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IDENTIFYING DROUGHT TOLERANT TRAITS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) GENOTYPES: WATER MINING AND WATER USE EFFICIENCY

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ABSTRACT

Sunflower is predominantly grown in rainfed conditions where water limitation is the most prominent constraint for productivity. This necessitates development of drought tolerant lines to sustain productivity under rainfed condition. For a comprehensive importance in growth and productivity, introgressing traits associated with water mining (roots) and water use efficiency (WUE) have great relevance. To identify superior sunflower lines for these traits, 71 sunflower genotypes were evaluated in temporary cement root structures and in field. The genotypes showed significant variability in root traits, $\Delta^{13}C$, total leaf area (TLA), specific leaf area (SLA), total dry matter (TDM) and yield parameters like grain yield, harvest index (HI) and oil content enabling identification of promising trait as donor genotypes. All the genotypes were further classified as low and high types to select the contrasting genotypes based on root traits, $\Delta^{13}C$ and yield parameters. Genotypes viz., NDR-4, IB-97, IB-47, IB-79, IB-60, BGR-135-2, CMS-336A, CMS-597A, CMS-335A, RHA-272-I, RHA-275 and RHA-297 performed better to root traits, TDM and grain yield.

Key words: Carbon isotope discrimination, harvest index, leaf area, root traits, specific leaf area, total dry matter, water mining, water use efficiency.

INTRODUCTION

Among abiotic stresses, water stress is one the major limitations to crop yields worldwide and global climate change scenarios suggest a future increase in the risk of drought (IPCC 2001). Drought alone reduces 15-50% of the yield. Breeding crop varieties for improved water use efficiency is, therefore, of great importance (Condon *et al.,* 2004). One difficulty in obtaining drought tolerant varieties is the unequivocal evaluation of plant responses to water deficits. Thus, the intensity of the soil-water deficit depends largely on a genotype's leaf area and root development. Wery *et al.,* (1997) evaluated sunflower (*Helianthus annuus* L.) genotypes for their drought tolerance by withholding irrigation, and found that their response was solely associated with leaf area which is directly related to soil-water depletion rates. Evolving varieties/hybrids having drought tolerance is the effective and cheapest strategy to overcome the ill effect. Enhanced drought tolerance is achieved by incorporating some traits that help the plant to cope with drought stress effectively (Chaves *et al.,* 1991).Several strategies are considered in breeding of drought stress in a crop species. This includes developing early varieties/hybrids escaping drought especially in late season or terminal drought, modification of certain plant traits that lead towards drought resistance and introduction of drought tolerant traits (transpiration efficiency (TE), specific leaf area (SLA), SPAD chlorophyll content (SCMR), drought susceptibility index (DSI) and harvest index (HI) associated with yield. Root characteristics such as root length, root biomass and lateral root density are important in determining the water extraction capability from soil (Blum, 1988). Developing drought tolerance in sunflower through a focused trait based breeding has been receiving great emphasis in recent years. Success of such efforts depend on the identification of promising traits donor lines and their validation under water limited conditions. From this context, assessing the genetic variability in relevant traits such as root traits (water mining) and WUE assumes importance. With this background, a research was carried out for screening the sunflower genotypes for better root system and high water use efficiency.

MATERIALS AND METHODS

Selection of seed material

A set of 71 sunflower accessions comprising of cytoplasmic male sterile lines (CMS lines / A lines) and fertility restorer lines (RHA lines), inbreds and few hybrids were selected for screening drought tolerant traits such as roots characters, water use efficiency

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(WUE) and other physiological traits and the results were compared with two checks, KBSH-41 and KBSH-44, sunflower hybrids released from University of Agricultural Sciences, Bangalore for higher seed yield.

