Screening of drought tolerant genotypes of sugarcane through biochemical markers against polyethyleneglycole

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ABSTRACT

Sugarcane is a sugar producing crop widely distributed in the mostly areas of Pakistan. The present research was aimed to evaluate the potential antioxidant, lipid peroxidation, prolin, glycin betain and phenolic contents against polyethylene glycol. Aim of this study is to determine the antioxidant activity aginst PEG. Antioxidant activity was evaluated by different assays, antioxidant, 2,2-diphenlyl-1-picrylhydrazyl (DPPH) radical, lipid peroxidation (MDA), prolin, glycin betain and total phenolic contents. For this study leaves extracts were used and absorbance were checked on spectrophotometer.The genotype S-2003-US-114 exhibited strong antioxidant activity in the DPPH (IC50, 35.08±1.25µg/ml) assay. Drought stress imposed at various stages of crop growth resulted in an increase in lipid peroxidation and decrease in membrane stability, CPHS-35 and S-2003-US-694 had the lowest lipid peroxidation (malondialdehyde content) at control and showed highest membrane stability at PEG 12.5%. There was a significant enhancement in the Proline contents and the reduction in Proline oxidase activities under PEG treatment. Drought stress causes an increase in the amino acid and glycine betaine (GB) content. The results indicate that sugarcane possesses potential antioxidant against drought tolerant.

Key words: Sugarcane, Proline, Glycin betain, Lipid peroxidataion, Phenolic contents, DPPH and Polyethylene glycole**.**

1 INTRODUCTION

Drought stress residue an ever-growing crisis that severely limits crop production worldwide and causes important agricultural losses mainly in arid and semiarid areas (Boyer et al., 1982). Drought induced osmotic stress triggers a wide range of perturbations ranging from growth and water status disruption to the alteration of ion transport and uptake systems (Lutts et al., 1996; Bajji et al., 2000; Santos-Diaz and Ochoa-Alejo, 1994). Upon disclosure to water deficit, plants exhibit physiological, biochemical and molecular responses at both the cellular and whole plant levels (Greenway and Munns, 1980; Hasegawa et al., 2000). Generally, the plants accumulate some kind of organic and inorganic solutes in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake (Rhodes and Samaras, 1994). Among these solutes, proline is the most widely studied (Delauney and Verma, 1993). It have been suggested that the increase of free proline levels is an indicator of injury that results from imbalances in other pathways (Bhaskaran et al., 1985; Perez-Alfocea and Lahrer, 1995). Also, the beneficial roles of Proline in conferring osmotolerance have been widely reported (KaviKishor et al., 1995; Bajji et al., 2000). Reactive oxygen species (ROS) such as the superoxide radical, hydrogen peroxide and hydroxyl radical can cause lipid peroxidation and consequently membrane injury which leads to leakage of cellular content, protein degrading, enzyme inactivation, pigment bleaching and disruption of DNA strands and thus cell death (Scandalios, 1993). Enhanced production of oxygen free radicals is responsible for peroxidation of membrane lipids and the degree of peroxides damage of the cell was controlled by the potency of the peroxidase enzyme system (Sairam and Tyagi, 2004). Plants have developed a series of both enzymatic and non-enzymatic detoxification systems to counteract AOS, thereby protecting cells from oxidative damage (Sairam and Tyagi, 2004).

2 METHOD AND METRIALS

2.1 PLANT MATERIAL

Sugarcane (Saccharum officinarum L.) collected from different research station of sugarcane growing areas of Pakistan. The experiment was conducted on thirteen genotypes which were S-2003-US-778, CPHS-35, CPF-247, SPF-234, S-2003-US-114, SPF-238, S-2003-US-633, S-2002-US-160, CO-1148, CPF-246, S-2003-US-694, Rb-72 and CPF-237. All genotypes were cultivated in RCBD design in the glass-house of Faculty of agriculture, Rawlakot, Pakistan.

2.2 POLYETHYLENE GLYCOL TREATMENT

Plants were grown in pods in RCBD design. Four treatment of PEG along with control were applied to the plants. T1: control, T2: 5%, T3: 7.5%, T4:10% and T5: 12.5%. An equal concentration of fertilizer was applied to the plants. Newly growing leaves were collected for these experiments.

