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Response of sugar beet plant to proline application under heat stress

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Abstract

The effects of foliar application of different concentrations (25 mg, 50 mg, 100 mg^{-1}) of proline on the vegetative growth, and some metabolite of *Beta vulgaris* L were studied under adapted heat stress. The study was conducted in pot experiments in complete randomized block design with five replicates. All treatments of proline led to significant increases in the plant height, root length, root diameter, root size, number of leaves and fresh weight and dry weight of leaves and root, the effect was more pronounced with 100 mg proline at 50 ˚c. The proline treatments increased total pigments, total carbohydrates, total soluble sugar, total soluble solid, total phenols and total free amino acid especially at 50 ˚c.

Keywords: *Beta vulgaris* L.; Chloroplast pigments; Growth parameters; Proline; Carbohydrates, Total phenols.
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1. Introduction

Sugar beet (*Beta vulgaris* L.) is a glycophytic member of *Chenopodiaceae*, and it is considered the second sugar crop for sugar production after sugar cane in the world and especially in Egypt. It is an important crop that helps in establishing integrated agricultural-industrial societies, especially in the newly reclaimed areas and contributes in many industries such as sugar industry, highly-value animal feed resulting from processing waste [1].

The sugar beet plant is commercially and physiologically interesting because of its ability to store sucrose at high concentrations within its root. Although the developmental physiology of the plant has been studied, little is known of the factors that govern the sugar content of the root or the physiological changes that cause it to vary [2]. In recent years, improvements in sugar concentration of sugar beet and development of more heat-tolerant varieties has created interest in growing sugar beet in areas currently growing sugar cane for sugar production.

Egypt is one of the most vulnerable countries to the potential impacts and risks of climate change that constringe the production of crops and altered Food Security [3]. Consequently, sugar beet is deemed to be an important sugar crop in Egypt improving its productivity is an urgent demand to meet the consumption of the ever-growing population and to secure the food supplementation.

The increasing threat of climate change is already having a substantial impact on agricultural production worldwide as heat waves cause significantly yield losses with great risks for future global food security [4]. Climatological extremes including very high temperatures are predicted to have a general negative effect on plant growth and development, leading to catastrophic loss of crop productivity and resulting in wide spread famine. The susceptibility to high temperatures in plants varies with the stage of plant development, heat stress affecting to a certain extent all vegetative and reproductive stages. The observed effects depend on species and genotype, with abundant inter- and intra-specific variations [5, 6].

A general response of plants to various kinds of stresses is the accumulation of compatible osmolytes such as proline, glycine betaine, proline betaine, etc. which protect cells against damage caused by stress [7]. These small uncharged molecules at physiological pH are highly soluble in water, which allows them to accumulate at elevated concentration in the cytosol of plant cells without harming cellular structures [8, 9].

Proline accumulation occurs in plants in response to a biotic stress. The resulting osmotic stress causes closure of stomata, reduction in photosynthesis rate and growth inhibition [9]. Within the cell, osmotic stress results in the production of ROS and the accumulation of toxic ions such as Na + or Cl −, which can severely damage the membrane structures and other cell components. To maintain low levels of Na + ions in the cytoplasm, plants possess specific transporters such as the plasma membrane SOS1 Na + /H + antiproton which pump out Na + ions into the vacuole or outside the cell [10].

Proline biosynthesis is a reductive pathway and uses NADPH to reduce glutamate to P5C and P5C to proline and generates NADP + that can be further used as electron acceptor in the oxidative pentose phosphate pathway[9]. The exogenous proline sprayed at seedling and/or at vegetative stage of *Zea mays* enhanced its growth under the conditions of water deficiency conditions[11]. Despite the fact that exogenous application of proline to plants subjected to abiotic stresses usually prevents or recuperates the deleterious effects caused by stress, on the other hand, application of high concentrations of proline to plants may inhibit their growth or may have detrimental effects on cellular metabolisms of plants [12].

2. Material and Methods

Growth conditions and treatments

The present study was executed to appreciate the employment of proline in the important biochemical components of sugar beet plant (*cv.Gezella)* to enhance the plant tolerance to heat stress. These experiments were carried out in the green house of Botany Department at National Research Centre.