Construction of temporary cement root study structures

Since it is extremely difficult to extract the complete roots without damaging in the normal field condition, plants were raised in temporary cement root structures with the dimension of 1.2m height, 3m wide and 18m long were built using cement blocks on the ground surface. Red soil was filled completely up to a height of 105cm and compacted by watering the soil completely for 15 days, to mimic the natural condition that prevailed in the actual field. Top layer of the soil was mixed with farm yard manure. After compacting the soil, levelling was done. The seeds of 71 sunflower entries were sown separately in rows in two replications. Sowing was done at the recommended spacing of 30 cm between the plants and 60 cm between the rows and for each genotype six plants were maintained per row. Before sowing, 50% of the recommended nitrogen in the form of urea and entire dose of phosphorus and potassium fertilizers (60:90:60 kg/ha) in the form of super phosphate and murate of potash, respectively were applied as a basal dose and the remaining 50% of the nitrogen was top dressed at star bud stage. Once the seedlings emerged, thinning was carried out and one seedling per hill was maintained. On 30th day after sowing (DAS), earthing up was done with the addition of remaining 50% nitrogen in the form of urea. Regular watering and plant protection measures were taken up as and when required to raise healthy plants.

Observations recorded during active vegetative growth

Observations were recorded on 50% flowering, plant height, number of leaves per plant, total leaf area and chlorophyll content was measured by SPAD chlorophyll meter reading (SCMR) during the vegetative growth of the plant (60 DAS). Specific leaf area, an indication of leaf area per unit leaf weight and total leaf area were measured in the fully expanded leaf more specifically, fifth leaf from the top. After measuring the maximum length and breadth of the leaf, the leaves were kept in an oven at 80^{o}C for 3 to 4 days, the dry weight was recorded. Then the specific leaf area (SLA) was calculated using the following formula.

SLA (cm^2/g) = Total leaf area (cm^2/pl)/ Leaf dry weight (g/pl). Estimation of carbon isotope discrimination (Δ^{13}C) is a surrogate method for the measurement of water use efficiency as the plants discriminate against heavy isotope of carbon (Δ^{13}C) during photosynthesis. A small quantity of dried leaf sample was collected from the field and dried overnight at 80^{o}C, powdered and ground into a fine powder and is used for the analysis of Δ^{13}C using Isotope Ratio Mass Spectrometer (IRMS).

Harvesting of plants

When the plants were in flowering stage, the walls of temporary root structures were dismantled and with lot of water pressure, the soil surrounding the root system was carefully removed. Once the plants were harvested from the root structures, roots, leaves and stem were separated and measured them separately. Accordingly, in the roots, several root traits like root length, root volume by water displacement method (Pinkas *et al.,* 1964) *etc*., were measured. Similarly, all the leaves were harvested from the plant and the total leaf area was measured. Once the primary observations were made, all the plant parts (root, stem, leaves and flower heads) were put for oven drying and after their complete dryness, the dry weight of flower head and leaf dry weight were measured and finally arrived at the total dry matter of individual plant.

Simultaneously plants were also grown in the field and all the physiological parameters were recorded similar to the observations recorded for plants grown in cement root structures in addition to grain yield and oil content.

RESULTS AND DISCUSSION

Genetic variability for growth parameters

There was a wide genetic variability observed for all the traits recorded. Days to 50% flowering is an important trait that is used for the breeding in order to synchronize the flowering time and to know the duration of the crop growth after flowering (Alza and Fernandez, 1997). In the present study the range for days to 50% flowering was from 44 to 71 days with a mean of 63 in root study structure (Table 1) and 43 to 69 days with a mean of 60 days in field (Table 2). Only one entry, CMS-438A reached 50%

flowering early by taking 44 days in both temporary cement root study structure and in field and some of the CMS entries like CMS-17A, CMS-336A and CMS-62A took more number of days to reach 50% flowering (70 days) in structure and 15D-R, EC-512674 and M-1013 in field.

