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Comparative Evaluation of Biochemical Changes in Different Safflower Varieties (*Carthamus tinctorius* L.) under Water Deficit

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Abstract

Studies to determine drought induced biochemical changes in safflower and their utilization in identifying stress tolerant genotypes were conducted under water deficit (60% field capacity) conditions at Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. Nitrate Reductase (NRA) and Nitrite Reductase (NiRA) activities, total soluble proteins, DNA contents, fresh and dry biomass of plant and plant yield were adversely affected by drought stress in all safflower genotypes. However, genotypes Thori 78 and PI-387820 showed less reduction in these attributes. Total free amino acids, reducing, non reducing sugars and total sugars increased in all genotypes under drought stress. Comparison among safflower genotypes indicated that V1 (with greater biomass, yield, high NiRA, proteins and DNA level) performed best under drought stress followed by V6 (with high NiRA, proteins and unsaturation/saturation level). V3 proved itself poorer upon the basis of growth and biochemical parameters. From the results it can be concluded that biochemical markers can be used to select drought tolerant safflower genotypes.

Keywords: Nitrate and nitrite reductase activities; DNA; Osmoregulators; Fatty acids; Drought

Introduction

Pakistan has been constantly and chronically deficient in major food products including edible oil production. According to PARC [1] about 70% of the domestic requirements are met through imports and import of edible oil which is increasing at the rate of 12.5% annually in early 1970s and the trend will further not only continue but will also get worsen with increase in population. However, efforts have been being made to increase its local production.

Safflower (*Carthamus tinctorius* L.) is one of the prospective oil-seed crops, because it yields about 32-40% seed oil [2]. Its oil is widely utilized in industries mainly as edible and dying purposes. One of the most important aspects for safflower seed production is related to rapid emergence and good seedling establishment in the field [3]. Safflower is moderately stress tolerant crop and can withstand under extreme conditions of drought. It is an excellent forage plant, which is palatable and has feeding value (crude value and total digestible nutrients) and yields are similar to or better than cereals and alfalfa. Safflower stubble is highly desired by cattle, sheep and goat [4]. In Pakistan safflower is grown on residual moisture following a rice crop [5]. In recent years, considerable attention has been generated in the consumption and development of safflower seed oil as an excellent health care product and health benefits derived from it including prevention and treatment of hyperlipidemia, atherosclerosis and coronary heart disease [6].

Intensive use of natural resources by increasing the world population causes environmental problems (such as salinity and drought). These environmental stresses contribute significantly in reduction of crop yields well below the potential maximum yields [7]. Among various environmental stresses, water is the most important component of life and it is rapidly becoming a critically short commodity for humans and crop production [7,8].

Keeping in view the importance of safflower as an oil seed crop and drought as major constrains in getting its optimum productivity, studies were conducted to investigate the biochemical changes which can be used as markers to identify drought tolerant and high yielding safflower genotypes to fulfill the edible oil requirement of the country.

Materials and Methods

Studies were conducted in pots in wire-house under natural conditions at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan with six safflower genotypes (PI-387820, PI-251978, PI-170274, PI-387821, PI-386174 and Thori-78) using two water stress (100 and 60% field capacity) levels. Plastic pots having capacity of 8 kg filled with alluvial soil (analyzed according to the methods given in Hand Book No. 60 US Salinity Lab Staff; summarized in Table 1) were used in this study. After completion of germination two treatments i-e Control (with 100 % field capacity) and Drought (60% field capacity) were imposed. Drought was imposed by maintaining the field capacity of soil up to 60% through weighing the pots daily and adding the measured amount of evaporated water (characteristic of irrigation water are given in Table 1. This practice was carried out throughout the duration of study. When the plants were of 95 days old, leaf samples were collected for the determination of biochemical changes. For the estimation of fresh and dry biomass, one plant was uprooted carefully from each pot, washed with distilled water, dried with filter paper and fresh weight was measured then place in an oven

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	Soil Characteristics	Irrigation water Characteristics
Soil texture	**Clay loam**	-
EC_e(dS m^{-1})	2.41	0.77
pH_s	7.76	7.9
Organic matter (%)	0. 4	-
NO_3-N (mg kg^{-1})	14.7	7
P (mg kg^{-1})	11	-
K (mg kg^{-1})	78	0.7
Ca+Mg (meq L^{-1})	15	3
CO_3 (meq L^{-1})	Nil	Nil
HCO_3 (meq L^{-1})	3.5	2

Table 1: Characteristics of soil and irrigation water used in this study.

at 70 ± 2°C for 72 hours and dry weight was estimated on a scientific digital balance.

