Genetic analysis of leaf hydraulics in sunflower (*Helianthus annuus* L.) under drought stress

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ABSTRACT

Improvement in leaf hydraulics is directly related to the improvement of plant tolerance to drought stress. Therefore, a field and pot experiment was carried out to determine the type of genetic variability and selection of parental types on the basis of combining ability for leaf hydraulics. Genotypes showed similar performance in both experiments; higher values were shown by drought tolerant genotypes in all traits except for osmotic potential, which drought tolerant genotypes maintained lower. Osmotic adjustment in pot experiment showed the highest magnitude of additive type of genetic variability. Female showed a higher and significant contribution of general combining ability effects as compared to male; it suggests that within genotypes female rather than male mostly contribute for additive genes. AMES-10103 showed the highest general combining ability effects for traits such as turgor pressure and osmotic adjustment.

Keywords: leaf hydraulics; genetic analysis; sunflower; drought

Leaf hydraulics are important plant traits for discriminating drought tolerant and sensitive genotypes (Rauf 2008). Recent studies in sunflower showed their significant relationship with achene yield under drought stress, thus depicting their potential for improvement of yield under drought stress (Chimenti et al. 2002, Rauf and Sadaqat 2008a, b). Significant genetic variability was reported to exist between the sunflower genotypes for these traits (Chimenti and Hall 1993, 1994, Rauf and Sadaqat 2008a, b). However, leaf hydraulic traits are quantitative in nature and therefore it is also important to find out the type of genetic variability associated with these traits; such type was found to affect the selection procedure and further improvement in the traits (Rauf et al. 2008). Furthermore, environment has a significant impact on the type of genetic variability (Khan et al. 2007, Rauf et al. 2007, 2008).

The selection of parents for hybridization in order to utilize transgressive segregation, for the manifestation of heterosis phenomenon or for the development of molecular marker, is a crucial step (Jamaux et al. 1997, Hervé et al. 2001, Kiani et al. 2007). In this regard, line × tester mating design were extensively used for estimation of the type of genetic variability and the selection of the parents on the basis of their combining ability (Rahman 2006, Rauf et al. 2008). Its biometrical analysis is easy to perform because of freedom of rigid assumptions that are seldom fulfilled in case of other mating designs such as diallel.

Keeping the same objectives in mind six male and female lines were crossed in line × tester fashion to estimate the type of genetic variability associated with leaf hydraulic traits under multi-environment conditions and selection of superior parents on the basis of combining ability.

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MATERIAL AND METHODS

Development of plant material. Series of experiments were carried out during the years 2005–2007, for the selection of parental material (Rauf and Sadaqat 2008a, b) and estimation of the type of genetic variability on the basis of leaf hydraulics. Parental material comprising of six female and male lines were crossed in line × tester fashion. Every female was crossed with each of male parent to obtain thirty-six cross combinations. Seeds were planted in the field and pot experiments to obtain parental and F_1 generations.

Field experiment. The seeds of parents and F_1 generation were planted in the sunflower research area under irrigated and drought stress conditions in a sandy loam soil. The plots were fertilized with 150 kg N/ha, 50 kg P/ha and 0 kg K/ha. Treatments were allocated in split plot design with three replications where water levels were assigned to main plots while genotypes to subplots. Each subplot was 4.8 m wide and 6.0 m long having rows spaced at 60 cm and plants spaced at 30 cm. Differential water levels were developed by irrigating the non-stress plots at regular interval while drought stress was developed by holding water at the beginning of button stage to achieve moisture stress conditions at anthesis (70 days after sowing). The soil moisture content was measured every 8–10 days up to a depth of 3 feet during the whole growth season. Total rainfall during crop growth cycle was only 64.4 mm, of which 41.3 mm fell during the vegetative phase and 23.1 mm during the reproductive phase. Weeds were controlled manually, diseases were considered absent.

