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Classification of Sugarcane Genotypes Based on Heat Stress and Morphological Parameters

Syed Rizwan Abbas, Asad Hussain Shah, Aurangzeb Rao

Department of Plant Breeding and Molecular Genetics, Faculty of Agriculture

University of Azad Jammu and Kashmir, Pakistan

E-mail: drsyedrizwanabbas@gmail.com

Syed Dilnawaz Ahmad Gardazi

Vic Chancellor of University of Azad Jammu and Kashmir, Muzaffarabad Azad Jammu and Kashmir, Pakistan

Syed Mubashir Sabir

Faculty of Agriculture

University of the Poonch Rawalakot, Azad Jammu and Kashmir, Pakistan

Muhammad Rehan Abbas

Department of Computer Science, University of Azad Jammu and Kashmir, Pakistan

Attiya Batool, RizwanTaj Khan

Department of Botany, University of Azad Jammu and Kashmir, Pakistan

Nisar Ahmed Khan

School of Plant, Environmental & Soil Sciences, Louisiana State University Agricultural Center, USA

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Abstract

Leaves of thirteen genotypes of sugarcane were treated in the oven for heat stress. The treated leaves were used for the estimation of phenolic contents and the measurement of lipid peroxidation. Minimum moisture loss is showed by CSSG-668 and maximum moisture loss showed by NSG-45. Maximum phenolic contents were observed in HSF-240 (65.7 mg GAE/100 ml) and minimum results showed by Lho 83-153 and CP-43-33 (32.98 and 33.78 mg GAE/100 ml). Lho 83-153, HSF-242 and S-2002-US-133 were showed high tolerant against heat therefor, showed higher membrane stability, maintenance of high fv/fm ratio under heat stress and lower lipid peroxidation of membranes. Hence, the relative tolerance of genotype to heat stress as reflected by its lower lipid peroxidation, higher membrane stability and pigment concentration is related to the levels of activity of its antioxidant enzymes.

Keywords: Sugarcane, Heat stress, Lipid peroxidation, Phenolic contents, Morphological parameters

1. Introduction

Sugarcane (*Saccharum officinarum* L.), an important cash crop of Pakistan, plays a vital role in the economic of farmers and is a survival of the ever-expanding sugar industry in Punjab. Pakistan is a significant cane producing country; it is ranked fifth in the world cane acreage and is 15th in sugar production. Sugarcane is grown on over a million hectares and delivers the raw materials for Pakistan's 84 sugar mills, which comprise the country's second largest agro-industry after textiles (Rehman, 2009). Thus evolution of new high cane and sugar yielding varieties and improved production technology that is, better management practices (BMPs) are current need for improving livelihoods of sugarcane growers and other crops and ultimately betterment of mill owners also (Nasir, 2006; Iftikhar *et al.*, 2010). Addition of organic matter in soil improves the physicochemical and biological properties. Assessment of adaptation, performance of various cane varieties in different ecologies, and evaluation of agronomic characters of exotic cane varieties are necessary before a variety is introduced for commercial cultivation. Cane yield and cane sugar evaluation of varieties were generally done on stalk, stalk height, stalk girth, stalk weight and fiber contents (Akhtar *et al.*, 2000). The major commercial sugar estates in Pakistan are all located in its Punjab and Sindh region: an ecological zone that is often subject to varying periods of drought due to inadequate and erratic distribution of rainfall. This poses a serious problem for sugar-cane, which has a 10 to 12-month crop cycle. When drought occurs during the formative growth phase (0 to 120 days after planting), the crop experiences slow growth, which subsequently results in low cane yield (Barnes, 1974). In order to improve sugar yields, it is necessary to develop cultivars that remain productive under low soil moisture availability. Screening for drought tolerance can be achieved by simulating moisture stress at the critical growth stage of the crop, either under controlled atmosphere conditions or under field conditions.

