Front. Agric. China 2009, 3(3): 266–273
DOI 10.1007/s11703-009-0063-5

RESEARCH ARTICLE

Suriyan CHA-UM, Souvanh THADAVONG, Chalermpol KIRDMANEE

Effects of mannitol induced osmotic stress on proline accumulation, pigment degradation, photosynthetic abilities and growth characters in C_3 rice and C_4 sorghum

Abstract Osmotic stress is one of the most important abiotic factors which inhibit growth and development in both the vegetative and reproductive stages of many plant species. The aim of this investigation was to compare the biochemical and physiological responses in C_3 rice and C_4 sorghum to water deficit. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoid (C_{x+c}) contents in both rice and sorghum seedlings under osmotic stress were adversely affected, related to increasing osmotic pressure in the culture media. In addition, the chlorophyll's fluorescence parameters and net photosynthetic rate (P_n) decreased, leading to growth reduction. Also, a positive correlation was found between physiological and biochemical data, while proline accumulation showed a negative relationship. The Chl_b, P_n and fresh weight were maintained better in osmotic-stressed (-1.205 MPa) C_4 sorghum seedlings than those in C_3 rice seedlings. The growth and physiological responses of C_3 rice and C_4 sorghum decreased depending on the plant species, the osmotic pressure in the media and their interactions. Pigment content and P_n ability in C_4 sorghum grown under mannitol-induced osmotic stress increased to a greater degree than in C_3 rice, resulting in maintenance of growth.

Keywords chlorophyll a fluorescence, net photosynthetic rate, *Oryza sativa* L., pigment degradation, *Sorghum bicolor* (L.) Möench, water deficit stress

Received March 5, 2009; accepted May 5, 2009

Suriyan CHA-UM (✉), Chalermpol KIRDMANEE
National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Klong Laung 12120, Thailand
E-mail: suriyanc@biotec.or.th

Souvanh THADAVONG
Agriculture Research Center, National Agriculture and Forestry Research Institute, Vientiane, Laos

1 Introduction

Water deficit is a major problem worldwide, limiting plant growth and the productivity of crop species, especially in rain-fed agricultural areas ($>1.2\times10^9$ hm^2) (Chaves and Oliveira, 2004; Kjine, 2006; Passioura, 2007). Water use efficiency (WUE) in the crops grown under water deficit stress is a target to be evaluated for use as water-deficit resistance criteria (Quarrie et al., 1999; Passioura, 2006; Zhang et al., 2007; Ali and Talukder, 2008). There has been a large amount of literature reviewing the effects of water deficit on the biochemical, physiological, morphological and anatomical changes in plants at the seedling stage, prior to the development of reproductive processes (Chaves et al., 2003; Flexas et al., 2004; Barnabás et al., 2008; Shao et al., 2008). Rice and sorghum crop species are two important grain crop species for which there are complete genome sequences (Goff et al., 2002; Yu et al., 2002; Paterson, 2008; Paterson et al., 2009). They are important as a carbohydrate source in people's diets (Khush, 2005) and as an alternative source for bioethanol production (Xin et al., 2009). Rice, (C_3 photosynthesis (Choudhury, 2001)), has been identified as being susceptible to water deficit. There are many techniques that can be used to identify water-deficit tolerance traits from rice genetic resources which can then be used to produce transgenic rice in order to resist water limitation, especially in rain-fed areas (Fukai and Cooper, 1995; Lafitte et al., 2007; Kamoshita et al., 2008). In contrast, sorghum, (C_4 photosynthesis) (Choudhury, 2001), has been characterized as water deficit resistant and can grow well during under water deficit conditions (Buchanan et al., 2005; Sinclair et al., 2005; Sharma et al., 2006). In the case of rice, there have been several documents on changing photosynthesis from C_3 to C_4 using transgenic approaches, for the purpose of improving abiotic stress tolerance and crop productivity (Suzuki et al., 2000; Agarie et al., 2002; Chi et al., 2004; Suzuki et al., 2006; Bandyopadhyay et al., 2007; Wang and Li, 2008).

In water-deficit conditions, water availability in the nutrient solution is restricted by polyethylene glycol (PEG), mannitol or sorbitol in the growth medium or nutrient solution, causing low water use efficiency (WUE) in plants (Blum, 2005; Bloch et al., 2006; Costa et al., 2007; Shao et al., 2008). Low WUE is a primary effect of water deficit conditions on plants, and it leads to biochemical changes, including inhibition of Rubisco (ribulose-1,5-bisphosphatase carboxyase/oxygenase) activity, accumulation of osmolytes (proline, glycinebetaine, polyamine, glutathione, polyamines, sugars, sugar alcohols and α-tocolpherol) and increased levels of antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase) in order to reduce reactive oxygen species (ROSs) (Chaves et al., 2003; Nayyar, 2003; Selote et al., 2004; Nayyar and Gupta, 2006; Cha-um and Kirdmnaee, 2009). Physiological changes which also occur include the loss of membrane stability, reduced leaf water potential, pigment degradation, diminished chlorophyll a fluorescence, decreased stomatal conductance, reduced internal CO_2 concentration, net photosynthetic rate (P_n) reduction and growth retardation prior to plant death (Yordanov et al., 2003; Chaves and Oliveira, 2004; Reddy et al., 2004; Cattivelli et al., 2008; Shao et al., 2008). The aim of this study was to compare the photosynthetic pigments, chlorophyll a fluorescence, proline, P_n and growth of C_3 rice and C_4 sorghum in responses to water deficit stress.