Large pot experiment. In order to conduct the experiment, large plastic container of 120 cm in length and 40 cm in diameter were used. The pots were buried in the soil with 15 cm above the ground to avoid water infiltration from surrounding. All pots were irrigated to field capacity before sowing to achieve uniform germination. After germination, pots were equally divided into two regimes i.e. non-stress and drought regime. Moisture contents within pots were measured after every 2–3 days. Optimum level of moisture was maintained in the non-stressed regime by irrigating with measured quantity of water to keep moisture contents close to field capacity, while stress in the drought regime was provided by skipping irrigations so that plants had 50% water deficit compared to the total of water applied in the normal regime. The total of 214 litres of water in thirty splits was supplied to non-stressed regime while 107 litres of water were supplied in fifteen splits to stressed regime in each pot during the whole crop growth cycle. However, the plot in which pots were buried was irrigated at regular intervals to avoid heat stress injury within pot as a result of increase of soil temperature due to moisture stress. There were three pots per genotype in each regime with three replications, each pot containing four plants. During the experiment plants were fertilized with urea (40% N) applied to each pot in solution form (10g urea/l of water) at 30, 50 and 60 days after sowing.

Measurement of leaf hydraulics. At anthesis, measurements were recorded for different leaf hydraulic traits such as relative water contents, leaf water potential, osmotic potential, turgor pressure and osmotic adjustment. In field experiment, the middle two rows were used for measurements, eliminating one row at each side. Within each row eight competitive plants were used for measurements. In large pot experiment two plants were selected for measurements within each pot. Within plant, measurements were recorded on fresh, fully expanded leaf borne at the second node from the top of canopy. Leaf hydraulics i.e. leaf relative water contents, osmotic potential, water potential and osmotic adjustment were measured by the method according to Rauf and Sadaqat (2008b).

RESULTS AND DISCUSSION

Parental mean performance under drought stress of field and pot experiment

All leaf hydraulics traits were measured in both stress and non-stress conditions of field and pot experiments. However, because of their relevance to drought and low heritability under non-stress condition, performance of genotypes has only been shown under stress conditions of both experiments.

Mean values of leaf hydraulics are shown in (Table 1). Overall mean of all genotypes were lower in pot experiment (PE) when compared to field experiment (FE) for all traits under study (Table 1). Performance of genotypes was not similar across leaf hydraulics and experiments. However, drought tolerance of genotypes appeared in both experiments. Among genotypes, AMES-10103 showed relatively stable performance across both experiments; it showed the highest significant relative water content and osmotic adjustment in both experiments. In addition, it also showed the

Table 1. Mean values for water relations i.e. relative water content (RWC), leaf water potential (LWP), osmotic potential (OP), turgor pressure (TP) and osmotic adjustment (OA) within water levels in pot (PE) and field experiments (FE)

Parents	RWC		LWP		OP		TP		OA	
	FE	PE	FE	PE	FE	PE	FE	PE	FE	PE
AMES-10103	0.77	0.69	–1.81	–2.69	–2.70	–3.46	0.89	0.77	0.67	0.39
PEM-SR-88	0.72	0.60	–1.91	–2.70	–2.69	–3.43	0.78	0.73	0.57	0.30
CM-614	0.68	0.59	–1.84	–2.89	–2.61	–3.42	0.77	0.53	0.42	0.33
HA-407	0.62	0.52	–1.90	–3.23	–2.15	–3.36	0.24	0.13	0.09	0.07
ORI-16/B	0.61	0.51	–2.05	–3.32	–2.12	–3.39	0.07	0.07	0.07	0.03
HA-350	0.60	0.56	–2.29	–3.15	–2.35	–3.23	0.06	0.08	0.04	0.07
RL-57	0.72	0.67	–1.68	–2.71	–2.56	–3.43	0.88	0.72	0.58	0.36
RL-52	0.72	0.65	–1.62	–2.79	–2.53	–3.37	0.91	0.58	0.52	0.32
CM-815	0.69	0.62	–2.35	–2.82	–3.06	–3.37	0.71	0.55	0.44	0.28
CM-631	0.54	0.57	–2.31	–3.01	–2.43	–3.14	0.12	0.13	0.08	0.10
RL-37	0.57	0.55	–2.46	–3.03	–2.48	–3.14	0.02	0.11	0.09	0.05
CM-619	0.63	0.52	–1.99	–3.13	–2.10	–3.27	0.12	0.14	0.13	0.03
Average	0.66	0.59	–2.02	–2.96	–2.48	–3.33	0.46	0.38	0.31	0.20
LSD (P < 0.05)	0.03	0.04	0.04	0.05	0.02	0.02	0.04	0.03	0.03	0.04
Correlation*	0.12^{NS}	0.60*	$–0.03^{NS}$	0.62*	0.42*	–0.37*	–0.07*	0.63*	0.01^{NS}	0.68*

