] Western Mediterranean Agricultural Research Institute, Antalya, Türkiye

* Corresponding author e-mail: erkan.ozata@tarimorman.gov.tr

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ABSTRACT

The aim of this study is to present the yield and quality characteristics of ATASAM hybrid single-hybrid popcorn variety developed by pure line selection method to the scientific world. ATASAM is a new popcorn hybrid produced by single crossing of pure lines "TCK77" as the male parent and "Yerli Yug Sarı" as the female parent. It was registered under the name ATASAM at the STK (Vegetable Registration Committee) meeting in March 2022 on behalf of the Black Sea Agricultural Research Institute. This variety, which poppining in the shape of a butterfly, has an orange-yellow grain color and anthocyanin content in the cob tassel. In yield trials conducted in different regions of Turkey, it gave an average yield of 6610 kg/ha. The average popping volume is 33.3% and the rate of non-popping grain is 2.4%. The average number of flowering day of the ATASAM variety is 77 days, plant height is 215 cm, cob height is 85 cm, 1000 grain weight is 158 g, and hectoliter content is 79.8 kg/hl. The average protein content was measured as 11.2%, fat content as 3.6%, Ca content as 60.6 mg/kg, Fe content as 30.3 mg/kg, Zn content as 29.2 mg/kg, Cu content as 4.1 mg/kg and Manganese content as 11.4 mg/kg. ATASAM variety is 18.6% higher than the standards average in terms of yield and 12% higher in terms of popping volume.

Keywords: Popcorn, macro and micro elements, quality ratio and energy value

Introduction

Popcorn, which is among the oldest and most popular snacks consumed extensively in the world, can be easily poppined with different popping methods (oil, air and microwave). Popcorn is constantly increasing in popularity for breakfast and meals today, as it is a high-quality and concentrated source of nutrients with its chemical content (proteins, antioxidants, fiber, vitamin B). In the USA, where popcorn consumption is the highest in the world although the majority of consumption is at home, the intense work tempo of today's life contributes to the continuous development of the ready-made food industry. It is reported that the world popcorn market will be at the level of 5.54 billion US dollars in 2022. It is estimated that this market will reach 13.53 billion US dollars with an annual increase of 11.10% in 2030 (Anonymous, 2023a).

Although there are no reliable statistics and systematic production data regarding popcorn production and consumption in Turkey, it is reported that 50-60 thousand tons of the product is produced in an area of 8-10 thousand hectares. Turkey's annual popcorn consumption is 22-25 thousand tons. The remaining part is exported to 26 countries, which contributes to it being among the top ten countries in terms of exports in the world (Anonymous, 2023b). However, the production remains well below the market potential. However, the limited number of varieties with both satisfactory agronomic characteristics and high popping volume is one of the main obstacles to the expansion of Turkey's popcorn crop. Although 19 popcorns are registered in Turkey according to the Standard Seed registration list, only a few varieties can be produced due to the contracted farming model. The contract farming model is widely used in popcorn production in Turkey.

In this model, seeds and other inputs are provided by the companies, so the seeds provided by the company are used extensively. This situation partially restricts the availability of these varieties in the market. It is of great importance for the producer and the company that contracted farming companies give new varieties a chance. The high efficiency and/or high popping volumes that developed and registered varieties can offer can contribute to increasing the income of the producer and the company. The use of new varieties in production will contribute to increasing the income obtained from unit area (Anonymous, 2023c).

The aim of this study is to introduce the ATASAM hybrid popcorn variety, which ranks first in terms of yield and quality (popping volume and non-popping grain ratio, etc.), to the scientific world.