2.3 PHENOLIC CONTENTS

The total phenol content was determined by adding 0.5 ml of the aqueous extract to 2.5 ml, 10% Folin–Ciocalteau's reagent (v/v) and 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated at 45 0C for 40 min, and the absorbance was measured at 765 nm in the spectrophotometer. Gallic acid was used as a standard phenol (Singleton, Orthofer R & Lamuela-Raventos, 1999). The mean of three readings was used and the total phenol content was expressed as milligrams of gallic acid equivalents/ g extract.

2.4 DPPH RADICAL-SCAVENGING

Scavenging of the stable radical, DPPH, was assayed in vitro (Hatano, Kagawa, Yasuhara, & Okuda, 1988). The extract (10– 100 µg) was added to a 0.5 ml solution of DPPH (0.25 mM in 95% ethanol). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm in a spectrophotometer. Percent inhibition was calculated from the control. Vitamin C was used as a standard compound in the DPPH assay.

2.5 ESTIMATION OF PROLINE CONTENTS

The PRO contents were estimated by the method of Bates et al,. (1973). The explants material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10000 rpm. Supernatant was used for estimation of PRO contents. The reaction mixture consisted of 2ml acid ninhydrin and 2ml of glacial acetic acid, which was boiled at 100oc for 1h. After termination of reaction in ice bath, the reaction mixture was extracted with 4ml of toluene and absorbance was read at 520 nm.

2.6 ESTIMATION OF GLYSINEBETAINE CONTENTS

The amount of GB was estimated according to the method of Grieve and Grattan (1983). The plant tissues were finely ground, mechanically shaken with 20ml demonized water for 24 h at 25oc. The sample was then filtered and the filtrates were diluted 1:1 with 2N H2SO4. Aliquots were kept in centrifuge tubes and cooled in ice water for 1 h. Cold KI-I2 reagents were added and the reactants were gently stirred with a vortex mixture. The tube was stored at 4oc for 16 h and then centrifuged at 10000 rpm for 15 min at 0oc. The supernatants was carefully aspied with a fine glass tube. The periodide crystals were dissolved in 9ml of 1,2- dichloroethane. After 2 h, the absorbance was measured at 365 nm using GB as standard and expressed in mg/g dry mass (dm).

3 RESULTS

Antioxidant constituents of plant origin are very important substances that have the ability to defiend the body from injuries caused by free radical induced oxidative stress (Ahmad et al., 2010). Regenerated platelets can build up secondary metabolites similar to those found in mother plant. Antioxidant potential in regenerated leaves solutions of S. officinarum was determined by using DPPH°-free radical Significantly higher antioxidant potential showed by S-2003-US-633 was (61.85%) at a 25ul leaves solution, but increased when concentration of leaves solution was increased, viz; 50ul, 100ul, 150ul and 300ul showed 63.69%, 66.89%, 70.19% and 73.03% respectively. When PEG concentration was increased then scavenging percentage decreased as, 34.36% of 25ul sample solution. Significantly lower antioxidant potential (45.48%) at control PEG concentration and 25ul leave sample was observed in S-2003-US-694 and sample concentration increased then antioxidant potential increased i.e., S-2003-US-694 showed 45.48% at 25 ul sample but, at 300 ul sample showed 56.92% and reduced when PEG concentration increased it: at control showed 45.48% but, at 12.5% PEG concentration showed 18.73% at 25ul sample solution and 28.12% at 300ul sample solution (Figure.2).
GB contents mostly increased when PEG concentration increased. Drought stress has a profound effect on the GB accumulation in sugarcane genotypes. The GB contents increased under abiotic stresses Abdul et.al., (2007). Plants showed an increase in GB with increasing stress Abdul et al., (2007). Rb-72 showed maximum results against PEG at control is 28.6 µg/g and the concentration of GB increased when the PEG concentration increased, as at control 28.6 µg/g , PEG-5% (37.6 µg/g), PEG-7.5% (41.2 µg/g), PEG-10% (47.8 µg/g) and PEG-12.5% showed (49.8 µg/g) (Fig-3) and minimum result are shown by SPF-234 is15. 6 µg/g (Fig- 4). But, SPF-238, CPF-246 and CO-1148 show decline in GB contents when PEG concentration is 12.5%. These genotypes don't survive at high concentration of PEG (Fig- 3,4). Proline contents of sugarcane genotypes mostly increased but those genotypes which show a decline in GB also show the same results in Proline concentration. Increased Proline accumulation was reported in water stressed sorghum Abdul et.al., (2008). Increased Proline in the stressed plants may be an adaptation to overcome the stress condition supplies energy for growth and survival and thereby helps the plant to tolerate stress Abdul et.al., (2008). As PEG concentration increased then the concentration of Proline contents increased mostly (Fig-5, 6). Maximum Proline contents released in CPF-246 (44.4 µg/g) and minimum Proline contents released by SPF-234 (20.1 µg/g) in (Fig-5). In the present study, higher membrane stability showed by CPHS-35 (30.225 nmol/g) (Fig-7) and maximum level of malondialdehyde released by 60.605 nm/g in CPF-246 (Fig-8).
Maximum phenolic were released by S-2003-US-778 (58.78%) at control and when PEG concentration was increased then the phenolic contents were decreased gradually. Minimum phenolics were released by Rb-72 (36.17) at control and gradually decreased when PEG concentration was increased (Fig-9).

