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fect of water stress on morpho-physiological traits of sunflower *elianthus annus* L.) genotypes

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Abstract: An investigation was carried out to study the morphological and physiological mechanisms involved in the differential response of the sunflower genotypes to water stress. Water stress was imposed at early (45 to 60 days after sowing) and late (65 to 80 days after sowing) stages of plant growth. Various morophophysiological parameters *viz.* plant height, leaf area, leaf expansion rate, RWC, LWP, leaf membrane integrity, stomatal diffusive resistance and transpiration rate were studied. Among the genotypes, TNAUSUF 205, CO 4 and RHA 272 recorded more plant height, leaf area, leaf expansion rate, RWC and LWP and also recorded low leakage of solutes, less transpiration rate with high stomatal diffusive resistance under both the stress situations. This study has demonstrated the interplay of morphological and physiological adjustment that enabled sunflower to survive under conditions of water stress with less reduction in grain yield.

Key words : Water stress, Leaf area, Leaf expansion rate, Relative water content, Leaf water potential, Leaf membrane integrity, Stomatal diffusive resistance, Transpiration rate.

troduction

Sunflower is one of the important oil ed crops. The global area under this crop nds around 20 m.ha with the total production 21-24 million tonnes. In India, sunflower cultivated in approximately 2.16 m. ha counting for an annual production of 1.32 m.t. ing 1995-96. However, over the years the erage productivity is decreasing quite drastically, ich is mainly attributed to the intermittent iotic stresses, the crop experiences during growing season, such as, extreme water ess, high temperature, salinity etc. It is estimated at due to water stress, there is more than per cent reduction in biomass and seed eld (Uma Shaanker, 1991). To increase the oductivity in a limited land area, the best tion would be to develop efficient plants ich can grow and produce even under adverse vironmental conditions.

Sunflower is considered as a good crop rainfed conditions because of its certain characters, such as, quick recovery in RWC of leaves when moisture is available following a prolonged stress period. However, moisture stress at any stage of growth reduces the seed yield and oil content. Several scientists have studied morphological and physiological characters in relation to seed yield under moisture stress conditions. Lovett and Campbell (1973) have reported that photosynthetic area (leaf) and root shoot ratio were reduced when sunflower plants were subjected to moisture stress at six different stages of plant ontogeny. Survival of tissues under extreme and rapid stress may be modulated by more than one morphophysiological mechanisms, and may be different from governing the recovery of plants subjected to moderate and slow stress levels. Based on this background, an experiment was conducted during 2000-2002 in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore to identify the different morphological and physiological mechanisms involved in drought resistance and reduced yield reduction in sunflower.

Table 1. Effect of water stress on plant height (cm plant^{-1}) of sunflower genotypes

	Early stress			Late stress		
Genotypes	Control	(45-60 DAS)	Mean	Control	(65-80 DAS)	Mean
CO 1	115.7	82.2	98.9	113.3	90.8	102.1
CO 2	117.3	92.3	104.8	119.4	95.6	107.5
CO 3	110.2	91.4	100.8	112.4	97.3	104.9
CO 4	117.2	93.6	105.4	118.3	99.4	108.9
TNHA 2	93.8	65.1	79.5	95.8	73.1	84.5
TNHA 75	108.4	74.3	91.4	106.5	81.6	94.1
TNAUSUF 205	127.8	98.4	113.1	126.4	107.1	116.8
EC 68415	121.4	72.6	97.0	120.7	80.4	100.6
ARM 242	87.5	58.7	73.1	85.6	59.5	72.5
MORDEN	64.5	48.3	56.4	65.3	50.9	58.1
CMS 23B	75.4	58.4	66.9	75.7	52.5	64.1
RHA 272	105.6	80.2	92.9	104.5	87.4	95.9
RHA 6D1	125.3	94.1	109.7	126.2	90.7	102.9
MORDEN*	67.6	52.6	60.1	69.7	53.8	80.01
Mean	102.7	75.8	89.3	102.8	80.0	91.4