Materials and Methods

ATASAM hybrid popcorn variety was obtained by crossing the main line (Yerli Yug Sarı) and TCK 77 sire lines. The main line of the ATASAM variety was bred by the Batı Akdeniz Agricultural Research Institute, and the sire line TCK 77 was bred by the Black Sea Agricultural Research Institute according to the pure line selection method. The lines were obtained by allowing domestic and foreign interaction materials to open pollination and then by selecting them according to their agro-morphological characteristics and transferring them to the next generation. Hybrid combinations were made during the 2016 corn growing season in the Samsun location. The breeding population was created by obtaining from domestic and international sources. Initially, 2000 cobs were selected from approximately 200 populations obtained from different sources (During inbreeding, selection was made according to agro-morphological characteristics (number of days to flowering, tassel spikelet density, cob shape and firmness). Single ear selection method was used during the breeding period (Table 1) Lines selected according to agro-morphological characteristics were crossed with two testers to determine their General Combination compatibility. The resulting hybrids were put into the test hybrid yield trial. Here, efficiency, blasting volumes and heterosis rates were the main selection criteria. It was decided that TCK 77, one of the parents of ATASAM popcorn variety, would be used as the father line due to its high burst volume and tassel spikelet density, and the Yerli Yug Sarı line would be used as the main parent due to its high popping volume and ear structure.

ATASAM popcorn variety was tested together with standard varieties in 2017 in 4 different locations (Izmir, Isparta, Samsun and Amasya) to determine its yield and quality characteristics. In the trial, plantings were completed in May in all locations, harvests were finished in September in Izmir location and October in other locations. Cultural procedures were carried out on time (irrigation, fertilization, pesticide application, etc.). Planting was done with two seeds in the pits, 70 cm between rows and 20 cm between rows. At harvest, the cobs were collected by hand and grain yield was arranged according to grain moisture. Hectolitre analysis of corn was done gravimetrically with a hectolitre measuring cylinder. The protein amount of the corn and the total nitrogen (N) content of the samples were determined by the Kjeldahl method (Kacar, 1972). Multi-element content in corn products was made according to Kacar and İnal 2010. In order to determine the popping volume (cm^3/g) 50 g samples were weighed and the explosion was carried out process with 1100 W Kiwi KPM-7408 brand hot air blowing machines according to İdikut et al. (2015).

Results and Discussion

Data obtained from four different locations in 2017 are given in Figure 1-5. The average plant height of the standards was measured as 212.3 cm, and the ATASAM variety was measured as 215.6 cm, and the plant height is high The ear height is similar to the standard average at 85.6. (Figure 1). The average number of flowering days for the standards was determined as 77.3 days, and for the ATASAM variety as 77.9 days. ATASAM variety is in the mid-late group with the number of flowering days similar to the standards (FAO 550-580). The grain/cob ratio of ATASAM popcorn variety was measured as 80.3% and the standard average was 81.2% (Figure 1).

The thousand grain weight was 157.8 g, close to the average of the standards (160.6 g), and the thousand grain weight is in the large group. The hectoliter of the ATASAM variety was measured as 79.8 kg/hl, and the hectoliter of the standards was measured as 80.9 kg/hl (Figure 2). The popping rate of the standard varieties was measured as 29.5 g/cm^3 and that of the ATASAM variety was 33.3 g/cm^3. When evaluated in terms of popping volume, it was determined that the popping volume was 12% higher than the standards. The non-popping grain rate of the standards was measured as 10.6%, while it was measured as 2.4% in the ATASAM variety. The low rate of non-popping grains contributed to the high measurement of the popping volume (Figure 2).

The average yield of the standards was measured as 5571.7 kg/ha, and the yield of the ATASAM variety was measured as 6610.5 kg/ha (Figure 3). K content of ATASAM popcorn variety was measured as 2757.8 mg/kg, P 1283 mg/kg, Mg 1236.8 mg/kg. According to the standards, P and Mg content is determined as high and P content as low (Figure 3).

The protein content of the ATASAM variety was measured as 11.2%, the fat content was 3.6%, the Ca content was 60.6 mg/kg, the Fe content was 30.3 mg/kg, the Zn content was 29.2 mg/kg, the Cu content was 4.1 mg/kg, and the Mn content was 11.4 mg/kg. (Figure 3 and Figure 4). The average protein content of the standards was measured as 10.8%, fat content 3.6%, Ca content 58.2 mg/kg, Fe content 27.5 mg/kg, Zn content 28.7 mg/kg, Cu content 4.0 mg/kg, Manganese content 11.4 mg/kg. Similar results were obtained in terms of macro-micro nutrient content the chemical content of the grain (Figure 4 and Figure 5). Energy values were measured as 356.7 kcal, and ATASAM variety was measured as 355.2 kcal. When popcorn, which is a whole grain food, is popping in air blowing machines, its energy values are measured to be lower than its oily and sauced versions (Figure 5).