2 Materials and methods

2.1 Plant materials

Seeds of rice (*Oryza sativa* L. spp. *indica*) cv. RD10 (sticky or glutinous rice) provided by Pathumthani Rice Research Center (Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand) and seeds of sorghum (*Sorghum bicolor* (L.) Möench) were de-husked by hand, sterilized once in 5% Clorox® (5.25% sodium hypochlorite, The Clorox Co, Oakland, CA, USA) for 60 min and in 30% Clorox® for 30 min, respectively, and then rinsed three times with sterile distilled-water. Surface-sterilized seeds were germinated on the 0.25% Phytagel®-solidified MS medium (Murashige and Skoog, 1962) in a 250-mL glass jar vessel. The media were adjusted to pH 5.7 before autoclaving. Seedlings were cultured *in vitro* under conditions of 25 ± 2°C ambient temperature, with 60% ± 5% relative humidity (RH) and 60 ± 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic proton flux density (PPFD) provided by fluorescent lamps (TDL 36 W/84 Cool White 3350 Illumination, Phillips, Bangkok, Thailand) with 16 $h \cdot d^{-1}$ photoperiod. Fourteen-day-old rice seedlings were aseptically transferred to MS sugar-free liquid media (photoautotrophic conditions) using vermiculite as supporting material for 7 d (Cha-um et al., 2007). The number of air-exchanges in the glass vessels was adjusted to 2.32 μmol $CO_2 \cdot h^{-1}$ by punching a hole in the plastic cap (∅ 1 cm) and covering the hole with a microporous filter (0.20 μm pore size; Nihon Millipore Ltd., Tokyo, Japan). The seedlings were subsequently cultured in a plant growth incubator with CO_2 enrichment at (1000 ± 100) $\mu mol \cdot mol^{-1}$. Mannitol induced osmotic stress in the culture medium was adjusted to −0.238 MPa (control), −0.392 MPa (100 $mmol \cdot L^{-1}$), −0.674 MPa (200 $mmol \cdot L^{-1}$), −0.939 MPa (300 $mmol \cdot L^{-1}$) or −1.205 MPa (400 $mmol \cdot L^{-1}$) for 14 days. Photosynthetic pigments, proline content, chlorophyll a fluorescence, net photosynthetic rate (P_n) and growth characteristics were measured.

2.2 Data measurement

Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) concentrations were determined following the method of Shabala et al. (1998) and total carotenoid (C_{x+c}) concentrations were determined following the method of Lichtenthaler (1987). One hundred milligrams of leaf material were collected, placed in a 25-mL glass vial, along with 10 mL 95.5% acetone, and blended using a homogenizer. The Chl_a, Chl_b, and C_{x+c} concentrations were measured using a UV-visible spectrophotometer (DR/4000; HACH, Loveland, Colorado, USA). A solution of 95.5% acetone was used as a blank. The pigment degradation percentage was calculated following Cha-um and Kirdmanee (2008).

The proline content of the leaves was extracted according to the method of Bates et al. (1973). Leaf tissue (100 mg) was ground in liquid nitrogen. The homogenate was mixed with 1 mL aqueous sulfosalicylic acid (3% w/v) and filtered through filter paper (Whatman #1). The extracted solution 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 $mol \cdot L^{-1}$ H_3PO_4) and incubated at 95ºC for 1 h. The reaction was terminated by placing the container containing the reagents in an ice bath. The reaction mixture was vigorously mixed with 2 mL toluene. After warming to 25°C, the chromophore was measured using a spectrophotometer (DR/4000, HACH, Loveland, Colorado, USA) at 520 nm. L-proline (Fluka, Switzerland) was used as a standard.

Chlorophyll a fluorescence emission from the adaxial surface of the leaves was monitored with a Fluorescence Monitoring System (FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode, as previously described by Loggini et al. (1999) and Maxwell and Johnson (2000).

Net-photosynthetic rate (P_n) was calculated by comparing the different concentrations of CO_2 inside and outside of the glass vessel containing the seedlings. The CO_2 concentrations inside and outside the glass vessel (C_{in} and

C_{out}) at steady state were measured by Gas Chromatography (GC; Model GC-17A, Shimadzu Co. Ltd., Japan). The detector (TCD; Thermal Conductivity Detector) and injector were set at 250°C. The temperature program of the GC capillary column (GS-Q, J & W Scientific®, Germany) was set at 30°C for 1 min at the initial state and increased to 100°C at a rate of 20°C per min and held for 1 min (Fujiwara et al., 1987).

Shoot height, root length, number of leaves, leaf area, fresh weight and dry weight of rice and sorghum seedlings were measured. Seedlings were dried in a hot-air oven (Model 500, Memmert, Buchenbach, Germany) for 2 d and then incubated in desiccators before measurement of the dry weight (Cha-um et al., 2006). The leaf area of seedlings was measured using a Leaf Area Meter DT-scan (Delta-Scan Version 2.03, Delta-T Devices, Ltd., Burwell, Cambridge, UK).

2.3 Experiment design

The experiment was arranged as 2×5 factorials in a Completely Randomized Design (CRD) with ten replicates and two seedlings per replicate. The mean values were compared using Bonferroni's correction analysis and analyzed with SPSS software (SPSS for Windows, SPSS Inc., Chicago, USA). The correlations between physiological and biochemical parameters were evaluated by Pearson's correlation coefficients.