Sugar beet seeds (*cv.Gezella)* were soaked in water till they obtained moisture 55% of their original dry weight to activate the embryo before exposure to heat hardening treatments which were carried out in a water bath previously adjusted to the required temperature treatments at 50 and 55 ^{0}C for 30 minutes. These pre-sowing treated seeds in addition to the untreated ones (attained the same moisture content and kept at room temperature).

Seeds of sugar beet were sown in pots (50 cm in diameter) filled with a soil mixture of sand and loamy clay soil (1:3 in proportion). Calcium super phosphate (15.5%p2o2) was added in a rate of 4g/pot, calcium nitrate (15.5%N) at 10 g/pot was applied at two equal doses, one month prior to sowing and two weeks after the first one, potassium sulfate (K_2O) (2g/pot) was added five weeks prior to sowing. Physical and chemical properties of the soil used in this study were determined according to Jackson (1973)[13] and Cottenie *et al.* (1982)[14]. Each experiment was laid out in complete randomized design with five replicates.

Eight weeks after sowing, the seedlings were thinned and three plants per pot were left. Then, the pots were divided into three main groups. The first group was seeds exposed to 50ºC and 55ºC for 30 minutes before sowing. The second group was sprayed with freshly prepared solutions of proline at concentration of 25, 50, 100 mg/L. The third control groups as seeds were untreated neither by heat hardening nor by proline. Tepol (1ml/L) was as a wetting agent to the prepared solutions of proline before spraying. The plants had been sprayed 30 days prior to sowing and after two weeks. Sugar beet plants that exposed to combined treatment of heat hardening and proline concentrations.

Growth criteria

For determination of growth parameters, the samples of plants were collected at full vegetative stage, plants were 90 days old from sowing. The criteria of vegetative growth were taken based on the following parameters; plant height (cm), number of leaves/plant, leaf fresh and dry weight/plant, leaf area (cm^2/plant). Yield and its components comprehending root length, root size, root diameter, root fresh weight/plant, root dry weight, and used sample to certain biochemical analysis.

Extraction and estimation of photosynthetic pigments

The photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) were determined by the spectrophotometer method recommended by Metzner *et al*. (1965)[15]. A known fresh weight of leaves was homogenized in 85% aqueous acetone for 5 minutes then centrifuged. The extinction was measured against a blank of pure 85% aqueous acetone at 3 wave lengths of 663, 644 and 452.5 nm using Spekol Spectrocolourimeter (Uni CAM UV300, USA). It was determine the concentrations of the pigments as µg / ml using the following equations:

Chlorophyll a =10.3 E663 - 0.918 E644

Chlorophyll b = 19.7 E644 – 3.870 E663

Carotenoids = 4.2 E452.5 – (0.0264 chlorophyll a + 0.426 chlorophyll b)

Finally, the pigment contents were calculated as mg g^{-1} fresh weight of leaves.

Extraction and estimation of carbohydrates

Total carbohydrate contents were extracted and determined according to the method described by Dubois *et al*. (1956) [16]. The amount of total carbohydrates was calculated from the standard curve of glucose as mg/100g dry weight.

The total soluble carbohydrates contents were extracted and estimated according to the method described by Dubois *et al*. [16]. Sample of 0.03 g mixed with 20 ml of ethanol (80%) were heated in digested tube, in a water bath for 20 min. After cooling at room temperature, a trace of barium carbonate was added to the digested sample, and then filtered through Whatman No.3 filter paper. The filtrate was completed to100 ml by distilled water. One ml of this solution was transferred to test tube (15 ml) and followed by addition of 1 ml of phenol solution (5 %) and 5 ml conc. sulfuric acid. After few minutes, the color –intensity of the solution was measured by using spectrophotometer at 490 nm (Model: PD-303S Company: Apel, Japan) against the blank. The amount of soluble carbohydrates was calculated from the standard curve of glucose as mg/100g dry weight. Then, the insoluble carbohydrates were calculated.

Determination of sucrose:

Sucrose contents were determined by first degrading reactive sugars present in 0.1 ml extracts with 0.1 ml 5.4 N KOH at 97 °C for 10 minutes [17]. Three ml of freshly prepared anthrone reagent were then added to the cooled reaction product, and the mixture was heated at 97 °C for 5 minutes, cooled, and read at 620 nm.