In studies conducted in different regions of Turkey (Aegean, Mediterranean and Black Sea), ATASAM variety ranks first in terms of yield. When evaluated in terms of morphological characteristics of the variety, the presence of anthocyanin in the top tassel is absent or weak. Anthocyanin density is high in the cob tassel, but there is no anthocyanin in the stem and leaves (Table 2). The top tassel, side branches and axis length are high, the top grain color is orange, and the popping volume is high. Introductory pictures of the ATASAM popcorn variety are given in Figure 6.

Conclusions

Türkiye has an important place in the world in popcorn production and consumption. Although popcorn trade in the world started in the last century, it has gained great momentum in the last decade. Although it is important to carry out productivity and quality together in breeding studies, this is even more important in popcorn. In popcorn breeding, high yield and popping volume and low non-popping grain rate are evaluated together. ATASAM hybrid popcorn variety stands out with its many advantageous aspects. ATASAM hybrid popcorn variety has high yield and popping volume and low non-popping grain ratio. Turkish plant variety protection has been applied for the variety. Breeder a foundation seed of the variety will be produced and maintained by Black Sea Agricultural Research Institute. ATASAM hybrid popcorn variety, with its high yield and popping volume, has the potential to provide additional income for producers and industrialists in contract farming. However, it will contribute positively to its place in the daily diet with its high content of macro and micro nutrients and low energy content.

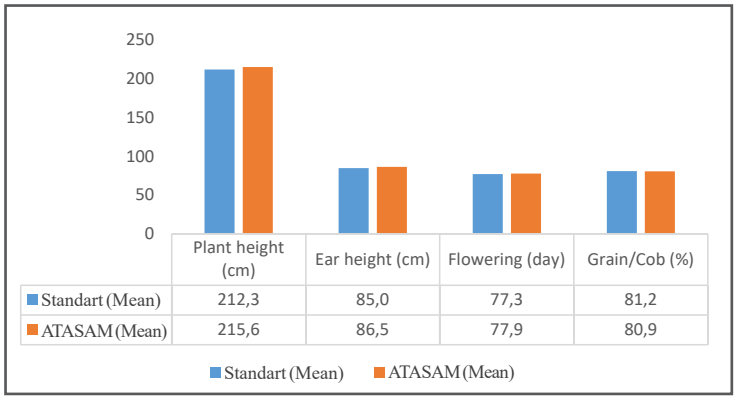

Figure 1. Averages of some agromophological charactetistics and genotypes.

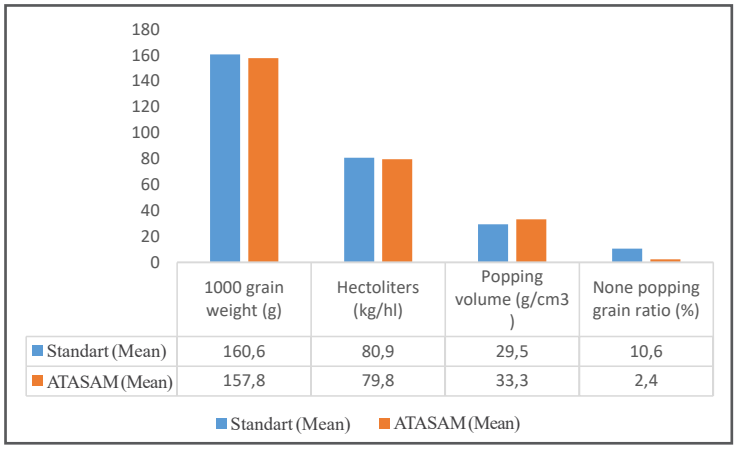

Figure 2. Averages of some yield elements and physical characteristics of genotypes.