3 Results

Shoot height (SH), root length (RL), number of leaves (NL), leaf area (LA), fresh weight (FW) and dry weight (DW) of rice (C_3) and sorghum (C_4) seedlings cultured under reducing osmotic potential in the culture media showed a decreasing trend in relation to the osmotic potential. These parameters in osmotic-stressed rice seedlings (−1.205 MPa) dropped to lower levels than in the control seedlings (−0.238 MPa) by 19.55%, 13.53%, 31.51%, 35.94%, 51.12% and 23.74%, respectively, whereas in sorghum they were 23.64%, 23.23%, 21.42%, 51.15%, 31.89% and 29.50%, respectively (Table 1). The different plant species, the osmotic potential in the culture media and their interactions strongly affected SH, RL, NL, LA, FW and DW ($P \leqslant 0.05$ or $P \leqslant 0.01$) (Table 1). Photosynthetic pigments, including chlorophyll a (Chl_a), chalorophyll b (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}), in osmotic stressed seedlings decreased significantly depending on plant species, osmotic pressure and their interactions. Photosynthetic pigments in −1.205 MPa osmotic stressed seedlings decreased by 28.86%, 72.56%, 47.98% and 29.85% respectively in rice, and by 49.22%, 54.76%, 50.73% and 34.80% respectively in sorghum, when compared to the control (−0.238 MPa) (Table 2). Degradation of Chl_a in osmotic stressed rice and sorghum was negatively related to the maximum quantum yield of PSII (F_v/F_m) with $r^2 = 0.62$ (Fig. 1(a)) and $r^2 = 0.73$ (Fig. 1(b)), respectively. In a similar way, the degradation of TC in osmotic stressed rice and sorghum was negatively related to the photon yield of PSII (Φ_{PSII}) with $r^2 = 0.95$ (Fig. 2(a)) and $r^2 = 0.93$ (Fig. 2(b)), respectively. The chlorophyll a fluorescence parameters, F_v/F_m and Φ_{PSII}, net photosynthetic rate (P_n) in osmotic stressed seedlings were drastically diminished according to plant species, osmotic stress and their interactions (Table 3). F_v/F_m and Φ_{PSII} in rice seedlings were reduced by 13.42% and 16.05%,

Table 1 Shoot height (SH), root length (RL), number of leaves (NL), leaf area (LA), fresh weight (FW) and dry weight (DW) of rice and sorghum seedlings exposed to mannitol induced osmotic stress for 14 days

species	osmotic potential/–MPa	SH/cm	RL/cm	NL	LA/cm^2	FW/mg	DW/mg
rice	0.238	12.38a	4.51b	3.65ab	20.17c	806.7b	85.1c
	0.392	12.38a	4.40b	3.65ab	17.14c	564.5c	83.2c
	0.674	11.02ab	4.39b	3.50b	16.72c	443.2c	74.0cd
	0.939	10.17b	4.36b	2.75cd	12.93c	437.7c	72.1cd
	1.205	9.96b	3.90b	2.50d	12.92c	378.2c	64.9d
sorghum	0.238	9.22bc	10.72a	4.20a	62.01a	1120.9a	153.7a
	0.392	8.81bc	9.93a	4.15a	54.95a	1016.9a	137.4a
	0.674	7.48c	9.84a	3.70ab	36.42b	843.2b	122.4ab
	0.939	7.13c	8.54a	3.35b	33.51b	773.6b	120.9ab
	1.205	7.04c	8.23a	3.30bc	30.29b	763.5b	108.9b
significant level	–	–	–	–	–	–	–
plant	–	**	**	**	**	**	**
osmo	–	**	*	**	**	**	*
plant ×osmo	–	**	*	**	**	**	*

Note: Different letters in each column show significant differences at $P \leqslant 0.01$, (**) by Bonferroni's multiple comparison.

Table 2 Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}) of rice and sorghum seedlings exposed to mannitol induced osmotic stress for 14 days

species	osmotic potential/–MPa	Chl_a/(μ·g^{-1} FW)	Chl_b/(μg·g^{-1} FW)	TC/(μg·g^{-1} FW)	C_{x+c}/(μg·g^{-1} FW)
rice	0.238	243.15a	189.24a	432.39a	75.17a
	0.392	242.29a	142.59b	384.88b	72.56a
	0.674	232.27a	95.86c	328.13c	65.24ab
	0.939	183.83bc	61.93d	245.76def	64.38ab
	1.205	172.97bc	51.92de	224.89ef	52.73b
sorghum	0.238	195.58b	73.92d	269.50de	54.96b
	0.392	200.40b	77.48d	277.88d	50.29b
	0.674	209.58b	70.00d	279.58d	47.30bc
	0.939	152.82c	46.67de	199.49f	47.12bc
	1.205	99.32d	33.44e	132.76g	35.83c
significant level	–	–	–	–	–
plant	–	**	**	**	**
osmo	–	**	**	**	**
plant × osmo	–	**	**	**	**

Note: Different letters in each column show significant differences at $P \leqslant 0.01$, (**) by Bonferroni's multiple comparison.