Extraction and estimation of free amino acids

The method used in this investigation was essentially similar to that adopted by Wasfi [18] . A known weight of the fresh tissue was transferred to a glass mortar followed by 20 ml distilled water. The tissue was ground thoroughly and occasionally over a period of one hour. The mixture was then quantitatively transferred to a boiling tube, and maintained at 80°C for 15 min. The insoluble residue was removed by filtration and the filtrate was made up to a certain volume and used for estimation of free amino acids. The tissue extract was deproteinized using ethanol / acetone mixture and the free amino-acids were then determined photometrically with ninhydrin. Under appropriate test conditions, (buffer, solvent and boiling time), the total free amino acids can be determined directly in the extract Muting and Kaiser (1963)[19]. The concentration of amino acids was determined finally from the standard curve of glycine and calculated as mg g^{-1} fresh weight.

Extraction and estimation of total phenols

The method applied for total phenols extraction was described by Danial and George(1972)[20] [21]A known volume of the extract (0.5 ml) was added to 0.5 ml Folin-Ciocalteu reagent. The mixture was allowed to stand for 3 minutes. One ml of saturated sodium carbonate solution (25 g Na_2CO_3 were dissolved in 1000 ml distilled water at 70-80 ° C, then cooled down, and filtered) was added to the mixture. The mixture was allowed to stand for 1 hr. The

optical density was measured at 725 nm using UV-spectrophotometer (BUCHI MODEL B 169, SWITZERLAND). The quantity of total phenols was calculated according to the standard curve of pyrogallol (99.5%) and expressed as microgram of pyrogallol equivalent per gram dry weight.

Total soluble solids (TSS %)

Total soluble solids of Sugar beet plant were measured using a hand refract meter[22]. The total soluble solids were expressed as a percent.

Statistical analysis

Continuous normally distributed data were notified using mean and standard deviation (SD), and its particular groups were compared using Analysis of Variance (ANOVA). The post-HOC least significant difference (LSD) test was used to appreciate the statically significant difference between the control and treatments groups. Categorical variables were expressed using number and was compared using *chi-*squared *test. The overall statistically significant difference was established at* $p < 0.05$ Statistical analysis was performed using SPSS software version 23 for Windows (SPSS Inc., Chicago, IL, USA). The figures were renovated using GraphPad Prism (GraphPad Software, Inc, San Diego) software version 7.

3. Results

Growth criteria

The values of growth parameters (plant height, root length, root diameter, root size, number of leaves/ plant and fresh and dry weight for leaves and root) of sugar beet at vegetative stage season in response to heat and/ or proline treatments are given in figure (1,2).

On the other hand, proline treatments, generally induced a highly significant increase in the values of all growth criteria (plant height, root length, root diameter, root size, number of leaves/ plant and fresh and dry weight for Leaves and root) and the maximum value recorded at treatment 100 mg of proline at 50 ˚c were calculated by 158.5%, 206.6%, 241.6%, 193.7%, 196.5%, 256.6%, 256.1%, 251.1% and 257.4%, respectively as compared with control plants.

