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EFFECT OF MOISTURE STRESS ON BIOCHEMICAL ATTRIBUTES OF SUNFLOWER GENOTYPES

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ABSTRACT
The experiment entitled "Effect of moisture stress on biochemical attributes of sunflower genotypes" was carried out at student's farm and Department of Crop Physiology, College of Agriculture, PJTSAU, Rajendranagar. Four genotypes of sunflower were screened for their performance under field conditions during *rabi*, 2013-14. Stress assigned to main plots *viz.,* control and water stress and constituted sub plots, four genotypes *viz.,* GMU-337, GMU-437, EC-602063 and DRSF-113. Moisture stress was created for a period of 35 days starting from 30 DAS to 65 DAS and biochemical parameters were studied. Water stress has negatively influenced biochemical parameters such as chlorophyll stability index and membrane stability index, while proline content increased under moisture stress. The chlorophyll stability index was affected by water stress and it has increased up to 55 DAS (90.5) and there after decreased. Membrane stability index significantly varied between control and water stress treatments during the stress period. And membrane stability decreased in stress (65) compared to control (73) at 75 DAS. Stress imposed during flower bud initiation stage resulted in significant difference in proline content between treatments throughout stress period. Maximum proline content was observed in moisture stress treatment (5.9 ì g per g. fresh weight) as compared to control. Based on performance of biochemical traits, out of the four genotypes studied, two genotypes viz., DRSF-113 and EC-602063 were concluded as tolerant to moisture stress and other two genotype viz., GMU-337 and GMU-437 were less tolerant to water stress.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) occupies a prominent place among oilseed crops as it contributes about 12% to the edible oil production. Only 25% of sunflower area is under irrigation and 75% rain fed. Among several biotic and abiotic stresses constraining crop productivity, drought is the most important one. As stress affects different physiological parameters and processes, it causes up to 50% reduction in biomass (Jolliffe, 1986) and more than 50% reduction in yields (Umashaanker, 1991). Drought is the most prominent of all abiotic stresses and it affects well over one third of the soils worldwide. Plants that manage to survive the effects of drought stress show a decrease in fertility, yield and product quality (Monti, 1986).

Hossain *et al.* (2010) reported that the drought had reduced the CSI in sunflower leaves and he observed that the CSI of leaves has reduced gradually with the increase of drought treatments. Oraki *et al.*, in 2012 reported that water deficits significantly increased proline, soluble sugars and chlorophyll b but decreased chlorophyll a, total chlorophyll and grain yield in all sunflower hybrids. Sayed and Gadallah in 2001 studied the plants of sunflower which were salt-stressed and results shown that the discs of salt-stressed plants were more injured and have high membrane leakage than those of unstressed plants.

Ghaffari *et al.* (2012) reported that proline content had a significant positive correlation with plant height, osmotic adjustment and it is an adaptive behavior of plants when they are subjected to different environmental stresses (Oncel *et al.*, 2000).

Sunflower is grown in a number of countries on so-called marginal soils, often in semi-arid conditions where almost every year an abiotic stress. However, of all field crops, sunflower withstands stress conditions. Several traits have been reported to play a role in imparting drought tolerance. The most important physiological parameters among them are biochemical traits like chlorophyll and membrane stability, accumulation of proline, osmoregulation and protecting enzymes from oxidative stress. Each of these traits either helps to maintain plant water status or enable the plant to tolerate stress.

Sunflower has good potential for drought tolerance because of well developed root system and to withstand temporary wilting. Although sunflower is moderately tolerant to water stress yet its area and production is greatly affected by drought. If drought resistant cultivars are developed, sunflower can be grown successfully in areas where water is a limiting factor. The present study was undertaken with an objective to assess the biochemical traits associated with drought tolerance in sunflower genotypes which will help development better genotypes suitable for water deficit areas.

MATERIALS AND METHODS

A research project entitled "Studies on drought tolerance in sunflower genotypes" was conducted with two experiments namely "Drought tolerance of sunflower parental lines at seedling stage" and "Understanding the mechanism of drought tolerance". The first experiment was carried out as a lab experiment, in which thirty nine sunflower genotypes were screened by subjecting them to water stress by treating with PEG (Poly ethylene glycol) solution at different concentrations *viz*., 0.0, -0.2, -0.4, -0.6 and -0.8 MPa. Based on the Germination percentage and seedling growth parameters, sunflower lines DRSF-113 and EC-602063 were selected as tolerant and GMU-337 and GMU-437 were identified as less tolerant to moisture stress. These genotypes were studied in field experiment, and biochemical parameters were observed.