^{NS} – not significant

highest turgor pressure and leaf water potential in PE. RL-52 showed the highest turgor pressure but it was statistically similar to AMES-10103 and RL-57. In addition RL-52 also showed the highest significant leaf water potential. Drought tolerant genotypes showed lower osmotic potential as compared to drought susceptible genotypes. In PE, AMES-10103 showed the lowest osmotic potential while under FE, CM-815 showed the lowest osmotic potential.

Combining ability variations across water levels within pot and field experiments

Analysis of variance showed significant (P < 0.01) differences between genotypes and within genotypes component such as parents, crosses and parents vs. crosses (P vs. C) (Table 2). Within crosses, female and female × male (F × M) interaction was also significant for all traits. However, variation due to female was non-significant (P > 0.05) for relative water contents (RWC) and osmotic potential (OP) in PE and leaf water potential (LWP) in FE. Variation due to male was non significant (P > 0.05) in all traits and experiments.

Interactions such as genotypes × water levels (G × W), parents × water levels (P × W), crosses × water levels (C × W) and parents vs. crosses × water levels (P vs. C × W) were also significant for all traits. Within crosses, interaction between female and water levels (F × W) was found significant (P < 0.05) for turgor pressure in both experiments, LWP in PE and OP in FE. However, interaction between male and water levels (M × W) was found non-significant (P > 0.05) in all traits and experiments. Female × male × water levels (F × M × W) were significant for all traits under study.

Combining ability variation under drought conditions of field and pot experiment

Analysis of variance was also carried within drought stress of both experiments (Table 3). It showed significant variation (P < 0.05) due to genotypes and within genotype components. Within crosses, female and F × M components were sig-

Table 2. Abstract of the analyses of variance of combining ability in sunflower for water relations i.e. relative water content (RWC), leaf water potential (LWP), osmotic potential (OP), turgor pressure (TP) over water levels in field (FE) and pot experiments (PE)

Source of variation	*df*	RWC		LWP		OP		TP	
		FE*	PE	FE	PE	FE	PE	FE	PE
Water level (W)	1	2.68**	3.87**	70.57**	128.16**	19.30**	31.32**	16.06**	31.42**
Genotypes (G)	47	0.01**	0.01**	0.10**	0.84**	0.12**	0.53**	0.20**	0.27**
Parents (P)	11	0.01**	0.00**	0.14**	0.09**	0.13**	0.08**	0.28**	0.18**
P vs. C	1	0.00**	0.05**	0.04**	7.69**	0.51**	3.25**	0.27**	0.81**
Crosses (C)	35	0.01**	0.01**	0.09**	0.88**	0.10**	0.60**	0.17**	0.28**
Females (F)	5	0.02*	0.01^{NS}	0.25**	1.74**	0.31**	1.09^{NS}	0.52**	0.92**
Males (M)	5	0.01	0.00^{NS}	0.09^{NS}	0.80^{NS}	0.14*	0.66**	0.15^{NS}	0.12^{NS}
F × M	25	0.01**	0.01**	0.06**	0.72**	0.05**	0.49**	0.10**	0.18**
G × W	47	0.01**	0.01**	0.07**	0.65**	0.05**	0.49**	0.11**	0.10**
P × W	11	0.01**	0.01**	0.10**	0.11**	0.12**	0.08**	0.19**	0.03**
P vs. C × W	1	0.00**	0.03**	0.11**	2.41**	0.19**	5.30**	0.01**	0.46**
C × W	35	0.01**	0.01**	0.08**	0.77**	0.04**	0.48**	0.09**	0.10**
F × W	5	0.01^{NS}	0.01^{NS}	0.14^{NS}	1.57**	0.08*	0.97^{NS}	0.22**	0.27**
M × W	5	0.01^{NS}	0.01^{NS}	0.09^{NS}	0.57^{NS}	0.04^{NS}	0.27^{NS}	0.02^{NS}	0.11^{NS}
F × M × W	25	0.01**	0.01**	0.06**	0.65**	0.03**	0.42**	0.07**	0.07**
Phenotype		0.02	0.03	0.28	2.33	0.29	1.56	0.49	0.63
Genotype		0.01	0.02	0.17	1.49	0.17	1.02	0.29	0.36
h^2 (broad sense)		0.65	0.67	0.63	0.64	0.59	0.66	0.60	0.62

