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Identification of physiological traits and genotypes combined to high achene yield in sunflower (*Helianthus annuus* L.) under contrasting water regimes

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Abstract

Field experiments were conducted to determine the effect of different leaf hydraulic traits on achene yield in sunflower under irrigated and drought regimes. Among leaf hydraulics, stomatal conductance (g_s) showed highest relationship with achene yield (a_y) under irrigated regime while in drought; osmotic adjustment (OA) showed highest positive relationship with a_y. Presence of highly strong relationship between OA and relative water contents (RWC) indicated that RWC contents may be utilized as physiological marker of OA when source material comprises of different genotypes. Further more RWC contents also showed highly significant relationship with a_y. OA also showed significant relationship with turgor pressure (TP) suggesting its role in maintenance of turgor. Genotype AMES-10107 showed highest a_y which was accompanied by the higher g_s in non stress regime. However under drought stress, AMES-10103 showed highest a_y. This genotype also showed highest OA, RWC and TP.

Keywords: Drought; Osmotic adjustment; Relative water contents; Stomatal conductance; Turgor pressure.

Abbreviations: OA- Osmotic adjustment; RWC- Relative water content; TP- Turgor pressure

Introduction

Over the years drought has been proved as major yield limiting factor through out the world. Scientists have achieved significant yield progress in different crops and under optimum condition yet. However success for breeding under stress condition is limited (Hollington and Steele, 2007). Over the past decades plant breeders have focused on some traits that were incorporated to plant survival under stress condition such as lower leaf canopy and reduced transpiration (Karamanos and Papatheohari, 1999, Fischer and Wood, 1979). These traits are not necessarily associated with high yield. As a result breeders continue to evolve cultivars with poor yield under stress condition. Therefore analysis of plant traits with significant effects on drought tolerance and high yield potential under stress condition seems to be necessary (Richards, 2004).
Leaf hydraulics is one of the most important characters which can be used in breeding programs for increasing drought tolerance in plants. The actual value of leaf hydraulics has not been clearly defined in high-yield and drought-tolerant genotypes yet. There are some antithetical reports about the association between; plant yield and achene under drought stress condition (Kirkham, 1988; Subbarao et al., 2000) although positive associations have also been shown (Lu and Zeiger, 1994, Subbarao et al., 2000; Chimenti et al., 2002). Therefore, the role of leaf water and hydraulic traits in plant yield production is still unknown and further investigation to define the value of these traits under drought stress is needed.

We conducted this experiment to study the effect of some leaf hydraulic traits on yield under irrigated and drought stress condition in sunflower and subsequently identifying high yielding and drought tolerant genotype using different sunflower lines.

Materials and Methods

Field experiments were conducted during February 2006 at the sunflower experiment field of the Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad, under irrigated (W_1) and non-irrigated conditions (W_2). The soil was a sandy-loam soil with low water retention capacity. Soil properties such as field capacity and wilting point were measured in laboratory, as averaged 14% and 7% by weight respectively. Other soil properties like pH 7.5, organic matter 0.91%, available phosphorous 28.6 ppm, and potassium 140 ppm were also measured. The plots were fertilized with 150 kg N ha^{-1} and 50 kg P ha^{-1}; no K was applied. Achenes of thirty six genotypes of sunflower belonging to different countries were obtained from North Dakota Sunflower Research Station, USA and Oil Seed Research Institute, Faisalabad-Pakistan at free of cost (Table 1).

A split-plot randomized block design (replications =3) was used, where levels of water availability were assigned to main plots and genotypes to subplots. Each subplot was 4.8 m wide and 6 m long, consisting of eight rows of a single genotype. The inter-row spacing was 60 cm and interplant spacing was 30 cm. Non stress condition wasmaintained, by irrigating the plots during the entire growth cycle to maintain the soil water content close to field capacity. While drought stress was developed , when the plots were irrigated at the same time and with the same amount of supplemental water as in the non-stress regime during the vegetative phase; supplemental irrigation was completely withheld beginning with the button stage (R1, 50 days after sowing) to achieve low soil moisture content during anthesis. The soil moisture content was measured every 8-10 days. The total rainfall during crop growth cycle was only 75.6 mm, of which 51.6 mm fell during the vegetative phase; 20 mm fell very late and during the reproductive phase. Leaf diseases were not presented, and weeds were controlled manually. Maturity measurements were made by eliminating two rows at each sides of plot. All physiological traits were measured at the time of anthesis (74 days after sowing).