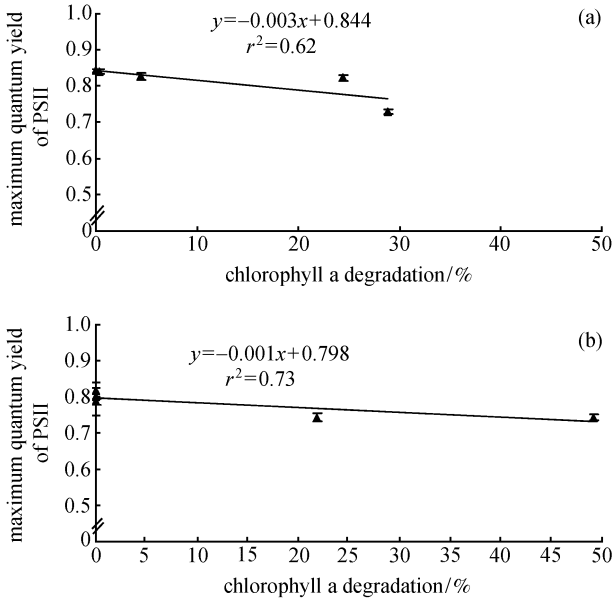

Fig. 1 Relationship between chlorophyll a degradation and maximum quantum yield of PSII (F_v/F_m)
Note: Rice (a) and sorghum (b) seedlings exposed to mannitol induced osmotic stress for 14 days. Error bars represent ±*SE*.

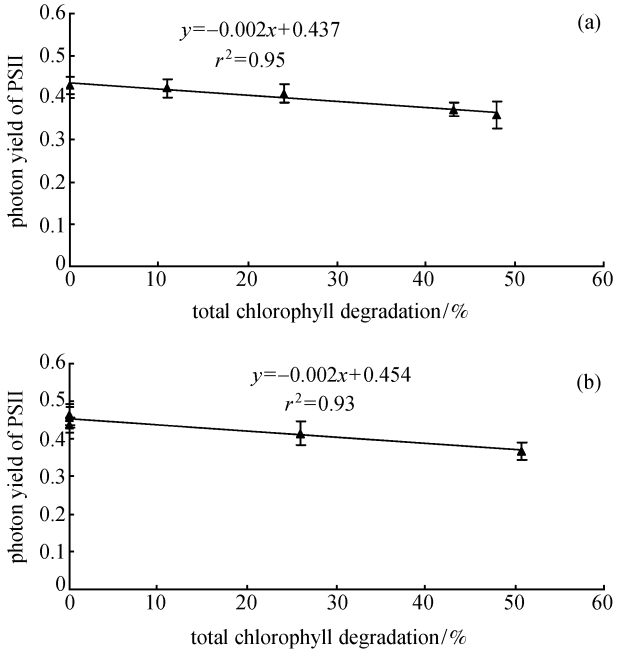

Fig. 2 Relationship between total chlorophyll degradation and photon yield of PSII (Φ_{PSII})
Note: Rice (a) and sorghum (b) seedlings exposed to mannitol induced osmotic stress for 14 days. Error bars represent ±*SE*.

respectively, and in sorghum by 8.94% and 20.86%, respectively, when exposed to osmotic stress (–1.205 MPa), while the proline content was enriched by 6.52 times in both rice and sorghum (Table 3). The reduction of P_n in osmotic stressed rice seedlings decreased sharply when compared to that in sorghum seedlings (Fig. 3). A correlation between physiological parameters i.e. Chl_a, Chl_b, TC, C_{x+c}, F_v/F_m, Φ_{PSII}, P_n and proline was demonstrated, except in the cases of C_{x+c} and Φ_{PSII}, as C_{x+c} and P_n were uncorrelated (Table 4). In addition, the diminished Φ_{PSII} in osmotic stressed seedlings was positively related to P_n in rice (r^2= 0.99) and sorghum (r^2= 0.96) crop species (Fig. 4), leading to dry mass reduction (r^2= 0.91 and r^2= 0.81, respectively) (Fig. 5).

Table 3 Maximum quantum yield of PSII (F_v/F_m), photon yield of PSII (Φ_{PSII}), net photosynthetic rate (P_n) and proline contents of rice and sorghum seedlings exposed to mannitol induced osmotic stress for 14 days

species	osmotic potential/–MPa	F_v/F_m	Φ_{PSII}	proline/(μmol·g^{-1} FW)
rice	0.238	0.842a	0.430a	208.23e
	0.392	0.841a	0.424ab	257.12e
	0.674	0.828a	0.411ab	496.84d
	0.939	0.823a	0.373b	763.48c
	1.205	0.729b	0.361b	1532.28a
sorghum	0.238	0.817a	0.465a	158.12f
	0.392	0.802a	0.459a	130.99g
	0.674	0.789ab	0.437a	304.17e
	0.939	0.744b	0.415ab	585.46d
	1.205	0.744b	0.368b	1030.43b
significant level	–	–	–	–
plant	–	**	**	**
osmo	–	*	*	**
plant × osmo	–	**	**	**

Note: Different letters in each column show significant differences at $P \leqslant 0.01$, (**) by Bonferroni's multiple comparison.

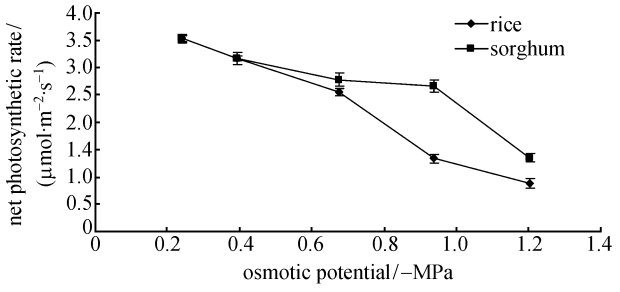

Fig. 3 Net photosynthetic rate (P_n)
Note: Rice and sorghum seedlings exposed to mannitol induced osmotic stress for 14 days. Error bars represent ±*SE*.