The second experiment was conducted on sandy loam soil at student's farm and Department of Crop Physiology laboratory, College of Agriculture, PJTSAU, Rajendranagar, during late *rabi* 2013-14 in a strip plot design. Water stress imposed from 30-65 DAS of crop for stress plot and control plots were given irrigation as usual. The data recorded was subjected to statistical analysis in a strip plot design by following the standard methods described by Panse and Sukhatme (1978). Critical difference for examining significance was calculated at 5 per cent level of probability.

The laboratory method was described by Kaloyereas (1958) that determine the drought hardiness based on the thermo-stability of chlorophyll pigments when kept in a hot water bath for an hour. The more stable the chlorophyll, the hardier the plant.

$$CSI \quad \frac{\text{OD value of heated sample} \quad 100}{\text{OD value of unheated sample}}$$

Membrane stability index (MSI) was determined according to the method of Premachandra *et al*. (1990) as modified by Sairam (1994) and was calculated by using the following formula.

$$MSI = [1\text{-}C1/C2] \times 100$$

Where C1 and C2 are EC values recorded with EC bridge at 40ºC and 100ºC.

Proline estimation was done by ninhydrin- based colorimetric assay which has been improved by Bates et al. (1973) which is standard method for many laboratories. Principle behind this is at acidic pH, ninhydrin can form a red product with proline and ornithine which can be used for estimation of concentration of amino acids in pure solution. Aqueous sulphosalicylic acid extracts the free proline of plant samples, also causes the precipitation of proteins. Extracted proline reacts with ninhydrin under acidic conditions (pH 1.0) to form the red colour cromophore, whose intensity can be read at 520 nm.

RESULTS AND DISCUSSION

Chlorophyll stability index

The data pertaining to chlorophyll stability index as influenced by moisture stress is given in the table 1. These data indicated that in sunflower genotypes chlorophyll stability index increased up to 55 DAS and there after decreased.

The control and moisture stress treatments have shown significant difference in chlorophyll stability index at 40, 55, 65 and 75 DAS. The maximum CSI of 91 was recorded in control against 85 in the stress at 55 DAS (25 days after imposition of stress). Whereas, the average CSI of 89 and 78 were recorded in control and stress respectively at 40 DAS. The percent reduction of CSI in moisture stress as compared to control was more (54.2%) during 75 DAS and least reduction in CSI was recorded at 55 DAS (6.6%).At 40, 55, 65 and 75 DAS there is significant difference shown among the studied sunflower genotypes. The genotype DRSF-113 recorded highest CSI of 90.5 at 55 DAS.The interaction between genotypes and moisture stress was significant at 40, 55 and 75 DAS. At 40 DAS genotype DRSF-113 in control (91) and DRSF-113, EC-602063 in stress conditions (79) showed superior CSI over remaining less tolerant genotypes and these both genotypes were on par with each other. At 55 DAS highest CSI of 91 was recorded in treatment combination of DRSF-113 and EC-602063 with control and in stress also same genotypes performed well. Similar reduction in the CSI in sunflower leaves due to water stress (Hossain *et al.,* 2010).

Membrane stability index

The data on membrane stability index is given in table no 2. Membrane stability index decreased in stress compared to control. The results pointed out significant variation between control and moisture stress treatments during the stress imposition period i.e. 45, 55 and 65 DAS. At 65 DAS, control treatment exhibited highest membrane stability index (74). Minimum reduction in membrane stability index in moisture stress treatment, was recorded at 75DAS (11%) and maximum

Table 1: Chlorophyll stability Index (CSI) of sunflower genotypes as influenced by moisture stress

Treatments		40 DAS			55 DAS			65 DAS			75 DAS	
	Control	Stress	Mean	Control	Stress	Mean	Control	Stress	Mean	Control	Stress	Mean
GMU-337	86	77	81.5	90	79	84.5	83	72	77.5	51	22	36.5
GMU-437	90	78	84.0	90	83	86.5	81	69	75.0	49	30	39.5
EC-602063	90	79	84.5	91	87	89.0	85	74	79.5	56	23	39.5
DRSF-113	91	79	85.0	91	90	90.5	84	77	80.5	79	34	56.5
Mean	89	78		91	85		83	73	59		27	
CD (1)		0.67			1.31			2.07			0.87	
CD (2)		2.36			1.81			2.86			0.74	
CD (3)		1.73			2.38			NS			1.12	
CD (4)		1.64			2.43			NS			1.30	

CD (1): Comparing means of moisture levels (stress and control), CD (2): Comparing means of genotypes, CD (3): Comparing means of moisture levels (stress and control) at the same genotype CD (4): Comparing means of genotypes at the same moisture level (stress and control)