4 DISSCSION

Antioxidant constituents of plant origin are vital substances that possess the ability to protect the body from damage caused by free radical induced oxidative stress (Ahmad et al., 2010). DPPH is usually used as a substracte to evaluate anti-oxidative activity of antioxidant. The method is based on the reduction of the maternal DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. The extract was able to reduce the stable radical DPPH to the yellow colored diphenylpicrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, poly-hydroxy aromatic compounds (hydroquinone, pyrogallol, gallic acid, etc.) reduce and decolorize DPPH by their hydrogen donating ability (Blois, 1958).

The proline contents increased in all samples of sugarcane under PEG concentrations. My result coincides with preceding works. Increased proline accumulation was stated in water stressed sorghum (Abdul Jaleel et al., 2008). Increased proline in the stressed plants may be an adaptation to overcome the stress conditions. Proline accumulated under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress (Abdul Jaleel et al., 2008). Under abiotic stress like ultra violet light the proline contents showed an increase in wheat (Abdul Jaleel et al., 2007). Proline accumulation in plants might be a scavenger and acting as an osmolyte. The reduced proline oxidase may be the reason for increasing proline accumulation.

Glycine betaine is a quaternary ammonium compound having the primary chain supplemented with methylated nitrogen (McNeil et al., 1999) and has been stated to accumulate in the plantlets undergoing drought stress. It has been found not only acting as a compatible osmolyte, but also as an osmoprotectant. The molecule is chloroplastic in origin and is responsible for maintaining photosynthetic efficiency during drought stress, besides having a protective ability against heat or cold shock (Ashraf and Harris, 2004; McNeil et al., 1999). The molecule has been stated to decrease the water potential in the cells during the stress conditions resulting in delayed wilting in tolerant varieties as against their susceptible counter-parts. In my study SPF-234 showed minimum results against GB and maximum result were obtained by Rb-72.

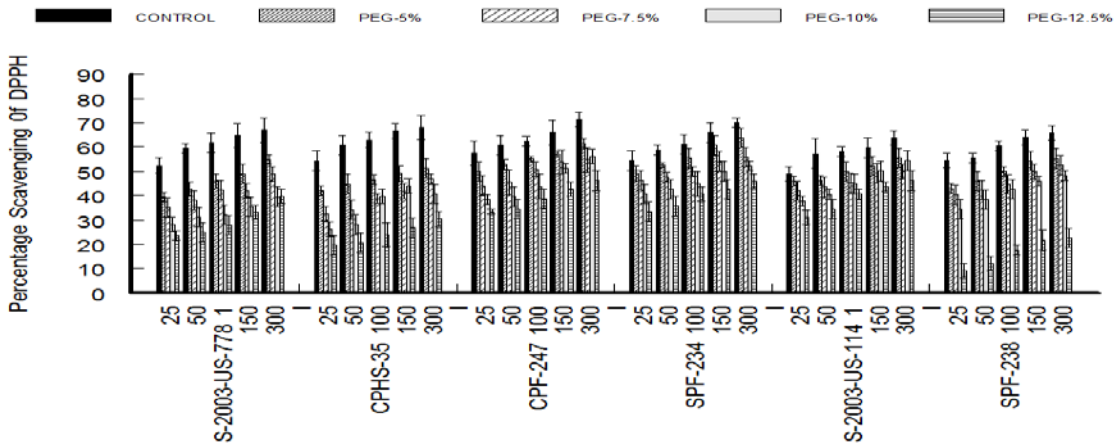
The stress induced increase in leaf membrane damage, reduced uptake of CO2 as a result of closer stomatal, decreased hydrolytic enzyme activity and increased lipid peroxidation level, it is due to stimulate formation of AOS such as superoxide, hydrogen peroxide, and hydroxyl radicals. Among AOS, superoxide is converted by the SOD enzyme into H2O2. (Noctor and Foyer,1998). Allen (1995) also reported that much of injury to plants caused by various stresses is associated with oxidative damage at cellular level such as cell membrane damage.