4 Discussion

Mannitol induced osmotic stress has been widely investigated in many plant species, i.e., rice (Zang and Komatsu, 2007), sorghum (Sharma et al., 2006), sugarcane (Cha-um and Kirdmanee, 2008), *Sesuvium portulacastrum* (Slama et al., 2007), *Fraxinus angustifolia* (Tonon et al., 2004) and soybean (Neto et al., 2004). In the present study, the photosynthetic pigments in osmotic-stressed C_3 rice seedlings were damaged in relation to increased osmotic pressure in the culture media, especially in the case of Chl_b content, which was lower than in C_4 sorghum. The findings are quite similar to those of a previous investigation in which the chlorophyll pigments in the leaf tissues of C_3 wheat (cv. C306) grown under water deficit stress using PEG6000 dropped significantly depending on osmotic stress (-0.4 to -1.5 MPa) and to a greater extent than in C_4 maize (cv. Sartaj) (Nayyar and Gupta, 2006). Similarly, the chlorophyll a fluorescence parameters, F_v/F_m and Φ_{PSII}, in osmotic stressed leaves were significantly diminished, mainly in response to extreme osmotic stress (-1.203 MPa), resulting in low P_n. The F_v/F_m and Φ_{PSII} in the leaf tissues are the most popular parameters used to

Table 4 Relationship between physiological and biochemical parameters of rice and sorghum seedlings exposed to mannitol induced osmotic stress for 14 days

parameter	Chl_a	Chl_b	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	P_n	proline
Chl_a	1	–	–	–	–	–	–	–
Chl_b	0.714**	1	–	–	–	–	–	–
TC	0.920**	0.931**	1	–	–	–	–	–
C_{x+c}	0.667**	0.502**	0.628**	1	–	–	–	–
F_v/F_m	0.411**	0.355**	0.413**	–0.274**	1	–	–	–
Φ_{PSII}	0.193*	0.199*	0.282*	0.027	0.455**	1	–	–
P_n	0.522**	0.536**	0.571**	0.168	0.292**	0.304**	1	–
proline	−0.548**	−0.483**	−0.555**	−0.228*	−0.354**	−0.394**	−0.878**	1

Note: Significant levels at $P \leqslant 0.01$ and $P \leqslant 0.05$ are represented by ** and *, respectively using Pearson's correlation coefficients.

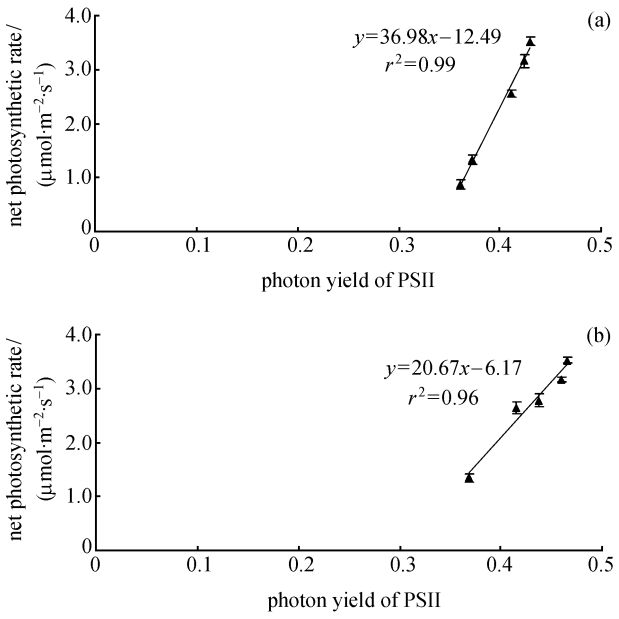

Fig. 4 Relationship between photon yield of PSII (Φ_{PSII}) and net photosynthetic rate (P_n)
Note: Rice (a) and sorghum (b) seedlings exposed to mannitol induced osmotic stress for 14 days. Error bars represent ±*SE*.

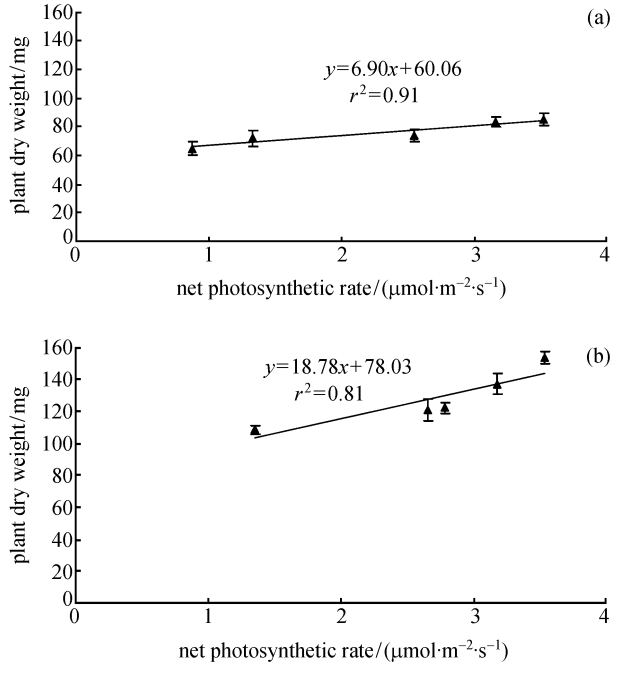

Fig. 5 Relationship between net photosynthetic rate (P_n) and plant dry weight
Note: Rice (a) and sorghum (b) seedlings exposed to mannitol induced osmotic stress for 14 days. Error bars represent ±*SE*.