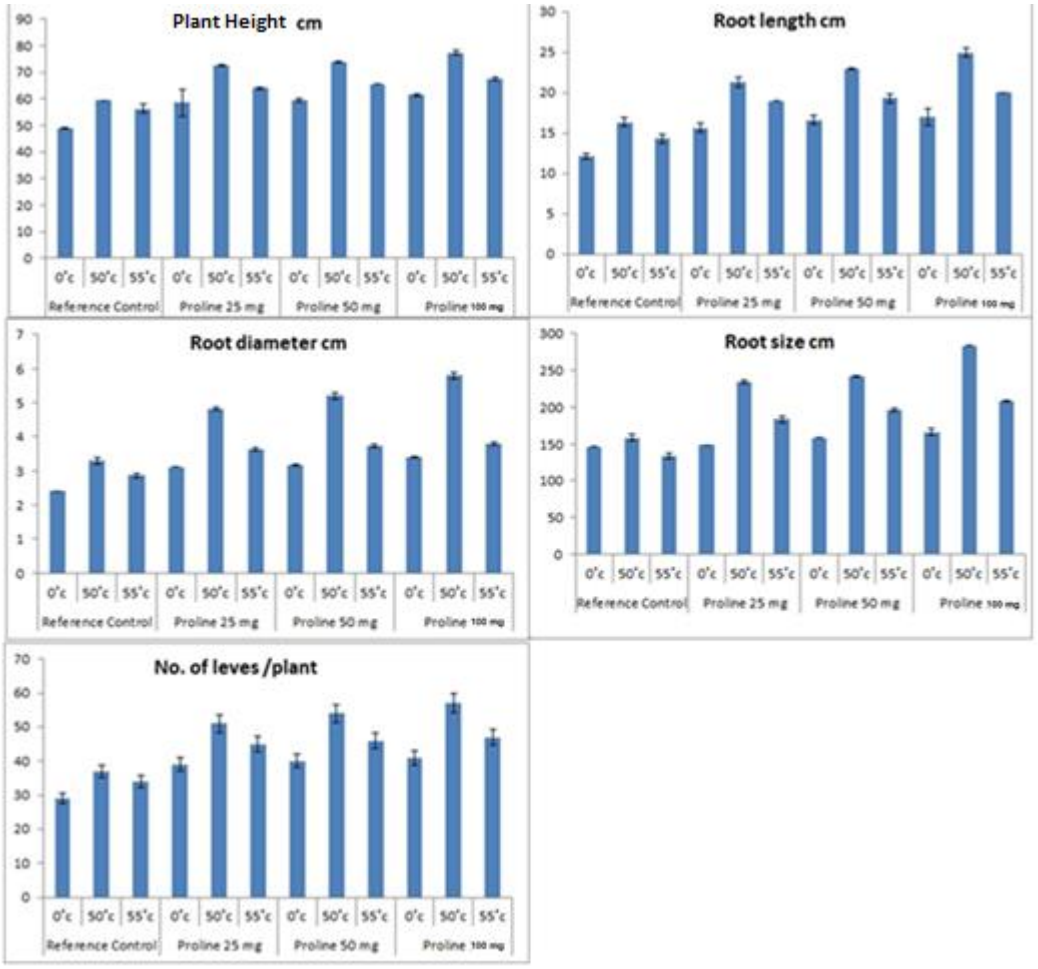

Fig (1): Effect of different temperature degree either alone or in combination with different concentrations of proline on plant height, root length, root diameter, root size and number of *Beta vulgaris* L. at vegetative stage. Each value is a mean of 5 replicates.

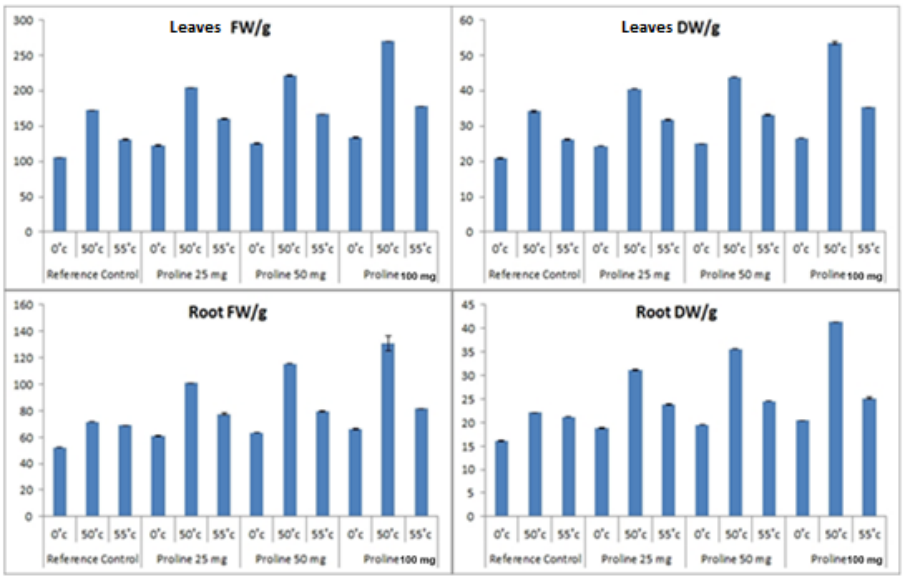

Fig (2): Effect of different temperature degree either alone or in combination with different concentrations of proline on fresh and dry weight for Leaves and root and TSS of *Beta vulgaris* L. at vegetative stage. Each value is a mean of 5 replicates.

