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Genetic Analysis of Yield and Physiological Traits in Sunflower (*Helianthus annuus* L.) under Irrigation and Drought Stress

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Abstract

Implementing appropriate breeding strategies for sunflower, alongside dependable information on heritability and gene effects upon yield and related traits under drought conditions, are all necessary. Thirty sunflower hybrids were produced by line × tester cross of six male-sterile and five restorer lines. Their hybrids were evaluated in three levels of irrigation, as follows: (1) non-stressed plots, irrigated at regular intervals (W_1); (2) mild water stress (W_2), irrigated from the beginning of the button stage (R_4) to seed filling initiation (R_6); (3) severe water stress (W_3) started from the beginning of button stage (R_4) to physiological maturity. Based on observations and specific methods for determination, canopy temperatures, chlorophyll index, relative water content and proline content, were studied by additive effects, under the different irrigation conditions. Canopy temperatures, chlorophyll index, relative water content, leaf water potential, proline content and yield were controlled by additive effects under mild stressed conditions. Under severe stress conditions however, canopy temperatures, leaf water potential and proline content were controlled by additive effects, while chlorophyll index and relative water content were controlled by both additive and dominant effects, as seed yield was mainly influenced by the dominant effects. The narrow sense heritability ranged from 47-97% for all traits, except for chlorophyll fluorescence. Yield correlated positively with chlorophyll index and relative water content, and negatively with canopy temperature and leaf water potential. Therefore, under drought stressed conditions in breeding programs, canopy temperatures, chlorophyll index and relative water content can be reliable criteria for the selection of tolerant genotypes with prospect to higher yields.

Keywords: gene action, general combining ability, heritability, line × tester analysis, water deficit

Introduction

Drought presents serious limitations to crop growth and productivity. Water stress decreases plant growth and productivity by slowing the rate of cell division and expansion, mainly due to loss of turgor and afterwards the effect of reduction of the water status components of the plant cells (Tezara *et al.,* 2002).

Plant breeding approaches however, are looking for ways to hinder the effects of drought-driven problems. The selection of suitable genitors, with a good general combining ability, for example, is one of the most important steps in a hybridization program.

Identification of the gene(s) responsible for the desired characteristics, like of drought resistance at different stages of plant growth and development, is of great importance (Dhanda *et al.,* 2002).

General combining ability is an average performance of a line in a particular series of crosses, in searching for heterotic performance (Kadkol *et al.,* 1984). From combining ability of parental lines, thus, we can project the ultimate usefulness of the lines via estimating additive gene actions (Sprague and Tatum, 1942). Also, the selection of parents for hybridization in order to utilize transgressive segregation, for the manifestation of heterosis phenomenon, is a crucial step (Kiani *et al.,* 2007).

Breeding programs can take advantage of such information on combining ability to find better selection strategy for developing high yielding lines and hybrids (Skoric, 1992). Also breeding programs and environmental conditions including drought stress can influence the evaluation of combining ability of sunflower genotypes (Petakov, 1996). To develop cultivars yielding better under drought stress, breeder needs information about the gene action of the traits related to yield and quality responsible for drought tolerance (Eshghi *et al.,* 2010).

Because direct selection based on agronomic traits in sunflower were shown to be complicated by low heritability of yield, indirect selection via yield components and other traits could be more efficient if these traits are related to yield and have a higher heritability than yield (Blum, 1988). An ideal secondary trait should be simple to measure, highly heritable, genetically correlated with grain yield under stress and identify genetic variation in the target species (Lafitte *et al.,* 2003).

Seed yield and canopy temperature have been reported to be involved in sunflower performance under drought conditions (Alza and Fernandez Martinez, 1997). In general, breeding for drought tolerance involves combining potential yield well in the absence of stress, and the selection of highly heritable traits that provide drought stress tolerance (Jones, 2007).

In sunflower, a number of studies regarding gene action of agronomic traits (Bajaj *et al.,* 1997; Hussain *et al.,* 1998; Radhika *et al.,* 1999; Ghaffari *et al.,* 2012) are reported; however, only a few studies are conducted under drought stress. Leaf hydraulics, such as relative water content (RWC) and leaf water potential (LWP) are important plant traits for discriminating drought tolerant and sensitive genotypes (Rauf and Sadaqat, 2008).