identify the water oxidation and photon yield harvesting in the light reaction of osmotic stressed plants (Ripley et al., 2007; Cha-um and Kirdmanee, 2008). In *Alloteropsis semialata*, the chlorophyll a fluorescence parameters including CO_2 assimilation (ΦCO_2) and Φ_{PSII} in drought-stressed C_4 types are better than those in C_3 types, leading to high P_n (Ripley et al., 2007). In addition, the growth performance of osmotic-stressed seedlings of C_3 rice declined significantly when compared to that of C_4 sorghum, demonstrated especially by fresh weight. There have been several reports comparing the growth characters of higher plants which have C_3 and C_4 photosynthetic pathways when cultivated under osmotic stress. The growth characteristics of the C_4 type are superior to those of the C_3 type when grown under osmotic stress (Nayyar, 2003; Nayyar and Gupta, 2006). On the other hand, the proline content was high in both C_3 rice and C_4 sorghum when exposed to osmotic stress. In the present study, the accumulation of proline in osmotic stressed C_4 sorghum occurred at a lower rate than in C_3 rice. Proline content, a biochemical stress indicator (Ashraf and Foolad, 2007), was regulated in C_3 wheat by −0.4 MPa PEG induced osmotic stress while in C_4 maize it was enhanced by −0.8 MPa PEG induced osmotic stress (Nayyar, 2003). Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) generated in osmotic stressed C_4 maize were lower than in C_3 wheat whereas antioxidant substances (ascorbic acid and glutathione) and antioxidant enzyme activities (ascorbate peroxidase and glutathione reductase) in C_4 maize increased to a greater extent than in C_3 wheat (Nayyar and Gupta, 2006). It is possible that the osmotic-stress defense mechanisms in C_4 plants are more efficient than those in C_3 plants when exposed to stress. Also, a positive relationship between physiological and biochemical data was demonstrated, with the exception of proline content, which was negatively correlated with the other factors. These findings are similar to those of a previous publication which presented a positive correlation in the physiological data, especially in the photosynthesis system in C_3 and C_4 plants exposed to osmotic stress (Ripley et al., 2007).

5 Conclusions

In conclusion, the growth characteristics and physiological responses of C_3 rice and C_4 sorghum decreased, depending on plant species, osmotic pressure in the media and their interactions. The parameters of fresh weight, Chl_b and P_n in osmotic stressed rice seedlings decreased to a greater extent than those in sorghum. Pigment stabilization, P_n maintenance and growth performance in C_4 sorghum grown under osmotic stress were demonstrated to be greater than those in C_3 rice. These parameters may be employed as criteria for assessing osmotic stress tolerance (sorghum) or osmotic stress susceptibility (rice).

Acknowledgements The authors are grateful to the National Center for Genetic Engineering and Biotechnology (BIOTEC) for funding source (BIOTEC; No. BT-B-02-RG-BC-4905) as well as for the Human Resource

Development of Short-term Training in Biotechnology Programm-2007 and to Dr. Teeraporn Busaya-angoon at Pathumthani Rice Research Center, for providing rice seeds.

References

Agarie S, Miura A, Sumikura R, Tsukamoto S, Nose A, Arima S, Matsuoka M, Miyao-Tokutomi M (2002). Overexpression of C_4 PEPC caused O_2-insensitive photosynthesis in transgenic rice plants. Plant Science, 162: 257–265

Ali M H, Talukder M S U (2008). Increasing water productivity in crop production—A synthesis. Agricultural Water Management, 95: 1201–1213

Ashraf M, Foolad M R (2007). Roles of glycinebetaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany, 59: 206–216

Bandyopadhyay A, Datta K, Zhang J, Yang W, Raychaudhuri S, Miyao M, Datta S K (2007). Enhanced photosynthesis rate in genetically engineered *indica* rice expressing *pepc* gene cloned from maize. Plant Science, 172: 1204–1209

Barnabás B, Jäger K, Fahér A (2008). The effect of drought and heat stress on reproductive processes in cereals. Plant, Cell and Environment, 31: 11–38

Bates L S, Waldren R P, Teare I D (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205–207

Bloch D, Hoffmann C M, Märländer B (2006). Impact of water supply on photosynthesis, water use and carbon isotope discrimination of sugar beet genotypes. European Journal of Agronomy, 24: 218–225

Blum A (2005). Drought resistance, water-use efficiency, and yield potential–are they compatible, dissonant, or mutually exclusive? Australian Journal of Agricultural Research, 56: 1159–1168

Buchanan C D, Lim S, Salzman R A, Kagiampakis I, Morishige D T, Weers B D, Klein R R, Pratt L H, Cordonnier-Pratt M M, Klein P E, Mullet J E (2005). *Sorghum bicolor's* transcriptome response to dehydration, high salinity and ABA. Plant Molecular Biology, 58: 699–720