In present study, higher membrane stability and lower level of lipid peroxidation in resistant genotypes (CPHS-35) might be due to the increased activities of antioxidant enzymes (APO, GR, POX, CAT and SOD) which act as a damage control system and thus provide protection from oxidative stress, otherwise, which would cause destruction of cell membrane and protein, DNA structure and inhibit the photosynthesis as stated under water stress condition (Sairam and Saxena, 2000; Sairam and Tyagi, 2004). Inferior lipid peroxidation and higher membrane stability (lower ion leaching) have also been stated in tolerant genotypes of wheat (Kraus et al., 1995) and Rice (Tijen and Ismail, 2005).

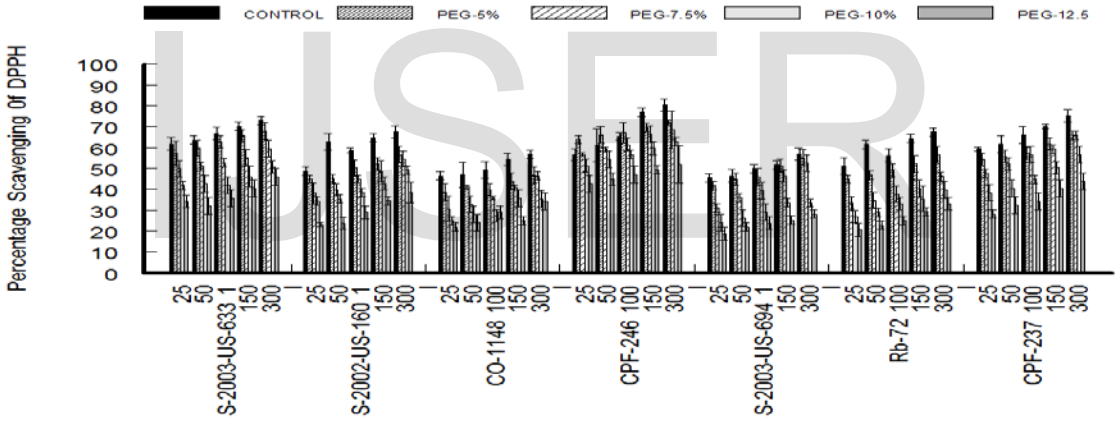
Phenolic compounds present in these extracts are stated to have beneficial effects on other chronic diseases such as coronary heart disease (Forester & Waterhouse, 2009). These health effects are reported to be due to antiradical and the antioxidant properties of phenolics in plants and plant derivatives (Lurton, 2003).

