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EFFECT OF SALINITY ON GROWTH, BIOCHEMICAL PARAMETERS AND FATTY ACID COMPOSITION IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.)

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Abstract

The aim of the present project is to investigate the effect of salinity on growth, biochemical parameters and fatty acid composition in six varieties of safflower as well as identification of stress tolerant variety under saline (8 d Sm^{-1}) condition. It was observed that salinity significantly decreased the dry weight and fresh weight of safflower varieties. Nitrate reductase (NRA) and nitrite reductase (NiRA) activities were also reduced in response to salinity in all safflower genotypes but Thori-78 and PI-387820 showed less reduction which could be a useful marker for selecting salt tolerant varieties. Under salinity stress, total free amino acids, reducing, non reducing sugars and total sugars increased in all varieties. Accumulation of sugars and total free amino acids might reflect a salt protective mechanism and could be a useful criterion for selecting salt tolerant variety. Comparison among safflower genotypes indicated that Thori-78 and PI-387820 performed better than the others and successful in maintaining higher NRA, NiRA and other metabolites thus were tolerant to salinity. Differential effect upon fatty acid synthesis was observed by different varieties under salinity stress but PI-170274 and PI-387821 varieties better maintained their fatty acid composition. It can be concluded from present studies that biochemical markers can be used to select salinity tolerant safflower varieties.

Introduction

Edible oil consumption is around 1.95 million tons in Pakistan. Seventy percent of the total oil requirement is met through imports. Edible oil import is next to petroleum and its demand is increasing day by day. Oilseed crops are among the 5% of total imports and 50% of agricultural imports of Pakistan (Anon., 2012). Therefore, it is of vital importance to enhance productivity of oilseed crop by using our natural resources. Safflower (*Carthamus tinctorius* L.) is oil-seed crop yielding 32-40% seed oil (Soliman *et al*., 2011). Its oil is widely utilized in many industries for edible and dying purposes (Sadeghi *et al*., 2011). Safflower is moderately stress tolerant crop and can withstand extreme conditions of abiotic stress. Safflower is an important oil seed crop due to its rapid emergence and good seedling establishment in the field (Siddiqui *et al*., 2007; 2010). In Pakistan safflower is grown on residual moisture following a rice crop (Soliman *et al*., 2011).

Among various environmental stresses, soil salinity has become a critical problem worldwide due to its dramatic effect on plant physiology and performance (Ahmad *et al*., 2012). These environmental stresses contribute significantly in reduction of crop yield below the potential maximum yield (Warraich *et al*., 2011; Abbas *et al*., 2013). Salinity delays the germination events, resulting in reduced plant growth and final crop yield (Azzedine *et al.,* 2011; Basiri *et al*., 2013). The deleterious effects of salinity on plant growth are associated with: 1) low osmotic potential of soil solution, 2) nutritional imbalance, 3) specific ion toxicity (salt stress) or 4) a combination of these factors (Ashraf *et al*., 2005). The growth and yield reduction in most crops under saline environments is known to cause an imbalance of the cellular ions resulting in hyper ionic and hyper osmotic stress in plants, leading to reactive oxygen species (ROS) production such as superoxide anion, hydrogen peroxide and hydroxyl radicals and metabolic toxicity (Tayefi-Nasrabadi *et al*., 2011). Salinity actually reduces the ability of plants to take up water and resulted in reductions of growth rate (Munns, 2002).

Keeping in view the importance of safflower as an oil seed crop and salinity as major constrains in getting its optimum productivity. Studies were conducted to investigate the effect on growth parameters, biochemical changes and fatty acid composition of safflower seed oil which can be used as markers to identify salinity tolerant and high yielding safflower genotypes.

Materials and Methods

Plant culture and treatment: Present experiments 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 salinity level of 8 dS m^{-1}. 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 soil salinity i.e. 2.14 dS m^{-1} and 100 % field capacity), salinity (8.0 dS m^{-1}) was imposed. Salinity was developed by mixing AnalR grade NaCl. This practice was carried out throughout the duration of study.