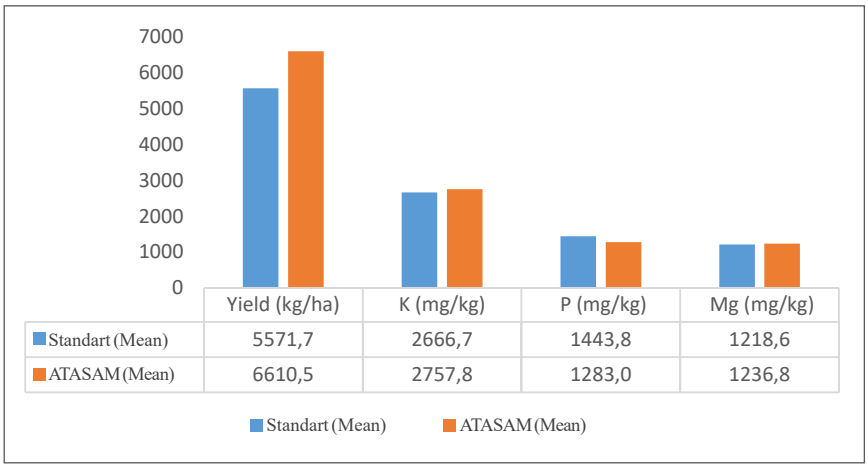

Figure 3. Yield (kg/ha) and macro nutrient content of genotypes.

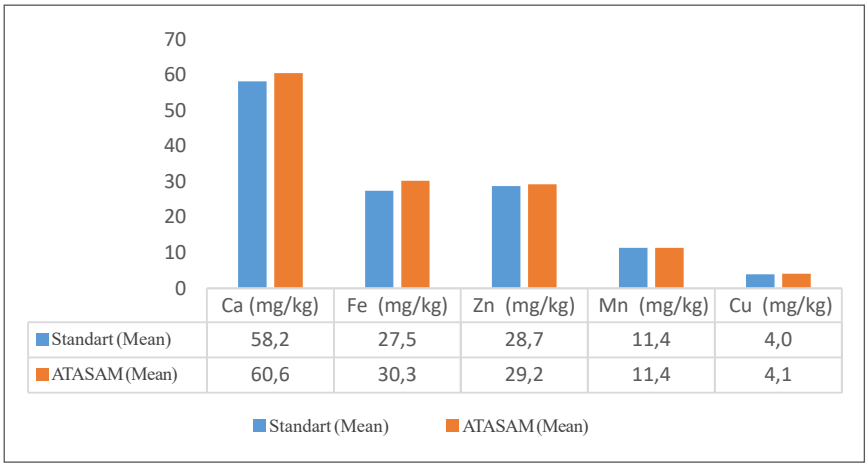

Figure 4. Micro nutrient content of genotypes.

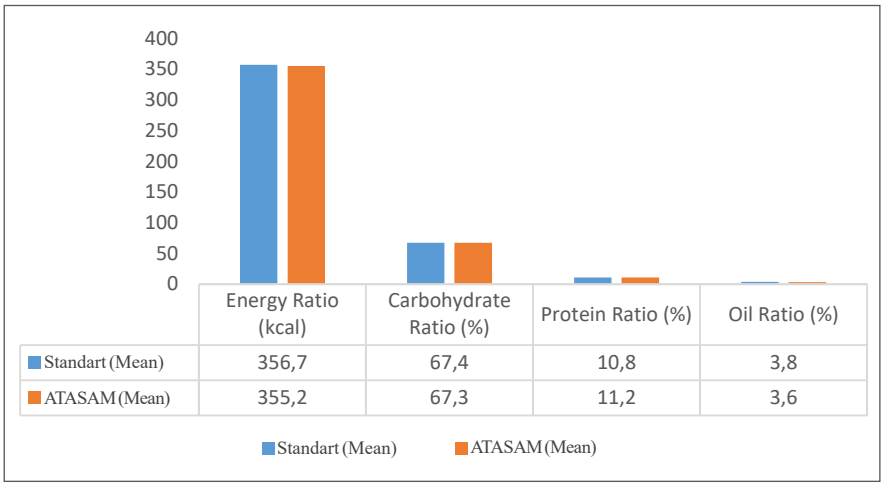

Figure 5. Grain Chemical Content of Genotypes.

Figure 6. Plant, 1000 grains, ear and popped grain view (Original).

Table 1. List of self-made materials.

