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Original Paper

Evaluating sugarcane (*Saccharum* sp.) cultivars for water deficit tolerance using some key physiological markers

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Abstract Seven sugarcane (*Saccharum* sp.) commercial cultivars, viz., UT-94-2-483, LK92-11, K84-200, K97-32, K95-84, K88-92 and K 92-80, originally derived from meristem cuttings were subjected to simulated osmotic stress (as 200 mM mannitol) under controlled environmental conditions. Proline content in the leaf tissues of all cultivars except K92-80, increased in plants subjected to mannitol-induced osmotic stress. Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), total chlorophyll (TC), maximum quantum yield of PSII (F_v/F_m), and photon yield of PSII (Φ_{PSII}) of all seven cultivars decreased under osmotic stress resulting in a reduction in net-photosynthetic rate (P_n). A positive correlation was found between F_v/F_m and Φ_{PSII}, proline content and non-photochemical quenching (NPQ), Φ_{PSII} and P_n, and P_n and plant dry weight in the sugarcane cultivars. Based upon Ward's multivariate cluster analyses of data for proline content, photosynthetic capacity, chlorophyll fluorescence, and growth inhibition, three cultivars (K88-92, K92-80 and UT-94-2-483) were identified as water deficit sensitive, whereas four (K84-200, K95-84, K97-32 and LK92-11) as water deficit tolerant. These observations on different cultivar's sensitivity/tolerance were confirmed by growth and yield attributes measured in a field trial. The plant dry weight (*in vitro*) correlated positively with total stalk weight of sugarcane cultivars

Key words: Sugarcane, osmotic stress response, growth, photosynthetic abilities, free proline, Ward's cluster analysis.

Sugarcane (*Saccharum* sp.) is one of the most important sugar producing crops and plays a key role in ethanol production in most of the tropical and subtropical countries (Waclawovsky et al. 2010). Being a C_4 plant with long life cycle, it utilizes high amount of water, nutrients, CO_2 and light energy to produce a considerably high biomass (Carr and Knox 2011). Generally, sugarcane cultivation is carried out in areas with sufficient amount of good quality water available for irrigation. However, in most of the areas sugarcane crop does not receive adequate supply of water during its entire growth period resulting in reduced yield (Inman-Bamber 2004; Silva et al. 2008; de Silva and de Costa 2009; Ishaq and Olaoye 2009).

Water deficit is one of the most vital abiotic stresses limiting crop productivity (Ashraf, 2010; Ashraf et al. 2011), including that of sugarcane (Hemaprabha et al. 2004; Silva et al. 2008; Ishaq and Olaoye 2009). The reduction in plant growth is the first response of sugarcane to water deficit; however, enhanced production of organic osmolytes (such as glycinebetaine and proline), reactive oxygen species (ROS), and reduced photosynthetic capacity have been reported as physiological and biochemical responses of sugarcane to osmotic stress conditions (Azevedo et al. 2011; Queiroz et al. 2011; Thapa et al. 2011). Nevertheless, crop improvement for drought tolerance trait is a viable approach for sugarcane production. In this regard, the screening of germplasm for drought tolerance, though a simple and straight-forward approach, yet is costly and time-consuming practice. Alternatively, *in vitro* screening is a well established simple, rapid and low cost tool by which large populations of breeding lines can easily be screened (Rai et al. 2011; Suprasanna et al. 2011).

In most of previous studies, a single parameter has been used as a selection criterion for screening sugarcane germplasm for drought tolerance (Wagih et al. 2003; Hemaprabha et al. 2004; Hemaprabha et al. 2006) and the results have not been so encouraging in terms of identification of tolerant genotypes. Thus, the aim of the present investigation was to compare different (seven) sugarcane cultivars grown *in vitro* and field conditions for drought tolerance screening using the Ward's cluster analysis based on a number of physiological, biochemical

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and morphological parameters.

Materials and methods

In vitro *evaluation—Plant materials and osmotic stress treatments*

Seven sugarcane (*Saccharum* sp.) cultivars, viz., UT-94-2-483, LK92-11, K84-200, K97-32, K95-84, K88-92 and K92-80 (commonly grown in Thailand), derived from meristem cutting were grown on MS media (Murashige and Skoog 1962) supplemented with 3% sucrose, 8.88 μM benzyl adenine (BA), and 0.25% Phytagel® for 42 days. After separating the shoots from roots, the latter were placed on MS medium supplemented with 2.46 μM indole butyric acid (IBA) for 14 days. Plantlets were grown under ambient temperature (25±2°C), 60±5% relative humidity (RH), and 60±5 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) provided by fluorescent lamps with a 16 h day^{-1} photoperiod. After 14 days, the plantlets were shifted to MS sugar-free liquid medium in a growth incubator maintained at 25±2°C, 60±5% RH, and 120±5 μmol m^{-2} s^{-1} PPFD at 16 h day^{-1} photoperiod and CO_2 enrichment at 1,000±100 μmol CO_2 mol^{-1} (Cha-um et al. 2003). Mannitol (200 mM) was added to the culture medium to induce osmotic stress. After 14 days different physiological and biochemical parameters including proline content, photosynthetic pigments, chlorophyll fluorescence, and net-photosynthetic rate (P_n), and the growth performance was measured in sugarcane plantlets. A parallel set with no mannitol was maintained as control (no osmotic stress).