Little success has been achieved in screening plants for drought tolerance by selection for morphological features due to genotype × environment interactions (Bendelow *et al.*, 1955). Blum (1988) hypothesized that the simplest approach for drought tolerance would be to utilize the natural stress of the field environment to screen genotypes. Despite the relatively drought

tolerant nature of the sugar-cane plant, varieties differ markedly in their tolerance to drought (Moore, 1987). Sugar-cane varieties susceptible to drought are likely to have wilt, early reduction of cane production; while tolerant varieties remain turgid and maintain near-optimum growth for longer production (Moore, 1987). Tolerant varieties have the ability to reduce transpiration losses, maintaining at the same time adequate absorption of water from the soil.

The mechanism of the toxic effect of organophosphate compounds involves the inhibition of acetylcholinesterase and other non-specific esterases through phosphorylation at –OH serine in the esterase centre of the enzyme. This mechanism is the same for all insecticides of the group, irrespective of differences in their chemical structure (Lotti, 2001). The inhibition of the activity of cholinesterase enzymes causes an increase in the level of endogenous acetylcholine in the organism and results in its binding to muscarinic and nicotinic receptors in both the peripheral and central nervous systems. This increase in the *central nervous system* (*CNS*) disturbs the balance between neurotransmitters and causes the onset of acute intoxication symptoms (Lotti, 2001). The symptoms of acute intoxication with organophosphates have been well described, while the effects of chronic exposure to these compounds are not completely clear. Many authors postulate that they may have an effect on redox processes in a number of organs, thus, leading to disturbances in these processes and causing enhancement of lipid peroxidation, both in acute and chronic intoxication by these compounds (Abdollahi, 2004; Sharma, 2005). As increased generation of reactive oxygen species and lipid peroxidation induced by these species underlies many diseases, it is extremely important to determine the effect of organophosphate insecticides on lipid peroxidation processes (Mates, 1999; Yagi, 1987).

The objective of this study was to look for drought tolerance sugar-cane germplasm in Pakistan. Such screening will aid in the classification of genotypes, in the selection of commercial sugar-cane cultivars, and in the identification of parents to genetically improved drought tolerance in sugar cane cultivars developed for Pakistan.

2. Materials and Methods

Sugarcane genotypes were collected from sugarcane growing agencies of Pakistan and cultivated in the Glasshouse of Faculty of Agriculture, Rawalakot.

2.1 Morphological Parameters

Thirteen local sugarcane accessions or varieties namely HSF-240, SPF-213, CP-77-400, HSF-242, CP-43-33, NSG-45, NSG-60, CPF-198, CSSG-668, S-2002-US-133, S-2003-US-718, Lho 83-153 and NSG- 555 planted in the Glasshouse of Faculty of Agriculture, Rawalakot were used for this study. The experiment was laid out in a randomized complete block design (RCBD) with three replications. All agronomic practices were kept normal for all the 13 genotypes. Data on plant height (cm), number of tillers per plant, number of leaves, cane diameter (cm), leaf area (cm^2), intermodal distance (cm), number of nodes were collected from 10 randomly selected stalks from each replication.

2.2 Heat Stress in the Laboratory

Three leaves were selected randomly from 13 sugar-cane accessions for laboratory screening. The leaves were cut early in the morning from mature cane stalks between the 5th and 6th leaf from top visible dewlap. Leaf area was calculated based on 3/4 of length and breadth of the product (Barnes 1974). The leaves were weighed and kept in an oven at 35℃ for three hours. Percentage (%) of moisture loss from the excised leaves was determined using the formula of Blum (1988): {(Fresh weight -Dry weight) / (Dry weight) ×100}.

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℃ for 40 min, and the absorbance was measured spectrophotometrically at 765 nm. Gallic acid was used as a standard phenol (Singleton et al., 1999). The mean of three readings was used and the total phenol content was expressed as milligram of gallic acid equivalents/ g extract.

