

Physiological Evaluation of Sugar Beet Genotypes under Drought Stress

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Abstract. This experiment was performed to study the effect of drought stress on some physiological characteristics of 15 sugar beet genotypes in 2009. White sugar yield, antioxidant enzymes (SOD, CAT, GPX) and biochemical biomarkers (MDA, Dityrosine, Dihydroxy goanozin) were measured. A split plot statistical design based on complete randomized block design was used to analysis the data. Highest level of drought stress treatment showed higher SOD (1796.48 mg protein / unit) activity and minimum enzyme activity obtained in normal irrigation treatment (967.24 unit /mg protein). Cultivars showed no significant difference for this enzyme. Highest level of drought stress treatment showed higher catalase (111.90 mg protein / unit) activity and minimum enzyme activity obtained in normal irrigation treatment (82.96 unit /mg protein). Highest activity for CAT appeared in genotype (13) with 107.31 (unit /mg protein) and the lowest activity of CAT appeared in genotype (1). Highest drought stress level increased GPX about 181.99 (unit /mg protein) activity and the lowest level of this enzyme appeared in normal irrigation treatments 105.16 (unit/mg protein). Significant differences for GPX observed among cultivars. Highest amount of MDA 54.27 (nmol/mg protein) and the lowest amount 30.52 (n mol /mg protein) appeared in drought stress and normal condition, respectively. Between cultivars there were no significant difference for MDA. The highest percentage of protein degradation produced in the treatment of stress 15.49 (n mol /mg protein) and lowest degradation rate of proteins produced in the normal treatment 12.47 (n mol /mg protein). No significant differences observed among cultivars for dityrosin. Results of analysis of variance showed no significant difference between irrigation treatments for dihydroxy goanozin. There was significant difference between cultivars for dihydroxy goanozin. The highest Di hydroxy goanozin obtained in genotype (8) and the lowest in genotype (10). According to the results there was not significant difference between different levels of irrigation (normal and stress) for this trait. Analysis of variance for cultivars showed significant difference at 1% level for white sugar yield. Highest White sugar yield obtained in genotype (11) and the lowest was seen in genotype (5). Correlation between antioxidant enzymes (SOD, CAT, GPX) were positive and significant. Correlation between antioxidant enzymes and oxidative stress products (MDA, Dityrosin, Dihydroxy guanozin) was significant and positive. It seems that the drought resistant sugar beet plants have not necessarily higher yield.

Keywords: White sugar yield, drought stress, antioxidant enzymes, DNA damage.

1. Introduction

Drought stress is one of the several environmental factors greatly limiting crop production and plant distribution worldwide. A common consequence of drought stress is an increased production of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^{\cdot}). These ROS are all toxic [7] and very reactive and cause severe damage to DNA, proteins and lipids [5]. To eliminate or reduce toxicity of ROS, plants have evolved various protective mechanisms (includes enzymatic and non enzymatic antioxidant defense systems), which are effective at different levels of stress-induced deterioration [16]. The identification of attributes useful for the process of screening genotypes for drought tolerance is a major challenge to plant breeder. Thus, study of the agronomic and physiological

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characteristics associated to high yield potential under sub-optimal environmental conditions could be used as stress tolerance indexes in future elite germplasm. A large amount of the damage to plants exposed to drought stress is owing to oxidative damage at the cellular level [14, 12]. If there is a severe difference between the production of ROSs and antioxidant defense in any cell, oxidative stress and damage occurs [24]. Foyer et al reported that drought-tolerant/adaptable species enhanced their antioxidant enzyme activities and increased their antioxidant contents under drought stress conditions, but drought sensitive species were unsuccessful to do so [11]. To overcome oxidative damage under drought stress conditions, plants must have efficient antioxidant system [28]. Gunes et al and Manivannan et al, reported that drought stress increased CAT and SOD activities of the sunflower [13, 26]. Also, increase of SOD, CAT and GPX activities under drought stress in canola was reported by Tohidi-Moghaddam et al [16]. However, depending on crop plant, duration of drought stress and type of antioxidants, antioxidants may increase, decrease or remain unchanged [29]. Sugar beet is one of the most important crops [2]. Moreover, sugar beet yield are determined by genotype and environment [15]. It is also well recognized that drought stress is the main restrictive factor for sugar beet yield [25]. However, the response of sugar beet to drought stress has been insufficiently studied [22]. The aim of the study was to investigate the effect of different levels of irrigation on white sugar yield, activities of some antioxidant enzymes, lipid and protein oxidation (MDA and dityrosine content) and DNA damage for 15 cultivars of sugar beet.

