

Comparison of maize lines and their test crosses according to grain yield and some physiological properties

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Abstract: This study evaluated 7 inbred lines of maize (*Zea mays* L.), 3 testers, and 21 hybrids produced using the line × tester mating design. The study was conducted in Konya, in The Central Anatolia Region of Turkey. CD (coefficient of determination) values were calculated to detect the effect of inbred lines on grain yield and other physiological properties of the test crosses. The mean values of all the parameters of the genotypes were evaluated using the Duncan's multiple range test for grouping progenies and parent groups. The CD values between parent groups and progenies were statistically significant for photosynthetic efficiency ($r^2 = 0.11^{**}$; $P < 0.01$) and Mg content ($r^2 = 0.14^{**}$; $P < 0.01$). The CD value for grain yield of line 14.2 was 0.58; for chlorophyll content, anthocyanin content, and grain yield of line 14.20 CD values were 0.70, 0.25, and 0.55, respectively. Similarly, the CD values for total anthocyanin and Mg contents of line 14.21 were 0.36 and 0.48, respectively. The inbred line 14.20 was effective for the formation of chlorophyll, total anthocyanin content, and grain yield according to its high CD values, and progenies of the line were within the first group for other properties, according to the Duncan's multiple range test (14.20 × FRMo 17 [total anthocyanin content, grain yield], 14.20 × ADK 451 [chlorophyll content, grain yield]). A hybrid produced from an inbred line that has high CD values within the first group represents the genetic potential of the inbred line, supporting the use of CD values for parental selection methods in breeding studies.

Key words: Coefficient of correlation, coefficient of determination, maize breeding, physiological breeding

1. Introduction

Maize (*Zea mays* L.) is the most widely produced cereal and is the primary staple food in many developing countries. It is a versatile crop with high genetic diversity, which enables its cultivation in tropical, subtropical, and temperate climates around the world (Izhar and Ckahrabarty, 2013). The annual global production of maize is the highest of all grains (1 billion tons), followed by that of wheat (751 million tons), and rice (482 million tons). Maize production in Turkey was 6.4 million tons in 2016, which represents a 68% increase compared with its production in 2006 (TMO, 2017). Maize has a wider range of uses than any other cereal crop; it is used as human food, feed, and fodder, and for several industrial purposes. The wide utility of maize is due to its broad global distribution, low price compared with other cereals, diverse grain types, and wide range of biological and industrial properties.

Maize production has been increasing because its hybrids possess high yield potential (Assefa et al., 2017). Therefore, scientists have focused on breeding studies for maize. The choice of germplasm is an essential and crucial

step which decides the success or failure of a breeding program (Rodrigo et al., 2012). Germplasm selected for use as parents in a breeding program should be genetically diverse and exhibit the desired traits. Inbred lines are used for the development of hybrids, and their significance is determined by their performance in combination with other inbred lines (Aslam et al., 2017). Conventional breeding approaches are time-consuming and do not guarantee the desired results, so scientists often search for techniques to shorten the breeding duration. To achieve this goal, physiological traits are used, as they are easy to detect and provide reliable knowledge about the genotype (Sade and Özdemir, 2011). In previous years, the effects of inbred lines on the properties of test cross progeny were detected using multiple hybridization combinations. However, it was difficult to use this method with a large number of inbred lines. Therefore, scientists focused on developing easier and more practical methods for parental selection (Çeçen et al., 1998). In this study, we aimed to determine the effect of inbred lines on the grain yield and physiological characteristics of hybrids using statistical methods, including CC (correlation coefficient) and CD.

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2. Materials and methods

This study was conducted at the Selçuk University Agriculture Faculty Crop Science Department, Prof. Dr. Abdulkadir Akcın trial area during the 2016 growing season. Seven inbred lines of dent corn (*Zea mays indentata* Sturt.), 3 testers (FRMo 17, FRB 73, and ADK 451), and 21 hybrids resulting from crosses between inbred lines and testers were used (Table 1).

Maize accessions were grown in a randomized complete block design with 3 replications. Seeds of each genotype were sown by hand in the second week of May with a spacing of 70 × 20 cm. Each parcel included two 5-m long rows.