Table 2: Membrane stability index (EC) of sunflower genotypes as influenced by moisture stress

Treatments	45 DAS			55 DAS			65 DAS			75 DAS		
	Control	Stress	Mean	Control	Stress	Mean	Control	Stress	Mean	Control	Stress	Mean
GMU-337	37	25	31.0	38	28	33.0	71	55	63.0	75	61	68.0
GMU-437	42	28	35.0	41	34	40.5	69	49	59.0	59	63	61.0
EC-602063	48	34	41.0	63	35	49.0	79	66	72.5	77	67	72.0
DRSF-113	42	35	38.5	44	30	37.0	76	64	70.0	79	67	73.0
Mean	42	31		47	33	74		59		73	65	
CD (1)		2.65			0.04			7.42			NS	
CD (2)		1.99			0.10			6.64			NS	
CD (3)		NS			0.15			NS			NS	
CD (4)		NS			0.14			NS			NS	

CD (1): Comparing means of moisture levels (stress & control),CD (2): Comparing means of genotypes,CD (3): Comparing means of moisture levels (stress & control) at the same genotype
CD (4): Comparing means of genotypes at the same moisture level (stress & control)

Table 3: Proline content (μ g per g. fresh weight) of sunflower genotypes as influenced by moisture stress

Treatments	42 DAS			55 DAS			70 DAS		
	Control	Stress	Mean	Control	Stress	Mean	Control	Stress	Mean
GMU-337	1.2	3.3	2.25	3.2	5.1	4.15	4.0	5.6	4.80
GMU-437	0.9	3.3	2.10	3.6	5.5	4.55	3.8	6.1	4.95
EC-602063	2.7	4.0	3.35	4.7	6.5	5.60	5.1	6.5	5.80
DRSF-113	3.3	5.0	4.15	5.3	5.6	5.45	5.3	5.3	5.30
Mean	2.0	3.9		4.2	5.7		4.6	5.9	
CD (1)		0.067			0.085			0.113	
CD (2)		0.115			0.089			0.151	
CD (3)		0.122			0.127			0.24	
CD (4)		0.125			0.138			0.241	

CD (1): Comparing means of moisture levels (stress & control),CD (2): Comparing means of genotypes,CD (3): Comparing means of moisture levels (stress & control) at the same genotype
CD (4): Comparing means of genotypes at the same moisture level (stress & control)

reduction was recorded at 55 DAS (29.8%).Among the genotypes, there is significant difference for membrane stability index at 45, 55 and 65 DAS. EC-602063 recorded maximum membrane stability index (72.5) followed by DRSF-113 (70) at 65 DAS.At 55 DAS, the interaction between genotypes and moisture stress was significant. At this stage, among genotypes EC-602063 under control (63) and in stress (35) exhibited higher membrane stability index. Whereas genotype GMU-337exhibited lowest membrane stability index both in control (38) and moisture stress treatments (28). The low membrane stability values under stress condition may be attributed to reduced membrane permeability. Similar results were observed by Riaz *et al*. (2012).

Proline content

The data on proline content in sunflower genotypes as influenced by moisture stress treatment at 12 days, 25 days after imposition of stress and 5 days after release of stress are presented in table 3.

Stress imposed during flower bud initiation stage resulted in significant difference in proline content between control and moisture stress treatments throughout stress imposition period. Maximum proline content was observed in stress treatment at these stages of crop. At 70 DAS the proline content was 5.9 ì g per g. fresh weight in stress as compared to 4.6 ì g per g. fresh weight in control. The percent increase in proline content in moisture stress conditions was highest (95%) at 42 DAS.

At 42, 55 and 70 DAS, sunflower genotypes varied significantly in proline content. Genotype DRSF-113 at 42 DAS and EC-602063 at 55 and 70 DAS showed highest proline content than rest of the cultivars.

At 42, 55 and 70 DAS, the interaction between moisture stress treatments and genotypes exhibited significant difference in proline content. The treatment combination of EC-602063 under moisture stress (6.5 ì g per g. fresh weight) has recorded highest proline content at 70 DAS. In DRSF-113, proline content in stress condition has came down to the proline content in the control condition at 70 DAS, whereas in other three genotypes higher levels (28%) maintained till the end.

Drought stress significantly increased proline content throughout. The present findings are in agreement with the results reported by Iqbal (2005), Unyayar *et al*. (2004) and Oraki *et al*. (2012). The major role of proline is to protect enzymes against dehydration and salt accumulation rather than osmoprotectant (Thomas, 1990).

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