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Review

Sugarcane, Sugar Metabolism and Some Abiotic Stresses

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ABSTRACT

Sugarcane (*Saccharum officinarum* L.) is a multipurpose and commercial crop of Pakistan. Sugar for food remains the most important product of sugarcane, by-products of the crop, many of them from fiber, are a significant part of its economic production. Sucrose serves as the major form of carbohydrate storage in sugarcane. Metabolism of sucrose, a mobile source of energy and carbon, is an absolute requirement for the survival of heterotrophic plant organs. In these organs, different isoforms of invertase with discrete subcellular locations hydrolyze the disaccharide into hexoses and thereby, feed sucrose into various biochemical pathways. Different invertase isoforms exist in the vacuole, cytoplasm and apoplasm of plant cells. The acid isoforms, having activity optima at pH 4.5-5.5, are present in the vacuole and apoplast. The isoforms with activity optima near neutral pH and alkaline isoforms with activity optima at pH 8.0 are in the cytoplasm. In storage sinks, invertase activity is proposed to be important for creating a sucrose concentration gradient from the phloem to sink tissue and then maintaing the storage sink for promoting phloem unloading. Since sugarcane is the prime source of sugar production in Pakistan, it is imperative to investigate the physiological and biochemical basis of yield reduction under various abiotic stresses.

Key Words: Sugarcane; Sugar metabolism; Abiotic stresses; Invertase.

INTRODUCTION

Sugarcane is the common name given to the sucrose-storing members of the genus *Saccharum*. Cultivated sugarcane hybrids are robust, vegetatively propagated perennial grasses, generally limited to latitudes within 30 degrees of the equator or to ocean-warmed coastal areas lying outside this belt. The prolonged growing seasons of the tropics, the harvest of the plant body instead of fruit or seed, coupled with the high production efficiency of C_4 photosynthesis, result in extraordinarily high crop yields. Although sugar for food remains the most important product of sugarcane, by-products of the crop, many of them from fiber, are a significant part of its economic production (Moore & Maretzki, 1996).

Sugarcane, a glycophyte, is an important cash crop.Growth and development of sugarcane is hampered by various biotic and abiotic stresses. A large number of laboratories in the world are engaged in research on stress tolerance of halophytic and glycophytic plant species including sugarcane. A brief review of the reported work on different aspects of sugarcane is given below:

Taxonomy. The genus *Saccharum* belongs to the family Poaceae of the order Poales and class Monocotyledoneae. Possible taxonomic relationships among this group are obscured by the extensive prehistoric distribution of sweet canes by humankind and wide hybridization among the various forms. Classification of the species varies, but it is generally considered that *Saccharum* comprises four domesticated species and two wild ones. *Saccharum officinarum* L., the noble garden cane of New Guinea and type species for the genus, is the one species with continuous acceptance since Linnaeus's 1753 description. Although *S. officinarum* was dispersed throughout Malaya, China, India, Micronesia and Polynesia during prehistoric times, it was not known by Europeans until it was collected by them on their explorations of the Pacific. *Saccharum sinense* Roxb., the sugarcane of China, and *Saccharum barberi* Jeswiet, the sugarcane of India, were known through folklore and mythology from 1000 to 500 B.C. These "original" sugarcanes (probably derived from natural hybrids between *S. officinarum* and the wild canes of India and China) were spread by humans from the Orient through the Middle East, Northern Africa, and the Mediterranean to be delivered to the Americas by Columbus in 1493. The domesticated *Saccharum edule* Hassk., grown as a garden vegetable for its abortive inflorescence, is restricted primarily to Malanesia. The wild species *Saccharum spontaneum* L., has a wide distribution throughout the tropics of Africa, Asia and Oceania while the second wild species, *Saccharum robustum* Jeswiet and Brandes is restricted to Malanesia and parts of Indonesia (Moore & Maretzki, 1996). Modern sugarcane cultivars are multispecies hybrids, primarily of *Saccharum officinarum* L., *Saccharum spontaneum* L., and *Saccharum robustum* Brandes et Jeswiet ex Grassl (Zhu *et al*., 1997; Ming *et al*.,

2002), with high sucrose content, low fiber content, thick stalks, little pubescence, rare flowering, and limited tillering (Ming *et al*., 2001).

Botanical description. Sugarcane is a tall, robust, clump-forming grass with culms, usually called stalks or stems, devoid of leaves below. Aerial stalks are unbranched, stout to slender, differentiated into nodes and internodes with prominent, annular leaf scars; and adventitious root primordia in a several-tiered band just above each node; an intercalary meristem or growth ring above each root band; and an axillary bud prominent in the root band. The lateral buds are inserted alternately along the stalk in the axil of alternately borne leaves. Leaves are differentiated into long (1 to 2 m) blades and shorter (0.2 to 0.3 m), stalk-clasping sheaths (Artschwager, 1940).

The bud at each node is capable of sprouting into a new plant, providing the method used for crop propagation. The shoot roots arise from underground nodes, and the axillary buds at these nodes give rise to tillers. Depending on the clone and growing conditions, more than 100 stalks can be produced from one bud, but when densely planted under field conditions, only a small fraction of stalks survive the competition (Moore & Maretzki, 1996).

