Selcuk Journal of Agriculture and Food Sciences

https://dergipark.org.tr/tr/pub/selcukjafsci

Research Article

(2023) 37(3), 474-486 DOI:10.15316/SJAFS.2023.045 e-ISSN: 2458-8377 Selcuk J Agr Food Sci

Breeding Sunflower (*Helianthus annuus*) Assisted with Speed Breeding & Drough Tolerance Tests

Ayşe Nuran ÇİL[1*]

[1]Eastern Medıterranean Agrıcultural Research Instıtute, Ankara, Türkiye

HIGHLIGHTS

- In the experiments, it was 4-5 generations could be achieved in one year.
- The genotypes K26-33 and K78-100 are located closest to the center as the most ideal genotypes in terms of the examined characteristics.
- In the MS environment, in terms of the characteristics examined, the genotypes K105-100, K78-100, K105-66, K26-100 and K1-100 were the preferred genotypes as they are located close to the center.

Abstract

Sunflower production in the world is expending towards marginal areas, along with rapid changes in cultural practises like no-till planting and weed management. The frequency and severity of abiotic constraints also rise as a result of climate change. *Helianthus annuus* is well-known for its adaptability to a wide range of agronomic conditions, by its robust root system that is capable of absobing water from deeper soils. However, water stress lowers grain yields and fatty acid content with complex phenotypic, physiological and biochemical signs. In this study which was carried out to develop parental lines tolerant or high-tolerant to drought, physiological screenings were carried out on 8 sunflower genotypes. Genotypes were planted in pots in a greenhouse and grown at three different irrigation levels (I100, I66 and I33). The genotypes were watered together until they reached the 6-8 leaf stage. Then, each genotype was managed and irrigated solely. Number of days between sowing and floweing days, number of days between sowing and number of days between sowing and number of days to transfer the embryo to the nutrient medium, plant height, head diameter, number of seeds in the head was between 52-67 days; 65-80 days; 50-200 cm; 3.0-13.0 cm; 25-500 pieces, respectively. Plant weight, plant high, root length, number of leaf, nnmber of days from transplant to glasshouse, number of days from transplant to field was between 0.22-0.45 g; 2.09-4.62 cm; 1.70-5.27 cm; 3.60-5.87 pieces; 5 or 6 days; 10-12 days, respectively. In the experiments, it was found that two and a half generations could be achieved in one year. The genotypes K26-33 and K78-100 are located closest to the center as the most ideal genotypes in terms of the examined characteristics. In the MS environment, in terms of the characteristics examined, the genotypes K105-100, K78-100, K105-66, K26-100 and K1-100 were the preferred genotypes as they are located close to the center.

Keywords: : Sunflower, *Helianthus annuus*, parental lines, drought tolerance, speed breeding, embryo culture

1. Introduction

The yield potential and stability of the sunflower (*Helianthus annuus*) have steadily increased as a result of conventional breeding. This improvement has been made possible by both the direct manipulation of a number of genes

Citation: Çil AN (2023). Breeding Sunflower (Helianthus annuus) Assisted With Speed Breeding & Drough Tolerance Tests. *Selcuk Journal of Agriculture and Food Sciences*, 37(3), 474-486. https://doi.org/10. 15316/SJAFS.2023.045

Corresponding Author E-mail: aysenuran.cil@tarimorman.gov.tr

Received date: 01/01/2022

Accepted date: 18/07/2023

that control resistance to parasitic weeds, pests, and fungal diseases, as well as the indirect selection of quantitative trait loci that regulate heritable variability of the traits and physiological processes that control biomass production and its partitioning. Due to the distribution of sunflower production towards marginal areas, the rapid changes in cultural practises like no-till planting and weed management, and the rise in the frequency and severity of abiotic constraints as a result of climate change, this approach may no longer be sufficient because genetic progress has been slower in recent decades. Three key strategies were developed as a result of recent research to alter the goals and methods of sunflower breeding. In order to discover traits that are beneficial to increase selection efficiency, plant physiology has supplied new tools and models to analyse the complicated network of yield- and stress-related variables. Second, molecular genetics has helped identify numerous loci that influence yield under prospective and stressful situations or the development of characteristics connected to stress tolerance. Third, molecular biology has produced genes that can be used for transgenic methods or as candidate sequences to analyse QTL (Sala et al. 2012).

Sunflower is not an exception to the rule that drought stress is one of the major factors limiting agricultural yield in the twenty-first century, according to Salehi-Lisar & Bakhshayeshan-Agdam (2016). Drought stress significantly reduces sunflower productivity, which is a key barrier to worldwide sustainable crop production, especially in arid and semi-arid nations (Wasaya et al. 2021). *Helianthus annuus* L., the common sunflower, is well-known for its adaptability to a wide range of agronomic circumstances, particularly on soils with fluctuating water contents (Raineri et al. 2015).

Sunflower has a robust root system that is capable of taking up water from deeper soils (Hussain et al. 2013). Since the plant have stomata on both sides of its leaves, it also has a great capacity for photosynthetic growth. However, because it grows mostly in tropical and subtropical climates, it is more vulnerable to drought, which reduces the seeds and oil yields (Hussain et al. 2018). Additionally, water stress lowers grain yields and fatty acid content (Alberio et al. 2016, Howell et al. 2015).

The effects of drought on sunflowers are complex, ranging from phenotypic to physiological and biochemical signs. These changes include decreased plant height, leaf surface area, relative water content, closed stomata, and reduced levels of photosynthesis (Buriro et al. 2015); increased root length and the root-shoot ratio; shrinkage in cell volume; decreased water potential; and membrane stability disrupted the balance of various biochemical processes (Soleimanzadeh 2012).

