

Article

Exogenous Foliar Application of Glycine Betaine to Alleviate Water Deficit Tolerance in Two *Indica* Rice Genotypes under Greenhouse Conditions

Rujira Tisarum ¹, Cattarin Theerawitaya ¹, Thapanee Samphumphung ¹, Teruhiro Takabe ² and Suriyan Cha-um ^{1,*} 

¹ National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathum Thani 12120, Thailand; rujira.tis@biotec.or.th (R.T.); cattarin.the@biotec.or.th (C.T.); thapanee@biotec.or.th (T.S.)

² Research Institute, Meijo University, Nagoya 468-8502, Japan; takabe@meijo-u.ac.jp

* Correspondence: suriyanc@biotec.or.th; Tel.: +662-564-6700; Fax: +662-564-6707

Received: 3 February 2019; Accepted: 15 March 2019; Published: 18 March 2019



Abstract: The aim of this investigation was to enhance overall growth, yield attributes as well as physio-biochemical adaptive strategies by exogenous foliar application of glycine betaine (GlyBet) in two rice varieties against water deficit stress under greenhouse conditions. Rice crop cvs. RD43 (low amylose content) and SPR1 (high amylose content) grown in clay pots containing garden soil until booting stage were chosen as the test plant material, sprayed by 0 (control) or 100 mM GlyBet and subsequently subjected to: MWD (mild water deficit by 8 d water withholding; 24.80% SWC; Soil water content) or SWD (severe water deficit by 14 d water withholding; 13.63% SWC) or WW (well-watered conditions or control). Free proline content in cv. RD43 was rapidly increased in relation to the degree of water deficit and suppressed by exogenous GlyBet, while free proline in cv. SPR1 was lower than cv. RD43. Overall growth performances and yield traits in both cultivars under MWD were maintained by exogenous application of GlyBet; however, these parameters declined under SWD even after the GlyBet application. Degradation of photosynthetic pigments and chlorophyll fluorescence in pretreated GlyBet plants under SWD were prevented, resulting in elevated net photosynthetic rate (P_n). Interestingly, P_n was very sensitive parameter that sharply declined under SWD in both RD43 and SPR1 genotypes. Positive relationships between physio-morphological and biochemical changes in rice genotypes were demonstrated with high correlation co-efficiency. Based on the key results, it is concluded that foliar GlyBet application may play an important role in drought-tolerant enhancement in rice crops.

Keywords: free proline; growth characters; net photosynthetic rate; photosynthetic pigments; yield attributes

1. Introduction

Rice is an important carbohydrate source for the world population with nearly 3 billion people consuming rice as a staple food, especially in Asia [1]. In fact, rice cultivation consumes about 24%–30% of fresh water resources in its whole life cycle. Water-saving strategies and improved varieties with high water use efficiency [2] have been developed [3–5]. In the world, 79 million ha cultivation of low land rice provides 75% of the world's rice production [6]. In Asia, paddy fields (40%–46% of irrigated area) have been known to consume 2- to 3-fold the volumes of the fresh water used by other crops [7]. A water shortage in the irrigated paddy fields is evidently observed, depending upon the high evaporation rate or the limiting fresh water supply, resulting in reduced productivity [8–12]. Drought stress as a consequence of climate change in rainfed areas is a major constraint to high productivity [13–15].

A large number of anti-drought agents, i.e., compatible solutes (GlyBet, proline, trehalose) and plant growth regulators (gibberellic acid, paclobutrazol, cytokinins, salicylic acid) are investigated for the amelioration of the drought stress [16–19]. GlyBet is a member of quaternary ammonium compounds, and a neutral compatible solute that plays a key role in cellular osmotic adjustment, maintaining the organelles (mitochondria, chloroplasts) and water-use efficiency in the plants under water deficit [20,21]. Rice crop has been reported as a GlyBet non-accumulator plant with a very low amount of GlyBet [$<1 \mu\text{mol g}^{-1}$ dry weight (DW)] [22]. However, overexpression of GlyBet biosynthesis-related genes, i.e., CMO (choline monooxygenase) and BADH (betaine aldehyde dehydrogenase) in rice crop has improved abiotic tolerance in the plant [23–25]. Alternatively, exogenous application of GlyBet in the rice crop has been well established to improve plant growth, development and yield traits under drought stress [26–28]. For example, exogenous foliar of 100 mM GlyBet in Pathumthani 1 (PT1), a drought-sensitive cultivar for water deficit alleviation (physiological adaptation, growth retention and yield stabilization, has been published [27]. The uptake, translocation and accumulation of GlyBet by exogenous application have been reported in several other plant species as well [29–32]. In addition, regulation of antioxidative defense mechanisms and their physiological adaptation in rice crops to cope with drought stress using GlyBet seed priming has also been well investigated [26]. Consequently, the role of GlyBet in physiological and biochemical adaptation processes and hormonal regulation against a drought situation has been well studied [20,33]. However, information regarding the endogenous proline regulation in drought-stressed plants pretreated with GlyBet, especially in different rice genotypes, is limited.

Recently, a released rice variety, RD43 (SPR99007-22-1-2-2-1 registered by the Rice Department, Thailand, 5 October 2010) with medium glycemic index and low amylose content [34] and irrigated lowland variety, Suphan Buri 1 (SPR1; SPRLR85163-5-1-1-2 registered by the Rice Department, Thailand, 28 October 1993) with high amylose content [35,36] were selected to be tested for water deficit-tolerant abilities using exogenous foliar GlyBet. These cultivars have been reported as moderately salt tolerant [37], however, there is no study in context with their drought tolerance efficiency. The objective of this study was to improve the overall growth and yield traits as well as physio-biochemical strategies by exogenous GlyBet application in the selected rice varieties under water deficit stress.