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

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

	Soil characteristics	Irrigation water characteristics
Soil texture	**Clay loam**	-
EC_e(dS m^{-1})	2.41	0.77
pH	7.76	7.9
Organic matter (%)	0. 4	-
NO_3^- (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

Determination of biochemical changes: enzymes: Nitrate and nitrite reductases activities (NRA and NiRA) were studied by following the methods of Sym (1984) for NRA and Ramarao *et al*., (1983) for NiRA.

Sugar: Immediately after harvesting, fresh leaf samples are chilled out to 0°C and then frozen to -40°C. Sugars were extracted 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*., (1985).

Total protein and amino acid: Fresh leaves were homogenized in phosphate buffer solution (pH 7) and filtrate was used for the estimation of protein, TFA and DNA. Total proteins were estimated using the method of Lowery *et al*., (1951) and total free amino acids were determined as described by Hamilton& Van Slyke (1943).

Fatty acid: Oil from 1g 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 & Stute (2000) with some modifications. Gas chromatography (GC-17A Shamadzu) having conditions, DB-Wax column 30m long 0.25mm 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 30mL/min.

Statistical analysis: The Data was analyzed by applying two way analysis of variance (ANOVA). Treatment means and varietal means were compared by LSD and the significance level was calculated at $p \leq 0.050$ (Steel *et al*., 1997).

Result

Growth: Fresh and dry biomass and yield were significantly reduced due to both the stresses in all the safflower genotypes. Under saline condition maximum reduction over control in fresh biomass was recorded in safflower genotype V_4 (50%) while it was minimum V_5 (21 %) closely followed by V_6 (25 %) (Table 2).

Dry weight was also affected by salinity in all safflower genotypes (Table 2). Under saline conditions minimum reduction over control in dry biomass was recorded in V_6 (16%) closely followed by V_5 (23%) while maximum reduction was noted in V_4 (35%).

Seed yield was significantly reduced in all safflower genotypes due to salinity (Table 2). Under saline conditions minimum decrease was recorded in V_1 (5%) and it was maximum in V_5 (53%).

Biochemical changes: It is evident from present study that activity of nitrate reductase was significantly reduced due to salinity. However, different genotypes responded differently to salinity. Salinity affected NRA of all the six genotypes but in PI-386174 (V_5) the reduction in NRA was less as compared to other genotypes. The reduction was upto (12%) in variety V_5 (26%) in variety V_3 under salinity conditions while it was (36%) V_1 under salinity conditions respectively (Fig. 1A).

Nitrite Reductase Activity (NiRA) was also reduced in all the varieties under salinity but among all the safflower genotypes PI-387820 (V_1) and THORI-78 (V_6) maintained the highest NiRA both under salinity conditions (Fig. 1B) while it was minimum in PI-387821 (V_4) closely followed by PI-386174 (V_5).

Concentrations of total free amino acid (TFA) were significantly ($p \leq 0.050$) affected by salinity in safflower genotypes. The safflower plants growing under normal conditions had less TFA contents than those growing under saline condition. All genotypes of safflower showed a significant increase in TFA. The concentration of TFA in safflower variety/genotype V_1 was significantly higher than all other genotypes under salinity condition. Safflower genotype V4 was next in performance regarding TFA (Fig. 2A).

Total soluble protein significantly ($p \leq 0.050$) decreased due to salinity in all safflower genotypes. The highest reduction as compared to control in soluble protein was noted in THORI-78 (V_6) under saline while the lowest was noted in PI-386174 (V_5) closely followed by PI-387820 (V_1) and PI-387821 (V_4) (Fig. 2B).

Table 2. Effect of salinity and drought on plant growth of safflower varieties

Genotype code	Designated name of genotype	Fresh weight plant^{-1} (g)		Dry weight plant^{-1} (g)		Seed yield plant^{-1} (g)	
		Control	Salinity	Control	Salinity	Control	Salinity
PI-387820	V1	33.69h	21.09k	10.795h	07.130k	1.479h	1.404k
PI-251978	V2	46.26d	31.79i	14.693d	10.030i	2.091f	1.612i
PI-170274	V3	52.11c	34.21g	15.917c	11.020g	3.623b	1.879g
PI-387821	V4	36.79f	18.24l	12.950f	8.467l	2.202e	1.416j
PI-386174	V5	52.35b	41.33j	14.703b	11.310j	2.631d	1.229l
Thori-78	V6	56.47a	42.45e	15.730a	12.800e	4.212a	2.659c