Although sunflower is a crop that tolerates modest amounts of drought, severe drought episodes reduce the seed and oil yields. Therefore, it is essential to know how the physiological, biochemical, genetic, and agronomic bases of drought interact in order to control it sustainably and ensure sustainable production of sunflower achene and oil. Drought stress has a major impact on sunflower's achene yield, oil quality, morphological and growth variables, as well as physiological and biochemical traits (including photosynthesis, water relations, nutrient uptake, and oxidative damage). Exogenous hormone and osmoprotectant sprays, seed treatments, soil nutrient management, and traditional or biotechnological breeding for drought resistance are a few examples of management strategies. In reaction to water stress, sunflower modifies its osmotic balance, preserves its turgor, maintains its capacity to absorb carbon, and regulates its hormones. The improvement of sunflower achene yield and oil quality under drought stress necessitates in-depth investigation of the fusion of several management techniques, including agronomic management, conventional breeding, and modern technological breakthroughs (Hussain et al. 2018).

Drought-related sunflower yield reductions are substantial (Prasad et al. 2008). Pekcan et al. (2015) claim that exposure, specifically during the anthesis and dough stages, can result in crop losses of up to 80% (Seiler et al. 2017). Any water stress screening experiment must include genotype characterisation based on relative water content, leaf water potential, photosynthetic efficiency, and proline concentration (Darvishzadeh et al. 2011). The amino acid proline is necessary for both osmotic adjustments and free radical scavenging during drought stress. For the plants to experience a fewer change in relative water content even with a drop in water potential, osmotic adjustments are crucial. This mostly aids plants in continuing to develop and expand their cells while under the stress of drought (Cechin et al. 2006).

Early- and mid-flowering phases are particularly impacted by water shortage produced by reduced irrigation, as opposed to seed filling, where restricted irrigation is tolerated (Karam et al. 2007). Rauf (2008) asserts that lower photosynthesis brought on by early leaf senescence during drought conditions results in a decrease in the weight of 100 achenes.

Development of new crop varieties requires time because it is based on the crop's generation period. Speed breeding, often known as rapid plant breeding, is a quick growing method used by plant breeders to develop new cultivars. Here, the plants are grown in controlled growth chambers or greenhouses with the best light quantity and quality, as well as a specific day length and temperature, which accelerates a number of physiological processes in plants, most notably photosynthesis and flowering, and cuts down on the amount of time that the generation process needs. 4-6 generations can be produced annually using fast breeding, as opposed to 2-3 generations under standard glasshouse conditions. Speed breeding protocols and processes are well-established and standardised for major crop species like wheat, barley, and canola. This strategy is already in use, and protocols for standardisation are being developed for new crops. For the purpose of improving the traits of agricultural species, speed breeding may serve as the essential building block for integrating high-throughput phenotyping and genotyping techniques, marker-assisted/genomic selections, and gene editing (Abdul Fiyaz et al. 2020).

Rapid plant breeding, often known as speed breeding, is a method employed by plant breeders to accelerate the production of new cultivars. For speed breeding, new methods that accelerate flowering, seed germination, embryo development, and other processes are required. Over time, speed breeding has evolved and can broadly be divided into three types: The plants in the first category were grown under controlled growth chamber circumstances with speed breeding criteria; the plants in the second category were grown in a glasshouse with speed breeding specifications; and the plants in the third category were produced in a specially designed home-built growth room for low-cost speed breeding programmes (Watson et al. 2018). 22 hours of photoperiod, 70% humidity, 22 °C during the day and 17 °C at night, as well as strong light intensity (360 to 650 mol m2 s) are all requirements for fast breeding. These requirements change depending on the stage of vegetative and reproductive plant growth (Pandey et al. 2022).

Rapid breeding is facilitated by the in vitro tissue culture technique known as "embryo rescue," which accelerates plant embryo growth. This method, which requires harvesting immature seeds and germination in the culture media, may or may not use the plant growth regulator (PGR). Numerous crop species have successfully used this strategy (Zheng et al. 2013; Castello et al. 2016; Bermejo et al. 2016; Yao et al. 2017). The genotype of the plant, the age of the embryo, its preparation, age homogeneity, sterilisation method, the composition of the medium (sugar, hormone, vitamin, other nutritional additives), environmental adjustments (humidity, photoperiod, and temperature), culture time, the medium to which the seedlings are transferred after culture, and the trial pattern all play a role in how sunflower embryos respond to the embryo rescue method (Çil et al. 2021).

2. Materials and Methods

The Studies were carried out to develop parental lines tolerant or high-tolerant to drought. For this purpose, the responses of different selected sunflower parent genotypes to drought stress were compared. Physiological screenings were carried out on genotypes and the necessary plantings and emergence were achieved.

2.1.Tested oil type sunflower genotypes and their resistance levels

- MAS RYM 17-17 (1) - Resistant restorer
- RYM 13-97/2 (19) - Tolerant restorer
- RYM 13-152/2/2 (26) - Susceptible restorer
- HA 430 (105) - Control Public Group 1
- DA-VB 16-39 (78) - Tolerant

- DA-VB 17-29 (88) - Susceptible

- HA 429 (109) - Control Public Group 2

- DA-VB 16-41 (79) – Resistant

As a part of the greenhouse studies of the project, genotypes were planted in 30x50 sized pots and grown at different irrigation levels (I100, I66 and I33) between January and June 2023. The properties of the soil used in the pots were analyzed and plant water consumption amounts were calculated.

The physical properties of the soil used in the pots were performed at "Soil Analyzes Tarsus Soil and Water Resources Research Station". The results obtained are given in Table 1.

Table 1. Physical properties of the soil used in the experiment

Volume weight (g/cm³)	Clay (%)	Sil (%)	San (%)	Structure Class
1,41	39,52	37,78	22,69	Clay Loam

The pots werefully filled with soil. During filling, every 1/3 of it was compressed with the help of a tamper and this process was repeated three times. Then, irrigation water was applied, pots were left for free drainage for two days and the seeding process was started. Following plant emergences, five plants were left in each pot. The genotypes were planted in February. Each genotype were in 15 pots. Total 75 plants of each genotype were followed. There were 6 pots for each irrigation application group (I33, I66 and I100) with 5 plants in each pot. Plants were grown in the trial greenhouse to test genotypes under windless and sunny weather conditions.