Reagents and standards

All analytical grade chemicals were purchased from either sigma-Aldrich (Buchs, Switzerland) or E. Merck (Darmstadt, Germany).

Determination of biochemical changes

Enzymes: Nitrate and nitrite reductases activities (NRA and NiRA) were studied using fresh leaf material of treated and untreated plants by following the methods of Sym [9] for NRA by Ramarao et al. [10] for NiRA.

Sugar: Immediately after harvesting, fresh leaf samples are chilled out to 0°C and then frozen to-40°C. Sugars were extracted in from 0.1 g chopped leaf sample in 10 mL of 80% ethanol (v/v) by shaking it overnight. Reducing, non-reducing and total sugars were estimated from the above extract as described by Riazi et al. [11].

Total protein, amino acid and DNA: Fresh leaves were homogenized in phosphate buffer solution (pH 7) and filtrate was used for the determination of protein, total free amino acids and DNA. Total proteins were estimated using the method of Lowry et al. [12] and total free amino acids were determined as described by Hamilton and Van Slyke [13]. DNA contents were estimated as according to Hoogendoom et al. [14].

Fatty acid: Oil from 1 g of seeds of each variety was extracted in n-hexane through mechanical method using metallic rod to press the seeds. Vials containing seeds were shaked for 30 minutes on a forward and back shaker and then centrifuged. Supernatant containing oil was recovered, solvent was evaporated and oil was esterified for gas chromatographic analysis. Methylation of fatty acids in the extracted oil sample was carried out according to the procedure described by Wang and Stute [15] with some modifications. Gas chromatography (GC-17A Shamadzu) having conditions, DB-Wax column 30 m long 0.25 mm inside diameter and flame ionization detector was used for fatty acid profile determination. The temperature of the thermostat was 140°C for 5 min 240°C at 4/min but the temperature at injection time was 260°C at 150psi pressure and Helium served as carrier gas with a flow rate of 30 mL/min.

Statistical analysis: Statistical significance of the differences between mean values was assessed two way analysis of ANOVA under CRD and DMR test using Minitab 2000 version 13.2 statistical software (Minitab Inc., Pennsylvania, USA). A probability value of $p \leq 0.05$ was considered to denote a statistically significant difference [16].

Results

Biochemical changes

Nitrite Reductase Activity (NiRA) decreased in all the varieties under drought stress but among all the safflower genotypes PI-387820 (V_1) and Thori-78 (V_6) maintained the highest NiRA under drought stress conditions (Figure 1A) while its minimum level was noted in PI-170274 (V_3) closely followed by PI-386174 (V_5).

Nitrate Reductase Activity (NRA) was significantly reduced due to drought stresses (Figure 1B). However, different genotypes responded differently to drought. Both the stresses had significant effect on NRA of all the six genotypes. Only PI-386174 (V_5) maintained under stress however all genotypes showed overall trend of reduction. The minimum reduction was in PI-170274 (V_3) where it is 24% followed by PI-387820 (V_1) and Thori-78 (V_6). Moreover maximum reduction was in PI-251978 (V_2) and PI-387821 (V_4) that is up to 55 and 47% respectively.

Total soluble protein significantly decreased due to drought in all safflower genotypes (Figure 2A). The highest reduction as compared to control in soluble protein was noted in V_4 and V_3 while V_1 and V_6 proved themselves better with minimum reduction in content of total soluble proteins.