Leaf anatomy. The sugarcane leaf consists of a sheath which overlaps itself, tightly enveloping the stem and a blade, which is connected to the sheath by a collenchymatous joint. The blade, about five times the length of the sheath, is widest at its midpoint and tapers toward a narrowed base and pointed tip. A prominent midrib projects from the basal two-thirds of the lower surface of the blade. Leaf veination is parallel in the sheath and nearly so in the lamina of the blade, where the major veins diverge at an acute angle from the midrib toward the leaf margin in an acropetal direction.

In transverse section, the sugarcane blade has three sizes of longitudinal vascular bundles (large, medium, and small), typical of grass leaves (Artschwager, 1925) The longitudinal bundles are interconnected by numerous small transverse bundles (Colbert & Evert, 1982). Commonly, the large and intermediate bundles are flanked by small bundles, but this alternating pattern of a single small bundle between larger bundle is not absolute. The lanceolate shape of the blade is associated with coalescing of longitudinal bundles into fewer major bundles at the basal end while coalescing into a single small bundle at the acropetal end. Thus, virtually all of the longitudinal strands in the sugarcane leaf intergrade structurally from one bundle type to another (Colbert & Evert, 1982). In spite of the reduction in number of bundles at the basal portion of a blade, the increase in phloem or sieve-tube cross-sectional size as the bundles enter the leaf sheath apparently prevents a bottleneck or restriction in translocation as was suggested by McDavid and Midmore (1980).

The sugarcane leaf has two distinct chloroplast containing cell types, mesophyll and bundle-sheath cells, arranged in concentric rings around the vascular bundles in a pattern now known as Kranz anatomy. The Kranz mesophyll (KM) cells are highly branched and loosely arranged with numerous intercellular spaces among them (Colbert & Evert, 1982). The thick-walled bundle-sheath (BS) cells are unbranched and tightly packed between the KM and the prominent thick-walled cells of the mestome sheath (MS) and the vascular tissues. The walls of both the chlorenchymatous BS cells and the MS cells contain suberin lamellae (Robinson-Beers & Evert, 1991). The large vascular bundles are surrounded by a complete outer chlorenchymatous BS and inner MS. The intermediate vascular bundles have a complete chlorenchymatous BS but only a partial MS which borders the phloem, whereas the small vascular bundles have a smaller amount of MS confined to the phloem area. Thus, more than one layer of suberized cells must often be traversed by photoassimilates during their passage from the chlorophyllous cells to the sieve tubes. The suberin lamellae are continuous in all walls of BS cells bordering the xylem. However, the suberin lamellae in the radial walls between adjacent BS cells do not merge and thus provide a potential pathway for apoplastic movement of substances through the compound middle lamella of these walls (Robinson-Beers & Evert, 1991).

Stem anatomy. The sugarcane stem is divided into short, complex node regions and long, relatively simple internodes (Artschwager, 1925). The node includes structures associated with the attachment of the leaf, the nodal root primordia, an axillary bud, and the intercalary meristem.

The internal anatomy of the node differs in each of the areas associated with a separate organ or function. The node is composed primarily of branching vascular bundles (the nodal plexus) with very little storage parenchyma tissue; the node thus has a high percentage of fiber and a low percentage of juice. The juice of the immature node has a higher percentage of brix than that of the adjacent internode but later partitioning of sucrose to the internode reverses this situation so that in the mature stalk sections the brix of a node is lower than that of its attached internode (Fernandes & Benda, 1985). As a consequence of the high fiber and low juice content of the node, it contains very little sucrose.

The internode consists of a uniform set of structures for support of the aerial part of the plant and the transport and storage of water, nutrients, and photoassimilates. Internal anatomy of the internode consists of numerous vascular bundles within parenchymatous tissue. The vascular bundles, scattered throughout the internode, increase the number and decrease in size from the center toward the periphery. The mature sugarcane stem typically has a diameter of 20-30 mm and contains about 1500 vascular bundles; approximately 50% of the total number are within the outer 1 mm and 75% are within the outer 3mm of the stem (Jacobsen *et al*., 1992). At the periphery, the bundles are so small and so close together that they form a nearly solid ring of sclerenchymatous tissue just below the narrow cortex, hypodermis and single cell layer of epidermis. These peripheral vascular bundles contain xylem

but sometimes lack phloem; when phloem is present, it consists of only metaphloem elements, typically 1-10 sieve tube members interspersed with diminutive companion cells (Jacobsen *et al*., 1992). Near the center of the stem, the vascular bundles and associated parenchyma cells are quite large. Here the vascular bundles tend to contain larger and more numerous cells of all types. Typically, there are two large vessels of the metaxylem, a large protoxylem lacuna, crushed protophloem, and complete metaphloem surrounded by pronounced sclerenchyma sheaths. Elements of the vascular bundles can be quite long; sieve tubes may exceed 1 mm (Jacobsen *et al*., 1992; Moore & Maretzki, 1996).