Data collection

Free proline in the leaf tissues was extracted and analyzed as per the method of Bates et al. (1973). Fresh leaf material (50 mg) was ground in liquid nitrogen, mixed with 1 ml of sulfosalicylic acid solution (3% w/v) and filtered. An aliquot of the filtrate was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 mg ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M H_3PO_4) and incubated at 95°C for 1 h. Then, the mixture was mixed vigorously with 2 ml of toluene. After cooling to 25°C, the absorbance of the chromophore was measured at 520 nm on a spectrophotometer (HACH DR/4000; Model 48000, HACH Company, Loveland, Colorado, USA) using L-proline as a standard.

Contents of chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total chlorophyll (TC) were determined as per the method of Shabala et al. (1998). Leaf material (100 mg) was using an electric homogenizer. The glass vials were sealed with parafilm and kept at 4°C for 48 h. The absorbance of the chromophore was measured at 662 and 644 nm using a UV-visible spectrophotometer.

Chlorophyll fluorescence emission from the leaf adaxial surface was measured using a fluorescence monitoring system (model FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode (Loggini et al. 1999). Original (F_0) and maximum (F_m) fluorescence yields were measured under weak modulated red light (<0.5 μmol m^{-2} s^{-1}) with 1.6 s pulses of saturating light (>6.8 μmol m^{-2} s^{-1} *PAR*). The variable fluorescence yield (F_v) was calculated as: $F_v=F_m-F_0$. The maximum quantum yield of PSII was determined as F_v/F_m. The photon yield of PSII (Φ_{PSII}) in the light was calculated as $\Phi_{PSII}=(F_m'-F_v)/F_m'$ after 45 s of illumination at steady state. In addition, non-photochemical quenching (NPQ) was calculated following the method of Maxwell and Johnson (2000).

Net photosynthetic rate (P_n; μmol m^{-2} s^{-1}) was calculated by comparing the different concentrations of CO_2 inside (C_{in}) and outside (C_{out}) the glass vessel containing the sugarcane plantlets. The CO_2 concentrations at steady state were measured by a gas chromatograph (GC; Model GC-17A, Shimadzu Co. Ltd., Japan). The P_n of *in vitro* cultivated plantlets was calculated as described by Fujiwara et al. (1987).

Data for various growth parameters such as shoot height, root length, fresh weight, dry weight and leaf area of sugarcane plantlets were recorded 14-days after the start of mannitol-induced osmotic stress treatment. Plantlets were dried at 80°C in a hot-air oven for 48 h and their dry weights were recorded. The leaf area was measured using a Root/Leaf Area Meter DT-scan (Delta-Scan Version 2.03, Delta-T Devices, Ltd., Cambridge, UK). Quantification of free proline, photosynthetic pigments, chlorophyll fluorescence, photosynthetic capacity and growth was done following the method given in Cha-um et al. (2009).

Field trial evaluation

Plantlets of seven sugarcane cultivars were directly transferred to plastic bags containing clay soil (EC=2.687 dS m^{-1}; pH=5.5; organic matter=10.36%; total N=0.17%; total P=0.07%; total K=1.19%) in 50% shading light intensity in the greenhouse for 1 month (i.e. acclimatization). Irrigation was applied as water spray. Acclimatized plants were directly transplanted into a field (30 cm plant to plant and 150 cm row to row distance) at two sites including well irrigation (control; WW) and rain-fed (554 mm $year^{-1}$) water deficit (WD) in Chaiyaphum, Northeast of Thailand (Latitude 16°35′N and Longitude 101°55′E; Fig. 1). In addition, SWC was calculated using the weight fraction: SWC (%)=[(FW−DW)/DW]×100, where FW was the fresh weight of a soil portion of the internal area of each pot and DW was the dry weight of the soil portion after drying in a hot air oven at 85°C for 4 days (Coombs et al. 1987). Chemical fertilizer (16 : 16 : 16; nitrogen: phosphorus: potassium) was applied three times, i.e., February, May and August at 0.0156 kg m^{-2} prior to stalk harvesting in November 2010. Plant height, stalk weight, number of stalks per plot and total stalk weight per plot were recorded.

Experimental design and statistical analysis

The experiment was arranged as 7×2 factorials in a Completely Randomized Block Design (CRBD) with 8 replicates (n=8). The mean values obtained were compared using the Duncan's New Multiple Range Test (DMRT) and analyzed with the

SPSS software. To classify the sugarcane lines into drought tolerant and sensitive categories, data for proline accumulation, photosynthetic pigments, chlorophyll fluorescence, photosynthetic capacity and growth under osmotic stress were subjected to the Ward's method of Hierarchical cluster analysis using SPSS software.