Concentrations of Total Free Amino Acid (TFA) were significantly affected by drought stress in safflower genotypes (Figure 2B). The safflower plants growing under normal conditions had less TFA contents than those growing under drought stress conditions. All

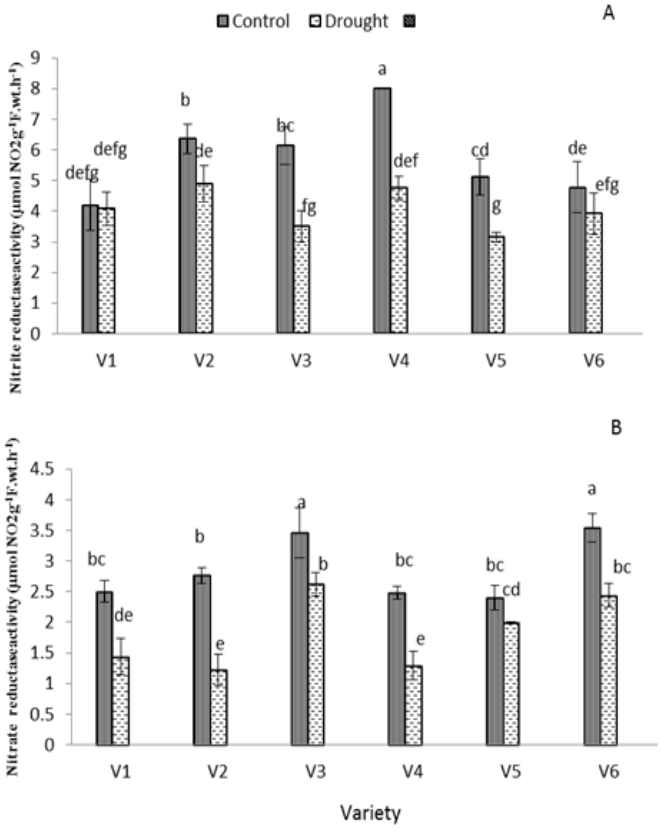

Figure 1: Comparison of nitrite (A) and nitrate reductase (B) activity in different safflower varieties under water deficit.

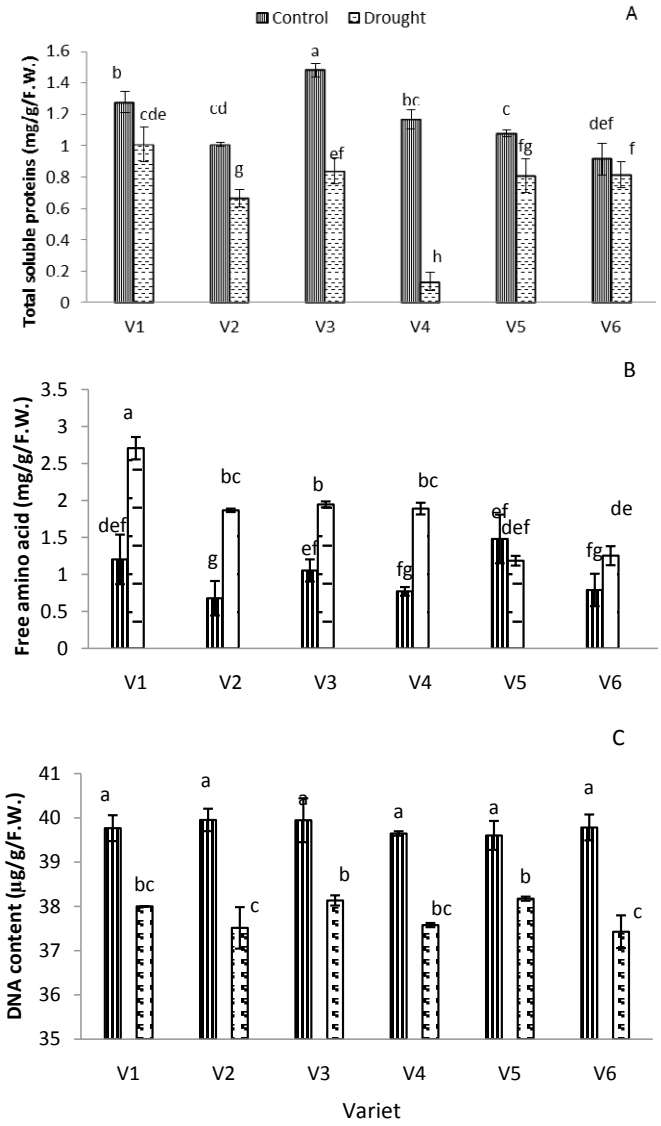

Figure 2: Comparison of total soluble proteins (A) free amino acids (B) and DNA content (C) in different safflower varieties under water deficit.