2.4 Lipid Peroxidation Estimation

The level of malonyldialdehyde, as a substance that reacts with thiobarbituric acid (TBARS), was determined in the homogenates of the organs and in the serum according to the method of Buege et al. (1978). 10% homogenates of tissues were added to 0.15 m KCl and centrifuged at 10000 x g for 30 min. To 0.5 ml of supernatant or 0.5 ml of serum, 0.5 ml of 50%, trichloroacetic acid was added and centrifuged again at 5000 x g for 5 min. After the final centrifugation, the tubes with 0.5 ml of supernatant and 0.5 ml of thiobarbituric acid covered with aluminium foil were kept in a water bath at 90℃ for 1 h. The absorbance was read at 540 nm at room temperature against the blank and then concentration of thiobarbituric acid reactive substances was read from standard calibration curve, which was plotted using 1, 1, 3, 3'tetra – ethoxy propane. The resulting concentration of TBARS was presented in micromoles of TBARS per dm^3 of serum or in nanomoles of TBARS on g of tissue.

3. Results and Discussion

3.1 Morphological Parameters

Three logical clusters I, II and III were based on morphological parameters. In cluster I, HSF-240, NSG-60 and CP-77-400 were present. In cluster II SPF-213, CP-43-33, NSG-45 and NSG-555 were present and HSF-242, CPF-198, CSSG-668, S-2002-US-133, Lho 83-153 and S-2003-US-718 were present in cluster III. HSF-240 and S-2003-US-718 showed maximum difference because total height of plant, leaves length, number of internodes and number of tillers vary from each other. Clusters II and I were connected on gene linkage 77 due to cane diameter and leaf size similarities; they had dissimilarities in plant height, number of tillers, internode distance and number of internodes as shown in Figure 5. Clusters I and II differ from cluster III due to plant height, internode distance, cane diameter and number of leaves; but their similarities are due to leaf size. Therefore, clusters I and II are connected to cluster III on 138 gene linkage as shown in Figure 1.

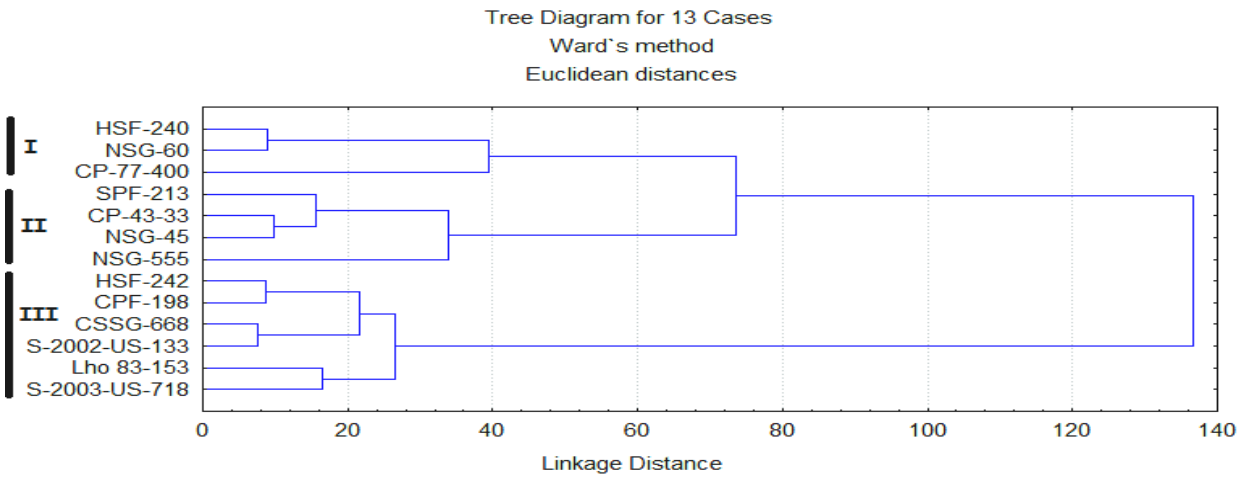

Figure 1. Dendrogramme of morphological data of thirteen sugarcane genotypes.

3.2 Total Moisture Loss

Mean percent moisture loss from excised leaves, which is an index of moisture stress, is presented in Figure 2. Minimum moisture loss is found in HSF-240 and CSSG-668 (74.99 and 74.64% respectively) and maximum moisture loss in CP-43-33 and NSG-45 (84.033 and 83.948% respectively); they were observed to lose more moisture than accessions with smaller leaf area, as a result of their larger evaporating surface for transpiration. Although leaves of some accessions with large surface area lost much moisture when subjected to oven temperature, some accessions with small leaf area also exhibited similar behaviour. Barnes (1974) attributed such response to the presence of many stomata and large bulliform cells in the leaves, whereas Meneses (1986) attributed it to the release of electrolytes from the cells. Varieties that are least damaged by heat (that is, low moisture loss) are likely to be tolerant to drought. This had been observed also in the study of Viqueira *et al.*, (1984).