Metabolism. In sugarcane, as in most plants, sucrose is the sugar translocated in the phloem (Hatch & Glasziou, 1964) to sinks, where it is used for cell growth, metabolism, respiration, or storage (Hawker, 1985). In the sugarcane stem, phloem unloading for the movement of sucrose from the source cells to the sink cells may occur through either the symplasm, the apoplasm, or both. On the basis of the pattern of lignifications and suberization of the vascular bundle-sheath and parenchyma cell walls, the particular path followed may be a function of the developmental stage or age of the tissue.

Upon arrival of sucrose in the stem, sucrose can be catabolized by sucrose synthase (Susy) or one of the three invertases: soluble acid invertase (high in apoplast and vacuoles of young internodes but virtually absent in mature tissue, pH optimum 4.4, K_m = 1.3 x 10^{-2} *M*), bound acid invertase (cell wall bound in all aged tissues, pH optimum 3.8, K_m = 8 x 10^{-3} *M*), and a neutral invertase (in cytoplasm at low concentrations in young tissue and greater concentrations in mature tissue, pH optimum 7.0, K_m = 3 x 10^{-4} *M*) (Hawker & Hatch, 1965; Glasziou & Gayler, 1972). After entry into the metabolic compartment of the parenchyma cells, the hexoses may be metabolized or resynthesized into sucrose by sucrose phosphate synthase (SPS) and sucrose phosphatase (SPase) (Hatch *et al*., 1963; Hatch, 1964). Potentially, Susy could also be involved in sucrose synthesis (Goldner *et al*., 1991), but the equilibrium is usually in the direction of degradation.

The close relationship between acid invertase activity and sucrose storage in young internodes suggested that sucrose uptake from the apoplast was dependent upon its hydrolysis prior to transfer to the storage compartment of the parenchymatous tissue (Glasziou, 1960; Sacher *et al*., 1963). Support for the phloem unloading of sucrose and its cleavage in the apoplastic space prior to uptake by the plasmalemma came from numerous studies on photosynthate uptake by fruit and seed of various plants (Hawker, 1985). In developing fruit, there is no physical connection between maternal tissue and seed of the next generation so that it is easy to understand the necessity for apoplastic transport in this system (Hawker *et al*., 1991). Although there is no known anatomical necessity for apoplastic transport in the sugarcane stem, the enzymatic, labeling, and membrane transport data supported this idea.

Role of invertases. Physiological studies correlating invertase types and levels in tissues after treatments to change the growth rates rapidly helped define probable roles for two of the invertases in regulating sucrose accumulation. Growth rates were reduced by application of the growth inhibitor glyphosate (Su *et al*., 1992), low temperatures and drought stress (Hatch & Glasziou, 1963); growth rates were increased with applications of gibbercellic acid during cold treatments (Gayler & Glasziou, 1972). In all these studies, the soluble acid invertase, occurring in the vacuole and apoplastic space of elongating internodes, disappeared when internode growth ceased and reappeared when growth resumed. The vacuolar form appeared to be involved with regulation of turgor and the internal sugar pools; the apoplastic-form appeared to be the major controller for dry matter import accompanying cell extension growth. The neutral invertase increased during maturation and appeared to be involved in controlling sugar flux in the mature storage tissue. In mature tissue, lacking a measurable soluble acid invertase, there was a cell wall bound acid invertase which functioned in cleaving sucrose in the apoplastic space to control dry matter import for sucrose storage (Hawker & Hatch, 1965).

Sugarcane cultivars vary in their potential of sucrose accumulation. Early masking of vacuolar invertase activity is responsible for high sucrose accumulation in internode tissue of high sugar and early maturing cultivars (Sehtiya *et al*., 1991; 2000; Dendsay *et al*., 1995). Sugarcane storage tissue accumulate sugar against a concentration gradient using energy provided by respiration (Bieleski, 1960; Burg & Bieleski, 1962). This is accompanied by a continuous cleavage and synthesis of sucrose during accumulation of sucrose in storage tissue (Hatch *et al*., 1963; Batta & Singh, 1986; Whittaker & Botha, 1997). Primary sucrose metabolism is governed by several enzymes (Quick & Schaffer, 1996). Despite recent advances in the study of sucrose accumulation into the vacuole of plant cells (Getz, 1991; Keller, 1992; Greutert & Keller, 1993; Getz & Klein, 1995; Milner *et al*., 1995; Echeverria *et al*., 1997), very little is known about its mobilization. From various studies, it has been inferred that sucrose is enzymatically hydrolyzed in the vacuole prior to mobilization (Leigh, 1984; Hawker, 1985; Martinoia, 1992).

The level and timing of sucrose accumulation in the whole stalk and within individual internodes was correlated with the down regulation of soluble acid invertase (SAI) activity above which high concentration of sucrose did not accumulate (Zhu *et al*., 1997). This low level of SAI activity was always exceeded in the internodes of the lower sucrose storing genotypes. The role of SAI has been linked to growth and differentiation and these observations suggest that CWI may also be intrinsically involved in these processes. NI appears to have a housekeeping role in

maintaining hexose concentrations within the cytosol (Albertson *et al*., 2001).