genotypes of safflower showed a significant increase in TFA with the exception of V_5 that maintained the level of TFA under stress. The concentration of TFA in safflower variety/genotype V_1 was significantly higher than all other genotypes both under controlled and stressed conditions. Safflower genotype V_4 was next in performance regarding TFA. Minimum rise in TFA level was noted in V_6.

DNA contents in all varieties of safflower significantly reduced by drought stress (Figure 2C). Genotypic comparison indicated that safflower genotype V_1, V_3 and V_5 showed less reduction in DNA content than others. The reduction in DNA content in V_1 was 1.43 µg g^{-1} FW under drought stress whereas it was more than 2 µg g^{-1} FW in V_2, V_4 and V_6.

Sugars accumulation significantly increased under stress as compared to controlled conditions in all the safflower genotypes (Figure 3). Under controlled conditions V_3 showed minimum level of total soluble sugars and non-reducing sugars but in stressed conditions it showed sharp rise in the level of sugar accumulation leading its level comparable to other genotype However, accumulation of sugars was significantly higher in safflower genotype V_3 followed by V_2 and V_5. All safflower genotypes showed an increase in reducing sugars under stressed conditions. However, V_1, V_2 and V_5 showed greater accumulation of reducing sugars.

Drought stress significantly influenced the concentration of total soluble sugars in safflower genotypes. Plants growing under environmental stresses generally showed increase in sugars, betaine and proline.

Fatty acid, oleic acid was the highest in V_3 while PI-251978 (V2) and V_3 have high linoleic acid but low oleic acid (Table 2). All varieties showed a decrease in palmitic, stearic, oleic and linoleic acid except V_3, which showed a remarkable increase in oleic acid contents under drought but over all varieties, exhibited a decrease in oil contents and change in fatty acid composition. Drought stressed significantly reduced unsaturation to saturation ratio in V1 and V5 however it was improved in V2, V3, V4 and V6 (Figure 4).