	SEd	CD (0.05)	SEd	CD (0.05)
G (Genotypes)	0.81	1.62	0.70	1.41
T (Treatment)	0.30	0.61	0.27	0.53
G x T	1.15	2.30	0.99	1.99

* Represented twice (seeds obtained from diffrent sources)

Materials and Methods

Fourteen sunflower genotypes viz. CO 1, CO 2, CO 3, CO 4, TNHA 2, TNHA 75, TNAUSUF 205, EC 68415, ARM 242, Morden, CMS 234B, RHA 272, RHA 6D1, Morden were used for screening drought resistance based on the morphophysiological characters. The field experiment was laid out in factoial randomized block design with three replications and three treatments viz. control (irrigated), early stress and late stress.

Irrigation and stress imposition

Uniform irrigation was given upto 45 days to all treatments. Thereafter, control plots received irrigation on every fourth day to maintain the normal soil moisture percentage. The irrigation was withheld between 45 and 60 days after sowing (DAS) for early stress treatment, and 65 and 80 DAS for late stress treatment. Once in two days, mineral soil moisture content (MSM) (m^3 m^{-3}) and organic soil moisture content (OSM) (m^3 m^{-3}) were observed by using ML 2 Theta Probe Moisture Meter (Delta T.Sensor type). Ten values were taken for each treatment in each replication and mean values were worked out. When wilting was clearly observed at 10-12 per cent of mineral and organic soil moisture contents, irrigation was resumed to the stressed field.

Observations on various morphological and physiological characters were taken at specific interval of 10 days after imposition of stress. The yield was taken at harvest. Five plants were selected at random from each plot in each treatment and each replication. Morphological characters *viz.* plant height, leaf area and leaf expansion rate, and physiological parameters *viz.* relative water content (RWC), leaf water potential (LWP), leaf membrane integrity, transpiration rate and stomatal diffusive resistance were assessed.

Table 2. Effect of water stress on leaf gas exchange parameters of sunflower genotypes

Genotypes	Leaf diffusive resistance (s cm^{-1})						Transpiration rate (m moles m^{-2} s^{-1})					
	Early stress			Late stress			Early stress			Late stress		
	Control	45-60 DAS	Mean	Control	65-80 DAS	Mean	Control	45-60 DAS	Mean	Control	65-80 DAS	Mean
CO 1	0.83	0.67	0.75	0.70	0.58	0.64	11.93	11.30	11.62	10.01	9.30	9.66
CO 2	0.83	0.52	0.67	0.74	0.49	0.61	11.82	11.00	11.41	9.85	9.20	9.53
CO 3	0.79	0.53	0.66	0.75	0.49	0.62	11.65	10.10	10.88	9.73	8.90	9.32
CO 4	0.84	0.69	0.76	0.76	0.56	0.66	11.92	9.60	10.76	9.91	7.91	8.91
TNHA 2	0.79	0.68	0.74	0.69	0.52	0.61	10.99	9.43	10.21	9.73	8.90	9.32
TNHA 75	0.82	0.69	0.76	0.70	0.57	0.64	11.35	9.61	10.48	9.72	8.90	9.31
TNAUSUF 205	0.83	0.63	0.73	0.72	0.59	0.65	11.46	8.87	10.17	9.65	7.32	8.49
EC68415	0.81	0.49	0.65	0.69	0.42	0.55	11.54	10.90	11.23	9.98	8.80	9.39
ARM 242	0.82	0.49	0.66	0.69	0.41	0.55	11.65	10.90	11.31	9.91	8.90	9.41
MORDEN	0.80	0.54	0.67	0.70	0.41	0.55	11.71	10.60	11.16	10.01	9.10	9.56
CMS 234B	0.79	0.59	0.69	0.70	0.52	0.61	11.82	10.40	11.11	10.02	9.30	9.66
RHA 272	0.79	0.68	0.74	0.69	0.59	0.64	11.81	9.70	10.78	9.95	8.00	8.98
RHA 6D1	0.75	0.54	0.64	0.70	0.58	0.64	11.68	10.10	10.89	9.87	8.10	8.99
MORDEN*	0.79	0.58	0.68	0.69	0.57	0.63	11.73	10.20	10.97	9.86	8.30	9.08
Mean	0.81	0.59	0.70	0.71	0.52	0.62	11.65	10.20	10.93	9.87	8.64	9.26