Note: Values sharing same letters in mean columns for genotypes and in rows for treatment did not vary significant at p≤0.01

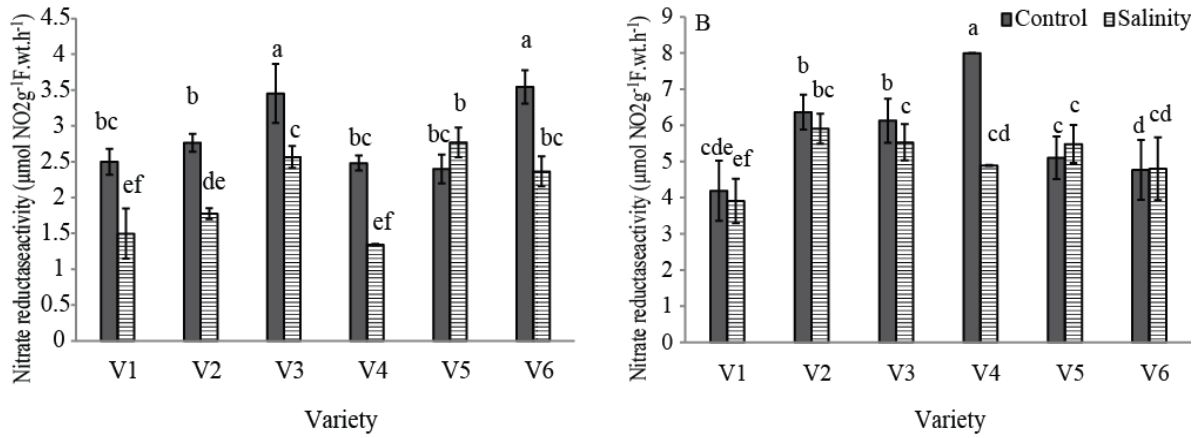

Fig. 1. Effect of salinity on Nitrate reductase (A) Nitrite reductase activity (B) in different safflower varieties.

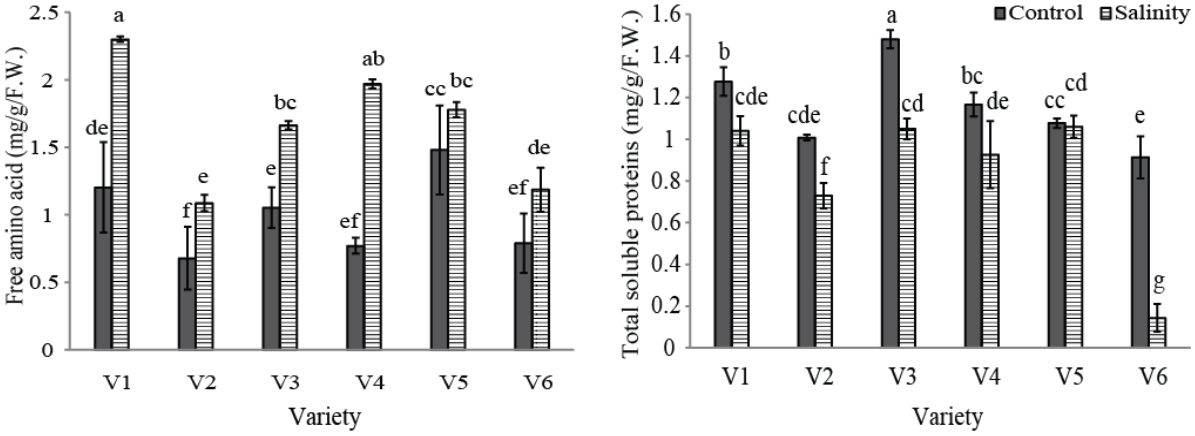

Fig. 2. Effect of salinity and drought on Total free amino acids (A) Total soluble proteins (B) in different safflower varieties

Sugars accumulation significantly (p≤0.050) increased under salinity as compared to non stress conditions in all the safflower genotypes. However, accumulation of sugars was significantly higher (p≤0.050) in safflower genotype PI-251978 (V_2) than other under salinity (Fig. 3A). All safflower genotypes showed an increase in reducing sugars under stressed conditions. However, PI-251978 (V_2) showed the maximum accumulation of non-reducing sugar than others (Fig. 3B).