2.2.Drought applicaqtion in greenhouse trials

Immediately after seed planting, all pots were irrigated to field capacity. Next irrigation practices were started when the plants were at 6-8 leaves stage when 50% of the available moisture in the soil was depleted. Irrigation was applied when wilting was observed on the plant leaves. Irrigation was repeated for five times during the entire growth season. On average, irrigation was applied every 7 days. The decreasing humidity level in the A-Pan evaporation container was used to determine the amount of irrigation water to be applied to cover every seven days. The lost moisture was delivered with each irrigation to reach field capacity.

The genotypes were watered together until they reached the 6-8 leaf stage. Then, each genotype was managed and irrigated solely. In this regard, plants with I100 irrigation criteria were checked daily and irrigation need of the genotypes were determined. Pots of the same genotype with I66 and I33 irrigation criteria were also irrigated with restriction. Restricted irrigation applied amounts were 6.5 liters for I100, 4.5 liters for I66 and 2.5 liters for I33 in each irrigation period.

2.3.Irrigation water amount calculations for pots

In the study, the amount of irrigation water applied every 7 days and evaporation (ETo) was determined by monitoring it from the evaporation container. For this purpose, a Class A evaporation pan was placed in the trial area. A-Pan evaporation vessel was made of galvanized sheet metal with a diameter of 121 cm, a height of 25 cm and a thickness of 2 mm. A 15 cm high wooden grill was placed under the container, allowing air flow. Water level changes in the container were measured. A wire cage has been placed over the container to prevent any animal from drinking water from the container. For measurements, water was applied up to 5 cm below the container edge height and the water level was not allowed to fall 7.5 cm below the container edge height. The water in the container was renewed at least every four days to prevent the water from becoming excessively dirty. All readings were conducted at 9:00 am each day. These measured values were used to calculate the amount of irrigation water to be applied every 7 days. The amount of irrigation water applied to the trial plots was calculated by taking into account the daily evaporation amount from Class A Pan.

Irrigation applications were made with measured beakers. Irrigation was not conducted with drip irrigation system due to the pressure drop towards the end of the line and reductions in amounts of applied water. In terms of full irrigation (I100), the water consumption of a plant in a pot was 30.6 liters.

Figure 1. Some photos from the experiment

The amount of irrigation water was calculated using open water surface evaporation and plant-pan coefficients, and determined according to the method of according to Gençoğlan et al. (2006). The amount of water given to parcels was calculated with the help of equations 1, 2 and 3.

$$I = Ep.\ kcp\ .\ P \quad (1)$$

$$V = A\ .\ I \quad (2)$$

I: amount of irrigation water (mm); Ep: evaporation from pan (mm); kcp: plant-pan coefficient (I33=0.33, I66=0.66 and I100=1.00); P: vegetation coverage percentage (%); A: parcel area (m2); V: water volume (L).

Equation 3 was used to determine the vegetation cover percentage (wetting factor) (Gençoğlan et al., 2006).

$$P = a / b \quad (3)$$

a: plant canopy diameter (cm); b: row spacing (cm).

Plant diameter (a) was measured from an average of five plant diameters before each irrigation. Irrigation applications were carried out in a controlled manner by passing three water meters to the irrigation areas. In the study, the water balance equation (method) was used to directly determine plant water consumption. The following water balance equation (equation 4) was used to calculate the plant's water consumption:

$$ETa = P + I - Rf - Dp \pm \Delta S \quad (4)$$

ETa: Evapotranspiration (mm); P: precipitation (mm); I: amount of irrigation water (mm); Rf: surface flux (mm); Dp: Deep infiltration (mm); ΔS (mm): Soil moisture change at the rhizosphere.

Since the drop flow rate preferred in the study was lower than the infiltration rate of the soil, surface runoff did not occur. Since no more water will be given to the soil than the field capacity during irrigation and the dripper flow rate is lower than the soil infiltration rate, deep percolation losses (Dp) are accepted as zero. Additionally, surface flow values (Rf) were considered unimportant and were not taken into calculation.

According to the water balance equation method in the early vegetative period, the weekly plant water consumption (ETa) value (per pot) in I100 irrigation, where the water need wasfully met, varied between 3.5 liters (per pot) week-1, while in the flowering period it was 9.0 liters (1.44 mm) week-1 and 36 liters month-1. Simply, it was 3.5 liters (2.16 mm) per week per pot in May and June, and 9 liters (2.16 mm) in July and August; as year 2022 was extremely hot. The ETa value decreased towards physiological maturity. According to the water balance equation method for sunflower lines during the growing season, ETa amount was determined as seasonal plant water consumption (ETa) as 128 liters (21 mm) pot-1.

2.4.Physiological measurements related to drought

Physiological screenings were carried out on drought tolerant and sensitive genotypes. Evaluations were made by taking physiological and morphological measurements in the pots before and after irrigation. The determination of excess water in pot irrigation was made by determining the amount of water filtered into the pot within 1 hour after irrigation. 5.5 liters of water was given to the pots, and the amount of water filtered under the pot was determined as 0.25 liters. Therefore, 4.5 liters of water was consumed.

According to the morphological observations, small leaves with narrow angles and a low rate of leaf wilting were determined to be more resistant. Physiological measurements also support each other. In infrared-meter measurements, high values showed sensitive and low values showed resistant genotypes, and in chlorophyll measurements, high values showed resistant and low values showed sensitive genotypes.

2.5.Embryo culture within the scope of speed breeding

With the "Growth Under LED Lights for Speed Breeding" system, required days to complete the vegetation period for the sunflower in embryo culture and the regeneration rate of the sunflower with limited water application were investigated by applying the LED light. In speed breeding, red light was applied as 343 lux, green as 69 lux, blue as 174 lux, a total of 586 lux until the flowering period. The temperature was 24 °C day and 18 °C night at 70% humidity for 22 hours light and 2 hours dark photoperiod. In the experiments, it was found that two and a half generations could be achieved in one year.