2. Material and Methods

2.1. Sample Preparation for Enzyme Assay and Protein Measurement

Leaves from each plant were washed with distilled water and homogenized in 0.16M Tris buffer (pH 7.5) at 4°C. Then, 0.5 mL of total homogenized solution was used for protein determination by the Lowery et al [17] method. The activity of following enzymes were expressed as specific activity (Activity/mg. protein).

2.2. Superoxide Dismutase Assay

For estimation of SOD (EC 1.15.1.1) activity, method of Beyer and Fridovich [7] was followed. SOD activity was assayed by measuring the inhibition of photo-reduction of nitroblue tetrazolium (NBT) at 560 nm using UV-Vis spectrophotometer. A unit of SOD is defined as that being present in the volume of extract that caused inhibition of the photo reduction of NBT by 50%, and was expressed in enzyme units (mg-1 protein).

2.3. Catalase Assay

Catalase (CAT) activity was determined by monitoring the disappearance of H₂O₂, measuring a decrease in the absorbance at 240 nm [1]. The reaction was carried in a reaction mixture containing 1.0 ml of the 0.5 M (pH 7.2) phosphate buffer, 3 mM EDTA, 0.1 ml of the enzyme extract and 0.3% H₂O₂, and allowed to run for 3 min. The enzyme activity was calculated using the extinction coefficient 0.036 mM⁻¹ cm⁻¹. One enzyme unit (U) determines the amount of enzyme necessary to decompose 1 μ mol of H₂O₂ per mg protein per min at 25°C and expressed as U mg⁻¹ protein.

2.4. Glutathione Peroxidase

Activity of GPX (EC 1.11.1.9) was determined as described by Rotruck et al [27]. The leaf sample was homogenized in 0.4 M Tris-HCl buffer (pH 7.0), and the reaction mixture contained 0.2 ml of tissue homogenate, 0.2 ml of 0.4 M Tris-HCl buffer (pH 7.0), 0.1 ml of 10 mM sodium azide, 0.2 ml of glutathione and 0.1 ml of 0.2 mM hydrogen peroxide. The contents were incubated at 37 °C for 10 min. The reaction was stopped by the addition of 0.4 ml of 10% trichloroacetic acid (TCA), and centrifuged. The supernatant was assayed for glutathione content by using Ellmans reagent. One unit of enzyme activity is the amount of glutathione consumed per minute at 37 °C.

2.5. Measurement of Dityrosine

1.2 grams of fresh tissue material were homogenized with 5 ml of ice-cold 50mM HEPES-KOH, pH 7.2, containing 10 mM EDTA, 2 mM PMSF, 0.1 mM p-chloromercuribenzoic acid, 0.1 mM DL-norleucine and 100 mg polyclar AT. The plant tissue homogenate was centrifuged at 5000 g for 60 min to remove debris.

Purification of o,o'-dityrosine in the clear tissue homogenized supernatant fluid was accomplished by preparative HPLC. o,o'-dityrosine was recovered by gradient elution from the C-18 column (Econosil C18, 250mm × 10 mm) [20]. The composition of eluent varied linearly from acetonitrile–water–TFA (1:99:0.02) to acetonitrile–water–TFA (20:80:0.02) over 25 min. The gradient was started 5 min after the injection. A flow rate of 4 ml/min was used. o,o'-dityrosine was analyzed by reversed-phase HPLC with simultaneous UV-detection (280 nm) and fluorescence-detection (ex. 280 nm, em. 410 nm). A phenomenexinertsil ODS 2 (150mm × 4.6 mm, 5µm)HPLC column (Bester, Amsterdam, the Netherlands)equipped with a guard column was used for these analyses. A gradient was formed from 10 mM ammonium acetate, adjusted to pH 4.5 with acetic acid, and methanol, starting with 1% methanol and increasing to 10% over 30 min. The flow rate was 0.8 ml/min. A standard dityrosine sample was prepared according to Amado et al [3]. Dityrosine was quantified by assuming that its generation from the reaction of tyrosine with horseradish peroxidase in the presence of H₂O₂ was quantitative (using the extinction coefficient $\epsilon_{315} = 4.5 \text{ mM}^{-1} \text{ cm}^{-1}$ at pH 7.5 [18]).