Blocking was non-significant in field experiment, therefore skipped from ANOVA; ^{NS} – not significant, * is significant at 0.05, ** significant at 0.01 probability level

nificant ($P < 0.05$) for all traits. However, variation due to female was found non significant ($P > 0.05$) for RWC in PE and OP in FE. On the other hand male component showed non-significant ($P > 0.05$) variation for all traits.

Contribution due to female, male and F × M to the total genetic variation was also calculated in both experiments (Table 3). Relative contribution of male + female to the total contribution was related to additive genes (general combining ability) and contribution of F × M was related to non-additive gene action (specific combining ability). Magnitude of contribution due to female, male or F × M was found similar in both experiment except in OP and osmotic adjustment (OA). In OP, genetic variation was largely due to additive gene action in FE while non-additive gene action was observed in PE. Conversely, OA showed predominance of non-additive gene in FE and higher contribution of additive gene in PE. Over all non-additive gene action was observed in RWC and LWP of both experiments while additive gene action was observed in turgor pressure (TP) of both experiments. Contribution due to female increased in PE except for traits related to leaf potential i.e. OP and LWP. Conversely, contribution due to F × M increased in PE for these two traits (Table 3). Contribution due to male decreased in PE for all traits.

Status of parental lines for general combining ability (GCA) effects under drought stress of both experiments

General combining ability effects (GCA) was caused by the additive genes (Table 4). Therefore, higher GCA value was a reflection of higher number of additive genes within particular genotypes. Relative performance of parental lines on the basis of GCA was not similar in both experiments

Table 3. Abstract of the analyses of variance of combining ability in sunflower for water relations i.e. relative water content (RWC), leaf water potential (LWP), osmotic potential (OP), turgor pressure (TP) and osmotic adjustment (OA) within drought regimes of pot (PE) and field experiments (FE)

Source of variation	*df*	RWC		LWP		OP		TP		OA	
		FE*	PE	FE	PE	FE	PE	FE	PE	FE	PE
Genotypes	47	0.01**	0.01**	0.18**	1.27**	0.16	0.92**	0.28**	0.23**	0.13**	0.05**
Parents	11	0.02**	0.01**	0.23**	0.09**	0.23	0.06**	0.44**	0.10**	0.18**	0.06**
Parents vs. crosses	1	0.02**	0.00**	0.33**	9.35**	0.96	8.43**	0.43**	0.02**	0.52**	0.00**
Crosses	35	0.01**	0.01**	0.16**	1.41**	0.12	0.98**	0.23**	0.27**	0.12**	0.05**
Females	5	0.02NS	0.02*	0.36*	3.14*	0.35	1.71NS	0.69**	1.02**	0.19**	0.20*
Males	5	0.01NS	0.01NS	0.18NS	1.05NS	0.14	0.80NS	0.13	0.08NS	0.10**	0.02NS
F × M	25	0.01**	0.01**	0.11**	1.13**	0.08	0.87**	0.15**	0.16**	0.11**	0.02**
Residual		0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Contribution (%)											
Females		21.18	27.29	32.57	31.81	40.92	25.01	43.40	53.08	23.45	58.63
Males		15.28	12.02	16.33	10.69	15.68	11.70	8.28	4.13	12.70	6.79
F × M		63.54	60.70	51.10	57.50	43.40	63.28	48.33	42.80	63.85	34.58
Broad sense heritability		0.91	0.94	0.88	0.93	0.83	0.91	0.86	0.91	0.86	0.96

Blocking was non-significant in field experiment, therefore skipped from ANOVA; NS – not significant, * is significant at 0.05, ** significant at 0.01 probability level