REFERNCES

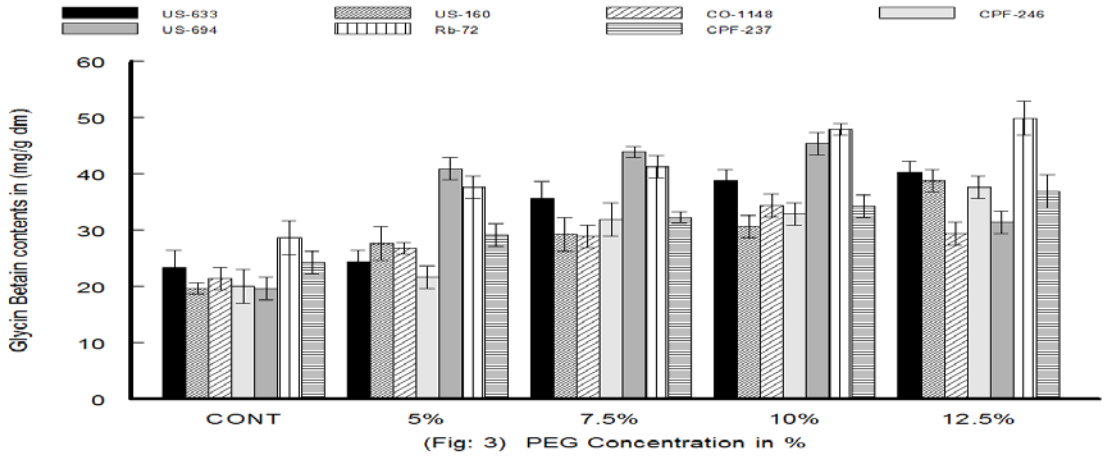
1. Ahmad N, Fazal H, Abbasi BH, Rashid M, Mahmood T, Fatima N. 2010. Efficient regeneration and antioxidant potential in regenerated-tissues of Piper nigrum L. Plant Cell Tiss. Org. Cult., 102: 129-134.
2. Ashraf M. and Harris PJC. 2004. Potential biochemical indicators of salinity tolerance in plants Plant Sci. 166: 3-16.
3. Allen RD. 1995. Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol., 107: 1049-1054.
4. Abdul Jaleel, C., R. Gopi, B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharan and R. Panneersevam. 2007. Alteration in germination, seedling vigour, lipid peroxidation and proline metabolism in Catharanthusroseusseedling under salt stress, S.Afr.J.Bot., 73:190-195.
5. Abdul Jaleel, C., B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharan and R. Panneersevam. 2007. Calcium chloride effect on salinity induced oxidative stress, Proline metabolism and indole alkaloid accumulation in Catharanthusroseus, C.R. Biol., 330:674-683.
6. Abdul Jaleel, C., P. Manivannan, A. Kishorekumar, R. Sridharan and R. Panneersevam. 2008. Salt stress mitigation by calcium chloride in Phyllanthusamarus, Acta. Bot. Croatica., 67: 53-62.
7. Abdul Jaleel, C., R. Gopi and R. Panneersevam. 2008. Biochemical alteration in white yam (DioscorearotundataPoir) under triazole fungicides; impacts on tuber quality, Czech J. Food Sci., 26: 298-307.
8. Blois MS. 1958. Antioxidant determination of the use of stable free radicals. Nature. 181:1199-2000.
9. Boyer JS. 1982. Plant productivity and environment. Science. 218: 443-448.
10. Bhaskaran S, Smith RH, Newton RJ. 1985. Physiological changes in cultured sorghum cells in response to induced water stress. Plant. Physiol 79: 266-269.
11. Bates, L.S., R.P. Waldern and Teare I.D. 1973. Rapid assay for determination of free Proline for water stress studies, Plant Soil, 39:205-207.
12. Bajji M, Lutts S, Kinet JM. 2000. Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in callus cultures issued from durum wheat (Triticum durum Desf.) cultivars differing in drought resistance. J. Plant. Physiol. 156: 75-83.
13. Delauney AJ, Verma DPS. 1993. Proline biosynthesis and osmoregulation in plants. Plant J. 4: 215-223.
14. Forester, S.C. and A.L. Waterhouse. 2009. Metabolites are key to understanding the health effects of wine polyphenolics. J. Nutr., 138: 1824S-1831S.
15. Greenway H, Munns R. 1980. Mechanisms of salt tolerance in nonhalophytes.Annu. Rev. Plant Physiol. 31: 149-190.
16. Grieve, C.M., Grattan S.R. 1983. Rapid assay for determination of water soluble quanternary ammonium compounds, Plant Soil, 70:303-307.
17. Hasegawa P, Bressan RA, Zhu JK, Bohnert HJ. 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Mol. Biol. 51: 463-499.
18. Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. 1988. Two new flavonoids and other constituents in licorice root; their relative astringency and radical scavenging effects. Chemical and Pharmaceutical Bulletin, 36, 2090–2097.
19. Kishor PBK, Hong Z, Miao GH, Hu CAA, Verma DPS. 1995. Overexpression of [delta] -Pyrroline-5-Carboxylate Synthetase Increases Proline Production and Confers Osmotolerance in Transgenic Plants. Plant. Physiol. 108 (4): 1387-1394.
20. Lurton, L. 2003. Grape polyphenols: New powerful health ingredients. Innov. Food Technol., 18: 28-30.
21. Lutts S, Kinet JM, Bouharmont J. 1996. Effects of various salts and of mannitolon ion and Proline accumulation in relation to osmotic adjustment in rice (Oryza sativa L.) callus cultures. J. Plant. Physiol.149: 186-195.
22. McNeil SD, Nuccio ML, Hanson AD. 1999. Betaines and Related Osmoprotectants. Targets for metabolic engineering of stress resistance. Plant Physiol. 120: 945-949.
23. Noctor G, Foyer CH. 1998. Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Bio., 49: 249-279.
24. Perez-Alfocea F, Larher F. 1995. Sucrose and proline accumulation and sugar efflux in tomato leaf discs affected by NaCl and polyethylene glycol 6000 iso-osmotic stresses. Plant. Sci. 107: 9-15.
25. Rhodes D, Samaras Y. 1994. Genetic control of osmoregulation in plants. In cellular and molecular physiology of cell volume regulation. Strange K. Boca Raton: CRC Press. pp: 347-361.
26. Sairam RK, Saxena DC. 2000. Oxidative stress and Antioxidants in wheat genotypes: Possible mechanism of water stress tolerance. J. Agron. Crop Sci., 184: 55-61.
27. Sairam RK, Tyagi A. 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci., 86: 407-421.
28. Santos-Diaz MS, Ochoa-alejo N. 1994. PEG-tolerant cell clones of chilli pepper (Capsicum annuum L.): Growth, osmotic potentials and solute accumulation. Plant Cell Tiss. Org. Cult. 37: 1-8.
29. Scandalios JG. 1993. Oxygen stress and superoxide dismutases. Plant Physiol., 101: 7-12.
30. Sairam RK, Tyagi A. 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci., 86: 407-421.