Photosynthesis capacity of sunflower is hindered subsequent to RWC and LWP reduction (Tezara *et al.,* 2002). Also, duration of canopy temperature seemed to be related to sunflower yield (Prietolosada, 1992). Moreover, Chlorophyll fluorescence parameter has been used to investigate the response of sunflower to water stress (Maury *et al.,* 1996). In sunflower, proline has been an important osmolyte under drought stress. Its content tended to increase in young leaves in response to drought stress (Cechin *et al.,* 2006).

The aforementioned studies highlighted some important physiological traits influencing final yield. However, breeding approaches promise a light future for finding lines that withstand drought stress. Until now, researchers have found that additive gene effects has more important roles in some traits such as relative water content and leaf water potential (Rauf *et al.,* 2009), but they only considered one level of stress.

Others observed that, in normal condition, non-additive genes affect dominantly including seed yield (Singh *et al.,* 1989; Bajaj *et al.,* 1997; Hussain *et al.,* 1998; Radhika *et al.,* 1999; Ghaffari *et al.,* 2012).

In present research, we extended the number of influential traits as well as developing two distinct stress levels in order to investigate gene actions in affected lines. We conducted line x tester analysis, involving 6 cytoplasmic male sterile lines and 5 fertility restorer lines, aimed to estimate the type of genetic variability and select parents on the basis of their combining ability under normal and drought conditions. Here, we succeeded to evaluate sunflower lines and testers for general combining ability and investigate gene actions in lines under different levels of irrigation and determine GCA of different female and male inbreed lines and finally find the best testers for testing F_1 hybrid combinations, for Physiological traits correlated with seed yield and high heritability.

Material and method

Plant material and water stress treatments

30 F_1 hybrids developed by crossing 6 cytoplasmic male sterile (cms), female lines (B110, B147, B221, B329, B343 and B355) to five male restorer lines (R19, R26, R46, R50 and R56) were used in this study. These hybrids were made from 2010 plantings in the Khoy Field Station, Khoy, Iran and evaluated in line × tester fashion. The 30 hybrids obtained in 2010 were planted at three levels of irrigation in the University of Tabriz Agricultural Research Fields-Iran in May 2011, 2012. The research station is situated at the altitude of 1360 meters and 46°, 17′longitude and 38°, 5′ latitude. In the spring and summer cultivation average precipitation was 184 mm and average temperature was 12 °C.

Field experiment

The seeds of F_1 generation were planted in the sunflower research area under irrigated and drought stress conditions in a sandy loam soil. Treatments were allocated in split plot fashion based on randomized complete block design with three replications. Each genotype was sown in one individual row of 3 m length and distance between rows of 60cm and the seeds were planted at 30 cm intervals. Two seeds of sunflower were planted in each hole by hand and thinned to single plant at seedling stage. Different water levels were developed by irrigating the non-stressed plots at regular intervals (W_1) while two levels of drought were developed by mild water stress, from the beginning of button stage (R_4) (Schneiter and Miller, 1981) to start seed filling (R_6), (W_2) and applying a severe water stress which started from the beginning of button stage (R_4) to physiological maturity (W_3). Weeds were controlled manually, diseases were considered absent.

Measured Traits

Infrared canopy temperatures were taken at midday, in both water stressed and irrigated experiments with a hand-held infrared thermometer (Teletemp AG-42) when sunflower was approximately 50% in bloom. For chlorophyll fluorescence, we used florometer (model: Opti Science, OS-30, USA). Chlorophyll index was determined by Chlorophyll meter (model: SPAD-502, Minolta, Japan). For Relative water content, the method of Morant-Manceau *et al.* (2004) was used. First, the Fresh Weight (FW) of samples was measured. Then we put the samples in distilled water for 24 hours, then the Turgid Wight (TW) was measured and after putting samples in 75 °C Aven the Dry Weight (DW) was measured. Finally, the leaf relative water content measured as percent by this theorem:

$$RWC = FW\text{-}DW/TW\text{-}DW*100$$

Leaf water potential was measured by Pressure Chamber; model: Soil Moisture Equipment Crop, Sanata Barbara, CA.