(Table 4). However, AMES-10103 showed stable GCA effects in both experiments. AMES-10103 showed positive effects for all traits except OP in which it showed negative effects in both experiments. This line showed the highest GCA effects in FE for all traits except OP while for TP and OA it showed the highest effects in both experiments. PEM-SR-88 showed the highest positive GCA ef-

Table 4. Estimates of general combining ability of the parental cultivar for water relations i.e. relative water content (RWC), leaf water potential (LWP), osmotic potential (OP), turgor pressure (TP) and osmotic adjustment (OA) within drought regimes of pot (PE) and field (FE) experiments

Parents	RWC		LWP		OP		TP		OA	
	FE	PE	FE	PE	FE	PE	FE	PE	FE	PE
AMES-10103	0.06	0.04	0.21	0.20	–0.05	–0.21	0.25	0.41	0.16	0.16
PEM-SR-88	–0.01	0.05	–0.04	0.64	0.04	0.46	–0.08	0.17	–0.08	0.09
CM-614	–0.02	0.00	–0.18	–0.18	0.03	–0.03	–0.21	–0.15	–0.03	–0.04
HA-407	–0.01	0.00	–0.11	0.19	–0.05	0.29	–0.06	–0.10	–0.05	–0.02
ORI-16/B	–0.01	–0.04	0.09	–0.41	0.23	–0.24	–0.14	–0.17	–0.10	–0.11
HA-350	–0.01	–0.04	0.04	–0.44	–0.20	–0.27	0.23	–0.16	0.10	–0.08
RL-57	–0.04	0.01	–0.15	–0.02	0.00	–0.01	–0.15	–0.01	–0.14	0.02
RL-52	0.00	0.00	0.09	0.36	0.07	0.37	0.02	–0.01	0.00	0.00
CM-815	–0.01	0.03	–0.05	0.08	–0.14	0.03	0.10	0.05	0.08	0.03
CM-631	0.00	0.00	–0.03	0.06	–0.06	–0.03	0.03	0.09	0.00	0.01
RL-37	0.01	–0.05	0.02	–0.38	0.05	–0.28	–0.03	–0.10	0.02	–0.07
CM-619	0.03	0.00	0.13	–0.10	0.09	–0.08	0.04	–0.02	0.04	0.00

fects for RWC, LWP and OP in PE. In addition, RL-52 was good general combiner for LWP and OP in both experiments. CM-815 showed good positive GCA effects for TP and OA in both experiments and for all traits in PE.

Broad sense heritability

Broad sense heritability estimates were low across the contrasting water levels of both experiments (Table 2). However, estimates within drought stress condition of both experiments were very high (Table 3). Broad sense heritability estimates were high in pot experiments as compared to field condition within drought stress and over contrasting water levels (Tables 2 and 3).

Correlations

Correlation between per se performance and GCA effects were estimated and are given in Table 1. Correlation between mean values and GCA effects were non significant ($P > 0.05$) for all traits except OP in FE. However, in PE correlations were significant for all traits. The direction of correlation in pot experiments was positive for all traits except OP in which it showed negative estimates.

Correlation between GCA values of a trait in field and pot experiments was non-significant ($P > 0.05$) except TP and OA (Table 5). Correlations between GCA values of different traits were significant within experiment especially in PE experiment. Correlations between GCA effects of leaf hydraulics were high in magnitude in PE as compared to FE. GCA effects of TP and OA in FE showed significant correlation with all traits except RWC and LWP of PE. Similarly, GCA effects of TP in PE showed significant ($P > 0.05$) correlation with all traits except OP of both experiment. OA in PE also showed significant correlation with all traits except LWP and OP of FE. The highest correlations were obtained between GCA effects of OA and TP or RWC.

Over all averages of all leaf hydraulics were lower in pot experiment (PE) as compared to field experiment (FE). This may be related to higher intensity of drought stress in PE. Repressing effect on leaf hydraulics has been observed previously. Rascio et al. (1998) also found a decrease in leaf water and osmotic potentials (OP) with increasing intensity of drought. They also showed that differences between genotypes disappeared with increasing intensity of drought for these two traits; there was minor fluctuation for relative performance across both experiments. In our experiments genotypes however performed similarly for drought tolerance and susceptibility in both experiments and higher values were shown by drought tolerant genotypes in all traits except for OP in which tolerant genotypes maintained lower OP. The lower OP has been shown to be beneficial as it helps for maintenance of turgor, growth and photosynthesis and resulted due to active or passive accumulation of certain osmolytes (Bolaños and Edmeades 1991).