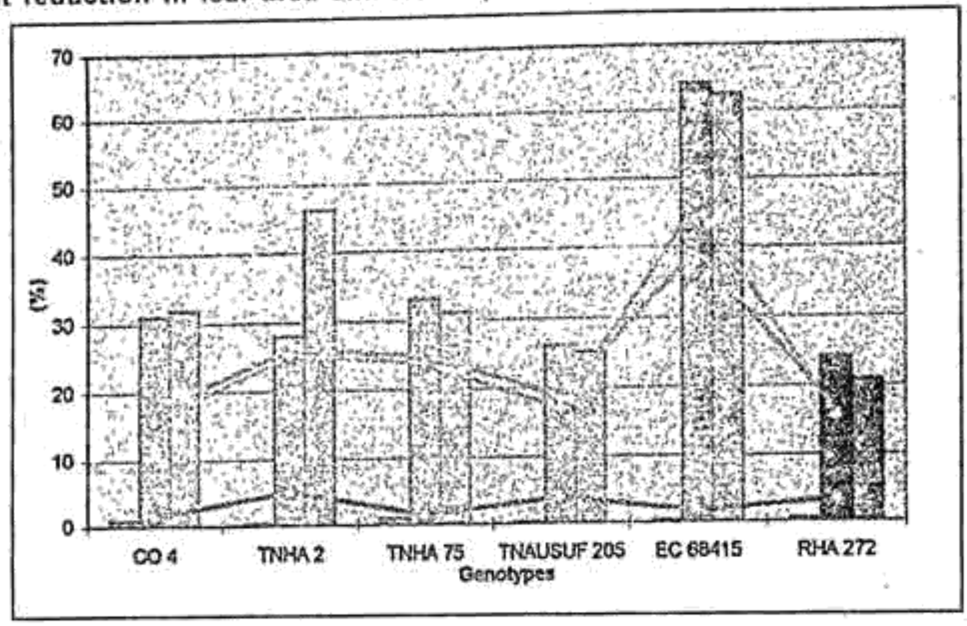
	SEd	CD (0.05)	SEd	CD (0.05)	SEd	CD (0.05)	SEd	CD (0.05)
G	0.0015	0.0031	0.0020	0.0040	0.0232	0.0465	0.1260	0.2527
T	0.0005	0.0012	0.0007	0.0015	0.0087	0.0176	0.0476	0.0955
T x T	0.0022	0.0044	0.0028	0.0056	0.0328	0.0658	0.1782	0.3574

* Represented twice (seeds obtained from different sources)