Photosynthetic pigments

The contents of photosynthetically active pigments (chlorophyll a, chlorophyll b and carotenoids), which estimated in leaves of sugar beet plant at vegetative stages are presented in (fig. 3). It can be seen that the contents of chlorophyll a, carotenoids and total pigments were increased with the rise of heat level as compared with normal plant. In contrast, the content of chlorophyll b was gradually lowered with the rise of temperature at 55 ˚c as compared with control plant.

The application of proline treatments did not only alleviate the heat stress on photosynthetic pigment contents, but also induced a significant stimulatory effect on the biosynthesis of pigment fractions when compared with those of the reference control plant. The increase in the values of total pigments in response to proline treatments ranged from 230.5 to 238.8 % at 50 ˚c as compared with control plants.

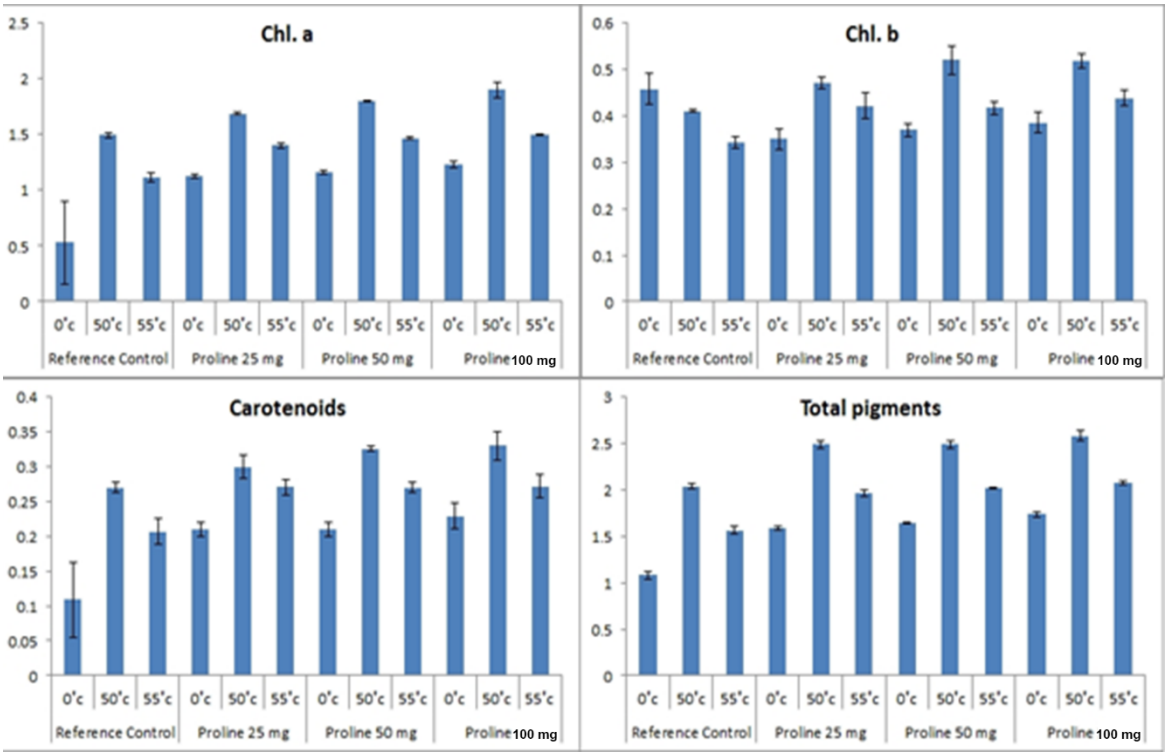

Fig (3): Effect of different temperature degree either alone or in combination with different concentrations of proline on chlorophyll pigments mg g^{-1} fresh weight of leaves per plant of *Beta vulgaris* L. at vegetative stage. Each value is a mean of 5 replicates.