Salinity significantly (p≤0.050) influenced the concentration of total soluble sugars in safflower genotypes. Plants growing under environmental stresses generally showed increase in sugars, betaine and proline. It was observed that PI-170274 (V_4) and PI-386174 (V_5) maintained the level of total soluble sugars (TSS) as compared to other varieties. However, it was the highest in PI-251978 (V_2) and PI-170274(V_3) (Fig. 3C).

Fatty acid, oleic acid was the highest in PI-170274(V_3) while PI-251978 (V_2) and PI-387821 (V_4) have high linoleic acid but low oleic acid (Table 3). All varieties respond differently in response to salinity. PI-386174 (V_5) and THORI-78 (V_6) showed a remarkable increase in oleic acid followed by palmitic acid and stearic acid but decrease in linoleic acid and PI-170274 (V_4) exhibited increase in linoleic acid and reduction in palmitic, stearic and oleic acid under salinity. It was observed that over all varieties showed a change in oil contents and fatty acid composition. However, minimum saturation level was found in PI-387821 (V_4) and maximum unsaturation level in PI-251978 (V_2) and PI-387821 (V_4) under salinity stress, While PI-387821 (V_4) showed highest ratio of unsaturation and saturation (Fig. 4).

Table 3. Effect of salinity and drought on fatty acids profile of different safflower varieties.

Genotype Code	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	Salinity	Control	Salinity	Control	Salinity	Control	Salinity
PI-387820	V_1	06.96j	7.66f	1.20k	0.67l	13.00g	13.78d	78.84f	77.89h
PI-251978	V_2	07.57g	7.35h	1.66h	2.65b	09.27l	13.60e	81.50b	79.68e
PI-170274	V_3	10.11a	6.42k	1.94e	3.21a	19.34a	11.07j	68.61l	79.30d
PI-387821	V_4	08.45c	6.29l	2.41c	1.72g	13.33f	11.83i	75.81j	80.15c
PI-386174	V_5	07.23i	9.08e	1.25j	1.72f	09.83k	16.05c	81.67a	73.14i
Thori-78	V_6	08.31d	9.56b	1.42i	2.38d	11.93h	14.59b	78.35g	73.47k