2.6. Determination of 8-Hydroxy-2-Deoxyguanosine (8-OH-2-DG) in Urine

8-hydroxy-2-deoxyguanosine levels in tissue extraction were measured essentially as described previously [4]. Briefly, an automated column switching LCEC method for 8-OH-2-DG is based on the unique selectivity of integral porous carbon column for purines. Samples were injected on to a C8 column and the band containing 8-OH-2-DG was then quantitatively trapped on a carbon column. The selectivity of the carbon column for 8-OH-2-DG allows elimination of interfering peaks by washing the column with a second mobile phase and then eluting 8-OH-2-DG to an analytical C18 column with an identical mobile phase containing adenosine to displace 8-OH-2-DG. Detection with series colorimetric electrodes provides qualitative certainty for 8-OH-2-DG peak by response ratios.

2.7. Malondialdehyde Analysis

Proteins of tissue homogenate were precipitated with 40% trichloroacetic acid (TCA), w/v. The MDA assay was based on the condensation of one molecule malondialdehyde with two molecules of thiobarbituric acid (TBA) in the presence of reduced reagent volumes to increase sensitivity, generating a chromogen with UV absorbance. The TBA + MDA complex was analyzed by HPLC essentially as described by Bird et al [5]. Briefly, the HPLC system consisted of a Hewlett + Packard 1050 gradient pump (Avondale, PA) equipped with an automatic injector, a 1050 diode-array absorption detector and a personal computer using Chem Station Software from Hewlett + Packard. Aliquots of the TBA + MDA samples were injected on a 5 mm Supelcosil LC-18 reversed phase column (30 × 4.6 mm). The mobile phase consisted of 15% methanol in double-distilled water degassed by filtering through a 0.5 µm filter (Millipore, Bedford, MA). The flow rate was 2 ml/min. MDA + TBA standards were prepared using tetraethoxypropane. The absorption spectra of standards and samples were identical with a characteristic peak at 540 nm. Measurements were expressed in terms of malondialdehyde (MDA) normalized to the sample protein content. Protein content was determined by the method of Bradford, with standard curves prepared using BSA [6].

2.8. White Sugar Yield

Samples of roots were collected from 4 m² and then washed. Sugar content measured with Betalyser (model D-3016). White sugar yield obtained by below equation:

$$\text{White Sugar Yield} = \% \text{SC} * \text{Root Yield}$$

2.9. Statistical analysis

Main and interaction effects of experimental factors were determined from analysis of variance (ANOVA) in SAS. The assumptions of variance analysis were tested by ensuring that the residuals were random and homogenous, with a normal distribution about a mean of zero. The LSMEANS command was used to compare means at a P<0.05 probability.

3. Results and Discussion

3.1. Superoxide Dismutase

Results showed significant difference between irrigation treatments at 1% level statistically. Highest level of drought stress treatment showed higher SOD (1796.48 mg protein / unit) activity and minimum enzyme activity obtained in normal irrigation treatment (967.24 mg protein / unit). Cultivars showed no significant difference for this enzyme.

3.2. Catalase

Results showed significant difference between irrigation treatments at 1% level statistically. Highest level of drought stress treatment showed higher catalase (111.90 mg protein / unit) activity and minimum enzyme activity obtained in normal irrigation treatment (82.96 unit /mg protein). Cultivars showed no significant difference for this enzyme. However, the highest activity for this enzyme appeared in genotype (13) with 107.31 (unit /mg protein) and the lowest activity of enzyme appeared in genotype (1). Between the interactions of the enzyme no significant difference appeared. The correlation between catalase enzyme activity and root yield was negative significant ($r^2 = -0.816$). This indicates that this plant, especially in the face of environmental stresses such as drought, with producing metabolites like antioxidant enzymes to deal with drought stress. Cost of this struggle will yield reduction. Therefore, it seems that the drought resistant plants have not necessarily higher yield. Correlation between superoxide dismutase and catalase were positive and significant.