Free proline contents were measured according to the method of Bates *et al.* (1973). Fresh leaves (0.2 g) were homogenized in 5 ml of 3% aqueous sulfosalicyclic acid and the residue was removed by centrifugation. Then, 1.0 ml of the extract was blended with 1.0 ml acid-ninhydrin and 1.0 ml of glacial acetic acid in a test tube. Then the blend was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 2.0 ml toluene, let cool to room temperature, and the absorbance was measured at 520 nm with a spectrometer (WPA model S2100). For yield, five randomly selected plants were taken from each plot to measure grain yield.

Statistical analysis

Statistical analysis was performed by MSTAT-C ver. 1.42 and SPSS ver. 17 software. Data for hybrids subjected to Line × Tester analysis (Singh and Chaudhury, 1995) to estimate general combining ability (GCA). The method described by Kempthorne (1957) was adopted for combining ability analyses.

Estimation of GCA effects

$$GCA(L) = \frac{X_i}{tr} - \frac{X}{ltr}$$

$$GCA(T) = \frac{X_j}{lr} - \frac{X}{ltr}$$

where l = number of CMS lines (female parent)
t = number of testers (male parent)
r = number of replications
Xi = Total of the F1 resulting from crossing ith lines with all the testers
Xj = Total of all crosses of jth tester with all the lines
X = Total of all crosses

Results and discussion

Analysis of variance showed there were significant ($p<0.01$, $p<0.05$) differences between hybrids for all traits except for chlorophyll fluorescence and proline content. Interactions such as line × tester (L × T) were significant ($p<0.01$, $p<0.05$) for canopy temperature and Relative water content. Interaction between line × water levels (L × W) was significant for relative water content, leaf water potential and proline. Interaction between tester × water levels (T × W) was significant ($p < 0.01$) for relative water content. However, line × tester × water levels (L × T × W) were not significant for all traits under study (Tab. 1).

The broad sense heritability ranged from 51-98% for all traits, except for chlorophyll fluorescence, implying the involvement of slightly greater genotypic variation than environmental variations in the phenotypic expression of traits. For narrow sense heritability the values ranged from 0.47 to 0.97. Here, the values of narrow sense heritability are due to a greater additive proportion of genes than non-additive. Estimates for heritability of canopy temperature were high (Tab. 1).

Since different levels of irrigation in all traits except for chlorophyll fluorescence, line and tester effect for all trait, interaction between line × water levels and tester × water levels for number of traits were significant, we performed a further line × tester analysis for different irrigation levels (Tab. 2). Some observations surprisingly differed between two Tabs 2.1. and 2.2. As we know significant line or tester effects indicate the importance of additive effects and if line × tester effect is significant it indicates that non-additive effects control the concerned traits (Singh and Chaudhary, 1995).

Here, in normal irrigation level (w_1) line and tester are significant ($p<0.01$). As a result, traits canopy temperatures, chlorophyll index, relative water content, proline content were under control of additive effect. In mild water- stressed level (w_2), line and tester are also significant ($p<0.01$), therefore, canopy temperatures, chlorophyll index, relative water content, leaf water potential, proline content and yield were controlled by additive effects.

In severe stress condition (w_3), however, line and tester effects were significant ($p<0.01$) for canopy temperatures, leaf water potential and proline content, so they were under control of additive effects. In this condition, line, tester and line × tester effects were significant for chlorophyll index, relative water content, so they were under control of both additive and dominant effects. For yield, however, line and tester were not significant; therefore, it was controlled by dominant effects (Tab. 2).

One important note from Tab. 2 is that under w_3, chlorophyll index, relative water content, in addition to additive effect, were controlled by dominant gene effect, while yield was under control of dominant gene effect. Combining ability analysis helps evaluate inbreeds in terms

Tab. 1. Analysis of variance in sunflower for canopy temperature (T), chlorophyll fluorescence (Fvm), chlorophyll content (Ch), relative water content (RWC), leaf water potential (LWP), proline content (P) and yield (Y)