Stomatal Conductance (g_s)

Stomatal conductance (g_s) was measured with porometer (Cam,Delta-T Device) starting at 01000 hour. A fully expanded leaf at the second node from top of canopy was inserted in the cup to take counts. Readings were taken until porometer became stable and gave tow consecutive similar readings.

Table 1. **List of the genotypes used in the experiments and their country of origin**

No	Genotypes	Country	No	Genotypes	Country
1.	B-FS-88	Spain	21.	ORI-10/B	Pakistan
2.	CM-614	Canada	22.	ORI-25/B	Pakistan
3.	B-Sin-82	Spain	23.	ORI-26/B	Pakistan
4.	PEM S-R88	Spain	24.	ORI-23/B	Pakistan
5.	HA-350	USA	25.	ORI-24/B	Pakistan
6.	HA-341	USA	26.	ORI-2/B	Pakistan
7.	HA-342	USA	27.	ORI-1/B	Pakistan
8.	Ames-10103	China	28.	ORI-19/B	Pakistan
9.	Ames-10107	China	29.	ORI-11/B	Pakistan
10.	CM-612	Canada	30.	ORI-9/B	Pakistan
11.	HA-G-P-13	USA	31.	ORI-27/B	Pakistan
12.	DM-2	USA	32.	ORI-28/B	Pakistan
13.	CM-628	Canada	33.	ORI-22/B	Pakistan
14.	HA-407	USA	34.	ORI-16/B	Pakistan
15.	HA-207	USA	35.	ORI-14/B	Pakistan
16.	HA-310	USA	36.	ORI-15/B	Pakistan
17.	ORI-13/B	Pakistan	-	-	-
18.	ORI-18/B	Pakistan	-	-	-
19.	ORI-21/B	Pakistan	-	-	-
20.	ORI-12/B	Pakistan	-	-	-