Cattivelli L, Rizza F, Badeck F W, Mazzucotelli E, Mastrangelo A N, Francia E, Marè C, Tondelli A, Stanca A M (2008). Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. Field Crops Research, 105: 1–14

Cha-um S, Kirdmanee C (2008). Effect of osmotic stress on proline accumulation, photosynthetic abilities and growth characters of sugarcane plantlets (*Saccharum officinarum* L.). Pakistan Journal of Botany, 40: 2541–2552

Cha-um S, Kirdmanee C (2009). Proline accumulation, photosynthetic abilities and growth characters of sugarcane (*Saccharum officinarum* L.) plantlets in response to iso-osmotic salt and water-deficit stress. Agricultural Sciences in China, 8: 51–58

Cha-um S, Supaibulwatana K, Kirdmanee C (2006). Water relation, photosynthetic ability, and growth of Thai jasmine rice (*Oryza sativa* L. ssp. *indica* cv. KDML105) to salt stress by application of exogenous glycinebetaine and choline. Journal of Agronomy and Crop Science, 192: 25–36

Cha-um S, Supaibulwatana K, Kirdmanee C (2007). Glycinebetaine accumulation, physiological characterizations, and growth efficiency in salt tolerant and salt sensitive lines of indica rice (*Oryza sativa* L. spp. *indica*) response to salt stress. Journal of Agronomy and Crop Science, 193: 157–166

Chaves M M, Maroco J P, Pereira J S (2003). Understanding plant responses to drought—from genes to the whole plant. Functional Plant Biology, 30: 239–264

Chaves M M, Oliveira M M (2004). Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. Journal of Experimental Botany, 55: 2365–2384

Chi W, Zhou J S, Zhang F, Wu N H (2004). Photosynthetic feature of transgenic rice expressing sorghum C_4 type NADP-ME. Acta Botanica Sinica, 46: 873–882

Choudhury B J (2001). Modeling radiation- and carbon-use efficiencies of maize, sorghum, and rice. Agricultural and Forest Meteorology, 106: 317–330

Costa J H, Jolivet Y, Hasenfratz-Sauder M P, Orellano E G, Lima M G S, Dizengremel P, de Melo D F (2007). Alternative oxidase regulation in roots of *Vigna unguiculata* cultivars differing in drought/salt tolerance. Journal of Plant Physiology, 164: 718–727

Flexas J, Bota J, Loreto F, Cornic G, Sharkey T D (2004). Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. Plant Biology, 6: 1–11

Fujiwara K, Kozai T, Watanabe I (1987). Fundamental studies on environment in plant tissue culture vessels. (3) Measurements of carbondioxide gas concentration in closed vessels containing tissue cultured plantlets and estimates of net-photosynthetic rates of the plantlets. Journal of Agricultural Methodology, 4: 21–30 (in Japanese)

Fukai S, Cooper M (1995). Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Research, 40: 67–86

Goff S A, Ricke D, Lan T H, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchison D, Martin C, Katagiri F, Lange B M, Moughamer T, Xia Y, Budworth P, Zhong J, Miguel T, Paszkowski U, Zhang S, Colbert M, Sun W L, Chen L, Cooper B, Park S, Wood T C, Mao L, Quail P, Wing R, Dean R, Yu Y, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller R M, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A, Briggs S (2002). A draft sequence of the rice genome (*Oryza sativa* L. spp. *japonica*). Science, 296: 92–100

Kamoshita A, Babu R C, Boopathi N M, Fukai S (2008). Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rain-fed environments. Field Crops Research, 109: 1–23

Khush G S (2005). What it will take to feed 5.0 billion rice consumers in 2030? Plant Molecular Biology, 59: 1–6

Kijne J W (2006). Abiotic stress and water scarcity: Identifying and resolving conflicts from plant level to global level. Field Crops Research, 97: 3–18

Lafitte H R, Yongsheng G, Yan S, Li Z K (2007). Whole plant responses, key processes, and adaptation to drought stress: the case of rice. Journal of Experimental Botany, 58: 169–175

Lichtenthaler H K (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology, 148: 350–380

Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F (1999). Antioxidant defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiology, 119: 1091–1099

Maxwell K, Johnson G N (2000). Chlorophyll fluorescence–a practical guide. Journal of Experimental Botany, 51: 659–668

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473–497

Nayyar H (2003). Accumulation of osmolytes and osmotic adjustment in water-stressed wheat (*Triticum aestivum*) and maize (*Zea mays*) as affected by calcium and its antagonists. Environmental and Experimental Botany, 50: 253–264

Nayyar H, Gupta D (2006). Differential sensitivity of C_3 and C_4 plants to water deficit stress: Association with oxidative stress and antioxidants. Environmental and Experimental Botany, 58: 106–113

Neto N B A, Satornino S M, Bomfim D C, Custódio C C (2004). Water stress induced by mannitol and sodium chloride in soybean cultivars. Brazilian Archives of Biology and Technology, 47: 521–529

Passioura J (2006). Increasing crop productivity when water is scarce—from breeding to field management. Agricultural Water Management, 80: 176–196

Passioura J (2007). The drought environment: physical, biological and agricultural perspectives. Journal of Experimental Botany, 58: 113–117

Paterson A H (2008). Genomics of sorghum. International Journal of Plant Genomics, doi: 10.1155/2008/362451