2. Materials and Methods

2.1. Plant Material, Exogenous Glybet Foliar and Water Deficit Treatments

Seeds of two rice genotypes, ‘Rice Department #43 or RD43’ (low glycemic index) and ‘Suphan Buri 1 or SPR1’ (high amylose content) obtained from the Pathumthani Rice Research Center, Thailand, were used as test materials. Two-week-old seedlings of rice were transplanted into clay pots ($\varnothing = 20$ cm) containing 2 kg garden soil (Electroconductivity (EC) = 2.687 dS m^{-1} ; pH = 5.5; organic matter = 10.36%; total nitrogen = 0.17%; total phosphorus = 0.07%; total potassium = 1.19%). The transplanted seedlings were incubated in a greenhouse under $500\text{--}1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photosynthetic photon flux density with a 10 h d^{-1} photoperiod, $25\text{--}37^\circ\text{C}$ ambient temperature and 50%–85% relative humidity, until booting stage. Rice plants were treated with 0 (water) and 100 mM foliar spray of GlyBet (Betaine; 50 mL plant^{-1}). Thereafter, 3 sets of drought treatment were set up. These included: well-watered condition (WW; 44.02% soil water content (SWC)), mild water deficit condition (MWD; 24.80% SWC) by withholding water for 8 days, and severe water deficit condition (SWD; 13.63% SWC) by withholding water for 14 days, (Figure S1). In addition, the relative humidity and air temperature during the water withholding period were recorded using a data logger (MicroLogPROII, Fourtec-Fourier Technologies Ltd., Rosh Ha’ayin, Israel) (Figure S2). Shoot height, number of leaves, number of tillers and flag leaf area in each treatment were measured. In addition, panicle weight (PW), panicle length (PL), seed fertility (SF) and hundred grain weight (HGW) at the time of harvest were also recorded.

2.2. Proline Quantification

Free proline in the flag leaf tissues of each treatment, collected at 0, 2, 4, 6, 8, and 14 days after water withholding and 3-days re-watering (recovery), was analyzed according to the method of Bates et al. [38]. Fifty milligrams of fresh material were ground with liquid nitrogen in a mortar. The homogenate powder was mixed with 1 mL of aqueous sulfosalicylic acid (3%, *w/v*) and filtered through filter paper (Whatman #1, Sigma-Aldrich, Darmstadt, Germany). The extracted solution was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 mg ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M H₃PO₄) and incubated at 95 °C for 1 h. The reaction was terminated by placing the container in an ice bath. The reaction mixture was mixed vigorously with 2 mL of toluene. After cooling to 25 °C, the chromophore was measured at 520 nm by spectrophotometer (HACH DR/4000; Model 48,000, HACH Company, Loveland, CO, USA) using L-proline as a calibration standard.

2.3. Plant Physiological Assay

Chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (TC) content in the flag leaf tissues were analyzed according to the method of Shabala et al. [39]. One hundred milligrams of leaf tissue were homogenized in a glass vial with 10 mL of 99.5% acetone, and blended using a homogenizer. The glass vials were sealed with Parafilm® to prevent evaporation, and then stored at 4 °C for 48 h. Chl_a and Chl_b concentrations were measured at 662 nm and 644 nm, respectively, using an ultraviolet–visible (UV–VIS) spectrophotometer (DR6000™ UV-VIS Spectrophotometer, HACH®, Loveland, CO, USA) against acetone (99.5%) as a blank.

Chlorophyll fluorescence emission was measured from the adaxial surface of flag leaf using a fluorescence monitoring system (model FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode [40]. A leaf, kept in dark for 30 min was initially exposed to the modulated measuring beam of a far-red light (LED; Light emitted diode source) with typical peak at wavelength of 735 nm. Original (F_0) and maximum (F_m) fluorescence yields were measured under weak modulated red light ($<85 \mu\text{mol m}^{-2} \text{s}^{-1}$) with 1.6 s pulses of saturating light ($>1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF; Photosynthetic photon flux density) and calculated using FMS software for Windows®. The variable fluorescence yield (F_v) was calculated using the equation: $F_v = F_m - F_0$. The ratio of variable to maximum fluorescence (F_v/F_m) was calculated as the maximum quantum yield of PSII photochemistry. The photon yield of PSII (Φ_{PSII}) in the light was calculated as: $\Phi_{\text{PSII}} = (F'_m - F)/F'_m$ after 45 s of illumination, when steady state was achieved [41].

Net photosynthetic rate (P_n ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) was measured using a portable photosynthesis system with an infrared gas analyzer (Model LI 6400, LI-COR® Inc., Lincoln, NE, USA). P_n parameter was measured by continuously monitoring the content of the air entering and exiting in the IRGA headspace chamber, according to Cha-um et al. [42].

2.4. Plant Morphological Characterization and Yield Traits

Shoot height, number of leaves, number of tillers and flag leaf area in each treatment were measured. In addition, panicle weight (PW), panicle length (PL), seed fertility (SF) and hundred grain weight (HGW) at the time of harvest were also recorded.

2.5. Statistical Analysis

The experiment was arranged as 3×2 factorial in a completely randomized design (CRD) with six replicates ($n = 6$) for each treatment. The mean values from six treatments were compared using Tukey's HSD (Honestly significant difference) and analyzed by SPSS software (version 11.5 for Window®, SPSS Inc., Chicago, IL, USA). In addition, the relationships between physiological and morphological characters were validated using Pearson's correlation co-efficiency method.