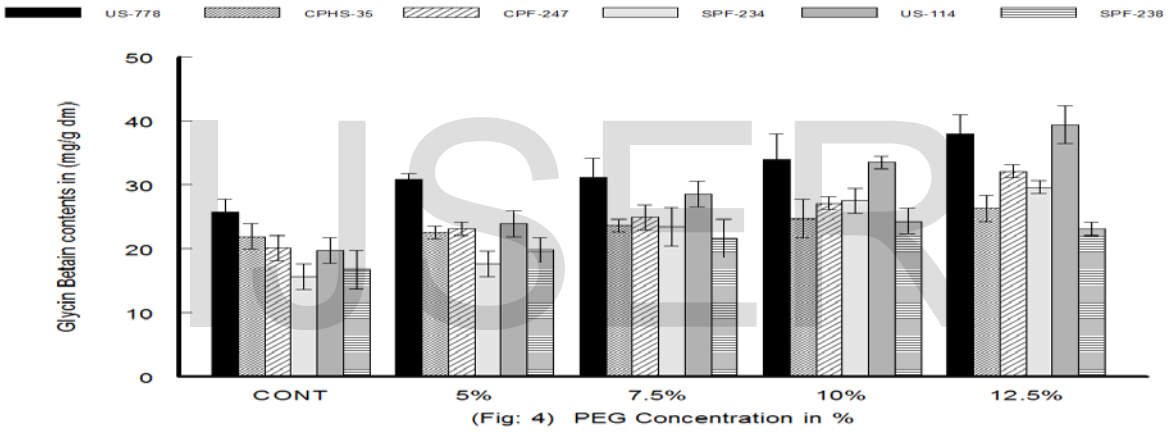
(Fig:1) Leaves extract concentration in ul/ml