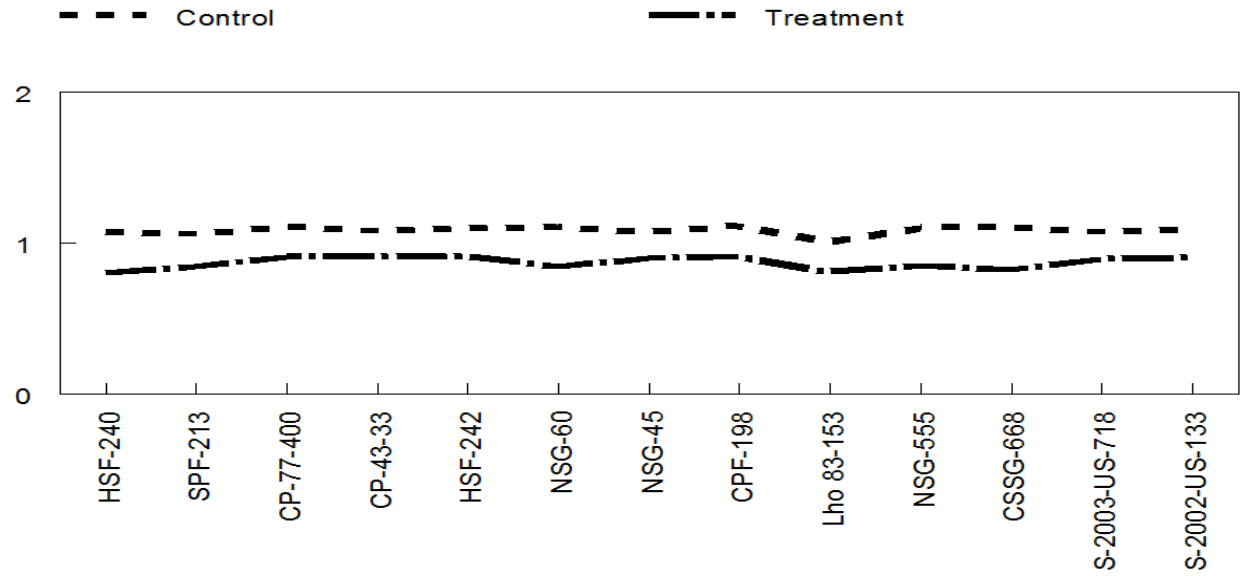

Figure 2. Result of thirteen genotypes of sugarcane (heat treatment).

3.3 Total Phenolic Compounds

The total phenolic contents in extracts obtained from the control and heat treated leaves of sugarcane are shown in Figure 3. HSF-240 showed high results (65.7 mg GAE/100 mL) at control and after treatment the absorbance decreased (49.56 mg GAE/100 mL). CPF-198 and NSG-45 showed the best results of 53.73 and 51.87 mg GAE/100 mL at control and gradually decreased when stress increased. After treatment these genotypes showed results of 37.88 and 34.65 mg GAE/100 mL. Lho 83-153 and CP-43-33 showed lowest values of 32.98 and 33.78 mg GAE/100 mL) at control and after treatment the values were 19.41 and 21.76 mg GAE/100 mL, as seen in Figure 3. Triantaphyllou *et al.*, (2001) stated that the extracts of *Mentha* species contained bound phenolic acids and flavonoids. The major phenolic acids reported in water-soluble *Mentha spicata* extract are eriocitrin, luteolin glucoside, rosmarinic acid and caffeic acid (Dorman *et al.*, 2003). Phenolic compounds present in these extracts are reported to have beneficial effects on other chronic diseases such as coronary heart disease (Forester and Waterhouse, 2009). These health effects are reported to be due to antiradical and antioxidant properties of phenolics in plants and their derivatives (Lurton, 2003).