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

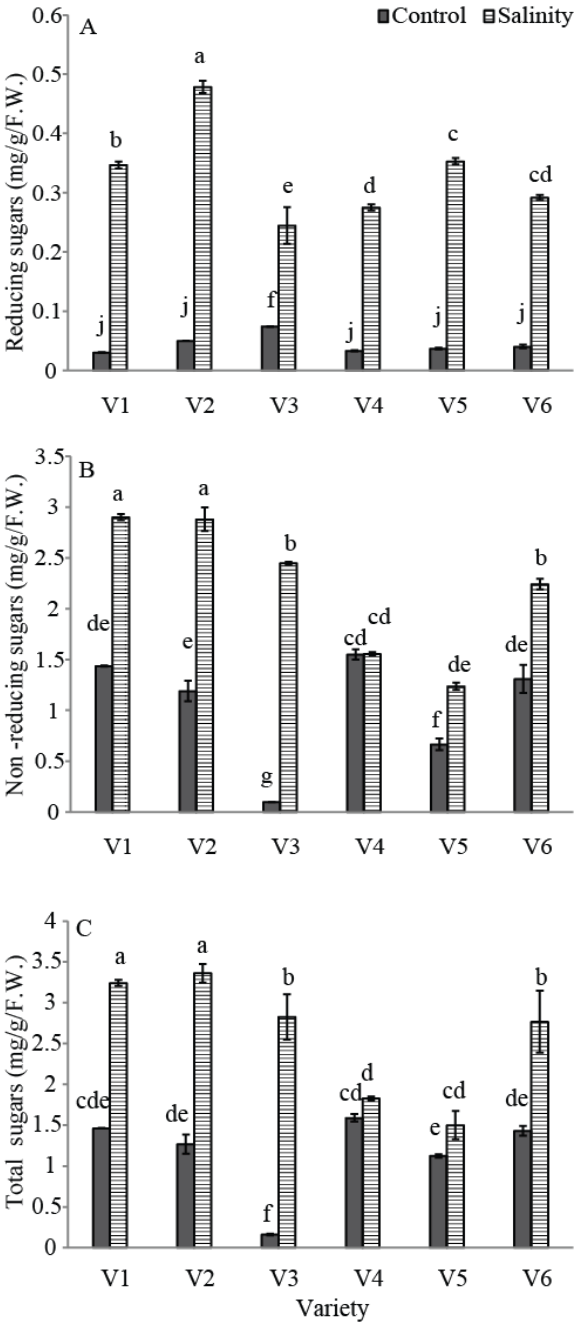

Fig. 3. Effect of salinity on reducing sugars (A) nonreducing sugars (B) and total sugars (C) in different safflower varieties.

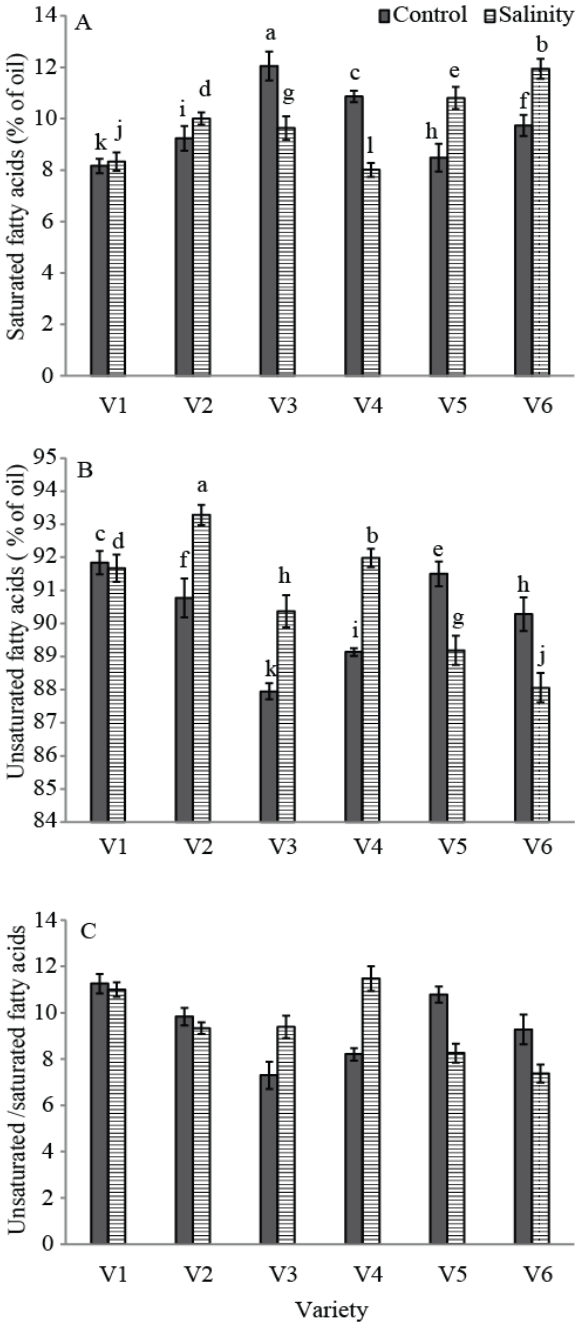

Fig. 4. Effect of salinity on saturated fatty acids (A) unsaturated (B) and unsaturated/ saturated (C) fatty acids in different safflower varieties.

Discussion

Investigations of plant responses to salt stress are very important in crop science, plant physiology and agricultural sciences because salinization of soil is progressive phenomenon. Plants adapt themselves by altering different physiological and biochemical processes to adjust the environmental stresses (Bohnert *et al*., 1995). These changes are: inhibition of plant growth and development, changes in soluble protein synthesis, accumulation of organic metabolites and altered ion relations (Hasegawa *et al*., 2000). Literature indicated that salt results in huge losses in plant productivity by reducing plant growth (Bohnert, 1995; Waraich *et al*., 2011; Ashraf *et al*., 2012; Kanwal *et al*., 2013) in almost all the plants. Salinity adversely affects plant growth and productivity of all the safflowers genotypes but it was minimum in tolerant crop varieties as observed in V_6 and V_1 in the present study (Table 3).