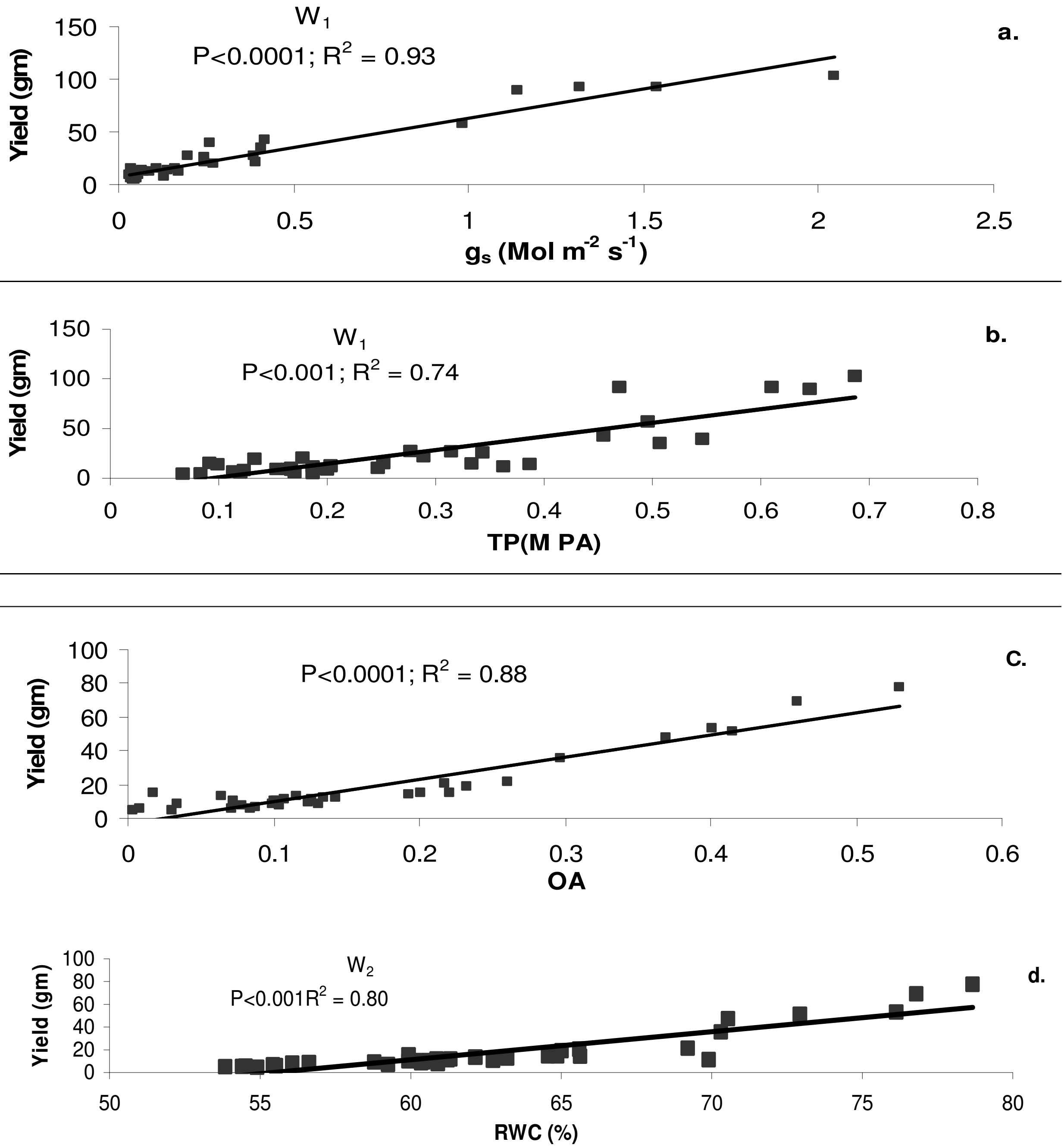

***Figure 1*. The response of achene yield (gram) to the variation in (a) stomatal conductance (g_s mol m^{-2} s^{-1}) under irrigated (W_1) treatment (b). Turgor pressure (TP MPa) (c.) Osmotic adjustment (OA) (d.) Relative water contents (RWC %) under drought (W_2) treatment across all genotypes.**

Turgor Pressure (TP)

Leaf sample were taken from eight plants per genotypes and replication in both regimes at 01000 hours. Turgor pressure (TP) was the difference between leaf water potential (ψ_w) and osmotic potential (ψ_s) in their respective regimes. Leaf water potential was measured by the pressure bomb method following Turner (1987) while osmotic potential was measured from the sap, extracted from frozen leaves by osmometer (Cam lab Robeling). Δ LT= ψ_w- ψ_s

Relative Water Contents (RWC)

Leaf Discs of 4cm^2 area in rectangle shape were taken from plants under irrigated and stressed regimes and the fresh weight was measured. Discs were then dipped in the glass vials containing 20ml deionized water. These vials were left for four hours at room temperature. After four hours, leaf disc were blotted and their turgid weight were recorded. The relative water content was calculated by the following formula:

$$RWC = \frac{\text{Fresh wt- Dry wt}}{\text{Turgid wt-Dry wt}} \times 100$$

Osmotic Adjustment (OA)

For the purpose of the measurement of osmotic adjustment (OA), leaf samples were collected from both regimes and their petioles were dipped in the deionized water for eight hours. OA was based on the difference between osmotic potential at full turgor ($100\psi_s$) of plants under non stress (W_1) and stressed regimes (W_2).

$$\Delta OA = 100\psi_s w_1 - 100\psi_s w_2$$

The osmotic potential at full turgor ($\psi_s 100$) was calculated according to equation given by Moinuddin and Chopra, 2004: $\psi_s 100$= (Corrected ψ_s x Relative water contents)/100
Where corrected ψ_s was $\psi_s + 0.1\ \psi_s$, for the dilution of symplastic sap by apoplastic water, assuming 10% apoplastic water (Kramer, 1983).

Achene yield (a_y)

Heads were harvested separately from each plant per genotype and replicate and achenes removed manually from dried receptacles and measured.

Statistical Analysis

The data was analyzed in factorial arrangement, water regime to have fixed effect and genotypes to have random effect. All data was analyzed using computer based program MSTAT-C (MSTA-C development Team, 1989).

Results

Analysis of variance detected significant variation ($P<0.01$) for all leaf traits with respect to genotype and field water level. The genotype x water regime was significant ($P<0.01$) for leaf hydraulics. This suggests that the relative ranking of various genotypes for all traits was not consistent across water regimes.