Table 1: Range and mean values for the different parameters of the plants grown in temporary cement root study structure

Traits	Range	Mean	CV (%)	SE m±	CD=P(0.05)
Days to 50% flowering	44.00-71.00	63.11	4.19	1.53	4.27
SCMR	28.72-42.88	36.25	9.48	2.01	5.58
Plant height (cm/plant)	82.00-182.00	124.41	7.98	5.73	15.89
Number of leaves/plant	16.00-33.00	23.50	9.76	1.32	3.70
Total leaf area (cm^2/plant)	2160.60-15767.08	5379.73	20.98	643.01	1797.92
Specific leaf area (cm^2/g)	82.37-475.44	228.45	22.04	32.69	90.61
Total dry matter (g/plant)	48.08-217.38	107.71	16.71	7.05	19.53
Root Length (cm/plant)	23.83-53.17	38.73	9.74	2.25	6.22
Root Volume (cc/plant)	33.33-253.00	97.65	21.29	12.00	33.26
Root dry weight (g/plant)	3.52-59.79	20.42	19.41	2.22	6.14
Root to shoot ratio	0.05-0.51	0.21	20.48	0.04	0.10
$\Delta^{13}C$ (‰)	19.70-22.75	21.39	3.77	0.47	1.29

There was a significant variability observed for SCMR readings among the 71 genotypes in both temporary cement root study structure and field. Both the checks KBSH-41 and KBSH-44 showed more SCMR values and it was less in CMS-lines and R-lines compared to inbreds and hybrids (Table 3 & 4). A significant genetic variability was found for plant height and total leaf area also.

Table 2: Range and mean values for the growth and yield parameters of 71 genotypes grown in field

Traits	Range	Mean	CV (%)	SE m±	CD=P(0.05)
Days to 50% flowering	43.00-69.00	60.85	4.32	1.52	4.20
SCMR	28.70-44.18	37.57	9.26	2.03	5.61
Plant height (cm/plant)	88.75-215.00	136.50	9.47	7.47	20.6
Number of leaves/plant	17.00-29.00	22.11	10.31	1.32	3.64
Total leaf area (cm^2/plant)	1355.25-16576.30	5358.37	17.52	541.97	1502.2
Specific leaf area (cm^2/g)	33.12-548.67	204.84	23.19	27.96	77.5
Total dry weight (g/plant)	41.08-191.40	93.54	24.11	7.78	20.5
$\Delta^{13}C$ (‰)	20.38-24.14	22.30	4.13	0.53	1.48
Grain yield (g/plant)	8.84-96.42	34.56	13.68	2.84	7.8
Oil content (%)	31.89-40.33	36.71	5.31	1.12	3.1
Harvest index	0.22-0.50	0.36	19.47	0.05	0.12

Leaf traits play an important role in carbon assimilation, water relations and energy balance. Among the selected 71 genotypes total leaf area TLA and SLA, were measured. Total leaf area is more in CMS-lines and inbreds but less in R-lines. The variability for SLA was observed and the variation in the SLA can be correlated with the drought tolerance. Genotype with lower SLA (due to thicker and/or denser leaves) contributes to long leaf survival, nutrient retention, and protection from desiccation (Mooney and Dunn, 1970). Specific leaf area is low in R-lines in both structure and field because of thick leaves and less leaf area. Total leaf area and SLA represent two facets of a functional strategy associated with low water and/or nutrient availability (Mooney and Dunn 1970; Cunningham *et al.,* 1999). Similar kind of variability among the genotypes was observed in other system like cotton (Ehab, 2006) and rice (Ayappa, 2004) and the association of TLA and SLA with the drought tolerant traits like water use efficiency (WUE), and relative water content (RWC) was reported.The association could be due to an adaptive strategy of the plant to the biotic and abiotic stress. In the current study TLA and TDM showed positive and significant correlation. This indicates that leaf area available

Table 3: Range and mean values for growth parameters in hybrids, CMS-lines, R-lines and inbreeds in plants grown in temporary cement root study structure