The photosynthetic efficiency (Fv/Fm), chlorophyll content (spad), stomatal conductance (mmol/m²s), total antioxidant content (%), total anthocyanin content (mg/100 g), and Mg content (%) of 5 plants per row were measured in each plot during tasseling. Maize leaves were enclosed in the dark for 30 min, and Fv/Fm values were recorded using a plant efficiency analyzer (Özdemir,

2012). Chlorophyll content (spad) was measured using a SPAD meter (Spad-502) (Seflek, 2010) and stomatal conductance (mmol/m²s) using a porometer (Özdemir, 2012). Mean values were calculated for each trait. The total antioxidant content (%) of plant samples was measured using the DPPH radical scavenging activity of phenolic compounds by measuring their capacity for bleaching a black-colored methanol solution containing DPPH radicals using spectrophotometry as previously described by Khampas et al. (2013). The total anthocyanin (mg/100 g) content in the plant samples was determined using the spectrophotometric method previously described by Leticia et al. (2009). To determine the Mg content, 0.5 g of dried and ground plant samples were treated with 15 mL of pure NH₃. The samples were then incinerated in a MARS 5 microwave oven at 200 °C. Distilled deionized water and ultrahigh-purity commercial acids were used to prepare all the reagents, standards, and samples. After digestion, the samples were filtered through Whatman filter paper No. 42, collected in 50-mL flasks, and analyzed using ICP-AES. The Mg content of samples was determined against that of standard solutions with known Mg concentrations; samples and standard solutions were simultaneously analyzed (Hamurcu et al., 2010). The grain yield (kg/ha) of each genotype was determined at a moisture content of 15%. SPSS version 20.0 was used for analyzing all the statistical data. Statistical analysis was performed using ANOVA for randomized complete block design. The data of lines, testers, and hybrids were separately analyzed. The mean value of each parameter for each genotype was grouped (first group and last group) according to the Duncan's multiple range test to determine the relative positions of lines, testers, and hybrids among all the genotypes. The CD, which is defined as the percentage of variation in 1 variable explained by another variable (Nargelkerke, 1991), was calculated according to Çeçen et al. (1998). Squares of CCs among genotypes for each trait were recorded as CD values. The CC values among genotypes were presented for each property to show the genetic basis of the population as well. Inbred lines with *r* > 0.50 were evaluated further.

3. Results and discussion

Results of variance analysis for all parameters are summarized in Table 2. For each parameter, the variations among genotypes were statistically significant (*P* < 0.01), suggesting a remarkable variation that promotes the investigation of the genotypes.

Significant differences were detected among test cross progenies for all the parameters (*P* < 0.01), except grain yield (Table 3). These differences probably resulted from heterosis and heterobeltiosis (Patil et al., 2012). Heterosis or hybrid vigor is defined as the progeny being

Table 1. Maize inbred lines and test crosses used in this study.

Lines	Test crosses
3.2	3.2 × FRMo 17 *
	3.2 × FRB 73 *
	3.2 × ADK 451 **
3.4	3.4 × FRMo 17
	3.4 × FRB 73
	3.4 × ADK 451
3.6	3.6 × FRMo 17
	3.6 × FRB 73
	3.6 × ADK 451
14.2	14.2 × FRMo 17
	14.2 × FRB 73
	14.2 × ADK 451
14.20	14.20 × FRMo 17
	14.20 × FRB 73
	14.20 × ADK 451
14.21	14.21 × FRMo 17
	14.21 × FRB 73
	14.21 × ADK 451
14.26	14.26 × FRMo 17
	14.26 × FRB 73
	14.26 × ADK 451

* Origin: USA.

** Origin: Turkey.

superior to both of the homozygotic parents in terms of multiple phenotypes. Many studies on heterosis have been conducted, but the concept remains unclear. Nevertheless, breeders continue to employ heterosis in breeding programs (Iqbal et al., 2010).

Significant differences were detected among the inbred lines for stomatal conductance ($P < 0.05$) and Mg content ($P < 0.01$); however, no significant differences were detected among testers for any parameter (Table 3).

The correlation analysis revealed a statistically significant correlation between inbred lines and test cross progenies for photosynthetic efficiency ($r = 0.33$; $P < 0.01$) and Mg content ($r = 0.37$; $P < 0.01$) (Table 4). Based on the derived CD values, inbred lines showed 11% and 14% of variation in test crosses for photosynthetic efficiency ($r^2 = 0.11$) and Mg content ($r^2 = 0.14$), respectively (Table 4). The CC values were not significant for any other physiological parameters: chlorophyll content ($r = -0.01$), stomatal

conductance ($r = -0.07$), total antioxidant content ($r = 0.11$), total anthocyanin content ($r = -0.05$), and grain yield (0.18) (Table 4).