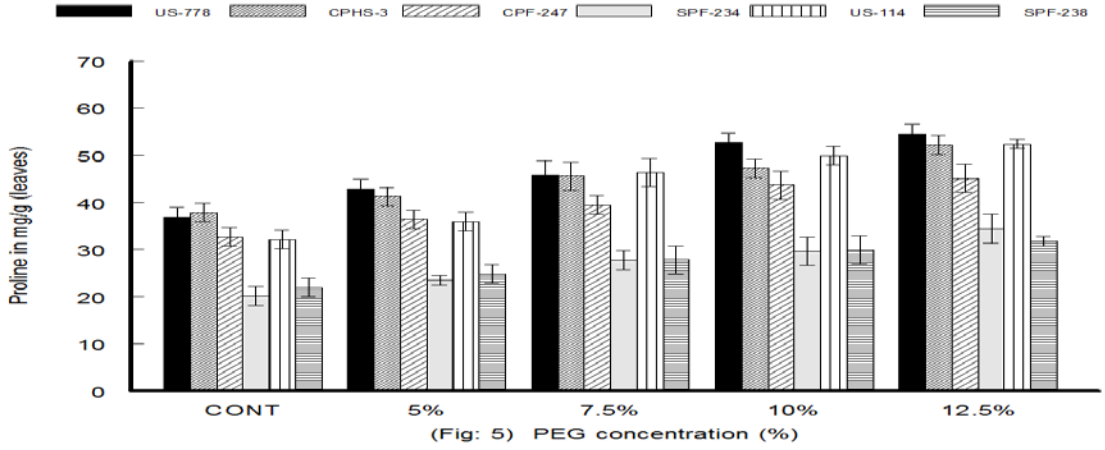
(Fig:2) Leaves extract concentration in ul/ml



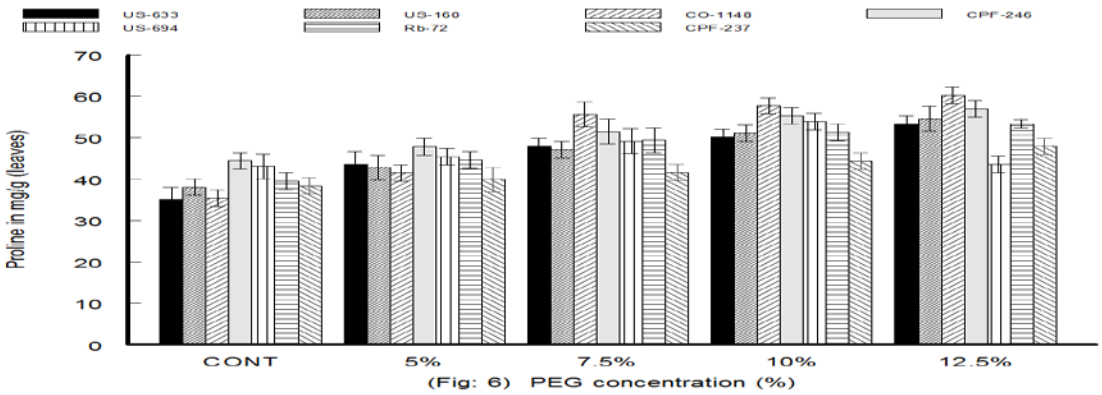
(Fig: 3) PEG Concentration in %



(Fig: 4) PEG Concentration in %



(Fig: 5) PEG concentration (%)



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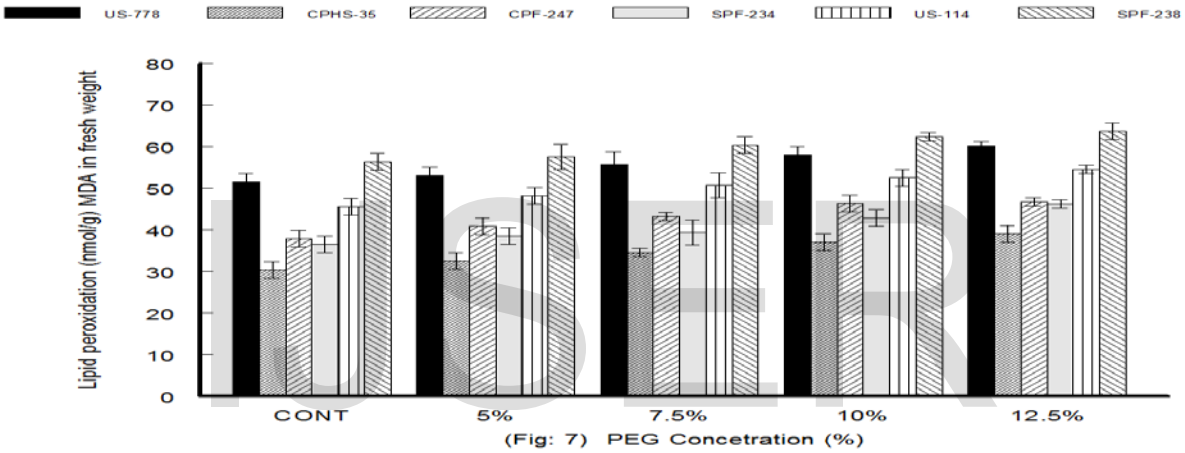