3. Results and Discussion

3.1. Free Proline Accumulation Pattern under Water Deficit Conditions

At booting stage, free proline in the flag leaf of *indica* rice cv. RD43 was increased in relation to the degree of water deficit and the maximum value obtained was 120.94 (29.14 folds high over well watering) and 172.32 $\mu\text{mol g}^{-1}$ FW (56.68-fold high over well watering) in MWD and SWD, respectively. The content declined by re-watering in the recovery process (Figure 1a). Similarly, free proline in cv. SPR1 under water deficit was accumulated by 1.80- and 20.93-fold in MWD and SWD, respectively, over WW conditions (Figure 1b). Interestingly, free proline accumulation rate in flag leaf of GlyBet-pretreated rice cvs. RD43 and SPR1 under water deficit declined when compared to untreated plants (Figure 1). Free proline was a major compatible solute in cv. RD43 to cope with water deficit stress, whereas this was not applicable in case of cv. SPR1, pointing towards the difference in their patterns of responses towards water deficit conditions (Figure 1). Moreover, free proline in GlyBet-pretreated rice cv. SPR1 under MWD was similar to that of well watering, and comparatively high under SWD (Figure 1b).

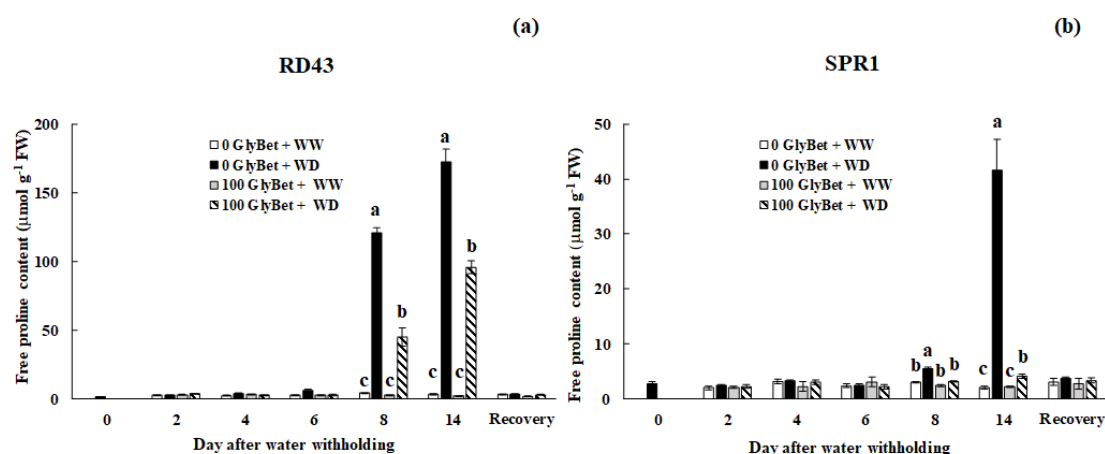


Figure 1. Free proline in two different rice genotypes, RD43 (a) and SPR1 (b), exogenously applied with (100 mM GlyBet) or without GlyBet, and subsequently subjected to well watering (WW) and water withholding for 0, 2, 4, 6, 8, 14 (water withholding), and 17 (recovery) days under greenhouse conditions. Error bars represent \pm SE. Different letters in each bar represent significant difference at $p \leq 0.05$ according to Tukey's honest significant difference (HSD).

An exogenous GlyBet foliar application in rice crop for abiotic stress tolerance, i.e., drought [26,27] and salt [43–45] has been well explored. Endogenous levels of GlyBet in several organs of monocotyledonous plant species, maize [46] and wheat [47–49], is enriched by exogenous GlyBet dosage application, water withholding period and their interaction. In addition, GlyBet uptake and translocation in higher plants, tobacco [50], sunflower [51] and *Carapa guianensis* Aublet [32], under drought conditions has also been reported. In the present study, free proline in both RD43 and SPR1 cultivars was regulated by reduction in the degree of SWC and it was decreased by exogenous foliar GlyBet application. In papaya cv. BH-65, free proline in the leaves of 18 d water deficit stress was recorded to be the maximum, but it was subsequently decreased by re-watering and repressed by GlyBet pretreatment [52]. Similarly, free proline in tobacco cvs. DHJ5210 (drought tolerant) and ZY100 (drought-sensitive) under drought stress was down-regulated by 80 mM GlyBet application [50]. In 19 wheat genotypes, free proline and endogenous GlyBet levels in the drought-stressed plants treated with exogenous GlyBet application increased in both tillering and anthesis stages [49]. Free proline in GlyBet pretreated rice cv. PT1 [27], maize cvs. S911 and S9 [17], sunflower [53] and pea [54], under water deficit conditions was the maximum. Moreover, free proline in leaf and panicle tissues of drought stressed rice cv. Basmati-385, irrespective of GlyBet foliar spray, enriched compared to cv. KS-282 [28].

The major role of free proline enrichment in the drought stress tolerance via osmotic adjustment and antioxidant activities at the cellular levels has been well established [26,48,50,54].