Carbohydrate contents

The interactive effects of heat and proline on total soluble sugar and total carbohydrates in leaves and roots as well as total soluble solid of beet sugar plants are given in figure (4).

The data clearly indicate that the contents of total soluble sugar and total carbohydrate increased with increasing temperature levels as well as with proline interaction at vegetative stage season, and it can see more effective in the case of roots than leaves when compared with those of normal plants.

proline treatments, generally, in a highly significant increase in the contents of total soluble solid in sugar beet plant. The maximum value was estimated by 253.8%, 260% and 263.1% at 50 ˚c with 25 mg, 50 mg and 100 mg proline, respectively as compared with control plants

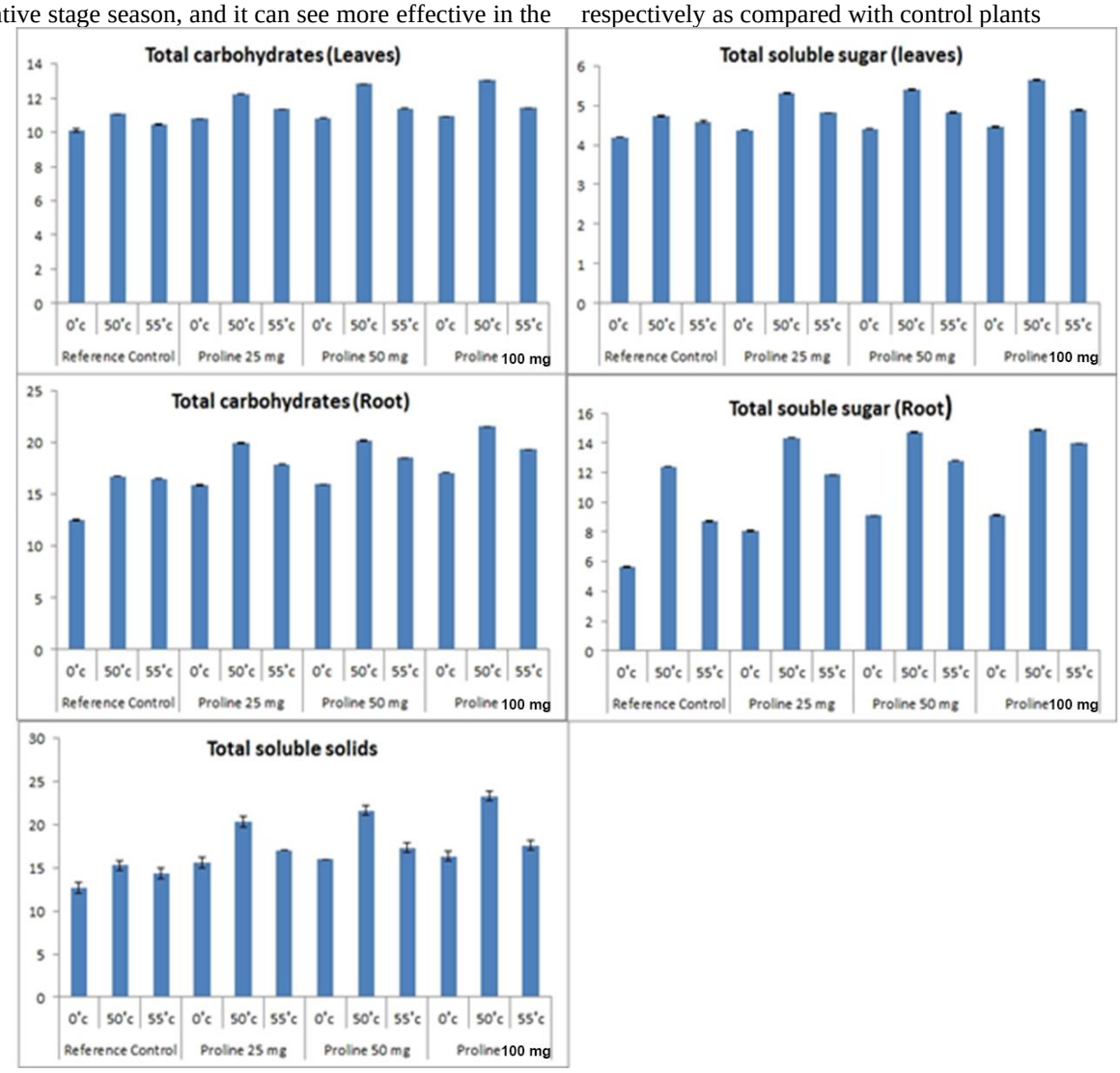

Fig (4): Effect of different temperature degree either alone or in combination with different concentrations of proline on total carbohydrate and total soluble sugar in leaves and roots and total soluble solid mg/100g DW of *Beta vulgaris* L. at vegetative stage. Each value is a mean of 5 replicates.