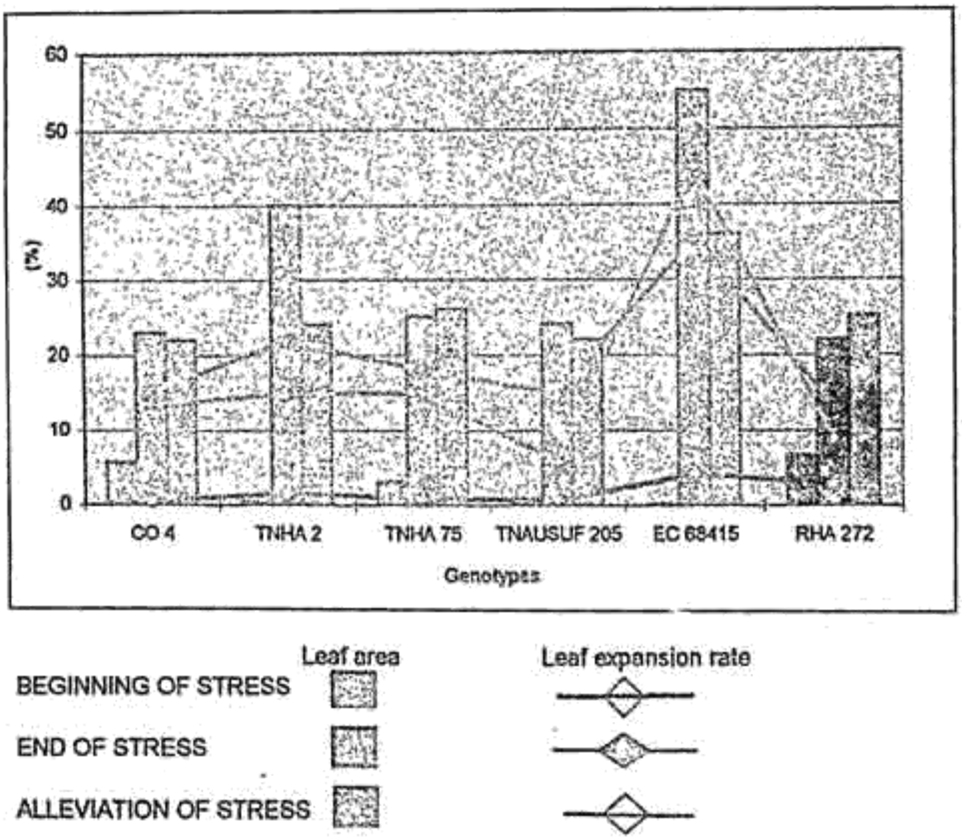
Height of the plant was measured from the base of the shoot to the capitulum and expressed in cm. Leaf area for the whole sampling unit was measured at beginning of stress, at the end of stress and 10 days after alleviation of stress by using Leaf Area Meter (LICOR model 3100) and expressed in cm^2 plant^{-1}. Leaf size of approximately 40 cm^2 was chosen and tagged at the beginning of stress and leaf expansion rate was recorded by measuring the length and breadth of tagged leaves at the beginning, at the end of stress and 10 days after alleviaton of stress. Relative water content (RWC) in leaves was measured as per the method described by Weatherly (1950). Leaf water potential (LWP) was measured by using Pressure chamber apparatus (Soil Moisture Equipment Corp., P.O.Box. No. 30025, Santa Barabara, CA 93105, U.S.A) between 11.00 a.m. to 1.00 p.m. during the stress period and expressed as M Pa. Leaf membrane inte-

Fig.1. Effect of water stress on leaf area and leaf expansion rate of selected sunflower genotypes

Per cent reduction in leaf area and leaf expansion rate under early stress condition