3.2. Overall Growth Performances and Yield Traits

Shoot height in rice cv. RD43 under MWD and SWD without GlyBet treatment was significantly declined by 3.53% and 5.23% over well watering, respectively, whereas shoot height under SWD with GlyBet application dropped by 4.31% over well watering conditions. In contrast, shoot height in cv. SPR1 under SWD without GlyBet was expressively declined by 14.43% over well watering and improved on GlyBet foliar application (Table 1). The number of leaves in cvs. RD43 and SPR1 irrespective of GlyBet treatment under SWD were decreased. The number of tillers per clump in cvs. RD43 and SPR1 without GlyBet treatment under MWD and SWD were significantly reduced; however, those were elevated by exogenous GlyBet application (Table 1). Interestingly, number of tillers in cv. RD43 under SWD was strongly raised up by GlyBet application, although it declined by 21.77% in cv. SPR1 (Table 1). In addition, flag leaf area in cvs. RD43 and SPR1 without GlyBet treatment under SWD was declined by >50% over well watering. Flag leaf area was found to be a good indicator to identify the cv. RD43 as water deficit tolerant over cv. SPR1, as it showed the reduction by 38.55% under MWD and by 55.50% under SWD (Table 1).

In the present study, overall growth performance, i.e. shoot height, number of leaves, number of tillers and flag leaf area, in rice cvs. RD43 and SPR1 grown under MWD were strongly improved by exogenous GlyBet application. Under SWD, number of leaves in both cultivars was significantly dropped even after exogenous GlyBet application. In rice cv. PT1 (drought sensitive), shoot height in plants under water deficit was deteriorated by 11.47% over the control, whereas it was maintained by 100 mM GlyBet foliar application (4.09% reduction of control) [27]. In tobacco, shoot height in cvs. DHJ5210 (drought-tolerant) and ZY100 (drought-sensitive) under drought stress was elevated by 80 mM GlyBet application [50]. In maize cultivars, Dongdan-60 and ND-95, shoot height and number of leaves under drought stress was elevated by GlyBet pretreatment and these traits in Dongdan-60 were more noticeable than in ND-95 [55]. In papaya cv. BH-65, the number of leaves was a very sensitive trait responding strongly to 18 d water deficit stress and declining by 65.27% to that of well watering (control), while it was improved by 50 mM GlyBet treatment (only 39.16% reduction to that of control) [52]. Moreover, plant height, number of leaves and leaf area of tomato cv. PS under drought stress (water withholding for 14 and 21d) were ameliorated by 10 mM GlyBet application [56].

Panicle weight of rice cv. SPR1 under MWD and SWD without GlyBet treatment was decreased by 28.92% and 63.45% over well watering, respectively. However, it declined by only 29.51% and 46.53%, respectively, in GlyBet-pretreated plants. In contrast, panicle weight of rice cv. RD43 under MWD was improved by GlyBet application; however, it was declined by 36.39% over well watering, when exposed to SWD (Table 2). Similarly, panicle length in cv. SPR1 under MWD and SWD without GlyBet pretreatment was decreased by 8.42% and 18.72%, respectively, over well watering, and decreased by 8.03% and 17.05%, respectively, over well watering in plants with GlyBet pretreatment. Interestingly, panicle length of cv. RD43 was strongly increased by foliar GlyBet application, leading to maintenance of the panicle weight and panicle length, especially under SWD (Table 1). Seed fertility in cv. SPR1 under MWD and SWD was significantly reduced by 30.37% and 74.96% over well watering. It was subsequently lifted-up by GlyBet pretreatment in cv. RD43 under MWD, whereas it declined by 33.14% under SWD with GlyBet pretreatment. Grain weight (100 grains) in cvs. SPR1 and RD43 under SWD without GlyBet application declined by 19.93% and 14.56% over the control, and it was strongly improved by GlyBet application (Table 2).

Table 1. Shoot height (SH), number of leaves (NL), number of tiller (NT) and flag leaf area (FLA) in two different rice genotypes, RD43 and SPR1, exogenously applied with (100 mM GlyBet) or without GlyBet, and subsequently subjected to well watering (WW) and water withholding for 8 (mild water deficit, MWD) and 14 days (severe water deficit, SWD) under greenhouse conditions.

Water Regimes	GlyBet	RD43				SPR1			
		SH (cm)	NL	NT	FLA (mm ²)	SH (cm)	NL	NT	FLA (mm ²)
WW	0	95.47a	3.67a	11.00a	8158ab	107.67a	3.33a	7.67a	8647a
	100	96.28a	3.50a	10.00ab	8865a	105.05a	3.17a	7.67a	10176a
MWD	0	92.10b	3.33a	9.33b	7183bc	105.70a	3.20a	6.00b	5314b
	100	93.17ab	3.50a	9.50b	9393a	107.88a	3.27a	6.33ab	8695a
SWD	0	90.48b	2.00b	9.00b	3857d	92.13c	1.60b	5.80b	3848b
	100	92.13b	2.67b	9.33b	6287c	106.84a	1.83b	6.00b	5962b
Significant level									
Water		*	**	**	**	*	ns	ns	**
GlyBet		ns	ns	ns	**	**	**	**	**
Water × GlyBet		ns	ns	ns	ns	*	*	*	**

^{ns}, *, and ** represent not-significant, and significant difference at $p \leq 0.05$ and $p \leq 0.01$, respectively. Different letters in each column represent significant difference at $p \leq 0.05$ according to Tukey's HSD.

Table 2. Panicle weight (PW), panicle length (PL), seed fertility (SF) and hundred grain weight (HGW) in two different rice genotypes, RD43 and SPR1, exogenously applied with (100 mM GlyBet) or without GlyBet, and subsequently subjected to well watering (WW) and water withholding for 8 (mild water deficit, MWD) and 14 days (severe water deficit, SWD) as well as recovery until harvesting stage under greenhouse conditions.