Leaf hydraulic traits showing significant ($P\leq0.01$) relationship with achene yield or osmotic adjustment are shown in Figures 1 and 2. Stomatal conductance (g_s) showed strong relationship with achene yield (a_y) ($P\leq0.0001$) under irrigated condition (Fig 1a). However this type of relationship was not observed under drought regime. Among genotypes, AMES-10107 showed highest a_y and g_s followed by B-SIN-82 (Table 2). All genotypes showed lower g_s under drought regime. In this regime genotype AMES-10103 showed highest a_y but this was not accompanied by higher g_s. In stressed regime B-SIN-82 showed highest g_s (Table 2).

Turgor pressure (TP) ($P \leq 0.001$) showed highly significant relationship with a_y under drought stress regime (Fig.1b). Local genotype ORI-25/B showed highest TP in irrigated regime. However AMES-10103 and AMES-10107 showed highest TP under drought stress (Table 2). Highly significant relationship between relative water contents (RWC) and a_y was observed under drought regime (Fig. 1c) but this relationship was failed to achieve under irrigated regime. AMES-10107 and PEM-SR-88 showed highest RWC under non stress regime. All genotypes retained lower RWC under drought regime in comparison to irrigated regime. In drought regime AMES-10103 showed highest RWC followed by AMES-10107 and CM-614 (Table 2). Osmotic adjustment (OA) showed highly significant ($P \leq 0.0001$) relationship with a_y of drought regime (Fig 1d). It also showed very strong relationship ($P \leq 0.0001$) with RWC (Fig. 2b) and significant relation ($P \leq 0.001$) with TP (Fig. 2a) under drought regime. Genotype AMES-10103 showed highest OA (Table 2).

Table2. **Mean performance of genotypes for achene yield (a_y), stomatal conductance (g_s), Turgor Pressure (TP), Relative water contents and Osmotic adjustment (OA) under control (W_1) and stress condition (W_2).**

Genotype	a_y gm		g_s mol m^{-2} s^{-1}		TP M Pa		RWC (%)		ΔOA
	W1	W2	W1	W2	W1	W2	W1	W2	$\Psi s100_{W1}$-$\psi s100_{W2}$
CM-614	57.24	53.14	0.98	0.23	0.64	0.50	85.23	76.12	0.53
B-Sin-82.	91.74	47.41	1.54	1.36	0.68	0.47	81.10	70.54	0.50
PEM S-R88	91.85	51.39	1.32	1.19	0.75	0.61	90.59	72.93	0.55
HA 341	25.97	15.36	0.25	0.17	0.73	0.34	79.66	59.93	0.11
HA 342	35.31	21.34	0.41	0.36	0.86	0.51	83.43	69.21	0.38
AMES-10103	89.91	77.63	1.14	0.78	0.68	0.65	84.99	78.66	0.68
AMES-10107	102.80	69.14	2.05	0.95	0.75	0.69	91.70	76.79	0.54
DM-2	42.60	35.51	0.42	0.25	0.81	0.45	86.08	70.31	0.42
CM 628	26.95	14.55	0.39	0.27	0.74	0.31	81.41	64.56	0.34
HA 310	20.62	7.94	0.39	0.17	0.65	0.18	78.77	60.90	0.20
B-FS-88	15.81	12.74	0.11	0.09	0.68	0.20	81.10	63.21	0.12
HA-350	16.93	10.26	0.12	0.05	0.49	0.17	81.18	60.14	0.07
CM-612	15.12	6.59	0.10	0.05	0.50	0.11	80.76	55.43	0.09
HA-GP-13	17.37	14.25	0.13	0.07	0.89	0.46	85.29	65.63	0.19
HA-407	7.12	4.44	0.05	0.04	0.42	0.07	84.69	54.91	0.01
HA-207	11.10	9.31	0.06	0.05	0.47	0.15	83.54	58.79	0.12
Local types									
ORI-21/B	27.39	13.44	0.20	0.13	0.76	0.28	82.71	62.15	0.16
ORI-12/B	14.86	7.07	0.16	0.13	0.58	0.25	77.52	59.25	0.18
ORI-25/B	39.40	20.59	0.26	0.13	0.97	0.55	85.73	65.60	0.25
ORI-23/B	19.62	10.63	0.27	0.13	0.64	0.13	79.16	62.74	0.23
ORI-24/B	15.14	11.22	0.11	0.05	0.70	0.09	75.92	61.23	0.21
ORI-9/B	21.79	19.11	0.25	0.18	0.80	0.29	80.22	65.02	0.35
ORI-11/B	13.84	10.42	0.14	0.03	0.58	0.10	77.32	60.57	0.20
ORI-19/B	11.80	11.16	0.17	0.02	0.56	0.36	83.70	69.89	0.29
ORI-13/B	16.33	11.74	0.13	0.07	0.61	0.19	80.51	60.86	0.15
ORI-18/B	12.03	5.62	0.07	0.04	0.39	0.12	81.85	54.52	0.01
ORI-10/B	10.83	8.55	0.06	0.03	0.69	0.2	88.45	56.63	0.03
ORI-26/B	11.79	5.27	0.07	0.05	0.66	0.19	81.75	54.42	0.07
ORI-2/B	12.31	5.88	0.07	0.05	0.58	0.17	79.85	55.53	0.08
ORI-1/B	12.69	10.18	0.08	0.05	0.55	0.25	82.74	59.94	0.10
ORI-27/B	10.65	8.06	0.06	0.13	0.85	0.12	83.54	56.07	0.13
ORI-28/B	17.29	14.69	0.13	0.04	0.91	0.40	84.73	64.89	0.20
ORI-22/B	13.54	8.33	0.08	0.04	0.69	0.17	83.82	60.35	0.10
ORI-16/B	7.142	4.93	0.05	0.04	0.84	0.08	84.84	53.84	0.03
ORI-14/B	14.78	11.74	0.10	0.04	0.59	0.20	85.39	61.32	0.14
ORI-15/B	14.07	9.36	0.09	0.06	0.55	0.16	80.70	60.42	0.12
***LSD* Value**	3.0		0.05		0.04		1.31		0.01