Traits	Hybrids		CMS-lines		R-lines		Inbreds	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Days to 50% flowering	63.7	60.7-66.3	62.7	44.0-70.7	63.4	55.0-69.0	62.97	47.7-69.0
SCMR	40.5	37.6-42.8	35.7	29.9-39.1	35.8	28.7-47.5	37.2	33.5-42.4
Plant height (cm/pl)	149.7	132-162	130.6	82.3-167.3	117.9	87.2-181.7	125.4	90.0-145.9
Number of leaves/pl	29.78	26.7-32.7	23.8	18.0-29.0	22.2	15.7-29.3	24.4	17.0-30.0
Total leaf area (cm^2/pl)	9714.1	2300.0-15767.1	5841.4	3302.6-10826.4	4873.3	2160-9554.4	5122.1	2731.4-10974.6
Specific leaf area (cm^2/pl)	266.7	109.1-375.6	234.3	100.9-475.4	220.0	82.4-468.2	234.3	124.9-436.4
Total dry matter (g/pl)	168.1	89.8-217.4	111.9	58.3-178.0	106.7	48.1-184.3	96.9	52.5-169.7
Root length (cm/pl)	43.1	40.3-44.8	36.9	23.8-51.7	38.6	30.8-53.2	39.8	26.7-51.7
Root volume (cc/pl)	148.8	118.7-208.3	108.8	41.6-253.0	87.3	43.2-179.2	96.6	33.3-190.5
Root dry weight (g/pl)	34.6	19.7-44.7	24.6	7.1-59.8	18.8	3.5-55.5	15.7	5.4-47.4
Root to shoot ratio	0.26	0.25-0.28	0.27	0.09-0.51	0.20	0.06-0.45	0.19	0.05-0.40
$\Delta^{13}C$ (‰)	21.6	20.7-22.1	21.5	20.5-22.2	21.3	19.7-22.7	21.4	20.4-22.3

for synthesis of photo assimilates contributes to the total dry matter. Total dry matter contributing parameters are total leaf dry weight, stem dry weight, head dry weight and root dry weight in temporary cement root study structure. Total dry matter included total leaf dry weight, stem dry weight and head dry weight in field grown plants. Genotypes with high TDM are KBSH-41, KBSH-44, BGR-135-2 and NDR-4. High production of biomass in any plants is strongly associated with water use and water use efficiency (Paussioura, 1986). Though several approaches are available to assess and quantify the water use efficiency one of that is carbon isotope discrimination technique. Earlier research workers have shown that, the plants are known to discriminate heavy isotope of carbon during photosynthesis (O' leary, 1981). Thus, one can resort to this approach to assess the genetic variability for water use efficiency (Farquahar *et al.,* 1999, Hubick and Farquahar, 1989). There is a genotypic variability for $\Delta^{13}C$ within the genotypes. RHA-23 and RHA-275 are low $\Delta^{13}C$ types and 15D-RHA is high $\Delta^{13}C$ type in both temporary cement root study structure and field. Low $\Delta^{13}C$ will have better photosynthetic mechanism. Thus selection for low $\Delta^{13}C$ will have better photosynthetic mechanism. Thus selection for low $\Delta^{13}C$ is an ideal approach for selection for better water use efficiency genotypes.

Table 4: Range and mean values for all the parameters in hybrids, CMS-lines, R-lines and inbreds in field