Water Regimes	GlyBet	RD43				SPR1			
		PW (g)	PL (cm)	SF (%)	HGW(g)	PW (g)	PL (cm)	SF (%)	HGW (g)
WW	0	3.63a	27.91a	66.18ab	2.61a	2.49a	27.08ab	70.34a	2.66a
	100	3.49ab	27.85a	72.08a	2.58a	2.88a	28.03a	70.27a	2.69a
MWD	0	2.61c	25.32b	65.58ab	2.56a	1.77bc	24.80cd	48.98b	2.58a
	100	3.13b	26.95ab	69.25ab	2.48a	2.03b	25.78bc	55.83a	2.55a
SWD	0	1.22e	20.82c	26.84d	2.23b	0.91d	22.01e	17.61c	2.13b
	100	2.22d	25.50b	48.19c	2.54a	1.54c	23.25de	31.86b	2.58a
Significant level									
Water		**	**	**	**	**	**	**	**
GlyBet		**	**	ns	**	**	*	**	**
Water×GlyBet		**	**	**	**	ns	ns	**	**

^{ns}, *, and ** represent not-significant, and significant difference at $p \leq 0.05$ and $p \leq 0.01$, respectively. Different letters in each column represent significant difference at $p \leq 0.05$ according to Tukey's HSD.

Panicle weight, panicle length, seed fertility and HGW in cv. RD43 under water deficit were better than those in cv. SPR1, and these were further improved by 100 mM GlyBet foliar application, especially under SWD. In rice cv. PT1 (drought sensitive), panicle length, panicle weight, seed fertility and HGW of GlyBet pretreated plants at booting stage under water deficit stress were significantly improved compared to untreated plants [27]. Likewise, number of seeds per panicle in 100 mM GlyBet pretreated rice cvs. KS-282 (coarse grain rice) and Basmati-385 (fine grain rice) under water deficit stress was greater than untreated plants [28]. Grain yield (g m^{-2}) of 100 mM GlyBet foliar sprayed wheat genotypes under drought stress was strongly elevated compared to untreated plants [57]. Moreover, yield traits, cob length, number of kernels per row, number of kernels per cob and grain yield per plant in GlyBet-pretreated maize cvs. Dongdan-60 and ND-95 under drought conditions were also better compared to the plants without GlyBet application [55].

3.3. Physiological Changes and Relationships between Those Traits

Chl_a and Chl_b contents in flag leaf of rice cv. RD43 without GlyBet pretreatment were sensitive to SWD, and decreased by 57.35% and 46.50% over the control (Table 3). Chl_a in flag leaf tissues of rice cv. RD43 without GlyBet pretreatment under MWD was degraded by 35.67% over control. Chl_a and Chl_b contents in cv. SPR1 under SWD without GlyBet application were found to be minimum at 130.38 and 170.63 $\mu\text{g g}^{-1}$ FW, respectively (Table 3). TC content in cv. RD43 without GlyBet pretreatment under SWD was significantly degraded by 51.13% (Figure 2a), as well as being lowest at 301.01 mg g^{-1} FW in cv. SPR1 under SWD (Figure 2b). Maximum quantum yield of PSII (F_v/F_m) and photon yield of PSII (Φ_{PSII}) of rice crop cv. RD43 without GlyBet pretreatment under SWD were diminished by 46.53% and 49.04% over the control, whereas these were retained upon exogenous GlyBet application. Moreover, F_v/F_m and Φ_{PSII} in cv. SPR1 without GlyBet pretreatment under SWD were reduced by 22.35% and 41.95% over control, respectively (Table 3). Interestingly, F_v/F_m in cv. SPR1 with GlyBet pretreatment under SWD declined by 11.49% over the control. P_n in rice cv. RD43 without GlyBet treatment under MWD, and SWD was reduced by 31.63% and 80.73% over the control, respectively; but it was elevated upon GlyBet pretreatment, especially under MWD (Figure 2c). However, P_n in cv. SPR1 was sharply declined when subjected to SWD in both with (77.68% reduction over control) or without (85.82% reduction over control) GlyBet application (Figure 2d). Positive relationships between Chl_a content and F_v/F_m in cv. RD43 ($r^2 = 0.6456$; Figure 3a) and SPR1 ($r^2 = 0.7568$; Figure 3b), and between TC content and Φ_{PSII} in cv. RD43 ($r^2 = 0.5354$; Figure 3c) and SPR1 ($r^2 = 0.5665$; Figure 3d) were demonstrated. Moreover, positive relationships between Φ_{PSII} and P_n in cv. RD43 ($r^2 = 0.8941$; Figure 4a) and SPR1 ($r^2 = 0.7298$; Figure 4b) as well as P_n and panicle weight in cv. RD43 ($r^2 = 0.9682$; Figure 4c) and SPR1 ($r^2 = 0.9332$; Figure 4d) were also established.

Table 3. Chlorophyll a (Chl_a; $\mu\text{g g}^{-1}$ FW), chlorophyll b (Chl_b; $\mu\text{g g}^{-1}$ FW), maximum quantum yield of PSII (F_v/F_m) and photon yield of PSII (Φ_{PSII}) in two different rice genotypes, RD43 and SPR1, exogenously applied with (100 mM GlyBet) or without GlyBet, and subsequently subjected to well watering (WW) and water withholding for 8 (mild water deficit, MWD) and 14 days (severe water deficit, SWD) under greenhouse conditions.