Application of Embryo Rescue Culture under LED Light

By combining the embryo culture method with CMS studies and restorer development studies, in previous studies conducted by (Çil et al. 2021), the embryo culture period was further accelerated and the "plant culture-field transfer" period was started. The period was reduced to 21-30 days. In this study, this period was reduced to 15 days under LED light with the same team. From the beginning of October to the flowering period, white light at 40 par, red light at 10 par, green light at 10 par, and blue light at 10 par were given. The temperature was 24°C during the day, 18°C at night, humidity was 70%, 22 hours during the day and 2 hours at night.

Embryo culture method

Seed surface sterilization process: Embryos in the fruits from the first five rows located at the outermost part of the table were used for embryo culture. The fruits separated from each head were subjected to surface sterilization with 70% alcohol for a very short time in a sterile cabinet in a glass container, then, alcohol was added and 20% bleach (5% Sodium hypochlorite, unperfumed) was added 3-5 drops. Sterilization solution containing Tween 80 was also added. The fruits were sterilized by shaking in the solution for 10 minutes (Dağüstü et al. 2010). Following this, surface sterilization was completed by rinsing the fruits with sterile distilled water by repeating 4-5 times.

Embryo isolation and culture of isolated embryos: The shells of the surface sterilized seeds were cut and removed. After the embryos were removed from the embryo sac and separated, five of them were placed in each petri dish of 60 x 15 mm before transferred to the embryo development medium.

Media and culture conditions: MS (Murashige and Skoog, 1962) medium containing 2% sucrose and 0.8% agar was used as the embryo medium in the experiments. The pH of the medium was adjusted to 5.8 using 1N KOH and 1N HCl. Sterilization of the medium was achieved by keeping it in an autoclave under 1.2 atmospheres of pressure and 121 oC for 15 minutes. All cultures were kept in growth cabinets providing a photoperiod of 22 hours of light and 2 hours of darkness and a temperature of 25 ± 2 oC.

Acclimatization to the external environment and transfer to the violet: The plantlets formed 6-7 days after the beginning of the culture were cleaned of agar residues with hot water. Well-developed plants were selected and transferred to glasses filled with soil. Plants developed in speedbreeding within 10-15 days were transferred to the outdoor environment.

Evaluation of data

Angle transformation was applied to the data obtained by the research and ANOVA (Oneway) test was applied with the JumpPro-13 statistical package program. Differences between means were compared

according to the Duncan test. In addition, comparison biplot analysis was performed in the Genstat 12th Edition package program to determine the ideal genotypes in terms of the examined characteristics in different environments. The relationship between features was interpreted with the scatterplot matrix produced by the JumpPro-13 package program.

3. Result and Discussion

3.1. Physiological results related to drought

Results of the examined properties of sunflower genotypes under greenhouse sowing are given in Table 1.

Table 2. ANOVA (Oneway) analysis of examined properties of sunflower genotypes under greenhouse sowing

Genotype	Sowing date	Number of flovering days	Embryo age	Number of days to transfer the embryo to the nutrient medium	Plant height (cm)	Head diameter (cm)	Number of seeds in the head (piece)
K26-100	21.03.2023	53	13	66	85.00±8.66 ıj	9.00±2.00 a-d	77.33±9.02 j
K26-66	21.03.2023	53	13	66	75.00±5.00 ıjk	8.00±2.00 a-d	69.00±9.00 jk
K26-33	21.03.2023	55	13	68	72.00±2.00 jk	8.00±2.00 a-d	63.00±3.00 jk
K78-100	21.03.2023	52	13	65	135.00±5.00 d	9.00±3.00 a-d	245.00±5.00 d
K78-66	21.03.2023	53	13	66	130.00±5.00 de	8.50±2.50 a-d	233.00±3.00 de
K78-33	21.03.2023	55	13	68	127.00±3.00 def	8.00±3.00 a-d	225.00±5.00 def
K19-100	21.03.2023	52	13	65	60.00±5.00 lm	5.00±1.00 cd	230.00±5.00 def
K19-66	21.03.2023	53	13	66	54.00±4.00 lm	4.50±1.50 d	214.00±4.00 ef
K19-33	21.03.2023	54	13	67	50.00±5.00 m	4.00±1.00 d	223.00±3.00 ef
K1-100	21.03.2023	52	13	65	80.00±5.00 ıj	4.00±2.00 d	67.00±2.00 jk
K1-66	21.03.2023	53	13	66	77.00±7.00 ıj	3.50±1.50 d	63.00±3.00 jk
K1-33	21.03.2023	55	13	68	69.00±4.00 jkl	3.00±1.00 d	58.00±3.00 k
K79-100	21.03.2023	58	13	71	170.00±5.00 bc	13.00±3.00 a	500.00±10.00 a
K79-66	21.03.2023	59	13	72	162.00±7.00 c	12.00±2.00 ab	456.00±6.00 b
K79-33	21.03.2023	60	13	73	157.00±7.00 c	11.50±1.50 ab	432.00±2.00 c
K88-100	21.03.2023	63	13	76	115.00±5.00 efg	8.00±1.00 a-d	118.33±2.89 h
K88-66	21.03.2023	64	13	77	111.00±6.00 fg	7.00±2.00a-d	116.00±6.00 h
K88-33	21.03.2023	66	13	79	108.00±5.00 g	7.00±1.00a-d	109.00±4.00 hı
K105-100	21.03.2023	64	13	77	200.00±5.00 a	11.00±2.00 abc	120.00±5.00 g
K105-66	21.03.2023	65	13	78	90.00±5.00 hı	6.00±1.00 bcd	195.00±5.00 f
K105-33	21.03.2023	67	13	80	85.00±5.00 ıj	5.00±1.00 c	53.00±3.00 k
K109-100	21.03.2023	64	13	77	180.00±5.00 b	11.00±3.00 abc	120.00±5.00 h
K109-66	21.03.2023	65	13	78	105.00±5.00 gh	8.00±3.00 a-d	96.00±6.00 i
K109-33	21.03.2023	67	13	80	90.00±5.00 hı	6.50±1.50 bcd	25.00±5.00 l
Mean		**58**	**13**	**71**	**107.77**	**7.52**	**171.15**
			Source	**DF**	**MS**	**MS**	**MS**
			Genotype	23	5268.26**	23.9660**	52204.3**
			Error	48	28.25	3.9688	27.0
			CV (%)	**4.92**	**26.42**	**3.03**	