Results and discussion

Growth performance of sugarcane cultivars

Mannitol-induced water stress caused a marked decrease in growth attributes such as shoot length, fresh weight, dry weight and leaf area in all the seven sugarcane cultivars (Table 1). However, the level of reduction varied in different genotypes. Cultivars LK92-11, K97-32, and K95-84 produced higher shoot fresh and dry weight than the other cultivars under osmotic stress conditions. Likewise, root length in decreased (20.97–42.5% reduction) in all cultivars under osmotic stress and the root length was the lowest in K92-80 (21.15% reduction) and K97-32 (20.97% reduction). These observations are paralleled by earlier studies reporting differential response of sugarcane genotypes under drought stress. For example, Wagih et al. (2003) screened 26 sugarcane cultivars for drought tolerance using plant height and biomass as selection criteria. They observed 15.21–33.33%, 68.34–81.70% and 61.89–77.43% reduction in plant height, fresh weight and dry weight, respectively, in 26 sugarcane cultivars (Wagih et al. 2003). Smit and Singels (2006) used leaf area reduction (as Leaf area index, LAI) as a potential indicator for drought tolerance in sugarcane cultivars. In sugarcane cultivar "NCo376" LAI was maintained during early drought stress (i.e. 28 days after drying); however, it dropped under dry conditions for long periods. In contrast, LAI in cultivar "N22" decreased quickly in early drought condition (14 days after drying) (Smit and Singels 2006).

Photosynthetic pigments

Chlorophyll *a* (Chl_a) content decreased in sugarcane cultivars, K88-92, K92-80, LK92-11, K95-84 and UT-94-2483, when subjected to osmotic stress, whereas it remained unchanged in K84-200 and K97-32 (Table 2). In general, the degradation of Chl_a in ranged from 13.28% (in K84-200) to 41.68% (in K92-80) over that in the control. Likewise, chlorophyll *b* (Chl_b) content declined in the leaf tissues of K88-92 (46.85%), K97-32 (27.31%), LK92-11 (35.58%) and UT-94-2483 (54.05%), while it remained unaffected in K84-200 (0%) under mannitol-induced stress. The reduction in total chlorophyll (TC) in followed a trend parallel to that of Chl_a and Chl_b (Table 2). TC content declined in the range of 6.57% (K84-200) to 39.61% (UT-94-2-483). The ratio of Chl_a to Chl_b was enhanced in the cultivars K88-92, K97-32, LK92-11 and UT-94-2-483, whereas a decline was observed in K84-200, K92-80 and K95-84 under 200 mM mannitol-induced stress (Table 2). In general, photosynthetic pigments in osmotically stressed plants are considered as one of the most sensitive parameters for assessing crop salt tolerance, especially in sensitive genotypes (Cha-um et al. 2012). For example, a greater decline in Chl_a content was recorded in drought sensitive sugarcane cultivar "Cadmus" compared to that in in drought tolerant 'Q77N1232', when exposed to drought conditions (Wagih et al. 2004). Similarly, a decline in TC content was seen in drought susceptible genotypes i.e. CP92-675, H99-295, and TCP02-4624, of sugarcane, whereas there was no change in TC content in drought-tolerant cultivars, −HOCP85-845, TCP02-4587, TCP02-4620, and US01-40, (Silva et al. 2007). In cultivar "K84-200", Chl_b content in osmotic stressed leaf tissues was high. Likewise, in spinach, chlorophyll content per fresh weight of leaf increased when there was a reduced relative water content (Gupta and Berkowitz 1988). In of late, studies have reported no change in TC content in drought tolerant cultivars (HOCP01-523, TCP89-3505, and RB867515) of sugarcane under osmotic stress (Silva et al. 2011; da Silva et al. 2012). Chlorophyll degradation in sugarcane exposed to osmotic stress may have a negative effect on photosynthetic capacity, especially in the PSII light harvesting complex (Cha-um and Kirdmanee 2008).

Chlorophyll fluorescence and photosynthetic capacity

Maximum quantum yield of PSII (F_v/F_m) decreased by 21.18% in the sugarcane cultivar "K95-84" under osmotic stress (Table 3), whereas the F_v/F_m in other cultivars did not change (1.19–10.71%). On the other hand, photon yield of PSII (Φ_{PSII}) decreased in the range of 8.77% (K97-32) to 26.32% (K88-92). A positive relationship was observed between F_v/F_m and Φ_{PSII} (Fig. 2A). In contrast, non-photochemical quenching (NPQ) was inhibited and it correlated positively with proline content (Fig. 2B). The Φ_{PSII} in sugarcane plantlets declined when exposed to mannitol-induced osmotic stress (Table 3), and it correlated positively with reduced net photosynthetic rate (P_n) (Fig. 3A). A sharp water stress-induced decline in P_n ranging from 42.7% (K88-92) to 80.0% (LK92-11) was observed under osmotic stress (Table 3), and it may have been one of the major factors of reducing plant dry weight (Fig. 3B). A decline in chlorophyll fluorescence in water stressed plants is a general response of many plant species. The observations made in the present study are corroborated by earlier findings. For example, F_v/F_m remained unchanged in the drought-tolerant genotypes (HOCP85-845, TCP02-4587, TCP02-4620 and US01-40) under drought conditions, whereas it declined significantly in susceptible genotypes (CP72-1210, CP92-675 and H99-295) (Silva et al. 2007). Likewise, a