The relationship between extractable invertase activities and sucrose accumulation in the sugarcane stalk, and *in vivo* invertase mediated sucrose hydrolysis was investigated to determine the significance of invertases in sucrose utilization and turnover. *In vitro* activities were determined by assaying the soluble acid, cell wall bound acid and neutral invertases from internodes 3-10 in mature sugarcane plants. Extractable activities were verified by immunoblotting. Sugarcane neutral invertase had a higher specific activity than soluble acid invertase (apoplastic and vacuolar) in the sucrose accumulating region of the sugarcane stem (Rose & Botha, 2000). Cell wall bound acid invertase was also present in significant quantities in both immature and mature tissue (Vorster & Botha, 1999).

Metabolism of sucrose, a mobile source of energy and carbon, is an absolute requirement for the survival of heterotrophic plant organs (Sturm *et al*., 1999). In these organs, different isoforms of invertase with discrete subcellular locations hydrolyse the disaccharide into hexoses and thereby, feed sucrose into various biochemical pathways. In contrast to the invertases with acidic pH optima and a vacuolar or extra cellular location, knowledge about the molecular nature of the cytoplasmic invertases (neutral and alkaline invertases) is still scarce (Sturm *et al*., 1999).

Isolation and purification of enzymes. Extraction and assay methods were developed for the determination of both soluble and cell wall invertase activity in sugarcane from minimal (0.5g) tissue (Albertson *et al*., 2001). Cell wall invertase (CWI) was measured using a pellet mix procedure and the pH optima ranged between pH 3.2 and 3.6. The pH optima for the soluble invertases were 4.5 and 7.3 for soluble acid invertase (SAI) and neutral invertase (NI) respectively. Invertase activity was examined in sugarcane tissues of varying ages. In leaves and stem, the SAI activity was greatly reduced in mature tissue extracts. Similarly, the CWI activity was reduced in old leaves and activity from stem extracts remained constant irrespective of tissue age.

Sugarcane neutral invertase (SNI), partially purified from a mature sugarcane stem tissue were found to be non-glycosylated and they exhibited catalytic activity in various forms such as a monomer, dimmer and tetramer but most of the activity eluted as a monmer of native MW 60 kDa. The enzyme displayed typical hyperbolic saturation kinetics for Suc hydrolysis. It has a K_m of 9.8 mM for Sucrose and a pH optimum of 7.2. An Arrhenius plot showed the energy of activation of the enzyme for Suc to be 62.5 kJ/mol below 30°C and –11.6 kJ/mol above 30°C. The end products of SNI inhibited its activity and fructose was stronger inhibitor than glucose. SNI is significantly inhibited by $HgCl_2$, $AgNO_3$, $ZnCl_2$, $CuSO_2$ and $CoCl_2$ but not by $CaCl_2$, $MgCl_2$ or $MnCl_2$ (Vorster & Botha, 1998).

The bound alkaline and acid invertases were isolated from the milky stage grains of rice. The bound isoforms of invertases (ITb) have been released by treating the grains with 1 *M* NaCl and purified to homogeneity through steps of polyethylene glycol 4000 fractionation, concanavalin-A Sepharose affinity chromatography, Sepharose Cl-6B gel filtration and F.P.L.C. on a Mono-Q column. The ITb having a pH optimum of 4.5 was found to be monomeric because the native molecular weight (32,000), estimated by gel filtration, and the subunit wt. (30,000), estimated on SDS/PAGE, were almost the same. The PI value of ITb was 6.45 by rod isoelectric focusing. The best substrate of ITb was sucrose, with a K_m of 5.39 mM. On the basis of their K_m values, PI values and protein patterns on SDS/PAGE, it was concluded that the NaCl released and the EDTA-released bound forms of invertase are identical (Sung & Huang, 1994).

Invertase from the crude extract of *Aspergillus* AS0023 was purified by successive chromatographies on DEAE-sephadex A-25, sepharose 6B, sephacryl S-200, and concanavalin A–sepharose 4B columns. On acrylamide electrophoresis the enzyme, in native and denatured forms, gave diffused glycoprotein band, with different electrophoretic mobility. On native PAGE and SDS-PAGE, invertase migrated as polydisperse aggregates yielding broad and diffused bands. This result is typical of heterogeneous glycoproteins and the invertase has proved its glycoprotein nature by its adsorption on concanavalin A lectin. Invertase showed higher mobility corresponding to a molecular range form 82-251 kDa, on native PAGE,and from 71-111 kDa on SDS-PAGE. Invertase showed optimum activity at pH 4.4 and 55 °C. The K_m and V_{max} values were 35.67 and 3.98 μmol ml^{-1} min^{-1} respectively. Invertase catalytic activity was dependent on sucrose concentration, which decreased markedly with increasing sucrose concentration. Further more, invertase exhibited only hydrolytic activity producing exclusively fructose and glucose from sucrose (Hocine *et al*., 2000).