**; significant at level 0.01, ±; Standard deviation. Levels not connected by same letter are significantly different.

Number of days between sowing and number of flovering days was between 52-67 days. Number of days between sowing and number of days to transfer the embryo to the nutrient medium was 65-80 days (Table 1). Plant height was between 50-200 cm with average of 107.8 cm. Plant height was lowest (between 50.0-60.0 cm) at K19-100, K19-66 and K19-33 genotypes, whereas highest (200.0 cm) at K105-100 genotype. Head diameter was between 3.0-13.0 cm. Number of seeds in the head was lowest (25 pieces) at K109-33 genotype whereas highest (500 pieces) at K79-100 genotype (Table 1). In comparison to other crops, *Helianthus annuus* is considered to be moderately drought resistant (Skoric, 2009). Additionally, there are numerous studies of genetic variation in drought responses as well as an array of genetic resources for sunflower (Poormohammad Kiani et al. 2007; Masalia et al. 2018).

Table 3. ANOVA (Oneway) analysis of examined properties of sunflower genotypes at *in-vitro*

Genotype	Plant weight (g)	Plant high (cm)	Root length (cm)	Number of leaf (piece)	Number of days from transplant to glasshouse	Number of days from transplant to field
K26-100	0.45±0.03 a	3.28±0.30 abc	4.10±0.44 a-f	5.07±0.23 abc	6	11
K26-66	0.33±0.03 a-d	2.90±0.26 abc	2.83±0.32 a-g	4.00±0.69 cd	6	11
K26-33	0.26±0.04 bcd	2.63±0.72 c	2.20±0.52 d-g	4.27±0.61 a-d	6	11
K78-100	0.36±0.06 d-d	3.57±0.65 abc	5.27±1.10 a	4.53±0.61 a-d	6	12
K78-66	0.23 ±0.01 cd	2.57±0.23 c	2.93±0.91 a-g	4.13±0.23 bcd	6	11
K78-33	0.29±0.05 a-d	2.73±0.47 c	3.70±1.21 a-g	3.67±0.12 cd	6	11
K19-100	0.28±0.03 a-d	3.63±1.16 abc	5.07±0.35 ab	4.13±0.23 bcd	6	12
K19-66	0.30±0.10 a-d	2.63±0.75 c	3.27±1.77 a-g	3.60±0.40 cd	6	11
K19-33	0.32±0.03 a-d	2.83±0.60 bc	2.30±0.70 c-g	3.73±1.01 cd	6	11
K1-100	0.40±0.04 abc	4.57±0.70 ab	4.87±0.92 abc	5.33±0.83 abc	6	12
K1-66	0.26±0.07 bcd	2.09±0.37 c	4.68±0.99 a-d	4.00±0.40 cd	6	11
K1-33	0.22±0.04 d	2.26±0.39 c	4.37±1.06 a-e	4.33±0.31 a-d	6	11
K79-100	0.43±0.02 ab	3.70±0.40 abc	4.33±0.75 a-f	5.07±0.46 abc	6	11
K79-66	0.32±0.02 a-d	2.30±0.36 c	2.50±0.36 b-g	3.87±0.61 cd	5	10
K79-33	0.36±0.18 a-d	2.43±0.51 c	2.30±1.32 c-g	4.13±0.46 bcd	5	10
K88-100	0.38±0.05 a-d	3.17±0.55 abc	3.50±0.75 a-g	4.80±1.06 a-d	5	10
K88-66	0.39±0.05 a-d	3.03±0.60 abc	3.27±1.31 a-g	5.20±0.80 abc	5	10
K88-33	0.28±0.01 a-d	2.27±0.15 c	1.70±0.10 fg	3.60±0.40 cd	5	10
K105-100	0.43±0.02 ab	4.62±1.05 a	4.80±0.60 a-d	5.87±0.61 ab	5	10
K105-66	0.38±0.06 a-d	3.50±0.40 abc	2.18±0.19 d-g	5.07±0.83 abc	5	10
K105-33	0.26±0.01 bcd	2.12±0.57 c	1.43±0.57 g	3.20±0.40 d	5	10
K109-100	0.42±0.03 ab	3.78±0.20 abc	4.23±0.98 a-f	6.00±0.40 a	5	10
K109-66	0.35±0.02 a-d	2.90±0.40 abc	2.00±0.10 efg	4.40±0.40 a-d	5	10
K109-33	0.28±0.01 a-d	2.08±0.13 c	1.34±0.24 g	3.73±0.61 cd	5	10
Mean	**0.33**	**2.98**	**3.29**	**4.41**	**5.5**	**10.7**
Source	**DF**	**MS**	**MS**	**MS**	**MS**	
Genotype	23	0.013845**	1.58756**	4.51102**	1.65903**	
Error	48	0.003052	0.31163	0.71786	0.34000	
CV (%)		**15.15**	**18.45**	**25.53**	**13.03**	

**; significant at level 0.01, ±; Standard deviation. Levels not connected by same letter are significantly different.

Plant weight was between 0.22-0.45 g (Table 2). Plant high was between 2.09-4.62 cm. Root length was between 1.70-5.27 cm. Number of leaf was between 3.60-5.87 pieces.

Number of days from transplant to glasshouse was 5 or 6 days. Number of days from transplant to field was between 10-12 days (Table 2).