Source of variation	df	T (°C)	Fvm	Ch	RWC(%)	LWP (MPa)	P (Mgr/gr)	Y(gr)
Repeat(R)	2	66.527^{ns}	0.092^{ns}	104.15^{ns}	243.63^{ns}	0.115^{ns}	97.32^{ns}	1453.1^{ns}
Water level (W)	2	335.25**	0.022^{ns}	1048.29*	2244.72**	323.55**	2912.2**	7541.02*
R × W	4	11.67	0.054	61.85	54.68	5.9	113.51	587.32
Cross	29	87.94**	0.019^{ns}	43.99**	50.44**	18.83**	57.22^{ns}	215.14**
Line(L)	5	24.13**	0.033*	76.03**	573.01**	36.1**	577.78**	546.17**
Tester(T)	4	583.86**	0.004^{ns}	175.65**	289.7**	26.67**	169.14**	144.48^{ns}
L × T	20	4.71*	0.014^{ns}	9.806**	53.66**	8.68^{ns}	52.52^{ns}	112.19^{ns}
L × W	10	10.97**	0.006^{ns}	3.148^{ns}	86.55*	16.791**	88.54*	64.47^{ns}
T × W	8	20.26**	0.007^{ns}	13.208**	77.7^{ns}	7.962^{ns}	63.37^{ns}	78.43^{ns}
L × T × W	40	1.85^{ns}	0.014^{ns}	5.271^{ns}	50.25^{ns}	7.37^{ns}	34.35^{ns}	71.638^{ns}
Erorr	175	2.63	0.012	4.387	42.34	6.391	42.57	94.25
h_b^2		0.983	0.194	0.905	0.521	0.587	0.753	0.511
h_n^2		0.973	0.149	0.867	0.477	0.539	0.732	0.487

ns - not significant, * is significant at 0.05, ** significant at 0.01 probability level of their genetic value and select suitable parents for hybridization (Singh and Chaudhary, 1995). Based on the combining ability analysis for 6traits, (Tab. 3), in normal condition irrigated lines B343 and R19, B221and R26, R19, B147, B147 and R50 and B147 had significant positive GCA for canopy temperatures, chlorophyll index, relative water content, leaf water potential, proline content and yield, respectively. In the mild stress condition, B147 and R19, B221 and R26, B221, R50 had significant GCA for canopy temperatures, chlorophyll index, leaf water potential, proline content respectively (Tabs. 3, 4).

In the severe stress condition, lines B147 and R19, B221 and R46, R26, B343 had significant GCA for canopy temperatures, chlorophyll index, relative water content, leaf water potential respectively (Tabs. 3, 4). This implies that these lines possess favorable alleles with additive effects develop these traits. This could be due to high variability in experimental conditions or similarities of genetic sources used for development of these lines. Correlations between traits were estimated and are given in Tab. 5. Correlations between canopy temperatures, chlorophyll index, relative water content and leaf water potential were significant for yield. The direction of correlation was positive for relative water content and yield. Therefore, selection for one of these traits should be accompanied by the associated traits, and this would provide the opportunity to exert multi-traits selection in sunflower breeding programs.

For the purpose of crop production, yield betterment and yield stability under water stressed conditions, the development of drought tolerant varieties is the best

Tab. 2.1. Analysis of variance of line × tester in sunflower for canopy temperature (T), chlorophyll fluorescence (Fvm), chlorophyll content (Ch), relative water content (RWC)) at the different levels of irrigation

Source of variation	df	MS								
		T			Ch			RWC		
		W_1	W_2	W_3	W_1	W_2	W_3	W_1	W_2	W_3
Repeat(R)	2	7.04**	57.58**	24.77*	182.64**	31.11**	14.5*	292.32^{ns}	7.07^{ns}	57.14*
Line(L)	5	3.95**	10.35*	13.79**	32.63**	23.33**	26.67**	45.45^{ns}	46.7**	33.56**
Tester(T)	4	164.1**	213.7**	210.4**	85.03**	43.29**	74.15**	293.2*	105**	49.11*
L × T	20	0.56^{ns}	3.18^{ns}	4.66^{ns}	4.97^{ns}	7.2^{ns}	8.18*	97.3^{ns}	16.1^{ns}	40.66**
Erorr	58	0.793	3.54	3.55	3.31	5.35	4.49	95.4	14.07	16.63

NS - not significant, * is significant at 0.05, ** significant at 0.01 probability level; normal irrigation (W_1), mild water stress (W_2), severe water stress (W_3)

Tab. 2.2. Analysis of variance of line × tester in sunflower for leaf water potential (LWP), proline content (P) and yield (Y) at the different levels of irrigation