Fv/Fm values are an indicator of the quantum yield of photosystem II (Lepedus et al., 2012). The measurement of chlorophyll fluorescence is a cost-effective, practical, and popular technique in plant physiology. Photosynthetic efficiency is linked with stress tolerance and crop yield (Adams and Demmig-Adams, 2004).

Plants with high photosynthetic efficiency are high-yielding (Hokmalipour and Darbandi, 2011; Lepedus et al., 2012), because high photosynthetic efficiency results in more carbon fixation and greater accumulation of carbohydrates.

Mg is an important nutrient for plants; approximately 75% of leaf Mg is involved in protein synthesis, and 15%–20% of total Mg is associated with chlorophyll pigments, acting mainly as a cofactor for a series of enzymes involved

Table 2. Analysis of variance for all investigated parameters.

	Photosynthetic efficiency (Fv/Fm)	Chlorophyll content (spad)	Stomatal conductance (mmol/m ² s)	Total antioxidant content (%)	Total anthocyanin content (mg/100 g)	Mg (%)	Grain yield (kg/ha)
Mean squares	0.000962	37.994	874.222	43.921	607.337	0.002	351406.061
F values	41.010 **	2.059 **	12.751 **	3.747 **	5.318 **	4.231 **	9.153 **
CV	0.59	7.82	15.81	4.58	13.84	9.02	20.94

** $P < 0.01$.

Table 3. Analysis of variance for all determined parameters at hybrids, lines, and testers.

Variation source	DF	Photosynthetic efficiency	Chlorophyll content	Stomatal conductance	Total antioxidant content	Total anthocyanin content	Mg	Grain yield
Hybrids	20	3.654 **	2.234 *	11.367 **	3.983 **	5.585 **	3.139 **	1.250
Lines	6	1.784	1.281	2.644 *	1.744	0.792	5.626 **	1.089
Testers	2	0.223	0.115	1.037	0.816	1.285	0.560	0.704

* $P < 0.05$.

** $P < 0.01$.

Table 4. CC and CD values between lines and testcrosses for all observed parameters.

	Photosynthetic efficiency	Chlorophyll content	Stomatal conductance	Total antioxidant content	Total anthocyanin content	Mg	Grain yield
r	0.33 **	-0.01	-0.07	0.11	-0.05	0.37 **	0.18
r ²	0.11	-	-	0.01	-	0.14	0.03

** $P < 0.01$.

in carbon fixation and metabolism (Guo et al., 2016). Mg also plays an important role in sucrose loading of phloem. Mg deficiency limits plant growth, resulting in short roots and shoots, and causes necrotic spots on leaves due to impaired carbon metabolism and fixation (Marschner, 2012).

The correlation between inbred lines and their test cross progenies was investigated. CC and CD values of inbred lines and test crosses for all parameters are summarized in Table 5.

The correlations between inbred line 14.20 and its test crosses (14.20 × FRMo 17, 14.20 × FRB 73, and 14.20 × ADK 451) were investigated for each parameter (Table 5).

The CC value of the inbred line 14.20 and its test crosses was 0.83; the CD value was 0.70 for chlorophyll content. Based on the CD value, inbred line 14.20 showed 70% variation in test crosses for chlorophyll content (Table 5). Cross 14.20 × ADK 451 was included in the first group according to the Duncan’s multiple range test for chlorophyll content (Table 6). These data suggested that inbred line 14.20 is potentially useful genetic material for studies based on yield and physiology because of its positive and strong correlation for chlorophyll content.

Similar results were obtained for total anthocyanin content; inbred line 14.20 showed 25% variation in test crosses for total anthocyanin content ($r = 0.50$; $r^2 = 0.25$) (Table 5). Cross 14.20 × FRMo 17 was included in the first group according to the Duncan’s multiple range test (Table 7).