The over-expressed extracellular sucrase (SacC) of *Zymomonas mobilis* from a recombinant *Escherichia coli* (pZSP62) carrying the SacC gene was purified partially by repeated cycles of freezing and thawing. This method separated the highly expressed recombinant protein from the bulk of endogenous *E. coli* proteins. The enzyme was further purified 14-fold with a 55% yield from the cellular extract of *E. coli* by hydroxyapatite chromatography. The purified enzyme had a MW of 46 kDa by SDS-PAGE. Its K_m value for sucrose was 86 mM and activity was optimal at pH 5.0 and at 36 °C (Sangiliyandi & Gunasekaran, 2000).

An invertase from the thermophilic fungus *Thermomyces lanuginosus* was immobilized on phenyl-sepharose and its properties were studied. Between the soluble and immobilized forms of the invertase, there were not much difference in their optimum pH, K_m and V_{max} for sucrose. In contrast, the K_m and V_{max} for raffinose changed significantly. The optimum temperature for the immobilized invertase was lower by 10°C. The immobilized invertase

showed stability at 50°C and was less sensitive to inhibition by metal ions (Basha & Palanivelu, 2000).

Productivity and yields. The world average of 61 mt cane and 5.82 mt sugar ha^{-1} represents fresh weight and product yields well above those for other crops. Factors which contribute to high yields of sugarcane are its perennial growth habit and continuous accumulation of sucrose in the vegetative plant structure. Crop cycles vary from less than 10 months in temperate areas such as Pakistan and Louisiana, where killing frosts set rigid seasons, to 24 months in Peru and South Africa; even longer cycles are sometimes used in Hawaii. Most of the world's remaining sugarcane is grown in 14 to 18 month plant crops and 12 month ratoons. Crop cycles average just over 15 months for the highest producing countries. Sugar yields are affected also by the length of the harvest and milling season. In most countries, milling is limited to the 5 or 6 months producing the ripest cane (Moore & Maretzki, 1996).

Specific Abiotic Stresses

Freezing or frost stress. Sugarcane susceptibility to cold injury, either by chilling or freezing, is the primary factor limiting the distribution of the crop to within 30° of the equator. In subtropical regions, freezing temperatures may terminate or even reverse the sucrose accumulation in the autumn or early winter. Freezing further reduces yields by delaying and suppressing crop development in the spring, resulting in a shortened growth season and producing poor crop stands (Moore, 1987). Resistance to freeze stress is required for different tissues at different crop development stages.

Mill cane. The majority of freeze resistance studies on sugarcane concern freeze-induced deterioration of mill cane. Preharvest freeze damage has been shown to depend on the intensity and duration of the freeze (Irvine, 1969), the post-freeze temperatures (Irvine, 1967), the resistance of the stalk tissue to freezing, and the rate of increase of acidity and gums following a freeze (Irvine, 1967; Miller & Gascho, 1975). Moderate preharvest freezes cause insignificant yield losses while severe freezes can result in a total crop loss (Moore, 1987).

Leaves and buds. Resistance of leaves to frost damage is important for prolonging the growth and harvest season while resistance of lateral nodal buds is important for assuring good germination of setts. Artificial freezing tests have been developed and used to evaluate leaf freeze tolerance of adult plants (Irvine, 1978), cloned seedlings (Breaux & Irvine, 1976), progeny of crosses (Irvine, 1968; Cesnik *et al*., 1978), parental clone selections (Irvine, 1978), and as a tool to preselect seedlings (Breaux & Irvine, 1976; Cesnik *et al*., 1978). Good agreement of artificial freezing tests with field observations has been reported only when care was taken to avoid hardening of test plants by acclimation with low light (Irvine, 1968; Moore, 1987).

Tiller resistance to freeze. In some sugarcane growing areas, a major factor determining the commercial success of a clone is its ability to tiller well following adverse winters (Edgerton *et al*., 1934; Kanwar & Kaur, 1978). A measure of yield reduction due to poor tillering following a severe winter showed a 78% decrease in shoot population and an 87% decrease in tones of cane per hectare when the underground buds were not protected from freezing (Kanwar & Kaur, 1978).

Chilling stress. Sugarcane is a tropical crop. It grows well only in the tropics or in subtropical areas where the climate is moderated by surrounding water masses. The optimum temperature for growth is about 35°C. Although sugarcane survives at minimum temperatures above zero, there is little growth at temperatures as low as 20°C and there may be tissue injury at temperatures below 15°C. Any temperature above freezing cool enough to produce an injury or to suppress growth and yield is referred to as a chilling temperature (Moore, 1987).

Each of the developmental and physiological process of the sugarcane crop has a temperature range from minimum through optimum and maximum in which it occurs (Moore, 1987; Chowdhury *et al*., 1998). Temperature-regulated processes which have been evaluated for variation in clonal resistance to chilling temperatures include developmental anomalies, rates of stem elongation and dry matter accumulation, set germination, flowering, and pollen fertility.