Paterson A H, Bowers J E, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti A K, Chapman J, Feltus F A, Gowik Udo, Grigoriev Igor V, Lyons Eric, Maher C A, Martis M, Narechania A, Otillar R P, Penning B W, Salamov A A, Wang Y, Zhang L, Carpita N C, Freeling M, Gingle A R, Hash C T, Keller B, Klein P, Kresovich S, McCann M C, Ming R, Peterson D G, Mehboob-ur-Rahman M, Ware D, Westhoff P, Mayer K F X, Messing J, Rokhsar D S (2009). The *Sorghum bicolor* genome and the diversification of grasses. Nature, 457: 551–556

Quarrie S A, Stojanović J, Pekić S (1999). Improving drought resistance in small-grained cereals: A case study, progress and prospects. Plant Growth Regulation, 29: 1–21

Reddy A R, Chaitanya K V, Vivekanandan M (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology, 161: 1189–1202

Ripley B S, Gilbert M E, Ibrahim D G, Osborne C P (2007). Drought constraints on C_4 photosynthesis: stomatal and metabolic limitations in C_3 and C_4 subspecies of *Alloterosis semialata*. Journal of Experimental Botany, 58: 1351–1363

Selote D S, Bharti S, Khanna-Chopra R (2004). Drought acclimation reduces O_2^- accumulation and lipid peroxidation in wheat seedlings. Biochemical and Biophysical Research Communications, 314: 724–729

Shabala S N, Shabala S I, Martynenko A I, Babourina O, Newman I A (1998). Salinity effect on bioelectric activity, growth, Na^+ accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. Australian Journal of Plant Physiology, 25: 609–616

Shao H B, Chu L Y, Jaleel C A, Zhao C X (2008). Water-deficit stress-induced anatomical changes in higher plants. Comptes Rendus Biologies, 331: 215–225

Sharma A D, Kumar S, Singh P (2006). Expression analysis of a stress-modulated transcript in drought tolerant and susceptible cultivars of sorghum (*Sorghum bicolor*). Journal of Plant Physiology, 163: 570–576

Sinclair T R, Hammer G L, van Oosterom E J (2005). Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. Functional Plant Biology, 32: 945–952

Slama I, Ghnaya T, Hessini K, Messedi D, Savouré A, Abdelly C (2007). Comparative study of the effects of mannitol and PEG osmotic stress on growth and solute accumulation in *Sesuvium portulacastrum*. Environmental and Experimental Botany, 61: 10–17

Suzuki S, Murai N, Arai J N (2000). Changes in photosynthetic carbon flow in transgenic rice plants that express C_4-type phosphoenolpyruvate carboxykinase from *Urochloa panicoides*. Plant Physiology, 124: 163–172

Suzuki S, Murai N, Kasaoka K, Hiyoshi T, Imaseki H, Burnell J N, Arai M (2006). Carbon metabolism in transgenic rice plants that express phosphoenolpyruvate carboxylase and/or phosphoenolpyruvate carboxykinase. Plant Science, 170: 1010–1019

Tonon G, Kevers C, Faivre-Rampant O, Graziani M, Gaspar T (2004). Effect of NaCl and mannitol iso-osmotic stresses on proline and free polyamine levels in embryogenic *Fraxinus angustifolia* callus. Journal of Plant Physiology, 161: 701–708

Wang J, Li R (2008). Integration of C_4-specific *ppdk* gene of *Echinochloa* to C_3 upland rice and its photosynthesis characteristics analysis. African Journal of Biotechnology, 7: 783–787

Xin Z, Aiken R, Burke J (2009). Genetic diversity of transpiration efficiency in sorghum. Field Crops Research, 111: 74–80

Yordanov I, Velikova V, Tsonev T (2003). Plant responses to drought and stress tolerance. Bulgarian Journal of Plant Physiology, 39: 187–206

Yu J, Hu S, Wang J, Wong G K, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, Cao M, Liu J, Sun J, Tang J, Chen Y, Huang X, Lin W, Ye C, Tong W, Cong L, Geng J, Han Y, Li L, Li W, Hu G, Huang X, Li W, Li J, Liu Z, Li L, Liu J, Qi Q, Liu J, Li L, Li T, Wang X, Lu H, Wu T, Zhu M, Ni P, Han H, Dong W, Ren X, Feng X, Cui P, Li X, Wang H, Xu X, Zhai W, Xu Z, Zhang J, He S, Zhang J, Xu J, Zhang K, Zheng X, Dong J, Zeng W, Tao L, Ye J, Tan J, Ren X, Chen X, He J, Liu D, Tian W, Tian C, Xia H, Bao Q, Li G, Gao H, Cao T, Wang J, Zhao W, Li P, Chen W, Wang X, Zhang Y, Hu J, Wang J, Liu S, Yang J, Zhang G, Xiong Y, Li Z, Mao L, Zhou C, Zhu Z, Chen R, Hao B, Zheng W, Chen S, Guo W, Li G, Liu S, Tao M, Wang J, Zhu L, Yuan L, Yang H (2002). A draft sequence of the rice genome (*Oryza sativa* L. spp. *indica*). Science, 296: 79–92

Zang X, Komatsu S (2007). A proteomic approach for identifying osmotic-stress-related proteins in rice. Phytochemistry, 68: 426–437

Zhang Z, Shao H, Xu P, Chu L, Lu Z, Tian J (2007). On evolution and perspectives of bio-watersaving. Colloids and Surfaces B: Biointerfaces, 55: 1–9