3.3. Glutathione Peroxidase

As can be seen between irrigation treatments at 1% level observed statistically significant difference. Highest stress level showed 181.99 (unit /mg protein) activity and the lowest level of this enzyme appeared in normal irrigation treatments 105.16 (unit/mg protein). Significant differences were observed between cultivars. Highest enzyme GPX observed in genotype (14) and the lowest enzyme in genotype (7). The interaction between cultivars and irrigation were not significantly different. Correlation between glutathione peroxidase and root yield was significant and negative ($r^2 = -0.617$). This shows this plant to deal with free radical oxygen from stress by producing antioxidant enzymes struggle with the work altogether, and therefore one of the consequences, including decreased production of dry matter yield of root will be happen. Correlations between glutathione peroxidase and SOD and CAT were positive and significant ($r^2 = 0.912$) ($r^2 = 0.844$). This shows that plants deal with drought stress increase all their antioxidant systems. This enzyme is also one of the enzymes that in the face of environmental stresses has important role that gives reduced hydrogen peroxide using reduced glutathione (GSH).

3.4. Malone Dialdehyde

Analysis of variance showed significant difference at 1% level on MDA level between irrigation treatments. Highest amount of MDA 54.27 (nmol/mg protein) and the lowest amount 30.52 (n mol /mg protein) appeared in drought stress and normal condition, respectively. Between cultivars there were no significant difference. The most damage showed in genotype (8) and the highest amount of MDA showed in this genotype (46.42). Lowest damage appeared in genotype (15) with the amount of (38.65) (n mol /mg protein). Difference between the interactions were significant. As is noted significant negative correlation exist between MDA(membrane fatty acid degradation product) and root yield. There were positive and significant differences between membrane degradation product (MDA) and SOD enzymes, catalase and glutathione peroxidase. This shows that antioxidant defense enzymes to combat oxidative stress in plants act altogether. When the antioxidant defense is reduced or increased formation of free radicals, in such cases the so-called oxidative stress created comes. Oxidative stress can lead to tissue injury. When oxidative stress occurs peroxidation of unsaturated fatty acids, lipids increases free radical attack of lipids, resulting malondialdehyde production [16].

3.5. Di Tyrosine

Oxygen free radicals to attack the protein and cause slight variation in the specific locations of amino acids, peptide chains. The sensitivity of amino acids in a peptide to oxidation attack is different and various forms of activated oxygen reactive potential, will vary with time. and mean comparison indicated significant difference between irrigation treatments at 1% level. So that the highest percentage of protein degradation produced in the treatment of stress 15.49 (nM /mg protein) and lowest degradation rate of proteins produced

in the normal treatment 12.47 (n mol /mg protein). No significant differences observed between cultivars. There was significant differences between interaction of irrigation and cultivars at 5% level. Highest amount of tyrosine observed in genotype (15) and consequently destroying most of the protein was obtained in this genotype which showed higher sensitivity to drought in genotype (15). The lowest amount of tyrosine 12.55 (nmol /mg protein) obtained in genotype (13) that shows more tolerance to drought. In fact, when considering the stress level, it will increase oxygen free radicals, proteins were more prone to degradation and production rate also goes up .Correlation between dityrozin and antioxidant enzymes SOD, CAT, GPX were positive and significant.

3.6. Di Hydroxy Goanozin

Results of analysis of variance showed no significant difference between irrigation treatments .There was significant difference between cultivars. The highest Di hydroxy goanozin obtained in genotype (8) and the lowest in genotype (10).Between the interactions there were significant differences in this character. Oxidative stress increase the rate of DNA degradation. There was positive correlation between dityrosine and Di hydroxy goanozin. Drought stress by increasing free radical production and secretion levels of Di hydroxy goanozin added DNA damage and mutations and other lethal genetic effects in the plants. Our results were consistent with Tohidi-Moghadam et al(2009)[16] indicated that this material increase in drought stress conditions.

3.7. White sugar yield

According to the results there was not significant difference between different levels of irrigation (normal and stress) for this trait. Analysis of variance for cultivars showed significant difference at 1% level .Highest White sugar yield obtained in genotype (11) and the lowest was seen in genotype(5). Analysis of variance between the interaction of irrigation and cultivar was significant at 1% level.

4. References

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