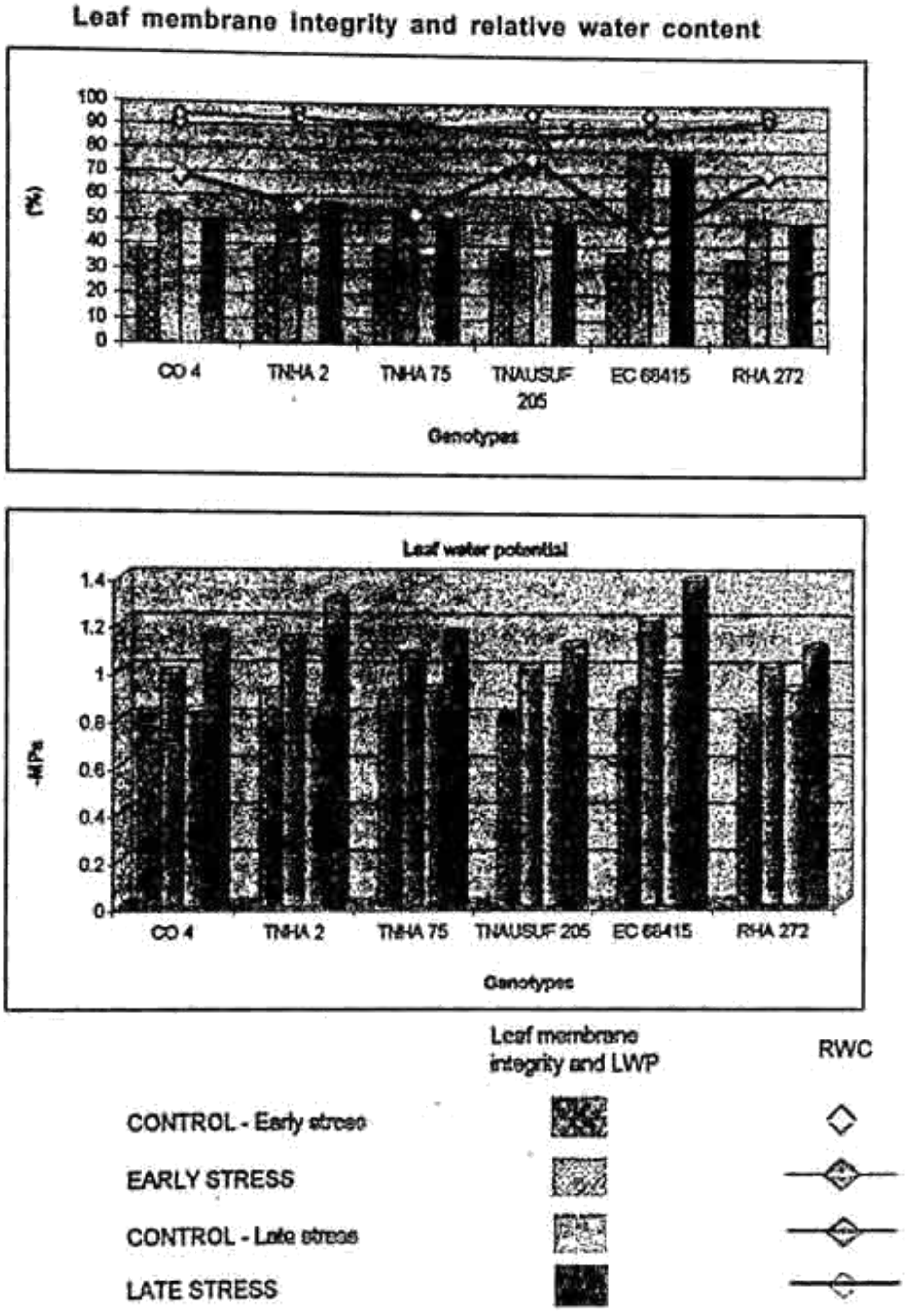


Per cent reduction in leaf area and leaf expansion rate under late stress condition



grity measurement was carried out to determine the extent of membrane damage in tissue subjected to moisture stress. The leakage of solutes was determined following the method of Leopold *et al.* (1981). Transpiration rate and stomatal diffusive resistance were measured using a Steady State Porometer (LICOR 1600) between 11.00 a.m. to 1.00 p.m. on the 10th day of the stress cycle. The measurements were recorded on the topmost fully expanded leaf from five plants. The average values were computed and expressed as m moles $m^{-2}s^{-1}$ (transpiration) and

Fig.2. Effect of water stress on physiological parameters of selected sunflower genotypes



s Cm^{-1} (diffusive resistance). The data were statistically analyzed as per the method of Panse and Sukhatme (1961).