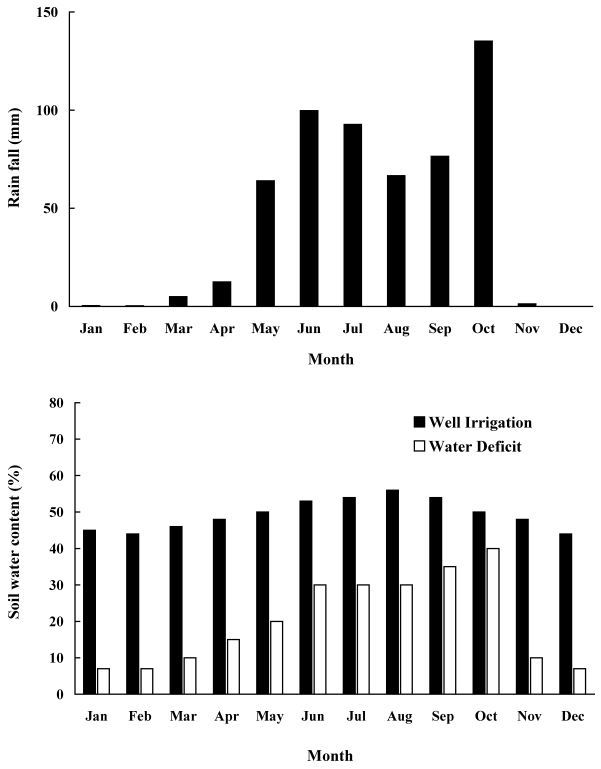

Figure 1. Monthly annual precipitation (A) and soil water content (B) of the field with well irrigation (WW) and without irrigation (WD) at Chaiyaphum province, Northeast of Thailand in year 2010.

decline was observed in F_v/F_m in the drought sensitive genotypes, viz., SP86-155, SP90-1638, TCP87-3388, HOCP93-776 and RB92579 under simulated drought stress; and it may one of the major causes of reduction in P_n under osmotic stress (Silva et al. 2007; Rodrigues et al. 2009; da Graça et al. 2010; Silva et al. 2011; da Silva et al. 2012). The efficiency of photochemical quenching in PSII relates to high value of F_v/F_m and Φ_{PSII} and it paralleled the observation made in water deficit tolerant genotypes (HOCP85-845, TCP02-4587, TCP02-4620 and US01-40) (Silva et al. 2007). In C_4 grasses, non-stomatal limitation in photosynthesis, including F_v/F_m and Φ_{PSII}, has been reported as a major barrier to growth under drought stress (Ghannoum et al. 2003). Aditionally, low NPQ has been reported to provide protection against photo-oxidative damage (Müller et al. 2001; Omasa and Takayama 2003). In a recent study, a reduction in F_v/F_m and Φ_{PSII} in some sugarcane cultivars was observed, and it resulted in reduced P_n (Rodrigues et al. 2009; da Silva et al. 2012). Previously, P_n has been demonstrated to be a very sensitive parameter for the classification of sugarcane germplasm for drought tolerance (de Silva and de Costa 2009; da Silva et al. 2012) and it has been found to hold good in the present study and useful in screening the sugarcane cultivars for drought tolerance.

Proline content

Proline content in the leaf tissues of water-deficit stressed plantlets increased over that in the control. However, it remained unchanged in K97-32 and K92-80. In K92-80, K97-32 and K95-84 proline content was very low ($<1.0\,\mu$mol g^{-1} FW) when plantlets were subjected to

Table 1. Shoot height (SH), root length (RL), fresh weight (FW), dry weight (DW) and leaf area (LA) in sugarcane cultivars grown under 0 (control) and 200 mM mannitol (osmotic stress) for 14 days. Percent reduction in each growth attributes of osmotic stressed plantlets of each cultivar is presented in each column.