Sunflower achene and oil output were discovered to be significantly influenced by the quantity and distribution of water (Krizmanic et al. 2003). It was demonstrated that irrigation at the flowering stage produced the highest achene yield. The effects of drought on plant growth have an impact on both the biological and economic benefits of the harvest. The main stem height, diameter, number of nodes or leaves, and leaf area are all decreased during vegetative development (Turhan and Baser 2004), whereas root length increases at the expense of above-ground dry matter. A larger root-to-shoot ratio acquired under drought stress has been used to confirm this. Lower plant surface area caused by the decline in vegetative biomass lowers photosynthesis and radiation usage efficiency (Germ et al. 2005). Finally, this limits photosynthetic assimilation during the reproductive phase, which decreases head diameter. Reduced head diameter also leads to a reduction in the number of rows and achenes per head and an association between yield components and the severity of the drought (Rauf and Sadaqat 2007a). On the other hand, stress during the flowering stage results in ovarian, embryonic, and pollen sterility abortions as well as a decline in the leaf area index. As a result, there are less achenes per head, less achenes per 100 grammes, and fewer fertile achenes per head. According to estimates, stress during the vegetative phase reduces production by 15–25%, and stress during the flowering stage might cause a yield drop of more than 50% (Reddy et al. 2003). If drought was applied during the achene filling stage, however, it was discovered that there would be minimal harm (Karam et al.

2007). As a result of the stress at this point, the plant responds by senescing its leaves prematurely and abruptly and mobilising stem reserves to support the growing achenes (Rauf and Sadaqat 2007b). However, an excessive amount of leaf loss at this stage could result in a reduction in the weight of 100 achenes due to lower photosynthate production (Rauf 2008).

Biplot analysis

In breeding studies, an ideal genotype should have both high average performance and high stability in different environments. The biplot analysis method is becoming frequently used in breeding studies in recent years (Yan and Tinker 2006; Tabrizi et al. 2011; Korkmaz et al. 2021; Koç and Güneş 2021). Figure 2 shows the ideal genotypes with the highest averages in terms of the examined characteristics (Yan and Tinker 2006).

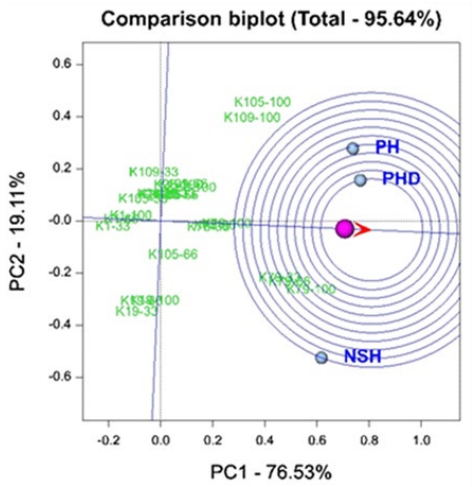

Figure 2. Relationship between the examined traits and genotypes by comparison biplot analysis under greenhouse. Abbreviations: PH: Plant high, PHD: Plant head diameter, NSH: Number of seed in head

In different studies conducted by other researchers, genotypes closer to the ideal genotype are preferred more than others. In the biplot model, based on the average data in our research, we obtained the variation rates as PC1: 76.53%, PC2: 19.11 and PC1+PC2: 95.64%. Highest variation was detected in the genotypes. The genotypes K26-33 and K78-100 are located closest to the center as the most ideal genotypes in terms of the examined characteristics. Genotypes K79-100, K79-66 and K79-33 are also located close to the center of the diagram.

In the MS environment, in terms of the characteristics examined, the genotypes K105-100, K78-100, K105-66, K26-100 and K1-100 were the preferred genotypes as they are located close to the center. Other genotypes were positioned outside the circle and was not preferred (Figure 3).

Based on the relationship between the examined traits, under greenhouse conditions, there was a positive and very significant relationship between plant head diameter and plant height ($r = 0.8599$, $P \leq 0.01$), between the number of seeds per head and plant height ($r = 0.4808$, $P \leq 0.01$), between the number of seeds per head and head diameter ($r = 0.5810$, $P \leq 0.01$). In MS medium, there was a positive and a very significant relationship between plant root length and plant height ($r=0.5327$, $P \leq 0.01$), number of leaves and plant height ($r=0.7128$, $P \leq 0.01$), number of leaves and plant root length ($r=0.5250$, $P \leq 0.01$) (Figure 4).

In different researches, some have found negative and significant relationship between head diameter and head number and plant height (Koç and Güneş 2021). In their study, Tabrizi et al. (2011) reported a positive and significant relationship between root length and plant weight. The findings obtained in our research were different from results of Koç and Güneş (2021) but similar to Tabrizi et al. (2011).

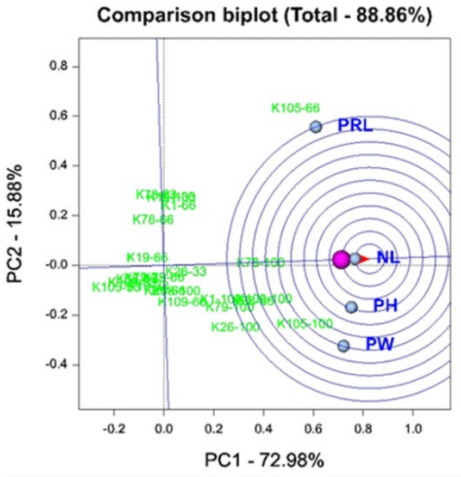

Figure 3. Performance of the genotypes for examined traits and genotypes by comparison biplot analysis at MS. Abbreviations: PRL: Plant root length, PH: Plant high, NL: number of leaf, PW: Plant weigh