Water Regimes	GlyBet	RD43				SPR1			
		Chl _a	Chl _b	F_v/F_m	Φ_{PSII}	Chl _a	Chl _b	F_v/F_m	Φ_{PSII}
WW	0	146.76a	197.09a	0.836a	0.675b	167.92ab	195.04ab	0.877a	0.789a
	100	162.15a	184.27a	0.845a	0.744a	185.44a	235.51a	0.888a	0.787a
MWD	0	94.41b	137.34ab	0.813ab	0.607b	142.35b	192.57ab	0.827a	0.780a
	100	126.62ab	141.35ab	0.879a	0.724a	170.18ab	223.35a	0.847a	0.784a
SWD	0	62.59c	105.44b	0.477b	0.344d	130.38b	170.63b	0.681c	0.458b
	100	135.45ab	149.70ab	0.809ab	0.453c	153.79ab	181.65ab	0.786b	0.786a
Significant level									
Water		**	**	**	**	**	**	**	**
GlyBet		ns	ns	ns	**	ns	ns	*	ns
Water×GlyBet		*	*	*	**	*	*	*	*

^{ns}, *, and ** represent not-significant, and significant difference at $p \leq 0.05$ and $p \leq 0.01$, respectively. Different letters in each column represent significant difference at $p \leq 0.05$ according to Tukey's HSD.

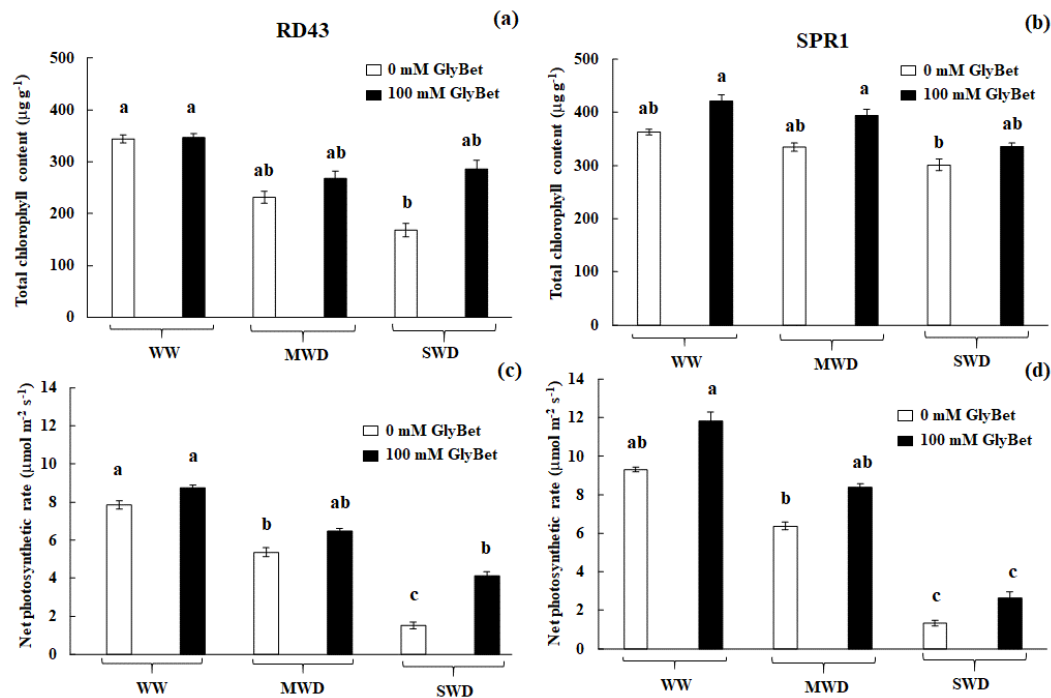


Figure 2. Total chlorophyll content (a,b), and net photosynthetic rate (c,d) in two different rice genotypes, RD43 and SPR1, exogenously applied with (100 mM GlyBet) or without GlyBet, and subsequently subjected to well watering (WW) and water withholding for 8 (mild water deficit, MWD) and 14 days (severe water deficit, SWD) under greenhouse conditions. Error bars represent \pm standard error (SE). Different letters in each bar represent significant difference at $p \leq 0.05$ according to Tukey's HSD.