Cultivars	Mannitol (mM)	SH (cm)	RL (cm)	FW (mg)	DW (mg)	LA (cm^2)
K84-200	0	24.3^{ab}	7.6^{b}	670^{bc}	103^{cde}	17.8^{b}
	200	12.9^{f}	5.1^{de}	295^{fg}	61^{fg}	11.2^{de}
		(46.91%)	(32.90%)	(55.97%)	(40.78%)	(37.08%)
K88-92	0	21.0^{bc}	4.0^{f}	568^{de}	77^{de}	18.9^{b}
	200	15.3^{def}	2.3^{g}	225^{g}	35^{gh}	9.7^{de}
		(27.14%)	(42.50%)	(60.39%)	(54.55%)	(48.68%)
K92-80	0	24.2^{ab}	5.2^{de}	801^{bc}	109^{bc}	24.4^{a}
	200	18.5^{cde}	4.1^{ef}	198^{g}	22^{h}	15.3^{bc}
		(23.55%)	(21.15%)	(75.28%)	(79.82%)	(37.30%)
K95-84	0	25.4^{ab}	5.6^{cd}	867^{b}	127^{b}	23.7^{a}
	200	14.9^{ef}	3.8^{f}	459^{ef}	71^{ef}	6.7^{e}
		(41.34%)	(32.14%)	(47.06%)	(44.10%)	(71.73%)
K97-32	0	26.3^{a}	6.2^{cd}	1126^{a}	131^{b}	26.8^{a}
	200	20.3^{bc}	4.9^{de}	602^{cd}	85^{de}	10.9^{de}
		(22.81%)	(20.97%)	(46.54%)	(35.12%)	(59.33%)
LK92-11	0	29.6^{a}	9.8^{a}	798^{bc}	113^{bc}	23.2^{a}
	200	15.3^{def}	6.7^{bc}	515^{de}	74^{ef}	13.0^{cd}
		(48.31%)	(31.63%)	(35.46%)	(34.51%)	(43.97%)
UT-94-2-483	0	27.7^{a}	7.8^{b}	815^{bc}	170^{a}	25.4^{a}
	200	16.8^{def}	5.5^{cd}	376^{fg}	65^{fg}	8.2^{e}
		(39.35%)	(29.49%)	(53.87%)	(61.77%)	(67.72%)

Different letters in each column show significant difference at $p \leq 0.01$ by Duncan's New Multiple Range Test (DMRT).

Table 2. Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), total chlorophyll (TC) and Chl_a:Chl_b ratio in sugarcane cultivars grown under 0 (control) and 200 mM mannitol (osmotic stress) for 14 days. Percent reduction in photosynthetic pigments of osmotic stressed plantlets in each cultivar is presented in each column.

Cultivars	Mannitol (mM)	Chl_a ($\mu g\,g^{-1}$ FW)	Chl_b ($\mu g\,g^{-1}$ FW)	TC ($\mu g\,g^{-1}$ FW)	Chl_a:Chl_b
K84-200	0	103.9^{de}	63.6^{cd}	167.5^{bc}	1.66^{bc}
	200	90.1^{e}	66.4^{cd}	156.5^{bc}	1.35^{bc}
		(13.28%)	(0%)	(6.57%)	
K88-92	0	157.1^{b}	125.5^{a}	286.6^{a}	1.32^{bc}
	200	107.8^{de}	66.7^{cd}	174.5^{b}	1.62^{bc}
		(31.38%)	(46.85%)	(39.11%)	
K92-80	0	91.9^{e}	64.5^{cd}	156.4^{bc}	1.44^{bc}
	200	53.6^{f}	47.8^{d}	101.4^{c}	1.12^{c}
		(41.68%)	(25.89%)	(35.17%)	
K95-84	0	122.3^{de}	73.7^{cd}	196.0^{b}	1.75^{bc}
	200	93.1^{e}	58.2^{d}	151.3^{bc}	1.61^{bc}
		(23.88%)	(21.03%)	(22.81%)	
K97-32	0	119.3^{de}	80.2^{bc}	199.5^{b}	1.58^{bc}
	200	93.8^{e}	58.3^{d}	152.1^{bc}	1.61^{bc}
		(21.38%)	(27.31%)	(23.76%)	
LK92-11	0	191.4^{a}	100.9^{ab}	292.3^{a}	1.95^{b}
	200	146.3^{bc}	65.0^{cd}	211.3^{b}	2.49^{a}
		(23.56%)	(35.58%)	(27.71%)	
UT-94-2-483	0	197.4^{a}	114.9^{a}	312.3^{a}	1.73^{bc}
	200	135.8^{cd}	52.8^{d}	188.6^{b}	2.65^{a}
		(31.21%)	(54.05%)	(39.61%)	

Different letters in each column show significant difference at $p \leq 0.01$ by Duncan's New Multiple Range Test (DMRT).