Inbred line 14.21 also showed significant values of CC and CD for total anthocyanin and Mg contents. It showed 36% and 48% of variation in total anthocyanin content ($r = 0.60$; $r^2 = 0.36$) and Mg content ($r = 0.69$; $r^2 = 0.48$), respectively, among the test cross progenies (14.21 × FRMo 17, 14.21 × FRB 73, and 14.21 × ADK 451). Cross 14.21 × ADK 451 was included in the first group according to the

Duncan’s multiple range test for total anthocyanin content (Tables 5 and 7).

Anthocyanins are the largest and most important group of water-soluble pigments with antioxidant, antimutagenic, and chemoprotective properties, which lower the incidence of chronic diseases. Interest in anthocyanins has increased, as they can potentially be used as natural alternatives to synthetic food colorants because of their color characteristics and health benefits (Jing, 2006).

Inbred line 14.21 showed high genomic efficiency for anthocyanin content. This feature of the inbred line has garnered the attention of breeders who focus on phenolic metabolism and other usage areas of this property.

Inbred lines were investigated according to grain yield. CC values of inbred lines 3.2, 3.4, 14.2, and 14.20 were lower than 0.50 for grain yield, with CD values of 0.26, 0.27, 0.58, and 0.52, respectively (Table 5). Çeçen et al. (1998) reported CD values ranging from 0.13 to 0.66 for yield and yield components of maize, which was consistent with this study. Among these lines, inbred line 14.20 was attractive because its CC value was lower than 0.50 for both chlorophyll content and grain yield. Inbred line 14.20 showed 52% and 70% of variation in test crosses for grain yield and chlorophyll content, respectively (Table 5). Guendouz and Maamari (2012) reported that an increase in biomass and photosynthetic efficiency is a major objective for improving the yield potential of maize germplasm.

The mean values of each parameter for different genotypes are listed in Tables 6 and 7. Features for each genotype included in the first group according to the Duncan’s multiple range test are summarized in Table 8.

Test crosses that were in the first group for other parameters according to the Duncan’s multiple range test were also included in the first group for grain yield

Table 5. CC and CD values of each line at all investigated parameters.

Parameters	Photosynthetic efficiency		Chlorophyll content		Stomatal conductance		Total antioxidant content		Total anthocyanin content		Mg		Grain yield	
	r	r ²	r	r ²	r	r ²	r	r ²	r	r ²	r	r ²	r	r ²
3.2	-0.07	-	0.44	0.20	0.23	0.05	-0.16	0.02	-	-	-0.57 *	0.33	0.51 *	0.26
3.4	-0.57 *	0.32	-0.04	-	0.12	0.01	-0.46	0.21	-	-	0.17	0.03	0.52 *	0.27
3.6	0.19	0.03	-0.36	0.13	-0.32	0.10	0.39	0.24	0.09	-	-0.31	0.10	0.40	0.16
14.2	0.42	0.18	0.16	0.02	-0.08	-	0.27	0.07	-0.28	0.08	0.39	0.15	0.76 *	0.58
14.20	0.13	0.01	0.83 *	0.70	0.21	0.04	-0.10	0.01	0.50 *	0.25	0.22	0.05	0.72 *	0.52
14.21	0.27	0.07	-0.14	0.02	0.04	-	0.06	-	0.60 *	0.36	0.69 *	0.48	0.13	0.02
14.26	-0.45	0.20	-0.01	-	-0.45	0.20	0.02	-	-0.32	0.10	0.01	-	0.19	0.03

* $r > 0.50$.

Table 6. Mean values of each genotype for photosynthetic efficiency, chlorophyll content, stomatal conductance, and total antioxidant content features.