Duncan and Cooke (1932) reported that lowering the root temperature from 28 to 21, 15, and 10°C caused a progressive decrease in water absorption. Mongelard and Mimura (1971) reported that the decrease in water uptake at a chilling temperature caused a corresponding decrease in dry matter yield. Sett germination and early shoot growth are chill-sensitive characters which significantly affect crop yield and therefore are important to subtropical breeding programs (Ishii, 1996; Singh & Ralham, 1999). Clones of subtropical origin showed a temperature optimum of 26 to 33°C while clones of tropical origin showed an optimum of 34 to 38°C (Whiteman *et al*., 1963; Moore, 1987; Chowdhury *et al*., 1998).

High temperature stress. High temperature stress is difficult to evaluate. Plant injury and yield losses associated with high temperature stress are more subtle than those associated with most other physicochemical stresses. Moreover, heat stress is usually associated with drought stress, which is much more devastating to the crop. Sugarcane survives at maximum temperatures approaching 45°C, however, there is little growth at temperatures above 40°C. This is not to say that the heat killing temperature lies between 40 and 45°C because when the time of treatment is limited, tissues survive much higher temperatures (Moore, 1987; Chowdhury *et al*., 1998).

Water deficit stress. If the water available to a plant is insufficient to meet its needs, the shortage of water may induce a water deficit stress. A water deficit is defined as any water potential below zero (Levitt, 1980). Note the water potential of pure water at atmospheric pressure is defined as zero. Thus, any water less than that in a saturated

soil causes a water deficit stress. Severe and moderate drought stresses decreased mean cane yields by 29.2 and 18.1%, respectively, compared to irrigated controls (Ramesh & Mahadevaswamy, 1999). In field trials, in, sugarcane grown under water stressed conditions, NAR and RGR were decreased by water stress (Singh & Singh, 1994). Morphological changes brought on by drought and thought to acclimate plants to it include reduced leaf area, thicker leaves, less responsive stomata, and increased ratio of roots to shoots. Cell size reduction with a concomitant increase in wall, thickness seems to be the most prevalent and earliest appearing anatomical acclimation. Biochemical acclimations include changes in enzyme activities, carbohydrates, and nitrogen pools and accumulation of stress indicators such as ABA, betaine, proline, and the metabolites of these compounds. Some of these changes are true acclimations allowing the plant to perform well under subsequent drought stress but other changes may be merely incidental and serve as indicators of the stress history of the plant (Hanson & Nelson, 1980; Moore, 1987).

Each of "drought stress acclimations" consists of characters which are present under non-drought stress conditions to various degrees among sugarcane clones (Quizenberry, 1982).

Root characters. From his work on the distribution and abundance of different root types in sugarcane, Evans (1935) suggested that rooting pattern was a clonal character which could be used to predict drought resistance. He noted that the more drought-resistant *S. spontaneum* clones had deeply growing "rope" root systems which were not present in the drought susceptible *S. officinarum* clones. The overall larger rooting system and greater drought tolerance of *S. spontaneum* have been repeatedly confirmed (Panje, 1972; Singh & Ramakrishnan, 1977) and suggested as a heritable trait (Moore, 1987).

Leaf characters. The potential rate of transpirational water loss is regulated by leaf size exposure, number, and structural modifications in the stomata, bulliform cells, and cuticle. Each of these characters exist in a quantity or configuration that can be classified as either a drought-resistant or a drought-susceptible trait on the basis of its predominance in xerophilic or hydrophilic plants. Xerophilic characters acting to restrict transpirational water loss include short and narrow leaves, a low density of stomata sunken below the epidermis, a narrow band of bulliform cells, and a thick cuticle (Gill & Singh, 1959; Moore, 1987).

Stomata. The most critical problem facing the plant is the relationship between the ability to assimilate carbon and the ability to minimize water deficit. Many stomatal characters which restrict water loss, such as low frequency and small size will similarly restrict assimilation and subsequently limit growth. Rate of stomatal action has been suggested as a drought resistance trait in sugarcane (Naidu & Bhagyalakshmi, 1967; Moore, 1987).

Osmotic adjustment. Osmotic adjustment to water deficit stress is considered an important physiological mechanism enabling plants to tolerate the stress. A leaf can increase its resistance to dehydration through a reduction in cellular osmotic potential by net accumulation of cellular solutes. Only limited work has been reported on osmotic adjustment of sugarcane. Koehler *et al.* (1982) reported osmotic adjustment in leaf and stem tissue of a single clone subjected to a five-week drought. Osmotic adjustment as a mean of combating water stress under saline conditions entails the accumulation and compartmentation of organic or inorganic osmotica into cytoplasm and vacuole respectively (Carpita *et al*., 1990).

Metabolic adaptations. Water deficit stress, if severe enough and of sufficiently long duration, will affect most functions of the plant. Proline, an amino acid prevalent in halophytes but generally in small amounts in unstressed glycophytes, has been postulated to increase the desiccation tolerance to plant cells (Singh *et al*., 1972; Hanson & Nelson, 1980). Proline accumulates significantly in stressed sugarcane leaves. Ashraf (1994) reported negative correlation between proline content and salt tolerance in mung bean because of its low content having no significant osmoregulatory role. Abscisic acid (ABA), known as a stress hormone, increased by about 75-fold in stressed sugarcane leaves (Kuhnle *et al*., 1979).