Plants require mineral nutrients especially nitrogen for their proper growth and integrity. Higher plants have mainly taken up nitrogen in inorganic form (NH_3 and NO^-_3) by roots. Stressed plants mostly exhibited nutrient imbalance which causes inhibition in protein synthesis delay in enzyme solubilization and reduction in enzymatic activities (Figs. 1, 2, 3). 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 (Carvajal *et al.,* 1999; Parida & Das, 2004; Ashraf *et al*., 2005; Akram *et al*., 2011). Sodium and chloride are the major ions, which cause many physiological disorder and poor plant productivity. Reduction in NO^-_3 uptake, NRA and NiRA under salinity has been reported by many researchers (Hamid *et al*., 2010; Jabeen & Ahmad, 2011). 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 (Lee, 1999). Reduction in NRA may lead the decrease in NiRA which is observed in the present study (Fig. 1A, 1B). Nitrate must be reduced to ammonia in order to synthesize the structural component of the biological system (Heuer *et al.,* 2005; Hamid *et al.,* 2010). Nitrate reductase is inactivated in response to stress and as a result nitrogen metabolism is hampered in plants.

It was observed that disturbance in N assimilation causes reduction in proteins in all safflower genotypes (Fig. 3). Decrease in soluble proteins is may be due to breakdown of proteins by proteolytic process under salinity or drought stresses (Parida& Das, 2004) consequently total amino acids increased in all safflower genotypes (Fig. 2). Proteins are structural component of the plant body. Stress induced reduction in protein synthesis may affect plant growth. Accumulation of amino acids reduces the osmotic potential which facilitates the inward movement of the water (Ashraf *et a*l., 2005; Balal *et al.,* 2011). Reports indicated that these amino acids are used to synthesize the necessary proteins and other molecules to support growth (Iqbal *et al*., 2011). However, some studies revealed a significant increase in soluble proteins in response to stresses (Hamid *et al*., 2010). Stress proteins may be developed in plants to cope with unfavorable environment conditions to protect certain enzymes and metabolic pathways.

In plants, under salinity stress conditions, accumulation of sugars (reducing, non-reducing) is reported which allowed the plants to adjust osmotically (Rolland *et al*., 2002; Wang & Stute, 2002). Plants have been attributed an adaptation by increase in carbohydrate level in response to stresses. In addition to osmoregulators soluble sugars may act as osmoprotectants for protein under stressed condition (Ashraf *et al*., 2005). In the present study, sugars contents increased due to imposition of stress in all safflower genotypes (Fig. 3). The salt tolerant genotype V_2accumulated more sugar, which is effective in maintaining turgor by decreasing osmotic potential, followed by genotype V_1 and V_3.

Two types of safflower oil are 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 linoleic acid (used as cold oil). Salinity modified fatty acids composition and it is considered to be very important in stress tolerance of plants (Malkit *et al.,* 2002). Under stress conditions, oil contents of olive were decreased and composition of fatty acids also changed Stefanoudaki *et al*., (2009). In present research differential effect upon fatty acid synthesis was observed by different varieties under both stresses (Table 3). The linoleic, oleic and linolenic acids are the fatty acid, which affect the quality of oil. According to Noreen & Ashraf, (2010) salt stress significantly increased seed oil palmitic, stearic acid contents but decreased seed oil linoleic acid contents in both lines of sunflower. Moreover, extent of unsaturation of fatty acids is correlated with salinity tolerance and potential of photosynthetic machinery to tolerate stress. Generally salinity stress induces inactivation of PSI and PSII (Allakhverdiev *et al*., 2000a). Unsaturated fatty acids in membrane lipids shelter PSI and PSII from inactivation as one of effective protective strategy. Where it affect dually; alleviating the salinity induced damage to PSI and PSII and improving the healing of injury (Allakhverdiev *et al*., 2000a; Allakhverdiev *et al*., 2000b; Allakhverdiev *et al*., 2001). Amongst genotypes unsaturation level was increased by PI-251978 (V_2) and PI-387821 (V_4) under salinity (Fig. 4).

Conclusion

It can be inferred from present findings that changes in the levels of biochemical metabolites, *i.e*. NRA, NiRA, sugars, soluble proteins and total free amino acids, fatty acid composition can be used to identify the safflower genotypes having potential to tolerate salinity.

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