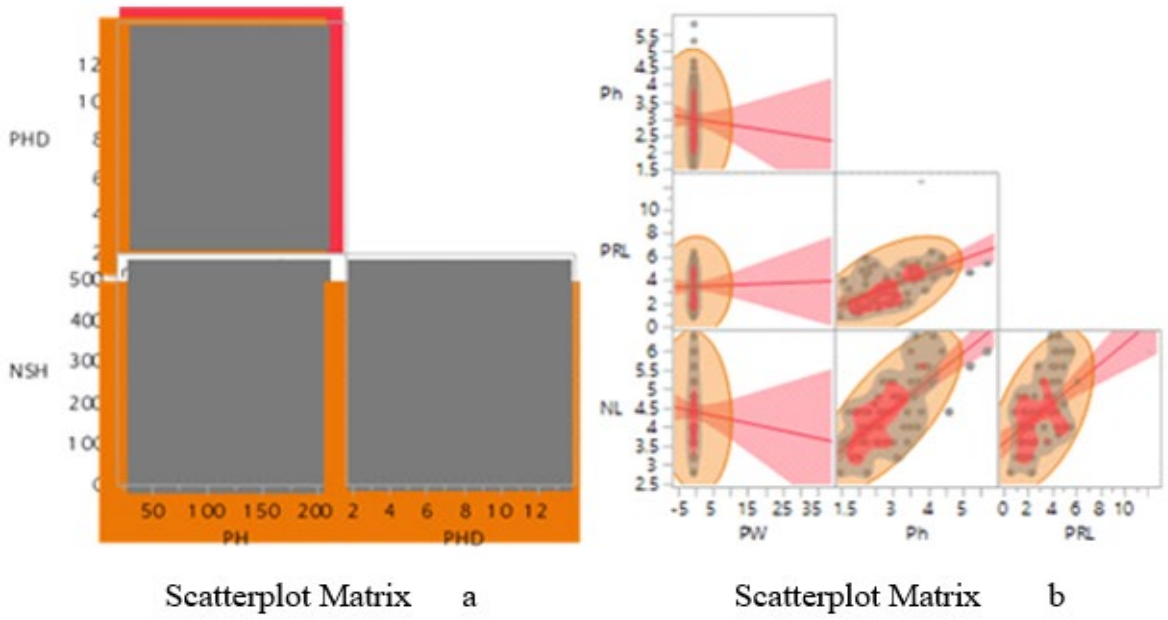

Figure 4. Correlation of traits under different conditions (a: under greenhouse, b: at MS) by scatterplot matrix. Abbreviations: PH: Plant high, PHD: Plant head diameter, NSH: Number of seed in head, PRL: Plant root length, PH: Plant high, NL: number of leaf, PW: Plant weigh.

4. Conclusions

Number of days between sowing and flovering days, embryo age and number of days to transfer the embryo to the nutrient medium, plant height, head diameter, number of seeds in the head was between 52-67 days; 65-80 days; 50-200 cm; 3.0-13.0 cm; 25-500 pieces, respectively.

In the experiments, it was found that 4-5 generations could be achieved in one year. The genotypes K26-33 and K78-100 are located closest to the center as the most ideal genotypes in terms of the examined characteristics. In the MS environment, in terms of the characteristics examined, the genotypes K105-100, K78-100, K105-66, K26-100 and K1-100 were the preferred genotypes as they are located close to the center.

Funding: Please add: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

Abdul Fiyaz R, Ajay BC, Ramya KT, Aravind Kumar J, Sundaram RM, Subba Rao LV (2020). Speed breeding: methods and applications. *Accelerated Plant Breeding*, Volume 1: Cereal Crops, 31-49.

Akinoğlu G, Korkmaz A, Boz E (2021). Ayçiçeği hatlarının demir beslenme kabiliyetleri yönünden gruplandırılması ve bu hatların en iyi demir beslenme özelliklerinin belirlenmesi. *Black Sea Journal of Engineering and Science* 4(4):153-159.

Alberio C, Izquierdo NG, Galella T, Zuil S, Reid R, Zambelli A, Aguirrezábal LA (2016). A new sunflower high oleic mutation confers stable oil grain fatty acid composition across environments. *European Journal of Agronomy* 73:25-33.

Bermejo C, Gatti I, Cointry E (2016). In vitro embryo culture to shorten the breeding cycle in lentil (*Lens culinaris* Medik). *Plant Cell Tissue and Organ Culture (PCTOC)* 127:585-590.

Buriro M, Sanjrani AS, Chachar QI, Chachar NA, Chachar S.D, Buriro B, Mangan T (2015). Effect of water stress on growth and yield of sunflower. *Journal Of Agricultural Technology* 11(7):1547-1563.

Castello M, Stefanova K, Nichols PGH, Nutt BJ, Revell CK, Croser JS (2016). In vitro reproduction in the annual pasture legumes subterranean clover (*Trifolium subterraneum* L.) and French serradella (*Ornithopus sativus* Brot.). *Grass and Forage Science* 71(1):79-89.

Cechin I, Rossi SC, Oliveira VC, Fumis TDF (2006). Photosynthetic responses and proline content of mature and young leaves of sunflower plants under water deficit. *Photosynthetica* 44:143-146.

Çil A, Çil AN, Burun HM, Sevilmis U, Hatipoglu R (2021). Ayçiçeğinde olgunlaşmamış embriyo kültürünün generasyon atlatma amaçlı kullanımında uygun embriyo yaşının saptanması. *MAS Journal of Applied Sciences* 6(3):531-538.

Darvishzadeh R, Pirzad A, Bernousi I, Abdollahi Mandoulakani B, Azizi H, Akhondi N, Sarrafi A (2011). Genetic properties of drought tolerance indices in sunflower. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science* 61(7):593-601.

Germ M, Berčič OU, Ačko DK (2005). The response of sunflower to acute disturbance in water. *Acta Agriculturae Slovenica*, *85*:135-41.

Ghobadi M, Taherabadi S, Ghobadi ME, Mohammadi GR, Jalali-Honarmand S (2013). Antioxidant capacity, photosynthetic characteristics and water relations of sunflower (*Helianthus annuus* L.) cultivars in response to drought stress. *Industrial Crops and Products* 50:29-38.

Hasan K, Güneş A (2021). Aspir Genotiplerinde Çiçek Verimi ile Bazı Morfolojik Özellikler Arasındaki İlişkilerin Belirlenmesi. *Türk Doğa ve Fen Dergisi*, 10(1):90-95.

Howell TA, Evett SR, Tolk JA, Copeland KS, Marek TH (2015). Evapotranspiration, water productivity and crop coefficients for irrigated sunflower in the US Southern High Plains. *Agricultural Water Management* 162:33-46.

Hussain M, Farooq S, Hasan W, Ul-Allah S, Tanveer M, Farooq M, Nawaz A (2018). Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agricultural Water Management* 201:152-166.