Results and Discussion

Effect of water stress on morphological aspects of sunflower

Under control, plant height of sunflower genotypes varied significantly from 64.5 cm in Morden to 127.8 cm in TNAUSUF 205 (Table 1). Plant height was reduced significantly under both early and late stress conditions. More reduction was observed under early stress compared to late stress condition. The less per cent reduction in plant height was observed in CO 4 (20.1%) followed by TNAUSUF 205 (23.2%) and RHA 272 (24.3%) under early stress situation. Significant differences were observed not only between genotypes but also between treatments. The interactions among genotypes and treatments were also significant.

Changes in leaf area in the beginning, end and after alleviation of stress stages were observed due to water stress and it was depicted in Fig.1. The mean per cent reduction in leaf area at the end of stress was 64.1 and at alleviation of early stress were 62.5. Irrespective of the genotypes, the reduction in leaf area was very high under early stress compared to late stress condition. Among the genotypes, RHA 272 maintained higher leaf area with less per cent reduction of 24.0 at the end of early stress and 22.0 at the end of late stress. This was closely followed by TNHA 2, TNAUSUF 205 and CO 4 under both stress situations. The highest per cent reduction was observed in EC 68415 (64.1) at the end of early stress and 55.0 at the end of late stress. Under both stress conditions, except Morden, CMS 234B and RHA 6D1, the rest of the genotypes showed significant interactions among genotypes and treatments with regard to production of leaf area during the stress period.

The effect of water stress on leaf expansion was monitored during early and late stress conditions, the per cent reduction of expansion was 52.02 compared to respective control. During alleviation of stress period, the rate of leaf expansion was less compared to that under control conditions for all the genotypes. RHA 272 showed its supremacy among the genotypes in recording lowest per cent reduction in leaf expansion (16.6) followed by CO 4 (17.7) and TNAUSUF 205 (18.95) at the end of early stress condition (Fig.2). Under late stress situation, the reduction in mean leaf expansion rate was 26.6 per cent at the end of stress and 25.5 per cent at alleviation of stress. The variety CO 2 was inferior to others at the end of stress, while RHA 272, CO 4, TNAUSUF 205 were superior over others. Statistical parity was observed between treatments in many genotypes at beginning of both stress situations. Significant differences were observed among genotypes and treatments at the end and alleviation of stress.

Irrespective of genotypes, plant height was reduced significantly due to water stress. Minimum reduction in plant height was observed in CO 4 followed by TNAUSUF 205 and RHA 272. Per cent reduction was high in early stress compared to late stress condition. Dezfouli (1994) observed drastic reduction in plant height in safflower and sunflower grown under moisture deficit conditions. The reduction was conspicuous when stress was imposed at early stage of plant growth. Vegetative growth phase is the grand growth phase of any crop and at this stage cells multiply, elongate and enlarge. Water stress at this period causes reduction in cell multiplication and cell wall enlargement (Kramer, 1983) ultimately leading to reduced growth. In the present study, the leaf area during early stress was less (791.8 cm^2 pl^{-1}) compared to late stress (883.6 cm^2 pl^{-1}) situations obviously so because, the early stressed crop was at the peak vegetative stage when the stress was imposed. Among the genotypes, RHA 272 maintained high leaf area at early (24.0% reduction) and late stress (22.0% reduction) situation. The recovery of growth on alleviation of stress was more in late stress situation than early stress. This ability to produce leaf area at post stress condition might be particularly important to yield and this trait was positively correlated with moisture stress tolerance. The expansion of leaf due to stress was monitored during early and late stress periods. Under both the stages stress, the leaf expansion was highly reduced. RHA 272, TNAUSUF 205 and CO 4 showed their supremacy in recording high leaf expansion rate under both the stress situations. Boyer (1970) reported that even small reduction in the leaf water potential caused considerable inhibition of enlargement. Leaf water potential below - 4 bars, completely suppressed leaf enlargement in sunflower. Maintenance of turgor resulted in increase in rates of cell enlargement, which was linearly correlated with drought tolerance. Similar results were reported in sunflower by Keshavamurthy (1995), Mukunda (1996) and Keerthi (2000).