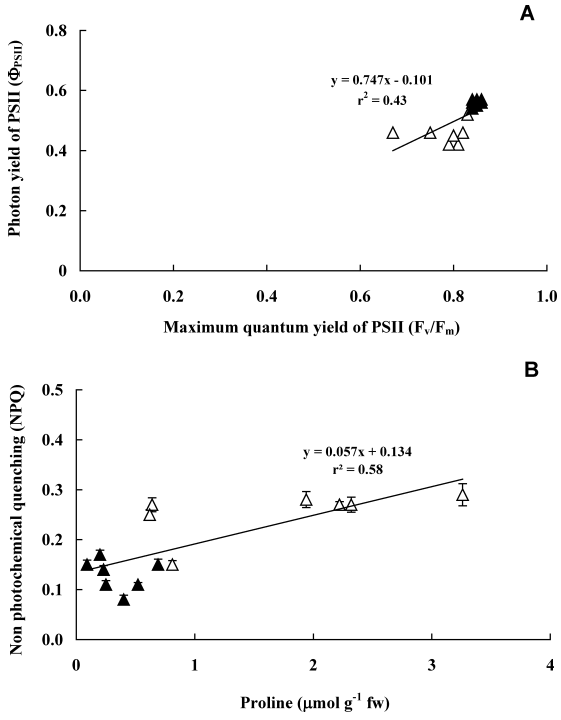

Figure 2. Relationships between maximum quantum yield of PSII (F_v/F_m) and photon yield of PSII (Φ_{PSII}) (A), proline and non-photochemical quenching (NPQ) (B) in sugarcane cultivars grown under 0 (control; dark symbol) and 200 mM mannitol (osmotic stress; light symbol) for 14 days. Error bars represent ±SE.

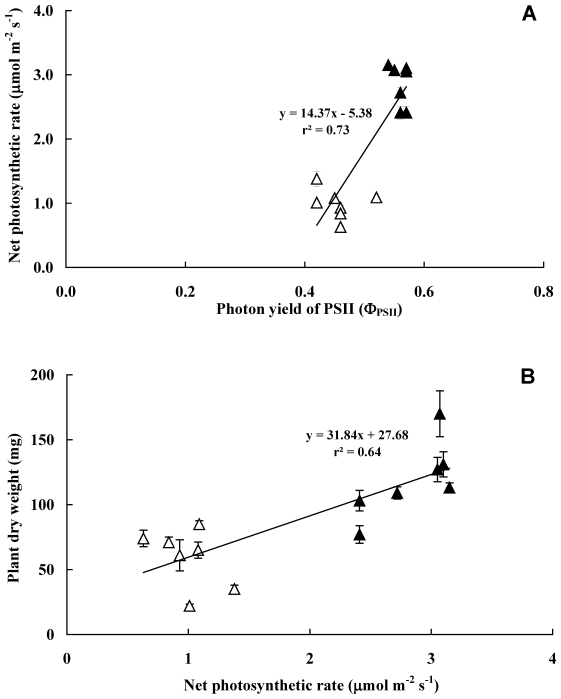

Figure 3. Relationships between photon yield of PSII (Φ_{PSII}) and net photosynthetic rate (P_n) (A), P_n and plant dry weight (B) in sugarcane cultivars grown under 0 (control; dark symbol) and 200 mM mannitol (osmotic stress; light symbol) for 14 days. Error bars represent ±SE.

200 mM mannitol-induced osmotic stress (Fig. 2B). Proline accumulation is a good indicator of drought tolerant genotypes in sugarcane (Errabii et al. 2006; Queiroz et al. 2011). Previously, enhanced proline content has been observed in sugarcane plantlets of cv. K84-200 under mannitol-induced osmotic stress (Cha-um and Kirdmanee 2008). Likewise, proline content

Table 3. Maximum quantum yield of PSII (F_v/F_m), photon yield of PSII (Φ_{PSII}), non- photochemical quenching (NPQ) and net photosynthetic rate (P_n) in sugarcane cultivars grown under 0 (control) and 200 mM mannitol (osmotic stress) for 14 days. Percent reduction in photosynthetic abilities of osmotic stressed plantlets in each cultivar is presented in each column.

Cultivars	Mannitol (mM)	F_v/F_m	Φ_{PSII}	NPQ	P_n (μmol m^{-2} s^{-1})
K84-200	0	0.86^{a}	0.56^{ab}	0.14^{cd}	2.41^{c}
	200	0.82^{ab}	0.46^{c}	0.27^{ab}	0.93^{ef}
		(4.65%)	(17.86%)	(1.93 folds)	(61.41%)
K88-92	0	0.86^{a}	0.57^{a}	0.17^{c}	2.41^{c}
	200	0.81^{bc}	0.42^{c}	0.29^{a}	1.38^{d}
		(5.81%)	(26.32%)	(1.71 folds)	(42.74%)
K92-80	0	0.84^{ab}	0.56^{ab}	0.15^{cd}	2.72^{b}
	200	0.79^{cd}	0.42^{c}	0.27^{ab}	1.01^{ef}
		(5.95%)	(25.0%)	(1.80 folds)	(62.87%)
K95-84	0	0.85^{ab}	0.57^{a}	0.15^{cd}	3.05^{a}
	200	0.67^{e}	0.46^{c}	0.25^{b}	0.84^{f}
		(21.18%)	(19.30%)	(1.67 folds)	(72.46%)
K97-32	0	0.84^{ab}	0.57^{a}	0.11^{de}	3.10^{a}
	200	0.83^{bc}	0.52^{b}	0.15^{cd}	1.09^{e}
		(1.19%)	(8.77%)	(1.36 folds)	(64.84%)
LK92-11	0	0.84^{ab}	0.54^{ab}	0.11^{de}	3.15^{a}
	200	0.75^{d}	0.46^{c}	0.27^{ab}	0.63^{g}
		(10.71%)	(14.81%)	(2.45 folds)	(80.0%)
UT-94-2-483	0	0.85^{ab}	0.55^{ab}	0.08^{e}	3.07^{a}
	200	0.80^{bc}	0.45^{c}	0.28^{ab}	1.08^{e}
		(5.88%)	(18.18%)	(3.5 folds)	(64.82%)