Hussain S, Ahmad M, Ahmad S, Iqbal J, Subhani MN, Nadeem SM, Ibrahim M (2013). Improvement of drought tolerance in sunflower (*Helianthus annuus* L.) by foliar application of abscisic acid and potassium chloride. *Pakistan Journal of Nutrition* 12(4): 345.

Karam F, Lahoud R, Masaad R, Kabalan R, Breidi J, Chalita C, Rouphael Y (2007). Evapotranspiration, seed yield and water use efficiency of drip irrigated sunflower under full and deficit irrigation conditions. *Agricultural Water Management* 90(3):213-223.

Krizmanić M, Lioviċ I, Mijić A, Bilandžić M, Krizmanić G (2003). Genetic potential of OS sunflower hybrids in different agroecological conditions. *Sjemenarstvo*, 20(5-6):237-245.

Masalia RR, Temme AA, Torralba NDL, Burke JM (2018). Multiple genomic regions influence root morphology and seedling growth in cultivated sunflower (*Helianthus annuus* L.) under well-watered and water-limited conditions. *PLoS One*, 13(9):e0204279.

Pandey S, Singh A, Parida SK, Prasad M (2022). Combining speed breeding with traditional and genomics-assisted breeding for crop improvement. *Plant Breeding* 141(3):301-313.

Pekcan V, Evci G, Yilmaz MI, Nalcaiyi ASB, Erdal SÇ, Cicek N, Kaya Y (2015). Drought effects on yield traits of some sunflower inbred lines. *Poljoprivreda i Sumarstvo* 61(4):101.

Poormohammad Kiani S, Grieu P, Maury P, Hewezi T, Gentzbittel L, Sarrafi A (2007). Genetic variability for physiological traits under drought conditions and differential expression of water stress-associated genes in sunflower (*Helianthus annuus* L.). *Theoretical and Applied Genetics*, 114:193-207.

Prasad PVV, Staggenborg SA, Ristic Z (2008). Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Ahuja LR, Reddy VR, Saseendran SA, Qiang Yu (Eds), Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes, s. 301-355.

Raineri J, Ribichich KF, Chan RL (2015). The sunflower transcription factor HaWRKY76 confers drought and flood tolerance to Arabidopsis thaliana plants without yield penalty. *Plant Cell Reports* 34:2065-2080.

Rauf S (2008). Breeding sunflower (*Helianthus annuus* L.) for drought tolerance. *Communications in Biometry and Crop Science*, 3(1): 29-44.

Rauf S, Sadaqat HA (2007a). Sunflower (*Helinathus annuus* L.) germplasm evaluation for drought tolerance. *Communications in Biometry and Crop Science 2*(1):8-16.

Rauf S, Sadaqat HA (2007b). Effects of varied water regimes on root length, dry matter partitioning and endogenous plant growth regulators in sunflower (*Helianthus annuus* L.). *Journal of Plant Interactions* 2(1):41-51.

Reddy GKM, Dangi KS, Kumar SS, Reddy AV (2003). Effect of moisture stress on seed yield and quality in sunflower. *Journal of Oilseeds Research* 20:282-283.

Sala CA, Bulos M, Altieri E, Ramos ML (2012). Sunflower: improving crop productivity and abiotic stress tolerance. *Improving Crop Resistance To Abiotic Stress* 1203-1249.

Salehi-Lisar SY, Bakhshayeshan-Agdam H (2016). Drought stress in plants: causes, consequences, and tolerance. Hossain MA, Wani SH, Bhattacharjee S, Burritt DJ, Tran LP (Eds), Drought Stress Tolerance in Plants, s. 1-16.

Seiler GJ, Qi LL, Marek LF (2017). Utilization of sunflower crop wild relatives for cultivated sunflower improvement. *Crop Science*, 57(3):1083-1101.

Škorić D (2009). Sunflower breeding for resistance to abiotic stresses/mejoramiento de girasol por resistencia a estreses abióticos/sélection du tournesol pour la résistance aux stress abiotiques. *Helia*, 32(50):1-16.

Soleimanzadeh H (2012). Response of sunflower (Helianthus annuus L.) to selenium application under water stress. *World Applied Sciences Journal*, 17(9):1115-1119.

Tabrizi H, Şahin E, Haliloğlu K (2011). Çimlenme ve erken fide gelişim aşamasında bazı f1 ayçiçeği melezlerinin temel bileşenler analizi. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 42(2):103-109.

Turhan H, Baser I (2004). In vitro and in vivo water stress in sunflower (*Helianthus Annuus* L.)/Estrés Hídrico En Girasol (*Helianthus annuus* L.) En Las Condiciones in vitro E in vivo/stress D'eau Du Tournesol (*Helianthus annus* L.) Dans Les Conditions in vitro Et in vivo. *Helia*, 27(40):227-236.

Wasaya A, Abbas T, Yasir TA, Sarwar N, Aziz A, Javaid MM, Akram S (2021). Mitigating drought stress in sunflower (Helianthus annuus L.) through exogenous application of β-aminobutyric acid. *Journal of Soil Science and Plant Nutrition*, 21:936-948.

Watson A, Ghosh S, Williams MJ, Cuddy WS, Simmonds J, Rey MD, Hickey LT (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. *Nature Plants*, 4(1):23-29.

Wu Y, Wang Y, Shi H, Hu H, Yi L, Hou J (2022). Time-course transcriptome and WGCNA analysis revealed the drought response mechanism of two sunflower inbred lines. *Plos One*, 17(4):e0265447.

Yan W, Tinker NA (2006). Biplot analysis of multi-environment trial data: Principles and applications. *Canadian Journal of Plant Science*, 86(3):623-645.

Yao Y, Zhang P, Liu H, Lu Z, Yan G (2017). A fully in vitro protocol towards large scale production of recombinant inbred lines in wheat (*Triticum aestivum* L.). *Plant Cell, Tissue and Organ Culture (PCTOC)* 128:655-661.

Zheng Z, Wang HB, Chen GD, Yan GJ, Liu CJ (2013). A procedure allowing up to eight generations of wheat and nine generations of barley per annum. *Euphytica*, 191:311-316.