Salinity and ionic stress. The terms salt and ion stress, as defined by Levitt (1980), are designated to refer to an excess only. While the effect of excess salt is due to its ions, we distinguish between salt and ion stress on the basis of concentration. If the soluble mineral concentration is not high enough to lower the water potential appreciably, the stress is called an ion stress. On the other hand, if the mineral concentration lowers the water potential appreciably (0.5 to 1.0 bars), the stress is called a salt stress (Moore, 1987). Plants differ greatly in their response to Na salts. Plants that cannot grow in the presence of high concentrations of Na salt are called glycophytes while those that can tolerate or require high salts are called halophytes.

Increased soil salinity has been taken as a noxious factor for most of the glycophytes. It induces specific changes at cell, tissue, and organ levels. These changes are morphological, physiological, and anatomical in nature (Cheeseman, 1988; Läuchli & Epstein, 1990; Shannon, 1997; Isla *et al*., 1998). Soil salinity reduces plant growth by perturbing different biochemical/physiological processes (Zeng & Shannon, 2000). As much of the world's soils are salt-affected to a considerable extent (Cheeseman, 1988), there has been keen interest in the development of crop plants displaying tolerance to the salinity (Rozeff, 1995; 1998).

Plants and salinity. Testing of clones to identify salinity resistance is usually done by comparing yields in saline fields (Mehrad, 1969; Thomas *et al*., 1981; Patil & Somawanshi, 1983) or comparing growth of plants irrigated with salinized water (Santo, 1980). Salinization of growth

media causes a decrease in plant growth (Sharma, 1995) by hampering various physiological phenomena. The growth of plant is affected at all stages of development, but sensitivity varies greatly in different crops (Steppuhn & Wall, 1997; Carvajal *et al*., 1998; Wilson *et al*., 2000).

Effect on germination. Dev and Bajwa (1972) noted that in general germination and early growth stages are considerably more resistant to salinity than is later growth. However germination of seed is adversely affected under high levels of salinity. When exposed to saline medium, seed experiences ion toxicity and reduction in water uptake (Allen *et al*., 1986; Katembe *et al*., 1998). Salinity alters the physiological and biochemical activities by inhibiting the anabolic and stimulating the catabolic processes (Corchete & Guerra, 1986; Torres-Schuman *et al*., 1989).

Effect on reproductive growth stage. Salinity tolerance is crucially important at reproductive stage of the plant growth (Francois & Kleiman, 1990). Salinity in the root zone of sugarcane decreases sucrose yield, through its effect on both, biomass and juice quality (Lingle & Wiegand, 1996). Saline soil reduces millable stalks per hectare, stalk length, and stalk weight (Wiegand *et al*., 1996). These reductions reduce the tonnage harvested from salt affected fields. The influence of soil salinity on sugarcane yield and quality may be due to physical factors, such as water potential of the tissue, rather than biochemical factors. Maas and Grieve (1990) found a marked reduction in spike and leaf development in salt stressed wheat. Grieve *et al*. (1992) found a reduction in tillering capacity, spike length, number of spikelets and kernels per spike of moderately salt stressed wheat, but increased kernels per spikelet which lead to the increased grain yield. It is likely that in salt affected areas, where germination or tillering is reduced, crop yield could be increased by increased planting density (Grieve *et al*., 1992; Wahid *et al*., 1999).

Effect on enzymes. Most of the enzymes isolated from the salt tolerant plants show similar behaviour as from sensitive species (Wyn Jones *et al*., 1977; Lingle & Wiegand, 1996). The increasing concentrations of NaCl and Na_2SO_4 in growth medium induce a progressive decrease in specific activity of mitochondrial enzymes in pea roots (Porath & Poljakoff-Mayber, 1964) or rapidly growing tissues at 50 to 150 mM Cl concentrations (Flowers, 1972).

Greenway and Osmond (1972) reported that malate dehydrogenase, aspartate transaminase, glucose-6-phosphate transaminase and isocitrate dehydrogenase extracted from halophytes and glycophytes showed similar NaCl sensitivity, despite great differences in the sensitivy *in vivo*. It has been reported that some marine microorganisms posses specific proteins due to which most of their enzymes show no absolute requirement for high ion concentration (Larsen, 1967; Lanyi, 1974) However, NaCl or KCl may induce conformational changes in the enzymes either by direct interaction with the protein or indirectly through interaction with the lipid component of the membrane (Ben-Hayyim & Rana, 1990). Thus growth of salt tolerant plants does not show sensitivity to the internal salts that would be expected from the response of the isolated enzymes (Flowers, 1972).

Specific ion toxicity. The stress of excess ions of specific elements has been recognized in sugarcane. McGeorge (1925) described soil and water culture experiments showing that the elements aluminum (Al) and iron (Fe) caused excess ion stress in sugarcane. Although the stresses occurred only under acidic rooting conditions and were alleviated with additions of phosphorus (P) and potassium (K), the stresses were proved due to the toxicity of Al and Fe ions and not due to a deficiency of H, K, or P ions. In addition to describing the symptoms of stress and the environmental conditions under which natural ion toxicity occurred, McGeorge (1925) reported clonal difference in resistance to ion stresses. According to Moore (1987), the concentrations of Al, Fe, Mg, and Mn are generally considered toxic for sugarcane. The toxic concentrations are lower in acid soils and soils deficient in K. The injuries due to ions involve specific effect initially on the plasmamembrane and then on the protoplasm (Kalaji & Pietkiewicz, 1993).