Source of variation	do	MS								
		LWP			P			Y		
		W_1	W_2	W_3	W_1	W_2	W_3	W_1	W_2	W_3
Repeat(R)	2	0.3^{ns}	5.79^{ns}	5.23^{ns}	19.75^{ns}	24.77^{ns}	277.08^{ns}	1997.9**	228.33^{ns}	629.18**
Line(L)	5	4.72^{ns}	44.77*	33.03**	41.27**	214.97**	494.51**	313.98^{ns}	358.87*	87.11^{ns}
Tester(T)	4	1.02^{ns}	32.37^{ns}	18.56*	31.12^{ns}	9.04^{ns}	251.04*	53.25^{ns}	214.59^{ns}	57.32^{ns}
L × T	20	2.85^{ns}	20.52^{ns}	8.06^{ns}	13.73^{ns}	42.3^{ns}	64.65^{ns}	89.04^{ns}	56.68^{ns}	137.53*
(R)×(LT)	58	3.36	13.6	5.27	9.3	30.955	88.65	138.85	122.88	63.59

ns - not significant, * is significant at 0.05, ** significant at 0.01 probability level; normal irrigation (W_1), mild water stress (W_2), severe water stress (W_3)

Tab. 3.1. Estimates of general combining ability of the female lines for canopy temperature (T), chlorophyll fluorescence (Fvm), chlorophyll content (Ch), relative water content (RWC) at the different levels of irrigation. Normal irrigation (W_1), mild water stress

	T			Ch			RWC		
	W_1	W_2	W_3	W_1	W_2	W_3	W_1	W_2	W_3
B110	-0.59*	-0.527^{ns}	-0.39^{ns}	-0.88^{ns}	-0.63^{ns}	-0.19^{ns}	0.327^{ns}	0.967^{ns}	0.67^{ns}
B147	-0.34^{ns}	-1.405**	-1.66**	-2.1**	-1.68**	-3.89**	-2.102^{ns}	-1.723^{ns}	-2.261*
B221	-0.40^{ns}	0.206^{ns}	0.01^{ns}	2.02**	1.91**	2.14**	-0.969^{ns}	-1.08^{ns}	0.397^{ns}
B329	0.19^{ns}	0.263^{ns}	0.26^{ns}	-0.36^{ns}	0.02^{ns}	1.4**	0.142^{ns}	-1.648^{ns}	-2.158*
B343	0.57*	0.649^{ns}	0.93^{ns}	1.2**	0.8^{ns}	1.27*	1.456^{ns}	1.140^{ns}	2.038^{ns}
B355	0.56*	0.813^{ns}	0.83^{ns}	0.12^{ns}	-0.42^{ns}	-0.73^{ns}	2.145^{ns}	2.343^{ns}	1.313^{ns}
SE(GCA)	0.229	0.486	0.486	0.469	0.597	0.538	2.522	32.983	1.052
$SE(g_i\text{-}g_j)$	0.325	0.687	0.688	0.664	0.844	0.773	3.566	46.645	1.489

ns - not significant, * is significant at 0.05, ** significant at 0.01 probability level

Tab. 3.2 Estimates of general combining ability of the female lines for leaf water potential (LWP), proline content (P) and yield (Y) at the different levels of irrigation. normal irrigation (W_1), mild water stress (W_2), severe water stress (W_3)

	LWP			P			Y		
	W_1	W_2	W_3	W_1	W_2	W_3	W_1	W_2	W_3
B110	-0.015^{ns}	-0.411^{ns}	-0.778^{ns}	-1.504^{ns}	0.793^{ns}	0.027^{ns}	1.24^{ns}	-1.706^{ns}	-0.407^{ns}
B147	0.957^{*}	1.042^{ns}	-0.965^{ns}	3.01^{**}	-0.928^{ns}	0.781^{ns}	7.026^{*}	2.220^{ns}	0.295^{ns}
B221	0.297^{ns}	2.602^{**}	1.301^{*}	-0.293^{ns}	-1.466^{ns}	2.486^{ns}	1.906^{ns}	3.944^{ns}	1.912^{ns}
B329	-0.562^{ns}	-1.411^{ns}	-1.338^{*}	-2.142^{**}	2.32^{ns}	-1.813^{ns}	1.575^{ns}	3.899^{ns}	1.922^{ns}
B343	-0.235^{ns}	-0.864^{ns}	2.641^{**}	-0.144^{ns}	2.104^{ns}	-1.442^{ns}	-5.171^{ns}	-6.856^{*}	-0.328^{ns}
B355	-0.442^{ns}	-0.957^{ns}	-0.858^{ns}	1.075^{ns}	-2.823^{*}	-0.039^{ns}	-6.576^{*}	-1.502^{ns}	-0.393^{ns}
SE(GCA)	0.473	0.952	0.592	0.787	1.311	2.219	2.777	2.612	3.879
SE(g_i-g_j)	0.669	1.346	0.838	1.113	2.031	3.438	4.302	4.047	2.911