Effect of water stress on physiological aspects of sunflower

An attempt was also made to understand the physiological mechanisms involved in the differential responses of the genotypes to water stress. Parameters, such as, relative water content, leaf water potential, leaf membrane integrity and leaf gas exchange parameters were assessed during different stress periods and discussed below.

The data on RWC of sunflower genotypes under control, early and late stress situations were taken. The RWC was very much reduced in both the stress conditions. Mean value of early stress was 56.6 per cent and late stress was 57.7 per cent compared to control (93.6 per cent). Among the genotypes, TNAUSUF 205 was superior in recording highest relative water content of 72.3 per cent under early and 76.5 per cent (Fig.2) under late stress conditions. Statistical parity was observed between genotypes of EC 68415, ARM 242, and RHA 6D1, and they recorded very low value of RWC under both stress conditions. The results of LWP indicated that there was a gradual reduction in water potential of the leaves under stress periods. The water potential reduced significantly under stress conditions, which accounted for 29.5 per cent in early stress and 24.0 per cent in late stress compared to control condition. The mean LWP in early and late stress conditions was -1.14 and -1.29 MPa compared to their respective control of -0.88 and 0.90 MPa (Fig.2). Under both the conditions, CO 4, TNAUSUF 205 and RHA 272 maintained higher LWP. Significant interaction was observed between genotypes and treatments. The amount of solutes leached into the bathing media (distilled water) from the leaf tissues, previously subjected to live wilting stress, was measured as a reflection of the maintenance of membrane integrity. At 60 per cent loss of fresh weight, EC 68415 showed significantly high per cent leakage of 79.8 in early and 77.8 in late stress conditions while TNAUSUF 205 recorded lowest per cent leakage of 47.5 and 48.2 in early and late stress conditions respectively (Fig.2). Mean value of leaf diffusive resistance of all the genotypes under early stress condition was high (0.598 s cm^{-1}) when compared to the late stress situation. A significant difference was observed among the genotypes and also between the treatments. Among the genotypes, TNHA 75 ranked first by recording 0.698 s cm^{-1} of leaf diffusive resistance followed by CO 4 (0.694 s cm^{-1}) and RHA 272 (0.685 s cm^{-1}) under early stress condition. RHA 272 and TNAUSUF 205 exhibited high value (0.593 s cm^{-1}) in late stress situatiion and were statistically on par with each other (Table 2). Lesser values were in EC 68415 and ARM 242 with regard to leaf diffusive resistance under early and late stress situation. Interaction between genotypes and treatments was significant. The mean value of transpiration rate was 10.2 m moles m^{-2} s^{-1} under early stress condition and 8.64 m moles m^{-2} s^{-1} under late stress condition. TNAUSUF 205 registered lowest transpiration rate of 8.87 and 7.32 m moles m^{-2} s^{-1} under early and late stress situatios respectively followed by CO 4 and TNHA 75. The highest value was observed in CO 1 and CO 2 under both stress conditions. No significant difference was observed in control condition among the genotypes.