Different letters in each column show significant difference at $p \leq 0.01$ by Duncan's New Multiple Range Test (DMRT).

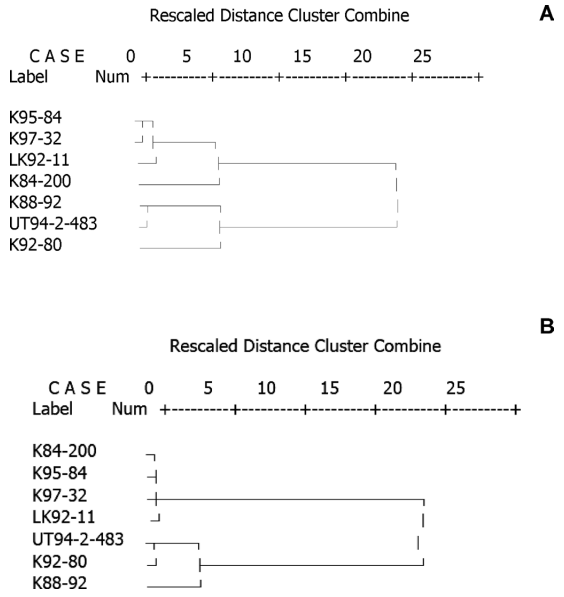

Figure 4. Ward's dendrogram for sugarcane cultivars to classify as (A) water-deficit sensitive, K88-92, K92-80 and UT94-2-483, and water-deficit tolerant, K84-200, K95-84, K97-32 and LK92-11, using proline accumulation, pigment content, chlorophyll fluorescence, net photosynthetic rate, and growth characteristics of *in vitro* classification as well as (B) water-deficit sensitive, K88-92, K92-80 and UT94-2-483, and water-deficit tolerant, K84-200, K95-84, K97-32 and LK92-11, using plant height, stalk weight, number of stalk per plot, and total stalk weight of field trial evaluation.

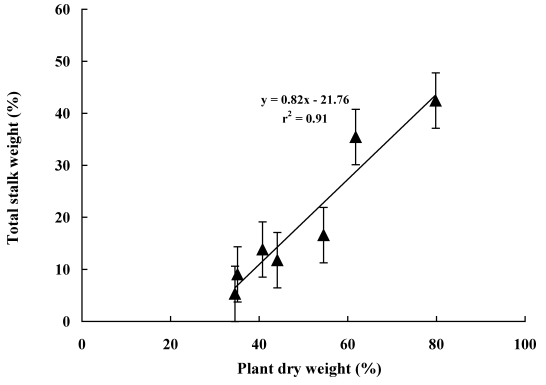

Figure 5. Relationships between plant dry weight (%) *in vitro* and total stalk weight (%) in a field trial of sugarcane cultivars. Error bars represent ±SE.

was greater in drought tolerant sugarcane cultivars (Q77N1232, N11, CP59-73 and ICA91-5155) than that in drought susceptible cultivars—Cadmus (Wagih et al. 2004), N12 (Rutherford 1989), R570 (Errabii et al. 2006), and ICA91-2195 (Queiroz et al. 2011) grown under osmotic stress. In fact, proline accumulation is a good indicator of identification of drought tolerant genotypes in sugarcane (Errabii et al. 2006; Queiroz et al. 2011).

Multivariate cluster analysis

Data for proline accumulation, photosynthetic pigments, chlorophyll fluorescence, net photosynthetic rate, and growth performance of the sugarcane cultivars were subjected to the Ward's cluster analysis. From this analysis it was possible to classify K88-92, K92-80 and UT94-2-483 as water-deficit sensitive and K84-200, K95-84, K97-32 and LK92-11 as water-deficit tolerant (Fig. 4A). Previously, a single parameter including lowest

Table 4. Plant height (PH), single stalk weight (SW), number of stalk per plot (NS) and total stalk weight per plot (TW) in sugarcane cultivars grown under well irrigation (WW) and rain fed water deficit stress (WD) in the field trial prior to harvesting period (10 months). Percent reduction in each growth and yield attribute of water deficit stressed plants in each cultivar is presented in each column.