***ΔOA**=Differences between osmotic potential at full turgor (ψs) of non stressed (W_1) and stressed plants (W_2)

Discussion

Among different leaf hydraulic traits, g_s showed highest relationship with a_y under irrigated regime. Fishscher et al. (1998) also found strong relationship of grain yield with g_s and concluded that g_s should be further investigated as potential indirect selection criterion for better yield. A probable explanation of this relationship could be that high gs was an indication of enhanced transpiration; it resulted in higher uptake of water and mineral nutrients resulting in better growth and yield. Masle et al. (1992) reported the positive correlation between transpiration and leaf mineral contents. It was also shown that potassium is one of the most important contributing elements to this relationship. Some studies has also suggested strong correlation of gs with canopy temperature (Lu et al., 1994; Fischer et al., 1998). These studies indicated that selection for higher yields in irrigated crops imposes indirect selection pressures for high g_s that lowers leaf temperature and appears to reduce deleterious effects of heat stress on critical flowering and fruiting stages, thus resulting in higher crop yields.

Under drought stress, OA showed highest positive relationship with a_y reflecting that yield under drought stress could be more explained by the OA. Similar types of judgments have also been made in different species. Moinuddin and Chopra (2004) divided chick pea cultivars into two groups on the basis of OA and found that high OA cultivars proved significantly superior to low OA ones in seed yield and most of other parameters. The yield benefit was 26 and 48% at moderate and severemoisture stress levels of the line source. Barary et al . (2002) showed significant negative