Genotypes	Photosynthetic efficiency (Fv/Fm)		Chlorophyll content (spad)		Stomatal conductance (mmol/m ² s)		Total antioxidant content (%)	
3.2 × T1	0.832	bcd	56.00	b-h **	37.33	j-m **	80.34	abc *
3.2 × T2	0.833	bc	55.33	b-h **	76.52	Bc	75.07	b-j
3.2 × T3	0.835	b	55.00	b-h **	47.50	f-k	75.37	b-j
3.4 × T1	0.827	cde	58.00	a-e *	80.44	abc *	74.98	c-j
3.4 × T2	0.832	bcd	53.67	c-h **	67.21	cde	73.39	d-j
3.4 × T3	0.827	cde	58.67	a-d *	46.70	f-k	77.36	a-f *
3.6 × T1	0.832	bcd	50.33	fgh	35.60	klm	71.10	ijk
3.6 × T2	0.825	de	58.67	a-d *	69.35	bcd	71.40	g-k
3.6 × T3	0.826	cde	49.00	h **	51.59	f-i	81.03	a *
14.2 × T1	0.826	cde	62.00	ab *	82.11	ab *	71.90	f-k
14.2 × T2	0.823	E	56.33	a-g *	92.15	a *	66.93	kl **
14.2 × T3	0.831	b-e	60.00	abc *	77.75	bc	75.47	a-j *
14.20 × T1	0.827	cde	54.00	c-h **	60.12	def	72.79	e-j
14.20 × T2	0.837	ab *	51.67	d-h **	36.21	j-m **	73.29	d-j
14.20 × T3	0.844	a *	56.67	a-f *	47.96	f-k	71.60	g-k
14.21 × T1	0.838	ab *	58.33	a-e *	59.82	d-g	64.85	l **
14.21 × T2	0.831	b-e	63.00	a *	59.58	d-g	74.08	d-j
14.21 × T3	0.826	cde	54.67	c-h **	52.04	f-i	71.30	h-k
14.26 × T1	0.838	ab *	50.33	fgh **	53.81	e-h	77.66	a-e *
14.26 × T2	0.833	bc	57.00	a-f *	46.33	g-k	78.35	a-e *
14.26 × T3	0.836	ab *	58.00	a-e *	52.90	fgh	75.77	a-i *
3.2 (1)	0.827	cde	50.67	fgh	37.40	j-m	75.32	a-g *
3.4 (1)	0.807	F	52.33	d-h **	49.23	f-j	75.47	a-j *
3.6 (1)	0.770	i **	53.33	c-h **	36.84	j-m **	78.65	a-d *
14.2 (1)	0.781	h **	49.33	gh **	28.47	lm **	80.14	abc *
14.20 (1)	0.783	h **	51.33	e-h **	25.07	m **	75.87	a-i *
14.21 (1)	0.793	G	54.67	c-h **	28.05	lm **	76.76	a-h *
14.26 (1)	0.826	cde	53.33	c-h **	42.51	h-k	80.64	ab *
FRMo 17 (2)	0.824	de	53.33	c-h **	48.29	f-k	69.91	jkl **
FRB 73 (2)	0.833	bc	55.33	b-h **	38.62	i-l	72.99	e-j
ADK 451 (2)	0.837	ab *	53.33	c-h **	56.18	d-g	74.38	d-j

(1) Lines.

(2) Testers.

* First group.

** Last group.

*** Significant variations were not observed among mean values statistically, which belong to the same group (* first group, ** last group) at each parameter.

(14.20 × FRMo 17 [12161.7 kg/ha; a-d], 14.26 × FRMo 17 [12605.9 kg/ha; a-d], 3.6 × ADK 451 [12666.7 kg/ha; abc], 14.20 × ADK 451 [13056.2 kg/ha; abc], 14.21 × FRMo 17 [13924.3 kg/ha; ab], and 14.26 × ADK 451 [14119.4 kg/ha; a]) (Tables 7 and 8).

In addition to grain yield, test crosses 14.20 × ADK 451, 14.21 × FRMo 17, and 14.26 × ADK 451 were included in the first group for both photosynthetic efficiency and chlorophyll content (Table 8). These data suggested that photosynthetic efficiency, chlorophyll content, and grain

Table 7. Mean values of each genotype for total anthocyanin, Mg content, and grain yield properties.