Traits	Hybrids		CMS-lines		R-lines		Inbreds	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Days to 50% flowering	60.6	58.0-63.0	59.6	42.7-68.0	60.5	55.0-69.0	62.5	58.0-69.0
SCMR	39.0	35.1-41.6	36.5	28.7-46.6	37.5	31.4-43.9	39.56	31.3-50.1
Number of leaves/pl	25.3	20.0-29.0	21.5	16.7-29.0	21.5	16.7-26.0	23.1	19.3-28.7
Plant height (cm/pl)	187.0	135-215.0	139.7	119.3-167.7	131.8	88.7-177.7	133.3	101.8-173.8
Total leaf area (cm^2/pl)	11496.4	2730.8-16576.3	5841.9	2197.0-13513.5	4092.8	1355.3-11743.8	5988.1	2200.6-155548.0
Oil yield (kg/ha)	1769.4	1491.5-1984.6	600.7	265.4-987.7	571.5	173.6-1356.9	674.3	376.6-873.5
Oil content (%)	37.6	36.5-39.3	36.3	33.9-38.4	36.9	31.9-40.3	36.8	31.9-39.0
Grain yield (g/pl)	86.6	70.3-96.4	32.8	15.0-54.3	29.6	8.8-66.3	35.8	21.3-50.0
Specific leaf area (cm^2/g)	189.3	75.4-288.7	222.3	33.1-543.1	172.5	54.0-412.2	232.4	6.3-548.7
Total dry matter (g/pl)	174.9	145.1-191.4	87.2	52.6-132.0	90.3	41.1-172.5	91.1	52.7-137.7
Harvest index	0.49	0.48-0.50	0.38	0.29-0.41	0.31	0.22-0.38	0.41	0.36-0.45
$\Delta^{13}C$ (‰)	22.5	22.3-22.6	22.4	21.5-23.8	22.2	21.0-24.1	22.3	20.4-23.4

Genetic variability in root traits

Genetic variability for root has been reported in different systems by several workers. Recent experiments conducted at our own lab have revealed a significant variability for root traits in recombinant inbred lines of Rice (Ayyappa, 2004) and mulberry germplasm accessions (Sudhakar, 2005). The genotypes which have high root dry weight have more total dry matter *viz.,* NDR-7, CMS-17A, CMS-135A and IB-56 were on par with checks. This indicates that genotypes which have more root dry weight will draw more water and contribute to more TDM. Changes in root to shoot ratios in response to environmental demand may provide an opportunity to exploit the observed variability to improve production for a wide range of growth conditions by altering the root development and function independent of shoot development (Rauf and Sadaqat *et al.,* 2008a). Difference in root to shoot ratio between genotypes were observed in our experiment, probably due to difference in environment and genetic characteristics. Root to shoot ratio was more in CMS-lines and less in inbred.

Table 5: Classification of sunflower genotypes as low and high types based on root dry weight, TDM and $\Delta^{13}C$ in temporary cement root structure

Root dry weight (g/plant)				Total dry matter (g/plant)				$\Delta^{13}C$(‰)			
Low types		High types		Low types		High types		Low types		High types	
CMS-10A	7.07	CMS-17A	59.79	RHA-275	63.20	KBSH-44	217.38	IB-97	20.78	GKVK-2	22.75
LTRR-83-273	6.67	NDR-7	55.45	PR-1	62.33	KBSH-41	197.06	DRSF-108	20.72	15D-RHA	22.67
EC-512674	6.54	NDR-4	47.94	EC-512674	61.91	NDR-7	184.34	EC-512674	20.63	IB-79	22.28
RHA-278	5.89	CMS-275A	47.68	RHA-297	61.63	CMS-17A	178.03	RHA-278	20.59	CMS207A	22.22
PR-1	5.81	IB-56	47.34	EC-512686	58.65	CMS-135A	171.21	CMS-302A	20.52	NDR-7	22.18
IB-35	5.50	CMS-135A	45.27	M-1013	58.47	IB-56	169.74	RHA-23	20.38	ACS-02/ DRM-34-2	22.18
RHA-275	5.45	KBSH-44	44.68	CMS-335A	58.33	NDR-4	168.40	EX-512686	20.35	GIGI/100× 9/M-55-1	22.15
IB-84	5.41	15D-RHA	42.80	CMS-7-IB	54.23	RCR-60-P	161.46	PR-1	20.28	KBSH-41	22.13
6D-5-3-5	4.00	RHA-296-I	42.15	IB-35	52.52	CMS-336A	157.76	RHA-297	19.85	CMS-17A	22.12
RHA-297	3.52	RES-834-1	41.86	6D-5-3-5	48.08	BGR-135-2	156.54	RHA-275	19.70	RHA-16	22.07

Genetic variability in yield attributing characters

Like plants grown in temporary cement root study structure, similar $\Delta^{13}C$ values were observed in CMS-lines, R-lines and inbreds. There was no variability between the genotypes. KBSH-41 (93.05) and KBSH-44 (96.42) showed more grain yield, whereas the mean values were similar in CMS-lines (32.28), R-lines (29.63), and inbreds (35.87). The following genotypes which had high TDM also showed more grain yield *viz.,* KBSH-44, KBSH-41, DRSF-108, 136-R, NDR-4, RHA-23 and BGR-135-2. This indicates genotypes capable of accumulating more biomass were also capable of translocating higher amount of biomass for seed development.