Breeding Materials	Number of inbred ear plants	Selection criteria	Number of plants selected
S_1	1000	Plant and cob appearance, Number of days to flowering	650
S_2	650	Plant and cob appearance, Number of days to flowering	742
S_3	742	Plant and cob appearance, Number of days to flowering	546
S_4	546	Plant and cob appearance, Number of days to flowering	283
S_5	283	Uniformity, Stability	112
S_6	112	Uniformity, Stability	94
S_7	94	Uniformity, Stability,	

Table 2. Some important characteristics of ATASAM popcorn variety.

Observations	Charasteristics	State of expression	Note
Tassel	Time of tassel	Medium to late	6
Tassel	Anthocyanin colorations of anters	Strong	7
Ear	Time of silk emergence	Late	7
Ear	Anthocyanin colorations of anters	Strong	7
Leaf	Anthocyanin colorations of leaf	Absent or very weak	1

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Released Varieties

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Registration of "Ekin" Winter Barley (*Hordeum vulgare* L.) Variety

İrfan ÖZTÜRK

Trakia Agricultural Research Institute, Edirne, Türkiye

* Corresponding author e-mail: irfan.ozturk@tarimorman.gov.tr

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Ekin is two rowed barley (*Hordeum vulgare* L.) variety developed by Trakya Agricultural Research Institute (TARI) and registered in 2023. Ekin is developed by crossing Coss/OWB71080-44-1H/3/Obz/Vic//Unk(1989-90AGBOreg.2-13)/Unk(1989-90AGBOreg.2-14) with TEA2507-0T-0T-10T-2T-8T-0T and segregating generations examined in pedigree method. Crossing was made in 2009 and yield test began in 2017-2018 growing year.

Ekin is a two-rowed variety (Figure 1) and its spike is long and compact. It resembles with the cultivar Hasat. Ekin is a tall cultivar, similar to Harman. Plant height is between 80 and 125 cm depending on the growing conditions. It is medium early and as it has good adaptation ability, it has been grown throughout Trakya-Marmara and the transitional zone region of Türkiye. It gives a high yield both on fertile and less fertile soils. It has resistance to winterkilling and is tolerant to medium drought conditions. Ekin is highly tolerant to net blotch (*Pyrenophora teres*), scald (*Rhynchosporium graminicola*), and powdery mildew (*Blumeria graminis* f. sp. *hordei*).

Its yield potential is high however, a high yield can be obtained if environmental conditions are favourable and good agronomic practices followed. The highest grain yield obtained was 10,031 kg ha^{-1} in the Edirne location in the 2020-2021 growing years. The mean yield of the variety testing experiment was 8041 kg ha^{-1} in Trakya growing conditions. The suggested planting rate is between 450-500 seeds/m^2.

Its grain-feeding quality is good. The mean values of some qualities of the variety testing experiment (2021 and 2022) are; test weight 73.0-74.9 kg/hl, thousand kernel weight 42.4-48.0 g, protein content 9.2-11.8%, and sieve value 87.3-94.5%. The highest quality values during the 2018-2019 growing seasons before the variety testing experiment were; 1000-kernel weight 49.2 g, test weight 75.0 kg, protein content 12.5%, and sieve value 94.2%.

Pre-basic and Basic seeds of the Ekin cultivar have been produced by Trakya Agricultural Research Institute (TARI) and UTEK Seed Company. Certified seeds of the Ekin cultivar are produced by both private companies and state farms.

Figure 1. Spike and grain of the Ekin variety (Original)

References and Notes

Anonymous, (2023). Trakya Bölgesi iki sıralı arpa tescil raporu, Ankara, 2023 (in Turkish).

Released Varieties

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Registration of "Poyraz" Winter Barley (*Hordeum vulgare* L.) Variety

İrfan ÖZTÜRK

Trakia Agricultural Research Institute, Edirne, Türkiye

* Corresponding author e-mail: irfan.ozturk@tarimorman.gov.tr

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Poyraz is a six-rowed barley (*Hordeum vulgare* L.) variety developed by Trakya Agricultural Research Institute (TARI) and registered in 2023. Poyraz is developed by crossing Aleli/Gob//E.Quebracho/3/Msel/5/Ataco/Alaloe//Lino/3/Mja/Brb2//Quina/4/Ciru with TEA2666-0T-0T-0T-14T-5T-0T and segregating generations examined in pedigree method. The crossing was made in 2010 and the yield test began in the 2018-2019 growing year.