Table 5. Correlation between general combining ability effects of different physiological traits i.e. relative water contents (RWC), leaf water potential (LWP), osmotic potential (OP), turgor pressure (TP) and osmotic adjustment (OA) in pot (*PE) and field (*FE) experiments

Traits	RWC*FE	RWC*PE	LWP*FE	LWP*PE	OP*FE	OP*PE	TP*FE	TP*PE	OA*FE
RWC*PE	0.17^{NS}								
LWP*FE	0.83*	-0.07^{NS}							
LWP*PE	0.10^{NS}	0.85^{NS}	-0.04^{NS}						
OP*FE	0.03^{NS}	-0.20^{NS}	0.20^{NS}	-0.06^{NS}					
OP*PE	-0.28^{NS}	0.57*	–0.32*	0.86*	0.05^{NS}				
TP*FE	0.64*	0.11^{NS}	0.65*	0.02^{NS}	–0.62*	–0.28*			
TP*PE	0.61*	0.78*	0.40*	0.64*	-0.20^{NS}	0.17^{NS}	0.48*		
OA*FE	0.73*	0.08^{NS}	0.58*	-0.09^{NS}	–0.55*	–0.38*	0.89*	0.41*	
OA*PE	0.47*	0.90*	0.21^{NS}	0.76*	-0.24^{NS}	0.35*	0.36*	0.95*	0.31*

^{NS} – not significant

Analysis of variance showed that drought regime of both experiments promoted varied type and amount of total of genetic variability. Rebetzke et al. (2003) indicated that genotype-environment interaction and differential gene and gene complexes expression caused the change in the results between environments. Drought stress of PE promoted additive genetic variation as contribution due to female, increased in PE except traits related to leaf potential i.e. OP and LWP. The positive correlation for GCA and parental means in PE further confirm that additive gene action was important in this experiment as compared to FE. In addition heritability estimates were high in PE experiment for all traits. Therefore, PE provided better environment for selection and screening of drought tolerant genotypes under drought stress. Among traits, turgor pressure (TP) was the most useful for enhancing drought tolerance as additive genes predominantly controlled genetic variability in both PE and FE. However, osmotic adjustment (OA) in PE showed the highest magnitude of additive type of genetic variability. Therefore, selection on the basis of this trait in PE may yield the highest response for the evolution of drought tolerant genotypes. Furthermore, GCA effects of OA in PE have shown significant correlation with RWC and TP of both experiments and with OA of FE. Therefore, improvement of OA in PE may also simultaneously improve these traits. Osmotic adjustment only results from the active accumulation of certain type of compatible solutes such as K^+, Ca^{2+}, proline and sugars in response to drought. Among osmolytes, sugar and proline are known to be important due to their utilization as a source of energy after relieve of the stress (Basu et al. 2007). Numerous available studies in sunflower show significant genetic variability and positive correlation between the yield of drought stress and osmotic adjustment (Chimenti et al. 2002, Rauf and Sadaqat 2008a, b), but none of them reports on the type of genetic variability associated with this trait in sunflower. However, the type of genetic variability for leaf hydraulics was previously estimated in other crops (Dhandha and Sethi 1998, Rebetzke et al. 2003, Bhutta et al. 2006). Dhandha and Sethi (1998) observed additive type of gene action in wheat for leaf trait such as relative water contents. A high magnitude of GCA effects was observed in comparison to the SCA effects, which were negligible for this trait (Dhandha and Sethi 1998).

AMES-10103, a drought tolerant female, and CM-631, drought susceptible male, both positive general combiner for TP and OA, may be used for the development of molecular marker for these two traits while AMES-10103 and CM-815, another good general combiner, may be crossed to produce trangressive segregants. Female showed higher and significant GCA effects as compared to male. This showed that within genotypes female rather than male mostly contributed for additive genes. AMES-10103 showed the highest GCA effects for traits such as turgor pressure and osmotic adjustment, and therefore it may be concluded that maximum additive genes were located in this drought tolerant female for these traits.

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