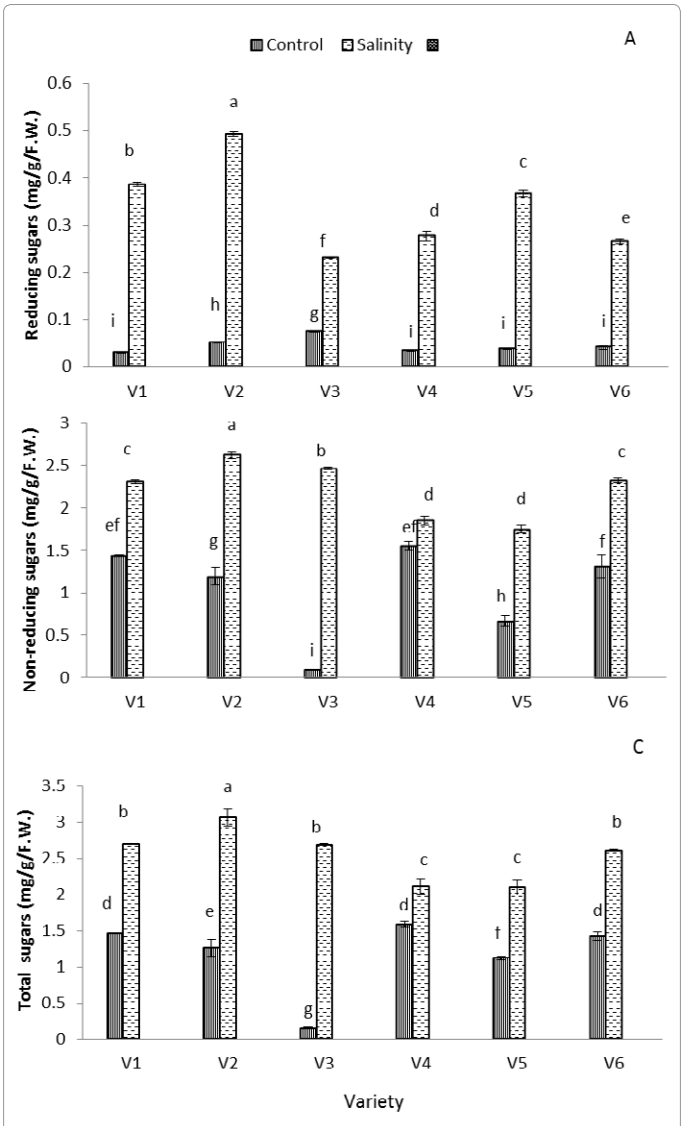

Figure 3: Comparison of reducing sugars (A) non-reducing sugars (B) and total sugars (C) in different safflower varieties under water deficit.

Genotypic Code	Designated Name of Genotype	Palmitic acid C16:1 (% of Oil Content)		Stearic Acid C18:0 (% of Oil Content)		Oleic acid C18:1 (% of Oil Content)		Linoleic Acid C18:2 (% of Oil Content)	
		Treatments		Treatments		Treatments		Treatments	
		Control	Drought	Control	Drought	Control	Drought	Control	Drought
PI-387820	V_1	06.96o	7.75h	1.20o	1.55j	13.00j	09.53p	78.84h	81.18d
PI-251978	V_2	07.57k	6.50p	1.66i	1.37m	09.27q	08.75r	81.50c	83.39a
PI-170274	V_3	10.11a	8.55d	1.94f	2.41c	19.34b	17.07c	68.61r	76.98m
PI-387821	V_4	08.45e	7.67i	2.41c	1.55j	13.33i	12.86k	75.81o	77.92j
PI-386174	V_5	07.23m	8.58c	1.25n	1.96e	09.83o	20.42a	81.67b	69.03q
Thori-78	V_6	08.31f	7.01n	1.42k	1.40l	11.93l	14.17f	78.35i	77.42l

Note: Values sharing same letters in mean columns for genotypes and in rows for treatment did not vary significant at $P \leq 0.05$.

Table 2: Comparison of fatty acids profile of different safflower varieties under water deficit.