Poyraz is a six-rowed variety (Figure 1) and its spike is long, and medium-compact. It resembles the cultivar Martı. Poyraz is a tall cultivar, similar to Martı. Plant height is between 88 and 126 cm depending on the growing conditions. It is medium early and as it has good adaptation ability, it has been grown throughout Trakya-Marmara and the transitional zone region of Türkiye. It gives high yield both on fertile and less fertile soils. It has resistance to winterkilling and is tolerant to drought conditions. Poyraz is highly tolerant to net blotch (*Pyrenophora teres*) and powdery mildew (*Blumeria graminis* f. sp. *hordei*), susceptible to scald (*Rhynchosporium graminicola*).

Its yield potential is high however, a high yield can be obtained if environmental conditions are favorable and good agronomic practices followed. The highest grain yield obtained was 12,122 kg ha^{-1} in the Tekirdağ location in the 2021-2022 growing years. The mean yield of the variety testing experiment was 8648 kg ha^{-1} in Trakya growing conditions. The suggested planting rate is between 450-500 seeds/m^2.

Its grain-feeding quality is good. The mean values of some qualities of the variety testing experiment (2020 and 2021) are; test weight 70.9-74.8 kg/hl, thousand kernel weight 41.0-46.4 g, protein content 9.9-12.1%, and sieve value 83.0-95.4%. The highest quality values during the 2018-2019 growing seasons before the variety testing experiment were; 1000-kernel weight 46.0 g, test weight 72.6 kg, protein content 10.5%, and sieve value 93.9%.

Pre-basic and Basic seeds of the Poyraz cultivar have been produced by Trakya Agricultural Research Institute (TARI) and UTEK Seed Company. Certified seeds of the Poyraz are produced by both private companies and state farms.

Figure 1. Spike and grain of the Poyraz variety (Original)

References and Notes

Anonymous, (2023). Trakya Bölgesi iki sıralı arpa tescil raporu, Ankara, 2023 (in Turkish).

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10(1):66, 2024

Registration of "Gizlenci" Rice (*Oryza sativa* L.) Variety

Serkan YILMAZ* **Rasim UNAN** **Melih ENGİNSU**

Black Sea Agricultural Research Institute, Samsun, Türkiye

* Corresponding author e-mail: yilmaz.serkan@tarimorman.gov.tr

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"Gizlenci" is a rice variety released by the Black Sea Agricultural Research Institute, Samsun, in 2021. Gizlenci rice variety was developed by cross breeding method among its parental lines (Halilbey × Nembo). Modified-bulk breeding method was conducted between 2008 and 215. Crossing was made in 2008, initiated as a $F_{1:3}$ bulk selection until 2011, sustained as a $F_{4:7}$ pedigree selection of a single panicle row between 2012 and 2015. Preliminary yield trial, yield trial, regional advanced yield trials conducted from 2016 to 2018. As a result of 2-year national registration trials, Gizlenci has grain yield potential as 800-900 kg da^{-1}, and it has 14% more yield of the standard varieties average. When the stability parameters based on repeated data were examined, it was ranked in the middle ranks under poor environmental conditions. The variety, which increases its yield as good environmental conditions are achieved, has been ranked at the top. The Gizlenci variety stands out with its 63% unbroken milled yield and 21.7 g rice thousand milled grain weight. It also has average values of 6.1 mm milled kernel length and 2.7 mm milled kernel width. In terms of agricultural characteristics; flowering day is 83days, maturity day is 128 days, and plant height is 97.3 cm. Rice grain (Figure 1) is the characteristic of its transparent appearance. (Anonymous 2019, 2021).

Turkish Plant Variety Protection has been applied for the variety. Breeder and foundation seed of the variety will be produced and maintained by Black Sea Agricultural Research Institute, 55300, Tekkekoy, Samsun, Turkey. Limited quantities of seed are available on request to the corresponding author for research purposes.

Figure 1. a) Field appearance, b) grain and c) milled whole rice of Gizlenci rice variety (Original)

References and Notes

Anonymous, (2019). Black Sea Agricultural Research Institute project reports, Samsun, Turkey (in Turkish).
Anonymous, (2021). Registration trials report, TTSM, Ankara, Turkey (in Turkish).