Epstein (1972) supported the notion that the membrane leakage was due to excess of Na^+. It weakens the membrane structure by displacing one or more divalent bridges, e.g., Ca^{2+} serves to bind phospholipids together and in the event of displacement, may limit membrane permeability (Cramer *et al*., 1989). It is difficult to separate the growth inhibiting components of salinity, i.e., water deficit or a specific ion effect. Available data indicate that the effect of both the components is similar (Bachman, 1990). Osmotic stress proceeds excess ion toxicity at higher concentration, while at low levels only water stress is prevalent (Munns & Termaat, 1986). NaCl treatment was found to be more inhibitory to water uptake, especially at high concentrations (Katembe *et al*., 1998).

All workers reporting ion toxicity show greater prevalence of the stresses under nutrient imbalance. It is frequently noted that ion stresses are alleviated by increasing K or Ca ions, neutralizing the soil acidity, and sometimes complexing the soil with silica additions (Moore, 1987).

Salinity and gene expression. Salinity tolerance is a complex process and is controlled by many genes (Gracia *et al.*, 1995). These genes are expressed only when plant is exposed to salinity. The selection of crop genotypes for tolerance to salinity is an important area of research (Hsiao *et al.*, 1984; Isla *et al.,* 1998). Many workers have explored diverse response of plants to salinity (Greenway & Munns, 1980; Flowers, 1985; Isla *et al.*, 1998). Sugarcane has been ranked as moderately sensitive to salinity (Shannon, 1997). However, genotypic differences are present in this species for salinity tolerance (Akhtar, 2001).

Cell wall bound invertases were shown to be specifically expressed under conditions that require a high carbohydrate supply to sink tissues. Substrate and reaction

products of invertases are not only nutrients, but also signal molecules like hormones and in combination with hormones and other stimuli, they can regulate many aspects of plant development from gene expression to long distance nutrient allocation (Roitsch *et al*., 2000).

Salt tolerance in sugarcane. Sugarcane, a major source of sugar production in Pakistan, undergoes substantial reduction in growth and yield above a threshold ECe of 5 dS m^{-1} (Maas, 1985; Rozeff, 1995; 1998). Sugarcane exhibits stunted or no growth under saline conditions, with its yield falling to 50% or even more of its true potential (Subbarao & Shaw, 1985). It is estimated that globally about one million hectares under sugarcane production are affected by salinity or sodicity. This is mainly assigned to the confinement of this crop to the tropical / sub-tropical areas.

Like many other crops, sugarcane sprouting and early growth are considerably more resistant to salinity than at later developmental stages (Maas, 1985; Wahid *et al*., 1997). There is a consensus that salt interferes with sugar production in two ways; first by affecting growth rate and yield of the cane and secondly by affecting the sucrose content of the stalk (Wahid *et al*., 1997). Sugarcane from saline soil shows also reduced total soluble solids and thus sucrose in whole stalk juice. Sugarcane sucrose accumulation is to balance increased salt concentration in the juice and maintain tissue water potential (Rozeff, 1995; Lingle *et al*., 2000).

Salt tolerant plants have adopted certain strategies of ion regulation at root (Wahid *et al.*, 1999), stem (Wolf *et al.,* 1992) or leaf level (Kumar *et al.*, 1994). Changes in physiological processes triggered by ion excess appear as changed morphology of the plant (Meinzer *et al.,* 1994). Another aspect is the selection of salinity tolerant plants at different growth stages (Maas *et al.*, 1985). This carries significance because incidence of salinity spell at any of the growth stages may lead to drastic reduction in crop yield or even complete crop failure.

Plant tolerance to salinity is usually ascertained by the yield response equation of Maas and Hoffman (1977). However, some other criteria have also been reported as its indicators, i.e., percentage of dead leaves (Ponnamperuma, 1977), visible growth and vigour (Srivastava & Jana, 1984), chlorophyll fluorescence (Belkhodja *et al.,* 1994) and plant growth and seed yield (Francois, 1996), but age and stage of plant growth also remains critical (Maas *et al.,* 1985; Ashraf, 1994; Wilson *et al.,* 2000).

The mechanisms and factors involved in salt tolerance are many and are not yet well understood. But salt tolerant characteristics appear to be related to either one or both of two conditions: (1) the ability of the plant to restrict the entrance of salts into its roots or (2) the ability to tolerate or adjust to salts after they are taken in by the plant. Salt sensitivity changes considerably during the development of plant (Akhtar *et al*., 2001). Four developmental stages, i.e., germination, vegetative, reproductive growth and grain filling can be distinguished with respect to salt tolerance. Developmental shifts in relation to salt tolerance also vary according to the genotypes (Akhtar *et al*., 2001; Ahmad *et al*., 2003).

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