Total phenol and total free amino acid

The results presented in figure 5 show the effect of heat levels alone or in combination with various concentrations of proline on the total phenol and total free amino acid of the tested sugar beet plant at vegetative stage. Total phenol and total free amino acid showed progressive increases by increasing the heat levels as well as interaction with proline concentrations in the case of leaves and roots. Although, the proline treatments were more effective in leaves in case total free amino acid especially at 50 ˚c as compared with control plant.

The maximum percentage recorded for total free amino acid in leaves at 50 c in proline concentrations 25 mg, 50 mg and 100 mg were 176.8% and 189.4% and 200%, respectively; when compared with normal plant.

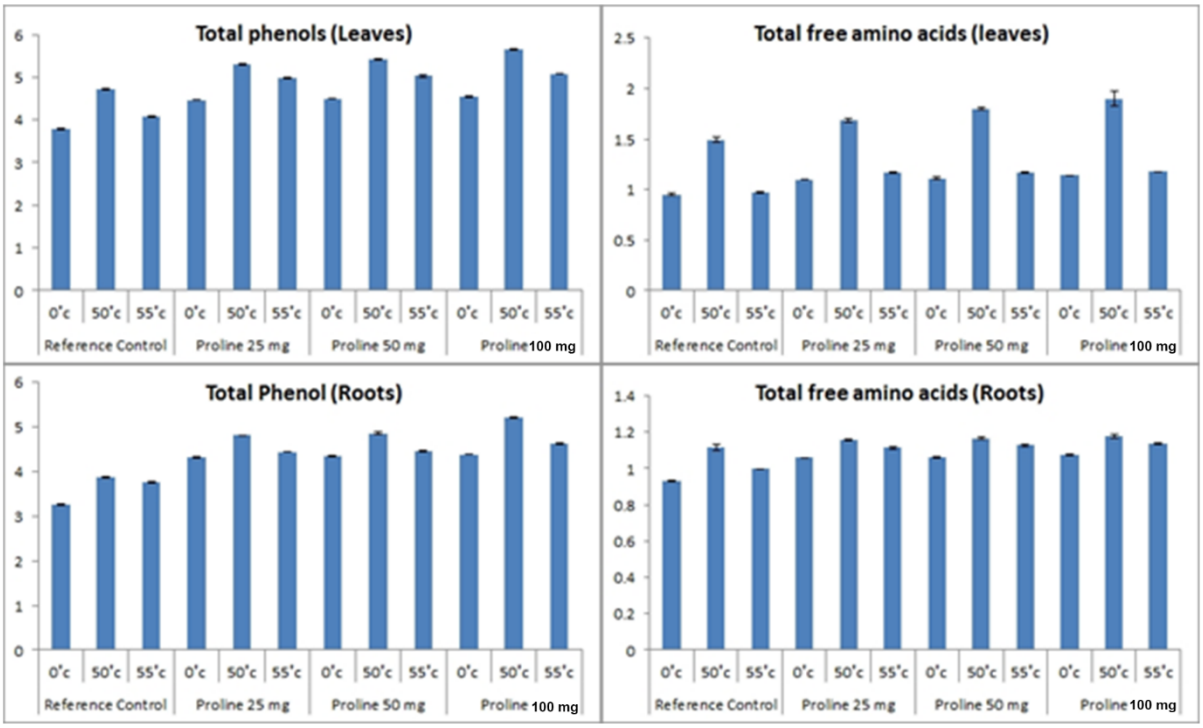

Fig (5): Effect of different temperature degree either alone or in combination with different concentrations of proline on total phenol µg of pyrogallol per g DW and total free amino acid mg g^{-1} FW for leaves and roots of *Beta vulgaris* L. at vegetative stage. Each value is a mean of 5 replicates.