The oil content in sunflower achenes mainly depends on the proportions of husk and kernel (Fick, 1978). According to Alexander (1963), the achene oil content is subjected to a complex polygenic control, with a strong genetic effect. In current study oil content of sunflower varies from 31.89% to 40.33%. Mean of oil content is 36% in CMS-lines, R-lines and inbreds. Even oil content was same in CMS-lines, R-lines and inbreds and oil yield was less in R-lines. ACS-02/DRM-34-2, GKVK-2 and CMS-10A have low oil content and RHA-16 and RHA-95-C-2 have high oil content.

Harvest index

Harvest index (HI) was more in inbreds, than in CMS-lines and R-lines. More preferential partitioning of dry matter to seed results in high HI. Remobilization of photosynthates from vegetative plant parts to the seed also results in high HI and seed yield..

Classification of genotypes as low and high types

Standard normal distribution was made to identify the contrasting genotypes based on

Table 6: Classification of sunflower genotypes as low and high types based on TDM, $\Delta^{13}C$, grain yield and oil content in plants grown in field

Total dry matter (g/plant)				$\Delta^{13}C$ (‰)				Grain yield (g/plant)				Oil content (%)			
Low types		High types		Low types		High types		Low types		High types		Low types		High types	
CMS-62A	62.43	KBSH-44	191.40	CMS-135A	21.50	15D-R	24.14	RHA-589	15.53	KBSH-44	96.42	IB-79	35.05	RHA-16	40.33
CMS-135A	62.33	KBSH-41	188.44	RHA95-C-2	21.44	NDOL-2	23.88	CMS-62A	15.03	KBSH-41	93.05	CMS-343A	34.94	RHA95-C-2	40.33
M-1013	61.54	RES-834-1	172.50	RHA-23	21.30	CMS-62A	23.81	RHA-856	14.58	DRSF-108	70.34	EX-512686	34.91	NDOL-2	39.97
RHA-278	59.88	BGR-135-2	166.75	RHA-275	21.28	RHA-856	23.71	GKVK-2	14.21	136-R	66.26	RCR-60-P	34.88	RHA6D-1	39.30
RHA-589	57.32	DRSF-108	145.07	RHA-272	21.26	IB-97	23.39	RHA-272	13.02	NDR-4	59.76	RHA-272	34.86	KBSH-41	39.28
EC-512674	52.68	M-307-2	137.70	RHA-348	21.21	IB-20	23.21	RHA-278	12.37	RHA-23	58.99	IMS-265-A	34.73	IB-35	39.00
CMS-10A	52.60	136R	136.90	RHA-278	21.07	CMS-234A	23.20	RHA-348	11.65	BGR-135-2	55.06	NDR-7	34.59	RHA-298	38.97
RHA-298	52.48	CMS-851A	132.00	RHA-298	21.00	CMS-335A	23.19	RHA-298	11.45	CMS-336A	54.27	CMS-10A	33.89	IB-56	38.72
6D-5-3-5	47.80	NDR-4	131.99	IB-84	20.89	ACS-02/ DRM-34-2	23.19	RHA-275	10.89	NDOL-2	53.32	GKVK-2	31.90	NDR-4	38.61
RHA-275	41.08	RHA-23	131.62	IB-47	20.38	6D-5-3-5	23.15	RHA-6D-1	8.84	PR-1	51.91	ACS-02/ DRM 34-2	31.89	RHA-17	38.49

root traits, TDM and Δ^{13}C in plants grown in cement root structure and grain yield, oil content and Δ^{13}C in field grown plants. Further they were classified as low and high types to select the promising sunflower entries for drought related trait (Table 5 & 6). A Few genotypes *viz.,* NDR-4, IB-97, IB-47, IB-79, IB-60, BGR-135-2, CMS-336A, CMS-597A, CMS-335A, RHA-272-I, RHA-275 and RHA-297 consistently showed the better performance with respect to root traits, TDM and grain yield.