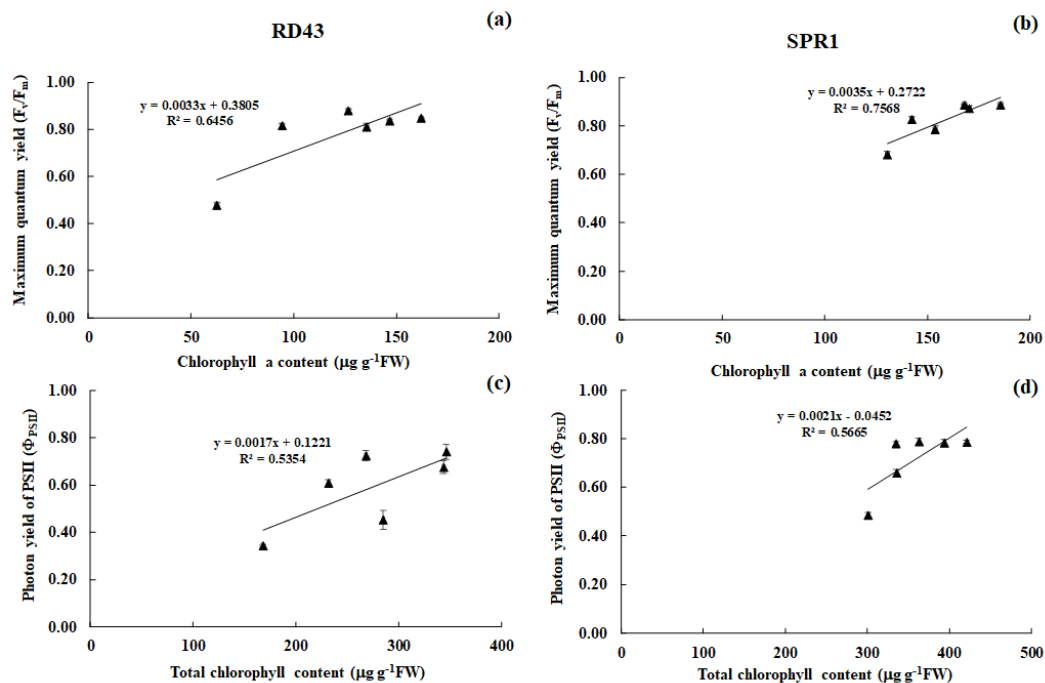


Figure 3. Relationships between chlorophyll a content (Chl_a) and maximum quantum yield of PSII (F_v/F_m) (a,b), and total chlorophyll content (TC) and photon yield of PSII (Φ_{PSII}) (c,d) in two different rice genotypes, RD43 and SPR1, exogenously applied with (100 mM GlyBet) or without GlyBet, and subsequently subjected to well watering (WW) and water withholding for 8 (mild water deficit, MWD) and 14 days (severe water deficit, SWD) under greenhouse conditions. Error bars represent \pm SE.

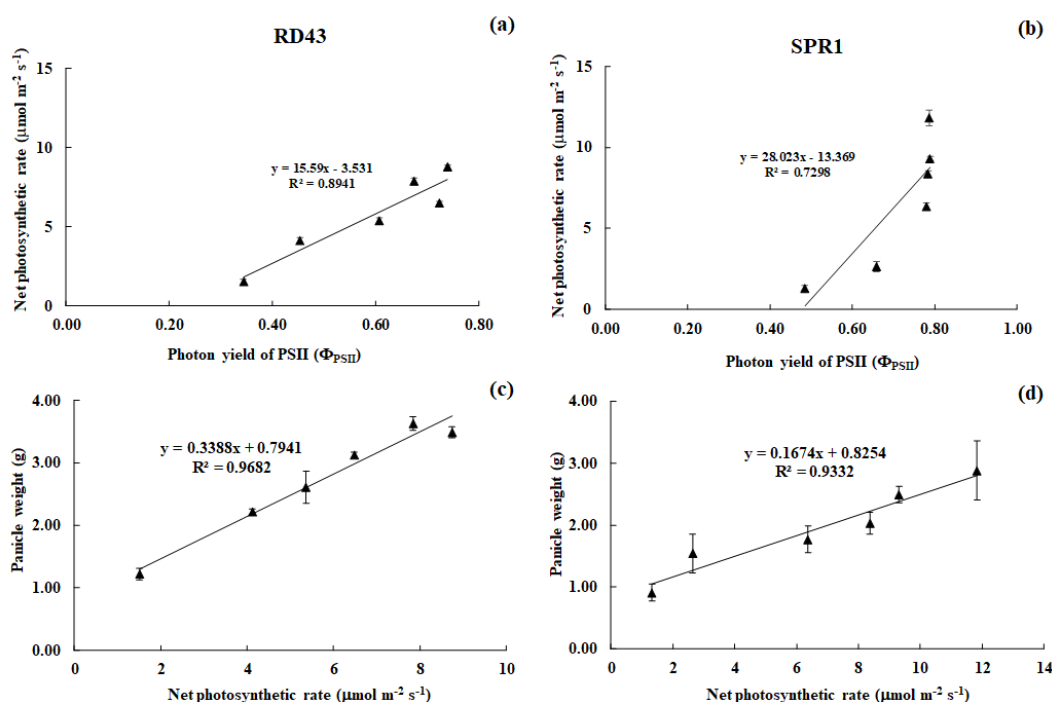


Fig. 4

Figure 4. Relationships between photon yield of PSII (Φ_{PSII}) and net photosynthetic rate (P_n) (a,b), and P_n and panicle weight (c,d) in two different rice genotypes, RD43 and SPR1, exogenously applied with (100 mM GlyBet) or without GlyBet, and subsequently subjected to well watering (WW) and water withholding for 8 (mild water deficit, MWD) and 14 days (severe water deficit, SWD) under greenhouse conditions. Error bars represent \pm SE.