The phenotypic expression of a character is the interplay of genetic factors and environmental influence, which readily alter the physiological mechanism of crop plants. Understanding of this altered physiology assumes importance to formulate crop management strategies, which shall be situation specific. Since the function of metabolic process and their rate in plants are related with water content (Barrs and Weatherly, 1962), it would be appropriate to monitor the parameters like relative water content, leaf water potential and leaf membrane integrity in terms of ion-leakage as indicators to be associated with water stress tolerance. Relative water content (RWC) represents the ability of the genotypes to retain tissue water status under water stress and the genotypes retaining more tissue water are expected to perform better. Results on RWC in the present investigations revealed that both early and late stress reduced the RWC in all the genotypes. Among the genotypes, TNAUSUF 205 recorded the highest RWC of 72.3 per cent under early and 76.5 per cent in late stress conditions. Decrease in RWC due to moisture stress in cotton was already reported (Janagoudar *et al.* 1983). In accordance to the results of present investigations, Begg and Turner (1976) and Izzo et al. (1992) have observed a sharp decline in RWC in sunflower when grown under water deficit conditions. Those genotypes that maintained higher per cent RWC recorded lower transpiration rate. Krishnayya and Murthy (1991), Lilley and Fukai (1994) and Chauhan et al. (1996) observed that in addition to reduction in transpiration loss, the tolerant genotypes also maintained higher leaf water status through extraction of more soil water and osmotic adjustment. The leaf water potential (LWP), a dependable indicator of plant water status, was measured under both the stress conditions. The effect of water stress on LWP was very well documented in both the stages. The mean LWP in early and late stress conditions was -1.14 and -1.29 M Pa compared to their respective control of -0.88 and -0.90 M Pa in sunflower. In the present study, the decrease in LWP noted due to water stress was less in the genotypes of CO 4, TNAUSUF 205 and RHA 272 as compared to other genotypes. This might be due to reduction in transpiration, which is in confirmation with the findings of Rajkumar (2001) in rice. As an index of the ability of species to withstand water stress, the loss of membrane integrity was estimated. At 60 per cent loss of fresh weight, EC 68415 showed significantly higher per cent leakage of 79.8 in early and 77.8 in late stress situations. Increase in per cent ion-leakage due to water stress was also reported by Izzo *et al.* (1993). This increase was due to crystallization of cellular components, which in sequence damaged the cellular structures (Ingram and Bartles, 1996). However, when the performance of genotyp was taken into account, results revealed th among the genotypes, TNAUSUF 205 followe by RHA 272 and CO 4 showed less per ce ion-leakage than EC 68415 in both the stre situations. This might be due to stable ce membrane even under stress condition. Stabl cell membrane that remains functional durin water stress, which appears central mechanis of adaptation to high moisture stress and foun related to drought tolerance (Sullivan and Ros 1979; Raison *et al.* 1980). Data on leaf diffusi resistance revealed that the genotypes particular TNHA 75, CO 4 and RHA 272 recorded high diffusive resistance in both the stress situato Higher diffusive resistance in response to wa stress is a characteristic behaviour of droug tolerant genotypes. It is well - established f that the decrease in stomatal diffusive resistan and turgidity loss due to the moisture stre causes a reduction in water loss (Pallas *et a* 1979. This finding provided a valid suppo for the result obtained in the present study In the present study, the genotypes RHA 272, TNAUSUF 205, and CO 4 recorded lower transpiration rate on acount of their highest diffusive resistance. Thus, these genotypes might have reduced the water loss. Transpiration rate was more in irrigated crop than in the crop grown under moisture stress condition. Transpiration rate was lowered due to restricted moisture availability and enhanced stomatal resistance, which induced stomatal closure (Tan *et al.* 1981). Balasubramaniam and Maheswari 1990) and Srivastava *et al.* (1996) also observed similar results in sunflower.

Water stress reduced the total grain yield significantly over control in all the genotypes. The decrease was 30.7 per cent under early stress and 23.7 per cent in late stress. Higher reduction in yield was observed in EC 68415 (43.75%), ARM 242 (41.03%) and CO 1 (38.0%) under early stress situation. Less reduction of 20.0, 21.0, 22.0, 22.9 and 25.0 per cent was observed in TNAUSUF 205 (from 1210.6 to 968.4 kg ha^{-1}), CO 4 (from 984.6 to 777.3

; ha^{-1}), RHA 272 (from 975.7 to 751.3 kg $^{-1}$), respectively (Fig.4). The same trend was ticed in late stress condition also.

The water relation parameters define definite ysiological responses at the level of leaf, hich can play an important role in the regulation f crop water status. This study has demonstrated e interplay of morphological and physiological ljustment that enabled sunflower to survive nder conditions of water stress.

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