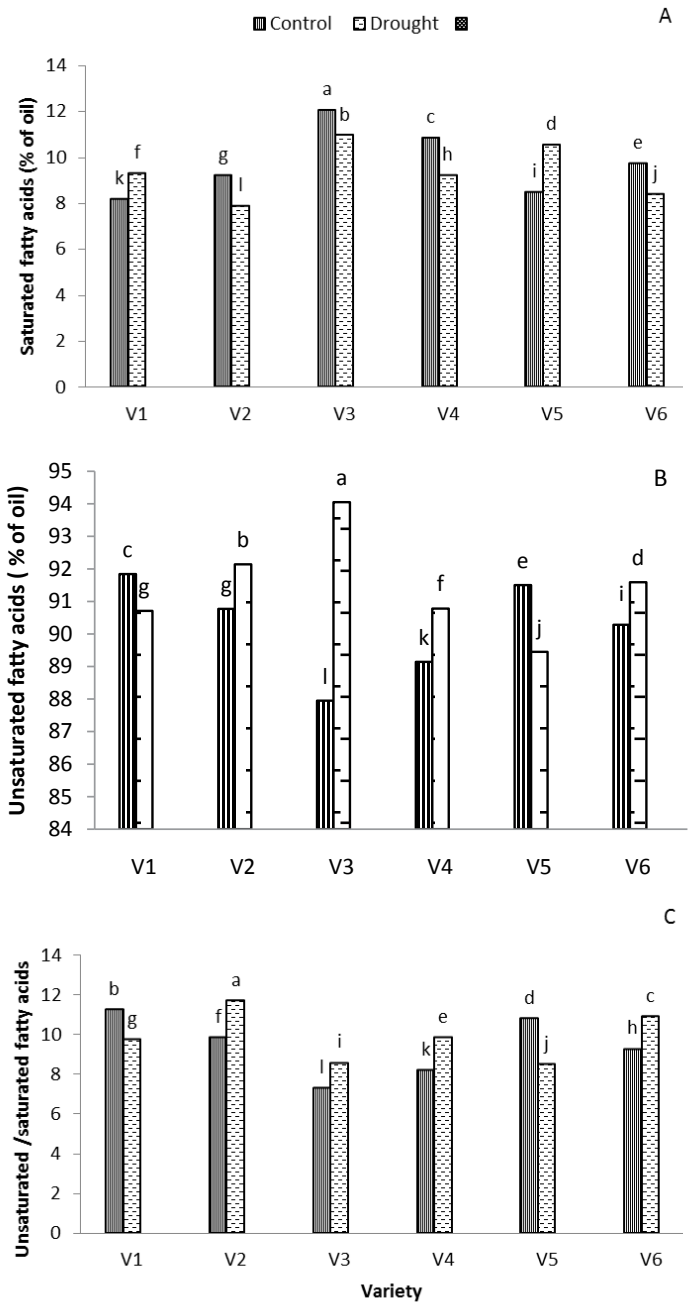

Figure 4: Comparison of saturated (A) unsaturated (B) and unsaturated/ saturated fatty acids (C) in different safflower varieties under water deficit.

Growth

Fresh and dry biomass and yield were significantly reduced due to stress in all the safflower genotypes. Under drought condition maximum reduction over control in fresh biomass was recorded in safflower genotype V_4 (56%) while it was minimum V_5 (27%) closely followed by V_6 (29%) (Table 3).

Dry biomass was also adversely affected by salinity and drought in all safflower genotypes (Table 3). Under drought condition reduction in dry biomass was very severe in all safflower genotypes but it was the highest in V_1 (62%) followed by V_3 (59%), V_6 (59%), V_2 (45%) and V_5 and V_4 (39%).

Seed yield was significantly reduced in all safflower genotypes under drought conditions (Table 3). The highest reduction in seed yield was noted in V_5 (64%) followed by V_4 and V_3 whereas minimum reduction was showed by V_6 (39%).

Discussion

Drought adversely affects plant growth and productivity of all the safflowers genotypes (Table 2). Plants adapt themselves by altering different physiological and biochemical processes to adjust the environmental stresses [17]. Literature indicated that salt results in huge losses in plant productivity by reducing plant growth [8] in almost all the plants. But it was minimum in tolerant crop varieties [8] as observed in V_6 and V_1 (Table 2).

Under drought stress nutrient imbalance was often observed in plants which cause inhibition in protein synthesizing, delay in enzyme solubilization and reduction in enzymatic activities (Figures 1A and 1B). Reduction in NO^{-3} uptake, NRA and NiRA under salinity has been reported by many researchers [18,19]. Reduction in NO^{-3} concentration and uptake is may be due to the antagonistic effect of Cl^- due to NaCl salinity and disruption of root membrane integrity [20-23]. Nitrogen assimilation is a fundamental biological process that occurs in plants and has marked effects on plant productivity and biomass. Nitrate reductase is the key enzyme that catalyzes the first reaction in the NO_3 assimilation pathway [24,25]. So, the reduction in NRA may lead the decrease in NiRA which is observed in the present study (Figures 1A and 1B). Nitrate has to be reduced to ammonia in order to synthesize the structural component of the biological system. The whole process is as follows:

Nitrate → Nitrite → Ammonia → Glutamine → Glutamate

So, reduction of NO_3 into NO_2 by NRA is the key and rate limiting step in nitrogen assimilation. In above indicated reactions the any disturbance in NRA and NiRA may affect the nitrogen metabolism [19,26].

The stresses cause disturbance in N assimilation resulting reduction in proteins which is observed in all safflower genotypes (Figure 3). Decrease in soluble proteins is may be due to breakdown of proteins by proteolytic process under salinity or drought stresses [27] consequently total amino acids increased in all safflower genotypes (Figure 4). Accumulation of amino acids reduces the osmotic potential which facilitates the inward movement of the water [28,29]. Reports indicated that these amino acids are used to synthesize the necessary proteins and other molecules to support growth [30]. However, some studies revealed a significant increase in soluble proteins in response to stresses [19]. These proteins are may be the stress proteins which are developed in plants cope with unfavorable environment conditions.