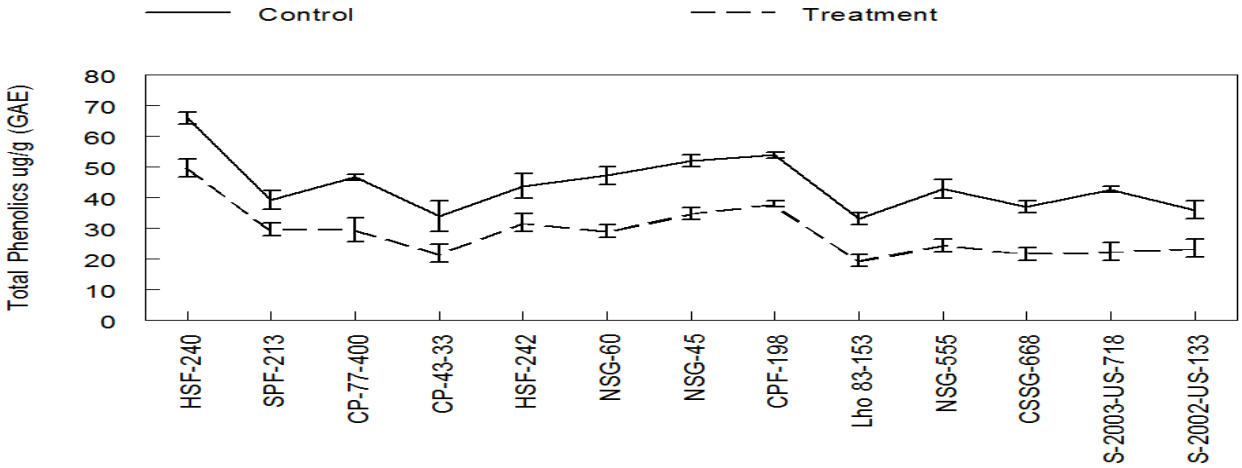

Figure 3. Thirteen genotypes of sugarcane (total phenolics).

3.4 Estimation of Lipid Peroxidation

Lipid peroxidation (LPO) as MDA content, estimated at formative and grand growth phase of the crop, showed increasing trend over the stage as well as heat stress condition (Figure 2). Under control, Lho 83-153, HSF-242 and S-2002-US-133 showed minimum values of 26.815, 32.395 and 34.125 (nmol malondialdehyde g^{-1}f.wt) respectively, and after heat treatment the values increased due to stress and showed value of 37.045, 38.905 and 43.555 nmol malondialdehyde g^{-1}f.wt, respectively. CP-77-400, HSF-240 and NSG-60 showed maximum values of 47.43, 45.57 and 41.075 nmol malondialdehyde g^{-1}f.wt. respectively; after treatment the values increased and moved to 56.42, 56.575 and 49.91 25 nmol malondialdehyde g^{-1}f.wt as seen in Figure 4. The stress induced increase in leaf membrane damage, reduced uptake of CO_2 because of closer stomatal, decreased hydrolytic enzyme activity and increased lipid peroxidation level; it may stimulate formation of AOS such as superoxide, hydrogen peroxide, and hydroxyl radicals. Among AOS, superoxide converted by SOD enzyme into H_2O_2, is further scavenged by CAT and various peroxidases. APOX and GR also play a key role by reducing H_2O_2 to water through the Halliwell-Asada pathway (Noctor and Foyer, 1998). Allen

(1995) also reported that much injury of plants caused by various stresses is associated with oxidative damage at cellular level such as cell membrane damage.