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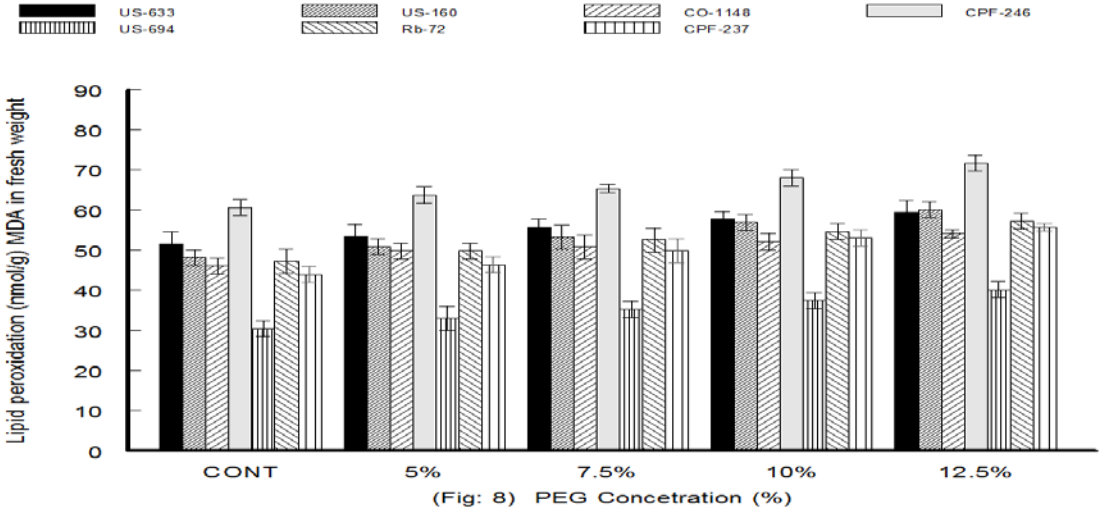

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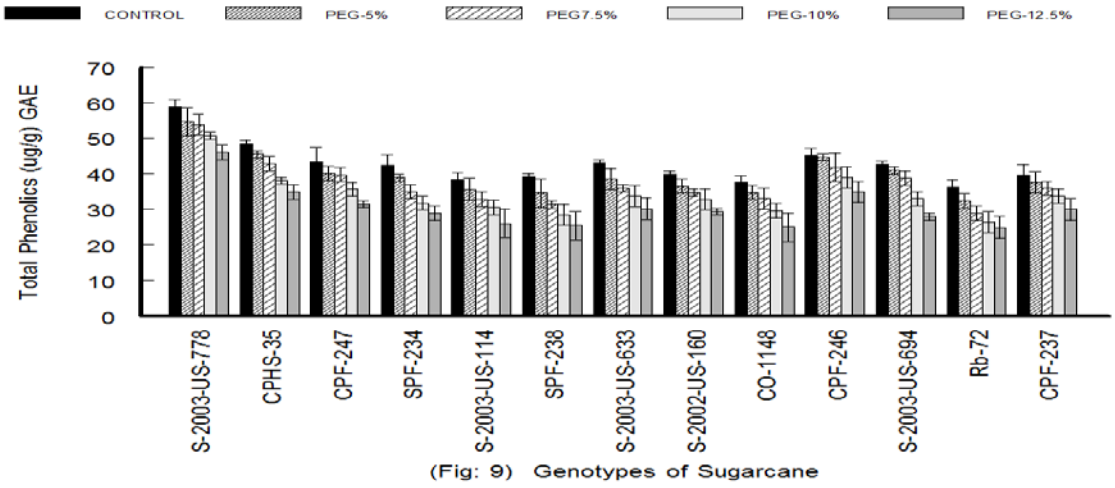

(Fig: 9) Genotypes of Sugarcane