ns - not significant, * is significant at 0.05, ** significant at 0.01 probability level

approach. Therefore, physiological and biochemical approaches are of great importance for a deeper understanding of the complex responses of plants to water deficiency, and the rapid development of new varieties.

In this study, the variation exhibited by the seven characteristics under consideration indicated that selection for some of these drought related characteristics could be effective in developing drought tolerant cultivars; however, the selection effectiveness is influenced by gene action, heritability and correlation with yield.

Canopy temperatures, chlorophyll index, relative water content, leaf water potential and proline content traits carried additive gene action under all three conditions, whereas non-additive type of gene action was observed for chlorophyll index, relative water content and yield under third moisture stress, which is desirable for heterosis breeding and may be exploited in hybrid seed production to water deficit conditions.

As intensity of drought increased, the effects of genes controlling the traits chlorophyll content, relative water content and yield altered. Rebetzke *et al.* (2003) indicated that genotype-environment interaction and differential gene and gene complexes expression changed as the result of environment's changes.

The type of genetic variability for physiological traits was previously estimated in other crops. Dhanda and Sethi, 1998; Rauf *et al.,* 2009, for example, observed additive type of gene action in wheat for leaf trait such as relative water contents and leaf water potential.

Additive gene action for specific traits will increase the selection success in a breeding program (Topal *et al.,* 2004). Kaur *et al.* (2010) reported that for proline content, additive and non-additive gene actions are involved. Farshadfar *et al.* (2011) using diallel mating design showed in bread wheat that chlorophyll fluorescence was controlled by additive type of gene action. Naroui Rad *et al.* (2013) reported that for proline content, additive gene effects and for RWC and plant grain yield, both additive and non-additive gene effects were important. The concept of combining ability is important in designing plant breeding programs; in particular, it is useful in testing procedures for the study and comparison of the performance of lines in hybrid combinations. In our study, breeding lines such as B147, B221, B343, R19, R26 and R50 (the good general combiners) could serve as base populations for intermating and recurrent selection. Plus, CMS lines B147 and B221 and restorer R19 and R26 were better general combiners for most of the traits at the all conditions. These parental materials could be better utilized as valuable basic materials in developing high yielding sunflower hybrids for water limited conditions.

Genetic gains as a parameter for selection efficiency are related to genetic variability, heritability and selection intensity. Low heritability obtained for grain yield, compared to most of the other characters, indicates that its phenotypic effect is mainly controlled by environmental difference. Therefore, for selection of the best genotype, we should concentrate mainly on physiological trait with high heritability such as canopy temperatures, chlorophyll index and proline content than yield, in both conditions of drought stress.

The correlations between seed yield suggest that a simultaneous selection for seed yield, in both water stressed and irrigated condition, alongside the selection for other traits with higher heritability correlated with yield, could be an efficient breeding strategy.

Conclusions

Based on these results, we can conclude that sunflower genotypes subjected to progressive drought at each growth stage of sunflower, as often happens in field conditions, presented a large variation and gene actions in terms of water status maintenance and photosynthetic potential. For canopy temperatures, chlorophyll index, relative water content, leaf water potential and proline content, exhibited additive gene action under all three conditions. Breeding lines such as B147, B221, B343, R19, R26 and R50 the good general combiners could serve as base populations for intermating and recurrent selection. Thus, CMS lines B147 and B221 and restorer R19 and R26 were better general combiners for most of the traits at the all conditions. According to heritability and correlation between trait, canopy temperature, chlorophyll content, relative water content and leaf water potential may be good criterias to identify drought tolerant lines with higher yield.