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Released Varieties

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Registration of "Toprak" Bread Wheat (*Triticum aestivum* L.) Variety

İrfan ÖZTÜRK

Trakia Agricultural Research Institute, Edirne, Türkiye

* Corresponding author e-mail: irfan.ozturk@tarimorman.gov.tr

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Toprak is a winter bread wheat (*Triticum aestivum* L.) variety developed by Trakya Agricultural Research Institute (TARI) and registered in 2023. Toprak is developed by crossing Basribey/Lagos-9/3/PBW343*2/Kukuna//Pastor/SLVS with TE7244-0T-0T-0T-43T-4T-0T through the pedigree method. The crossing was made in 2011 and the yield test began in the 2018-2019 growing year.

The spike of the Toprak (Figure 1) is long, white, smooth, with awn and medium compact. The flag leaf is medium light-green and with low glaucousity. Grain is oval, hard and red colour. Toprak is a tall cultivar, similar to Gelibolu. Plant height is between 84 and 108 cm depending on the growing conditions. It is early and as it has good adaptation ability, it has been grown throughout the Trakya-Marmara region and some other transitional-zone parts of Türkiye. It gives high yield both on fertile and less fertile soils. It has moderate resistance to winterkilling and is tolerant to medium drought conditions. Toprak is highly tolerant to stripe rust (*Puccinia striiformis* f. sp. *tritici*) and leaf rust (*Puccinia triticina*). It is tolerant to powdery mildew (*Erysiphe graminis* f. sp. *tritici*), and septoria leaf disease.

Its yield potential is high however, a high yield can be obtained if environmental conditions are favorable and good agronomic practices followed. The highest grain yield obtained was 9474 kg ha^{-1} in a variety testing experiment (Edirne location in the 2021-2022 cycle). The mean yield of the variety testing experiment was 8200 kg ha^{-1} in Trakya growing conditions. The suggested planting rate is between 550-600 seeds/m^2.

Its grain quality is extremely good. The mean values of some bread-making qualities of the variety testing experiment (2021 and 2022) are; test weight 75.9-78.0 kg hl, thousand kernel weight 34.1-36.4 g, protein content 11.5-14.1%, sedimentation (Zel) 53-56 ml, gluten index 90.3-98.6%, gluten value 24.4-31.8%, alveograph energy value (W) 235-290 and flour yield 73-75%. The highest quality values in 2019-2020 growing seasons application of the variety testing experiment were; thousand kernel weight 39.9 g, test weight 80.9 kg, protein content 13.4%, gluten value 43.0%, gluten index 97.2%, grain hardness 55 and sedimentation (Zel) 71 ml.

Pre-basic and basic seeds of the Toprak cultivar have been produced by Trakya Agricultural Research Institute (TARI) and Trakya Birlik Seed Company. Certified seeds of the Toprak are produced by both private companies and state farms.

Figure 1. Spike and grain of the Toprak variety (Original)

References and Notes

Anonymous, (2023). Trakya Bölgesi Ekmeklik Buğday Tescil Raporu 1, Ankara 2023 (in Turkish).

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Registration of "Değirmen" Bread Wheat (*Triticum aestivum* L.) Variety

İrfan ÖZTÜRK

Trakia Agricultural Research Institute, Edirne, Türkiye

* Corresponding author e-mail: irfan.ozturk@tarimorman.gov.tr

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Değirmen is a winter bread wheat (*Triticum aestivum* L.) variety developed by Trakya Agricultural Research Institute (TARI) and registered in 2023. Değirmen is developed by crossing Flm85/4/Sun371A*2/3/Chen/Ae.Sq//Weaver/5/Pehl//Rpb8-68/Chrc/3/SD-KM-44/Izgrev with TE7488-0T-0T-0T-9T-0T through pedigree method. The crossing was made in 2012 and the yield test began in the 2018-2019 growing year.