Genotypes	Total anthocyanin content (mg/100 g)		Mg (%)		Grain yield (kg/ha)	
	Value	Group	Value	Group	Value	Group
3.2 × T1	82.85	c-h	0.243	f-j	10312.4	cde
3.2 × T2	74.93	d-l **	0.278	a-f *	11784.3	a-d
3.2 × T3	68.89	f-l **	0.249	e-j	9448.3	def
3.4 × T1	94.80	abc *	0.227	ijk **	11201.4	a-e
3.4 × T2	61.23	jkl **	0.227	ijk **	11415.7	a-e
3.4 × T3	77.27	d-k	0.216	jk **	9871.4	cde
3.6 × T1	78.88	c-i	0.245	e-j	11568.5	a-d
3.6 × T2	60.14	kl **	0.267	b-h	10267.3	cde
3.6 × T3	101.84	ab *	0.257	c-i	12666.7	abc *
14.2 × T1	86.60	b-e	0.291	abc *	10772.7	b-e
14.2 × T2	67.40	g-l **	0.258	c-i	11349.9	a-e
14.2 × T3	62.84	i-l **	0.234	Hj	10378.4	cde
14.20 × T1	94.78	abc *	0.254	c-i	12161.7	a-d *
14.20 × T2	77.93	c-j	0.267	b-h	10693.6	cde
14.20 × T3	63.52	i-l **	0.266	b-h	13056.2	abc *
14.21 × T1	75.42	d-k	0.254	c-i	13924.3	ab *
14.21 × T2	89.15	a-d	0.235	g-j	11300.0	a-e
14.21 × T3	102.33	ab *	0.253	d-i	11737.5	a-d
14.26 × T1	104.14	a *	0.287	a-d *	12605.9	a-d *
14.26 × T2	101.68	ab *	0.299	ab *	10357.5	cde
14.26 × T3	75.53	d-k	0.296	ab *	14119.4	a *
3.2 (1)	86.16	b-f	0.222	ijk **	6440.1	fgh
3.4 (1)	83.75	c-g	0.224	ijk **	4644.1	hi
3.6 (1)	61.35	jkl **	0.272	a-g *	2753.5	i **
14.2 (1)	60.30	kl **	0.195	k **	3080.0	i **
14.20 (1)	62.35	i-l **	0.226	ijk **	4595.8	hi **
14.21 (1)	57.52	l **	0.237	g-j	3201.4	i **
14.26 (1)	73.03	d-l **	0.308	a *	4495.9	hi **
FRMo 17 (2)	70.33	e-l **	0.253	d-j	6469.1	fgh
FRB 73 (2)	70.39	e-l **	0.279	a-f *	8281.0	efg
ADK 451 (2)	65.78	h-l **	0.282	a-e *	5149.2	ghi

(1) Lines.

(2) Testers.

* First group.

** Last group.

*** Significant variations were not observed among mean values statistically, which belong to the same group (* first group, ** last group) at each parameter.

Table 8. Features of each genotype in the first group according to Duncan's multiple range test.

Genotype	Parameter
3.4 × T1	Chlorophyll content, stomatal conductance, total anthocyanin content
3.4 × T3	Chlorophyll content, total antioxidant content
3.6 × T3	Total antioxidant content, total anthocyanin content, grain yield
14.2 × T1	Chlorophyll content, stomatal conductance, Mg content
14.2 × T2	Chlorophyll content, stomatal conductance
14.2 × T3	Chlorophyll content, total antioxidant content,
14.20 × T1	Total anthocyanin content, grain yield
14.20 × T3	Photosynthetic efficiency, chlorophyll content, grain yield
14.21 × T1	Photosynthetic efficiency, chlorophyll content, grain yield
14.26 × T1	Photosynthetic efficiency, total antioxidant content, total anthocyanin content, Mg content, grain yield
14.26 × T2	Chlorophyll content, total antioxidant content, Mg content
14.26 × T3	Photosynthetic efficiency, chlorophyll content, total antioxidant content, Mg content, grain yield
3.6	Total antioxidant content, Mg content
14.26	Total antioxidant content, Mg content
ADK 451	Photosynthetic efficiency, chlorophyll content, Mg content

* Genotypes were considered to be in the first group with a minimum at 2 parameters.

yield were correlated. Lepedus et al. (2012) reported that high photosynthetic efficiency results in higher grain yield. This determination supported the fact that physiological elements were effective at increasing grain yield in these genotypes.

Additionally, crosses 3.6 × ADK 451, 14.26 × FRMo 17, and 14.26 × ADK 451 were included in the first group for grain yield, total antioxidant content, and total anthocyanin content (Table 8).

In this study, we identified inbred maize lines with high rates of formation of some features at crosses. Some of the combinations of these lines were in the first group according

to the Duncan's multiple range test for several parameters. These data suggested that CC and CD values can be used as statistical tools for the selection of germplasm to be used as parental lines in breeding programs. The results of this study highlighted the conformity between grain yield and physiological parameters of some genotypes; these genotypes comprise ideal genetic material for use in physiology-based studies.

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