Cultivars	Water stress	PH (cm)	SW (kg stalk^{-1})	NS	TW (kg plot^{-1})
K84-200	WW	261^{a}	1.88^{a}	84.5^{a}	156^{a}
	WD	259^{a}	1.62^{ab}	84.0^{a}	148^{a}
		(0.77%)	(13.83%)	(0.59%)	(5.43%)
K88-92	WW	219^{b}	1.87^{a}	74.0^{b}	103^{bc}
	WD	197^{bc}	1.56^{ab}	58.8^{de}	68^{d}
		(10.05%)	(16.58%)	(20.54%)	(34.21%)
K92-80	WW	225^{b}	1.72^{a}	68.3^{c}	117^{b}
	WD	160^{c}	0.99^{c}	44.5^{e}	45^{e}
		(29.01%)	(42.44%)	(34.85%)	(61.72%)
K95-84	WW	229^{b}	1.70^{a}	75.0^{b}	128^{ab}
	WD	225^{b}	1.50^{ab}	72.5^{bc}	116^{b}
		(1.88%)	(11.77%)	(3.33%)	(8.78%)
K97-32	WW	221^{b}	1.44^{b}	71.5^{bc}	115^{b}
	WD	218^{b}	1.31^{b}	66.0^{cd}	111^{b}
		(1.45%)	(9.03%)	(7.69%)	(3.57%)
LK92-11	WW	256^{a}	1.70^{a}	61.0^{d}	101^{bc}
	WD	241^{ab}	1.61^{ab}	60.0^{d}	90^{c}
		(5.66%)	(5.29%)	(1.64%)	(11.06%)
UT-94-2-483	WW	254^{a}	1.89^{a}	73.3^{bc}	142^{a}
	WD	210^{b}	1.22^{bc}	55.0^{de}	68^{d}
		(17.24%)	(35.45%)	(24.97%)	(52.42%)

Different letters in each column show significant difference at $p \leq 0.01$ by Duncan's New Multiple Range Test (DMRT).

reduction in biomass dry weight has been implemented to classify the drought tolerant genotypes (L6, 20, 9, 26 and 3) of sugarcane with best score (1–3) (Wagih et al. 2003). However, single parameter does not represent overall drought-tolerant defense mechanisms including water relations (Inman-Bamber and Smith 2005; Basra et al. 1999) and osmoregulation (Cha-um and Kidmanee 2008), required to maintain the biochemical, physiological and morphological characters (Silva et al. 2011) and yield attributes (Silva et al. 2007; Silva et al. 2008). Queiroz et al. (2011) employed multivariate analysis of biochemical (proline and trehalose osmolytes) and physiological characters to categorize IAC91-5155 as drought tolerant genotypes of sugarcane (Queiroz et al. 2011). Also, the multivariate parameters have been suggested as effective criteria for drought tolerant selection in sugarcane genotypes (Hemaprabha et al. 2004; Vasantha et al. 2005; Hemaprabha et al. 2006; Silva et al. 2008; Ishaq and Olaoye 2009).

Field trial evaluation

Yield traits, including single stalk weight, number of stalks per plot and total stalk weight per plot, in each cultivar of sugarcane declined when subjected to rain-fed conditions (water deficit stress), especially in water deficit susceptible cultivars (Table 4). For example, number of stalks per plot of water deficit susceptible cultivars, viz., K88-92, K92-80 and UT-94-2-483 declined by 20.54%, 34.85% and 24.97%, respectively, when grown in a field trial without irrigation for 10 months. In addition, total stalk weight per plot of water deficit susceptible cultivars, viz., K88-92, K92-80 and UT-94-2-483, decreased by 34.21%, 61.72% and 52.42%, respectively (Table 4). In contrast, yield traits in water deficit tolerant cultivars (K84-200, K95-84, K97-32 and LK92-11) were well maintained. From the cluster analysis, it was possible to classify K88-92, K92-80 and UT94-2-483 as water-deficit sensitive and K84-200, K95-84, K97-32 and LK92-11 as water-deficit tolerant genotpyes (Fig. 4B). These findings confirm the observations that data collected from *in vitro* screening may be used to accurately classify the drought tolerance in sugarcane, when compared to water deficit field trial. The productivity of sugarcane under drought stress conditions could be used as one of the key selection criteria for drought tolerance. In the present study, single stalk weight, number of stalks per plot and total stalk weight per plot of sugarcane cultivars were evaluated as field trial screening criteria. Additionally, a positive relationship was observed between plant dry weight of *in vitro* grown sugarcane plantlets and total stalk weight (Fig. 5).

These observations are supported by previous findings. For example, single cane weight of 55-high sugared genotypes was reported to decline to 66.10% when grown under drought stress conditions (Hemaprabha et al. 2004). Moreover, the stalk number and stalk weight of 80 sugarcane genotypes decreased by 15.93% and 22.47%, respectively, when subjected to limited irrigation (Silva et al. 2008). The survival percentage, single cane weight and sucrose percentage in 16 parental lines of sugarcane

have been used as criteria for drought resistant breeding program (Hemaprabha et al. 2006).

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