The spike of the Değirmen variety (Figure 1) is medium-long, white, smooth, with awn and medium compact. The flag leaf is medium dark-green and with medium glaucousity. Grain is oval, hard and red colour. Değirmen is a tall cultivar, similar to Gelibolu. Plant height is between 88 and 105 cm depending on the growing conditions. It is medium-early and as it has good adaptation ability; it has been grown throughout the Trakya-Marmara region and some other transitional-zone parts of Türkiye. It gives high yield both on fertile and less fertile soils. It has resistance to winterkilling and is tolerant to medium drought conditions. Değirmen is highly tolerant to stripe rust (*Puccinia striiformis* f. sp. *tritici*) and leaf rust (*Puccinia triticina*). It is tolerant to powdery mildew (*Erysiphe graminis* f. sp. *tritici*), and septoria leaf disease.

Its yield potential is high however, a high yield can be obtained if environmental conditions are favorable and good agronomic practices followed. The highest grain yield obtained was 10957 kg ha^{-1} in a variety testing experiment (Edirne location in the 2021-2022 cycle). The mean yield of the variety testing experiment was 8408 kg ha^{-1} in Trakya growing conditions. The suggested planting rate is between 500-550 seeds/m^2.

Its grain quality is extremely good. The mean values of some bread-making qualities of the variety testing experiment (2021 and 2022) are; test weight 73.8-77.6 kg hl, thousand kernel weight 37.7-45.4 g, protein content 11.5-15.0%, sedimentation (Zel) 49-69 ml, gluten index 93.3-99.8 %, gluten value 21-33%, alveograph energy value (W) 244-335 and flour yield 70-72%. The highest quality values in the 2019-2020 growing seasons application of the variety testing experiment were; thousand kernel weight 44.4 g, test weight 80.3 kg, protein content 14.3%, gluten value 42.5%, gluten index 93.8% and sedimentation (Zel) 67 ml.

Pre-basic and basic seeds of the Değirmen cultivar have been produced by Trakya Agricultural Research Institute (TARI) and Trakya Birlik Seeds Company. Certified seed of the Değirmen are produced by both private companies and state farms.

Figure 1. Spike and grain of the Değirmen variety (Original)

References and Notes

Anonymous, (2023). Trakya Bölgesi Ekmeklik Buğday Tescil Raporu 1, Ankara 2023 (in Turkish)

About the Journal

Ekin, Journal of Crop Breeding and Genetics, is an international journal owned and edited by the Plant Breeders Sub-Union of Turkey (BISAB). Ekin is aimed at keeping information among plant breeders about new advances in the plant breeding and genetics as well as genetic diversity of plant species. Ekin publishes research papers and critical reviews on all aspects of plant breeding, genetics and plant registrations cover; old and new cultivars, local populations and introduction materials, germplasm, resistance sources for biotic and abiotic stresses, parental lines, genetic stocks, breeding materials, mapping populations. All manuscripts submitted for publication are reviewed by at least two referees and accepted for publication by editors based on advice from referees.

The journal is peer reviewed journal and published twice a year by BISAB.

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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 For27: 277-283.

Toker C, Canci H and Ceylan FO (2006). Estimation of outcrossing rate in chickpea (*Cicer arietinum* L.) sown in autumn.Euphytica 151: 201-205.

Article by Digital Object Identifier (DOI) number:

Yasar M, Ceylan FO, Ikten C and Toker C (2013). Comparison of expressivity and penetrance of the double podding trait and yield components based on reciprocal crosses of kabuli and desi chickpeas (*Cicer arietinum* L.). Euphyticadoi:10.1007/s001090000086

Book:

Toker C (2014). Yemeklik Baklagiller. BISAB, Ankara.

Book chapter:

Toker C, Lluch C, Tejera NA, Serraj R and Siddique KHM (2007). Abiotic stresses. In: Chickpea Breeding and Management,Yadav SS, Redden B, Chen W and Sharma B (eds.),CAB Int. Wallingford, pp: 474-496.

Online document:

FAOSTAT J (2013) http://faostat.fao.org/site/567/default.aspx# anchor. Accessed 15 May 2013.

Dissertation (Thesis):

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

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Abbreviations

Abbreviations should be defined at first mention and used consistently.

bisab
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Adakale Street, No: 22/12 Kızılay, 06420 Çankaya/Ankara - TURKEY
Phone: +90 312 433 30 65-66 Fax: +90 312 433 30 06
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