Drought significantly decreased DNA contents in all tested safflower varieties (Figure 2C) which may be disturbance in protein and nitrogen metabolism as observed by [31] in different wheat genotypes. As DNA and RNA are responsible for protein synthesis, therefore, disturbance in nucleic acid metabolism may cause disturbance in protein metabolism which is very clear from the findings of present study (Figure 2C). However, there are a series of genes, such as those encoding for osmolytes and ion channels which prevent the damage in response to stresses [32]. Both types of stresses adversely affected all the safflower genotypes, but V_6 (THORI-78) had potential to tolerate the adverse environmental conditions, so it produced higher biomass and yield.

Sugars contents increased due to imposition of stress in all safflower genotypes. In plants, under abiotic stress conditions accumulation of sugars (reducing, non-reducing) is reported which allowed the plants to adjust osmotically [33]. Plants have been attributed an adaptation by increase in carbohydrate level in response to stresses. In addition to osmoregulators soluble organic compounds may act as osmoprotectants for protein under stresses [29].

Safflower oil comprised linoleic (approximately 75%), oleic (13%), palmitic (6%) and stearic (3%) acids [32]. Stressful environmental conditions not only lower the oil content it also alters the fatty acid composition [34]. In present research differential effect upon fatty acid synthesis was observed by different varieties. The linoleic, oleic and linolenic acids are the fatty acid, which affect the quality of oil. Safflower is an oil seed crop and two types of safflower oil were reported those containing high monounsaturated fatty acid such as oleic acid (used as heat stable cooking oil) and those containing high polyunsaturated fatty acids such as oleic acid (used as cold oil). Drought modified fatty acids composition and ultimately the food quality and it is considered to be very important in stress tolerance of plants [35]. Moreover extent of unsaturation of fatty acids is correlated with potential of photosynthetic machinery to tolerate stress. Generally abiotic stress induces inactivation of PSII and PSI [36] and unsaturation of fatty acids in membrane lipids shelter PSII and PSI as one of effective

Genotypic Code	Designated Name of Genotype	Fresh weight Plant^{-1}		Dry weight Plant^{-1}		Yield Plant^{-1}	
		Treatments		Treatments		Treatments	
		Control	Drought	Control	Drought	Control	Drought
PI-387820	V1	33.69j	18.55p	10.795h	4.095r	1.479l	0.843r
PI-251978	V2	46.26d	22.35n	14.693d	7.935n	2.091h	1.025o
PI-170274	V3	52.11c	30.84m	15.917a	6.437q	3.623b	2.121g
PI-387821	V4	36.79h	15.86r	12.950e	7.936m	2.202f	0.957p
PI-386174	V5	52.35b	38.09g	14.703c	8.937l	2.631d	0.944q
Thori-78	V6	56.47a	39.76f	15.730b	6.440p	4.212a	2.529e

Note: Values sharing same letters in mean columns for genotypes and in rows for treatment did not vary significant at $P \leq 0.05$.

Table 3: Comparison of plant growth of different safflower varieties under water deficit.

protective strategy. Where it affect dually; alleviating the damage to PSI and PSII and improving the healing of injury [36-38]. Amongst genotypes better unsaturation level was maintained by V2, 3, 4, 6 under drought conditions. Fatty acid composition is generally affected by genotype [39] and environmental conditions, particularly the level of unsaturation. Water stress causes a rise in oleic acid [40]. Similar were the findings of present research for V5 and V6 but reverse was true for other safflower genotypes. Amongst saturated fatty acid (palmitic and stearic acid) in sunflower, palmitic acid concentration has been noted to be increases in less water availability (with 0.39 to 0.74%) and stearic acid concentration lowers under drought (up to 1.33). Water stress lowers the level of oleic acid and raises the linoleic (up to 14%).

Conclusion

From the findings of present study it can be concluded that changes in the levels of biochemical metabolites, i.e. NRA, NiRA, DNA, sugars, soluble proteins and total free amino acids can be used to identify the safflower genotypes having potential to tolerate drought.

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