Tab. 4.1. Estimates of general combining ability of the male restorer lines for canopy temperature (T), chlorophyll fluorescence (Fvm), chlorophyll content (Ch), relative water content (RWC) at the different levels of irrigation. Normal irrigation (W_1), mild water stress (W_2), severe water stress (W_3)

	T			Ch			RWC		
	W_1	W_2	W_3	W_1	W_2	W_3	W_1	W_2	W_3
R19	3.8^{**}	-4.874^{**}	-4.661^{**}	-1.29^{**}	-1.27^{*}	-2.27^{**}	3.454^{*}	-0.928^{ns}	-2.469^{*}
R26	-2.117^{**}	-1.544^{**}	-1.799^{**}	2.7^{**}	1.86^{**}	1.45^{**}	0.707^{ns}	3.233^{ns}	3.323^{**}
R46	0.19^{ns}	0.188^{ns}	0.121^{ns}	1.65^{**}	1.47^{**}	3.44^{**}	1.708^{ns}	-0.251^{ns}	0.416^{ns}
R50	2.12^{**}	2.141^{**}	2.25^{**}	-2.64^{**}	-0.7^{ns}	-1.48^{**}	1.1^{ns}	0.69^{ns}	-1.079^{ns}
R56	3.606^{**}	4.089^{**}	4.088^{**}	-0.42^{ns}	-1.35^{*}	-1.13^{*}	-6.97^{*}	-2.743^{ns}	-1.19^{ns}
SE(GCA)	0.209	0.443	0.444	0.247	0.544	0.498	2.302	30.109	0.961
SE(g_i-g_j)	0.296	0.627	0.628	0.606	0.771	0.705	3.255	42.581	1.359

ns - not significant, * is significant at 0.05, ** significant at 0.01 probability level

Tab. 4.2. Estimates of general combining ability of the male restorer lines for leaf water potential (LWP), proline content (P) and yield (Y) at the different levels of irrigation. Normal irrigation (W_1), mild water stress (W_2), severe water stress (W_3)

	LWP			P			Y		
	W_1	W_2	W_3	W_1	W_2	W_3	W_1	W_2	W_3
R19	0.248^{ns}	0.799^{ns}	0.648^{ns}	-1.474^{**}	0.207^{ns}	4.154^{ns}	1.526^{ns}	-3.304^{ns}	-1.345^{ns}
R26	-0.212^{ns}	-0.655^{ns}	-1.356^{*}	-2.783^{**}	-2.492^{ns}	1.254^{ns}	-3.64^{ns}	3.894^{ns}	2.062^{ns}
R46	-0.045^{ns}	1.422^{ns}	0.609^{ns}	3.406^{**}	-0.494^{ns}	1.758^{ns}	-1.391^{ns}	1.275^{ns}	-1.25^{ns}
R50	0.248^{ns}	-0.266^{ns}	0.19^{ns}	4.441^{**}	2.9^{*}	-3.503^{ns}	3.178^{ns}	2.161^{ns}	2.064^{ns}
R56	-0.239^{ns}	-1.422^{ns}	0.287^{ns}	-3.59^{**}	-0.12^{ns}	-3.663^{ns}	0.328^{ns}	4.026^{ns}	-1.53^{ns}
SE(GCA)	0.432	0.869	0.541	0.718	1.311	2.219	2.777	2.612	1.879
SE(g_i-g_j)	0.611	1.229	0.765	1.016	1.854	3.138	3.927	3.695	2.658

ns - not significant, * is significant at 0.05, ** significant at 0.01 probability level

Tab. 5. Correlation between traits i.e. canopy temperature (T), chlorophyll content (Ch), relative water content (RWC), leaf water potential (LWP), proline content (P) and yield (Y)

Traits	T	Ch	RWC	LWP	P	Y
T						
Fvm	-0.012^{ns}					
Ch	0.077^{ns}					
RWC	-0.481^{**}	-0.197^{**}				
LWP	0.165^{**}	0.161^{**}	-0.3^{**}			
P	-0.148^{*}	-0.03^{ns}	0.087^{ns}	-0.042^{ns}		
Y	-0.279^{**}	0.121^{*}	0.43^{**}	-0.274^{**}	0.003^{ns}	

ns - not significant, * is significant at 0.05, ** significant at 0.01 probability level

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