REFERENCES

Alexander, D.E. (1963) The lisenko method of increasing oil content of the sunflower. *Crop science* **3**: 279-280.

Alza, J.O. and Fernandez-Martinez, J. M. (1997) Genetic analysis of yield and related traits in sunflower (*Helianthus annuus L.*) in dry land and irrigated environments. *Euphytica.* **95:** 243–251.

Ayyappa, R. (2004) Identification of molecular markers for WUE and associated traits in RILs of rice (*Oryza sativa*). M.Sc thesis, *University of Agricultural Sciences,* Bangalore, India.

Blum, A. (1988) Plant Breeding For Stress Environment. CRC Press Inc. Boca Raton, Florida.

Chaves, M.M. (1991) Effects of water deficits on carbon assimilation. *Journal of experimental Botany* **42**:1-16.

Condon, A.G., Richards, R.A., Rebetzke, G.J. and Farquhar, G. D. (2004) Breeding for high water-use efficiency. . *Journal of experimental Botany* **55:** 2447-2460.

Cunningham, S.A., Summerhayes, B. and Westoby, M. (1999) Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecological Monographs* **69**:569-588.

Ehab Abou Kheir (2006) Assessment of genetic variability in water use efficiency, root traits and intrinsic tolerance among the cotton hybrids and cultivars. M.Sc (Ag) thesis *University of Agricultural Sciences,* Bangalore.

Farquhar, G.D., Ehleringer, J.R. and Hubick, K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40:** 503–537.

Fick, G.N. (1978) Sunflower breeding and genetics. In: J.F. Carter (Ed.), Sunflower Science and Technology, pp. 279–337. *Agronomy* 19. ASA, CSSA and SSSA, Madison, USA.

Hubick, K.T. and Farquhar, G.D. (1989) Carbon isotope discrimination and the ratio of carbon gained to water lost in barley genotypes. *Plant Cell and Environment* **12**: 795-804.

IPCC. (2001) Climate change Synthesis report. A contribution of working group I, II & III to the Third Assessment Report of the intergovernmental panel on climate change. Cambridge University Press, New York

Mooney, H.A. and Dunn, E.L. (1970) Photosynthetic systems of Mediterranean-climate shrubs and trees of California and Chile, *The American Naturalist* **104**: 447-453.

O'leary, M.H. (1981) Carbon isotope fractionation in plants. *Phytochemistry* **20:** 553-567.

Passioura, J.B. (1986) Resistance to drought and salinity: Avenues for improvement. *Australian Journal of Plant Physiology* **13:**191-201.

Pinkas, L.L.H., Teel, M.R. and Swartzendruber. D. (1964) A method of measuring the volume of small root systems. *Agronomy Journal* **56**:90-91.

Rauf, S. and Sadaqat, H.A. (2008a) Identification of physiological traits and genotypes combined to high achene yield in sunflower (*Helianthus annuus L.)* under contrasting water regimes. *Australian Journal of Crop Science* **1(1):** 23-30.

Sudhakar, S. (2005) Development of an oxygen isotope enrichment method for the assessment of root biomass in plants. M.Sc. thesis, *University of Agricultural Sciences*, Bangalore, India.

Wery, J., Griveau, Y., Belhassen, E., Jameau, I. Seriyes, E. and Tardieu, F. (1997) Analyse de la re´ponse de diffe´rentes genoypes de tournesol au de´ficit hydrique. In: C. Riou (ed.), Compte Rendu du Programme Valorisation de la Ressource en Eau, 35-51. INRA, Paris.