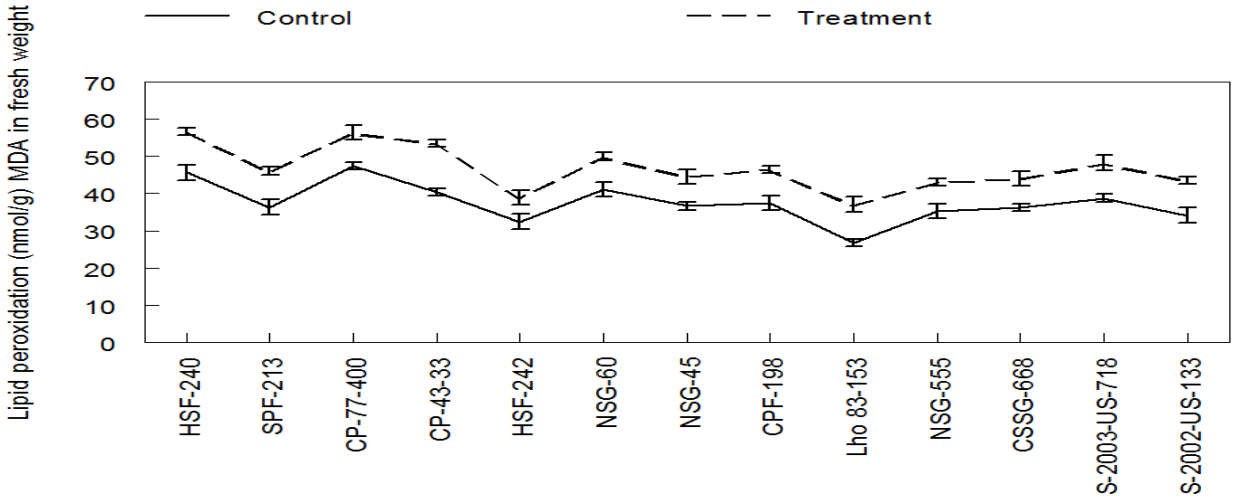

Figure 4. Thirteen genotypes of sugarcane (lipid peroxidation).

Cluster analyses can be used for the screening of thirteen genotypes of sugarcane by using MDA, phenolic and moisture loss through heat stress experiment. Cluster I having HSF-240, CPF-198 and CSSG-668, and cluster II having CP-77-400, NSG-60, NSG-45, NSG-555 and S-2003-US-718 were present. Cluster III has SPF213, HSF-242, CP-43-33, Lho 83-153 and S-2002-US-133. As a result, cluster III is more heat tolerant than cluster I. S-2002-US-133 and Lho 83-153 are more heat tolerant than HSF-240 in Figure 5.

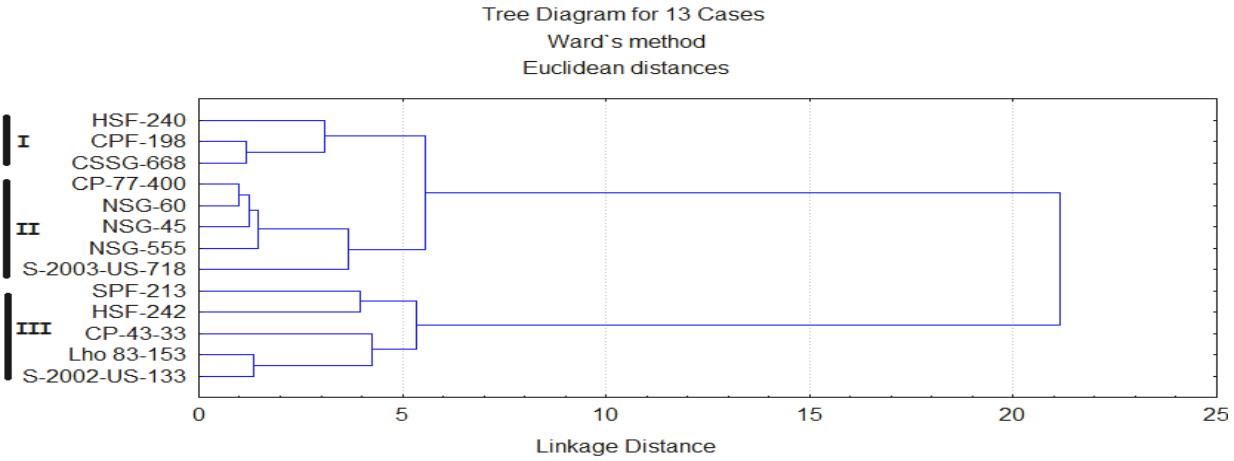

Figure 5. Dendrogramme of heat stress.

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