4. Discussion

Crop production in the arid and semi-arid environments is highly unstable and unsustainable due to uncongenial climate. Inhospitable climate such as heat stress due to increased temperature is an important agricultural problem in many areas of the world [23].

In arid and semi-arid countries such as Egypt there is a need to increase yields of important horticultural crops without use of excessive fertilization. Use of amino acids and cheap and biodegradable chemicals as foliar applications has been shown to been shown to increase yield and agronomic performance of several crops, use of proline could be effectively employed to increase growth criteria and physiological activities in sugar beet plant. Proline at different concentrations has been shown to simulate growth and yield of corn *Zea mays* L. [24], and *Urtica pilulifera* (L.)[23] This is likely due to the N-content of these amino acids, even though their metabolic functions are different.

It has been found in the present work that most growth parameters as plant height, root length, root diameter, root size, number of leaves, fresh and dry weights of Leaves and roots of *Beta vulgaris* L. plants were significantly increased with increasing the temperature level. And also, more effective increase when treated with proline applications especially at 50 ˚c with 100 mg proline. The proposed functions of accumulated proline are osmoregulation, maintenance of membrane and protein stability, growth, seed germination while carbon and nitrogen serve as an energy store [25].

Photosynthesis is one of the most promising physiological processes contributing to plant growth and productivity of crops for food [26]. The site of photosynthesis in plants is directly depends up on the chlorophyll bearing surface area, irradiance and its potential to utilize CO_2 [27].

Results showed that, chlorophyll a, carotenoid and total pigment were increased in leaves of *Beta vulgaris* plants treated by heat levels except chlorophyll b decreased at the same levels of heat. On the other hand, the applications of proline led to more improve for chlorophyll pigments. Ali and Hassan (2013) also found that the application of a mixture of amino acids could improve several yield-related properties and pigment content of *Ta-getes erecta*[28].

Wahba et al. (2007) [23] reported a positive effect on *U. pilulifera* growth and yield parameters by amino acid, is not only used for the synthesis of proteins, but also serves as an important precursor for natural products, including pigments, alkaloids[29]. Proline and tyrosine applied as plant growth regulators on leaves increase growth parameters and beetroot leaf pigments - carotenoids and chlorophylls. This can be used in practice as a simple but effective way to increase yield and improve the agronomic quality of this vegetable[30].

The increased in total carbohydrates, total soluble sugar in leaves and roots as well as total soluble solid of heat levels in *Beta vulgaris* plants concomitantly with arrested growth rate and increase in the leaf photosynthetic pigments led to the conclusion that exposed the seed to water moisture before heat treatment may adapted the plant to heat stress and this led to enhance the photosynthetic activity and/or increased the total carbohydrates, total soluble sugar through the metabolic pathways.

In the present results the observed significant increases in pigments content in leaves of the test plants foliar spray with proline either alone or/ and heat treated throughout the entire

period of the experiments are in good support to the increased growth rate specially leaves are as well as to the increase in carbohydrates content and also yield attributes. Proline act as protect the cell by balancing the osmotic strength of cytosol with that vacuole and external environment Kishor *et al.* (2005)[31]. Also, it could be a protective response, not only due to the osmoprotectant role, but also for the radical scavenger and protein stabilization properties of both compounds [32, 33].

It was observed that, enhanced in the both of total phenols contents and total free amino acid in leaves and roots either heat treated and/ or proline application as compared with untreated control plant. Leaf phenolic contents are important protective components of plant cells. The potential of phenolics to act as an antioxidant is mainly due to their properties to act as hydrogen donators, reducing agents and quenchers of singlet O_2 [34, 35]. The synthesis of phenolics is generally affected in response to different biotic/ abiotic stresses [36].

It was also noticed in this study proline alleviate the growth and development in sugar beet plant through the enhancing the biosynthesis of other amino acids and their incorporation into protein. Also, tolerance of plant to heat level due to presoaking seeds in water before heat stress as compared with normal plant.

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