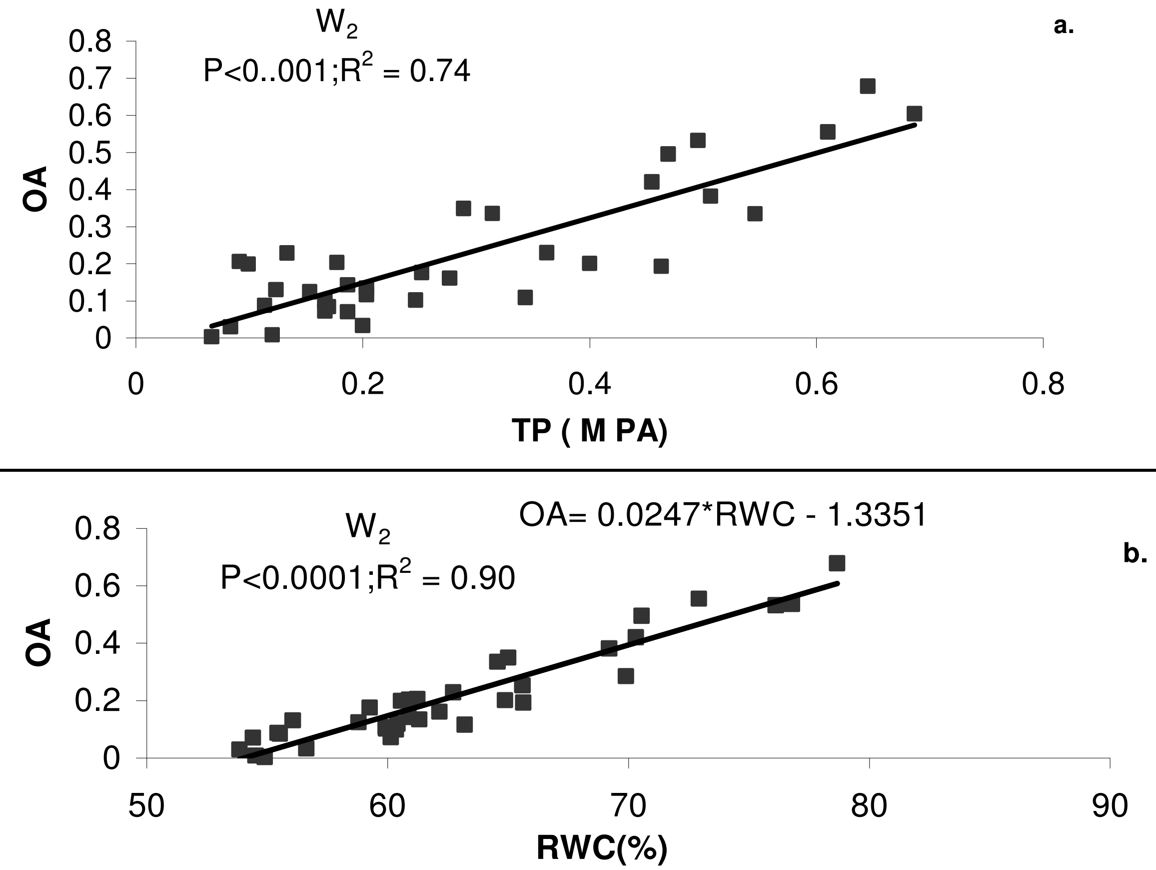

Figure 2. **The response of (a) turgor pressure (TP MPa) (b) Reltaive water contents (RWC %) to the variation in osmotic adjustment (OA) across all genotypes and drought (W_2) treatments.**

correlation between number of sterile tillers and OA, thus showing positive effect on yield. The positive impact on a_y may be attributed to the involvement of OA in bringing more water extraction from soil and thus maintaining turgor under stress regime.

This was indicated from highly significant positive relationship between OA and RWC and absence of negative relationship between g_s and RWC. Similarly, TP also showed significant relationship with OA, suggesting role of OA in maintaining turgor under drought stress. Among the traits allowing the evaluation of plant water-status, leaf relative water contents (RWC), gives best idea of the level of the water deficit in the plant at a specific time-point. As RWC is related to cell volume, when it is measured on the leaf, it may closely reflect the balance between water supply to the leaf and transpiration rate (Sinclair and Ludlow 1985). The positive relationship between OA and RWC in present studies is in corroborative with Lafitte, 2002. Presence of highly strong relationship between OA and RWC also indicated that RWC contents may be utilized as physiological marker of OA. RWC contents also showed highly significant relationship with achene yield.

The performance of genotypes for achene yield and leaf water traits was not consistent in both regimes. AMES-10107 showed highest achene yield accompanied by the higher stomtal conductance in non stress regime. However under drought stress, AMES-10103 showed highest achene yield. This genotype also showed highest OA, RWC and TP. All genotypes showed significant differences for OA. This is concurrent with previous finding showing higher intraspecific variations for OA in sunflower (Conroy et al., 1988; Chimenti and Hall, 1993, 1994). Foreign-origin genotypes showed higher OA (0.30 M Pa) in contrast to local lines (0.15MPa). High value of OA may be due to presence of more diversity with in these genotypes. However, overall mean of OA shown by all genotypes was 0.22 M Pa. Over all mean of OA expressed by all these genotypes was found slightly high in relation to previous report in sunflower. Chimenti et al. (2002) reported only 0.2MPa in high OA adjustment families of sunflower. However these values were found lower to those reported in rice (0.72MPa) (Babu et al., 1999).

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