The reduction rate of chlorophyll pigments, i.e. Chl_a , Chl_b and TC, in crop species depends on the degree of water deficit, GlyBet application, drought tolerant abilities and their interaction [27,55]. SPAD (Konica Minolta Chlorophyll Meter SPAD 502 PLUS) value or chlorophyll content in flag leaf of wheat was maintained in cv. HF9703 (drought tolerant) better than in cv. SN215953 (drought-sensitive) when subjected to drought stress [47]. In addition, similar results in cotton [19] and tobacco [50] have been established. Consequently, F_v/F_m and Φ_{PSII} in rice cvs. RD43 and SPR1 under SWD without GlyBet were sharply diminished and strongly improved by GlyBet pretreatment. In PT1 rice, F_v/F_m and Φ_{PSII} in flag leaf tissues were very sensitive to severe water deficit (25% soil water content) and diminished by 41.42% and 42.49% over the control, respectively, but promoted by exogenous GlyBet pretreatment, resulting in maintained P_n [27]. In wheat cvs. HF9703 (drought-tolerant) and SN215953 (drought-sensitive), the diminishing rate of F_v/F_m depended on their drought-tolerant abilities, intensity of drought stress, as well as strongly being enhanced by GlyBet application [48]. Moreover, Φ_{PSII} in tobacco cvs. DHJ5210 (drought-tolerant) and ZY100 (drought-sensitive) under drought stress conditions was retained in GlyBet treated plants [50]. Under SWD, P_n was a sensitive parameter in rice crop cvs. RD43 (80.73% reduction) and SPR1 (85.82% reduction) but it was stabilized in 100 mM GlyBet pretreated plants. Similar results of P_n stabilization under drought stress using exogenous GlyBet application in papaya [52], tobacco [50], cotton [19], and wheat [47] have been reported. In addition, a positive relation between physiological data and panicle weight of rice cvs. RD43 and SPR1 was validated. In an earlier study, relationships between Chl_a and F_v/F_m ($r^2 = 0.74$), TC and Φ_{PSII} ($r^2 = 0.72$), F_v/F_m and P_n ($r^2 = 0.82$), P_n and panicle weight ($r^2 = 0.67$) in GlyBet pretreated rice (cv. PT1) under water deficit have been established [27]. Likewise, positive relations between TC and Φ_{PSII} ($r^2 = 0.91$), photochemical quenching (qP) and P_n ($r^2 = 0.64$), P_n and plant dry weight ($r^2 = 0.75$) in GlyBet-pretreated rice (cv. KDML105) under salt stress have been validated [58].

4. Conclusions

Overall growth performances, yield attributes, physio-biochemical responses in *indica* rice cvs. RD43 and SPR1 coping with water deficit conditions, especially severe water deficit were ameliorated by 100 mM GlyBet foliar application at the booting stage. Under severe water deficit, chlorophyll pigments, chlorophyll fluorescence and net photosynthetic rate in 100 mM GlyBet-pretreated plants were maintained, leading to them retaining overall growth performances and yield traits. Foliar GlyBet application may play an important role as a simple short-gun technique for drought-tolerant improvement in the rice crop.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/9/3/138/s1>, Figure S1: Soil water content in the soil substrate after water withholding period and re-watering treatments, Figure S2: Relative humidity and air temperature of environmental conditions in whole experiment of present study.

Author Contributions: R.T. performed the physiological assay; C.T. performed the biochemical assay; T.S. performed morphological and yield attributes; T.T. performed the experiment layout consultation and manuscript preparation; S.C. conceived and designed the experiments. All authors were responsible for manuscript writing. All authors read and approved the final manuscript.

Funding: This research was funded by National Science and Technology Development Agency (NSTDA grant number P-18-51456).

Acknowledgments: The authors would like to sincerely thank National Science and Technology Development Agency (NSTDA grant number P-18-51456), Thailand, for funding support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Brar, D.S.; Singh, K.; Khush, G.S. Frontiers in Rice Breeding. In *The Future Rice Strategy for India*; Academic Press: Cambridge, MA, USA, 2017; pp. 137–160.
2. Mottaleb, K.A.; Rejesus, R.M.; Murty, M.V.R.; Mohanty, S.; Li, T. Benefits of development and dissemination of climate-smart rice: Ex ante impact assessment of drought-tolerant rice in South Asia. *Mitig. Adapt. Strat. Glob. Chang.* **2017**, *22*, 879–901. [[CrossRef](#)]
3. Bouman, B.A.M.; Humphreys, E.; Toung, T.P.; Barker, R. Rice and water. *Adv. Agron.* **2007**, *92*, 187–237.
4. Smith, M.L.; Mohanty, R.K. Monsoon, rice production, and urban growth: The microscale management of ‘too much’ water. *Holocene* **2018**, *28*, 1325–1333. [[CrossRef](#)]
5. Wu, S.J.; Chiueh, Y.W.; Hsu, C.T. Modeling risk analysis for rice production due to agro-climate change and uncertainty in irrigated water. *Paddy Water Environ.* **2018**, *16*, 35–53. [[CrossRef](#)]
6. Maclean, J.L.; Dawe, D.; Hardy, B.; Hettle, G.P. *Rice Almanac*; International Rice Research Institute: Los Baños, Philippines, 2002; p. 253.
7. Tuong, T.P.; Bouman, B.A.M.; Mortimer, M. More rice, less water—integrated approaches for increasing water productivity in irrigated rice-based system in Asia. *Plant Prod. Sci.* **2005**, *8*, 231–241. [[CrossRef](#)]
8. Tuong, T.P.; Bouman, B.A.M. Rice production in water-scarce environments. In *Water Productivity in Agriculture: Limits and Opportunities for Improvement*; Kijne, J.W., Baker, R., Molden, D., Eds.; CAB International: Wallingford, UK, 2003; pp. 53–67.
9. Kumm, M.; Guillaume, J.H.A.; de Moel, H.; Eisner, S.; Flörke, M.; Porkka, M.; Siebert, S.; Veldkamp, T.I.E.; Ward, P.J. The world’s road to water scarcity: Shortage and stress in the 20th century and pathways towards sustainability. *Sci. Rep.* **2016**, *6*, 38495. [[CrossRef](#)]
10. Jiang, S.; Wang, J.; Zhao, Y.; Shang, Y.; Gao, X.; Li, H.; Wang, Q.; Zhu, Y. Sustainability of water resources for agriculture considering grain production, trade and consumption in China from 2004 to 2013. *J. Clean. Prod.* **2017**, *149*, 1210–1218. [[CrossRef](#)]
11. van Oort, P.A.J.; Zwart, S.J. Impacts of climate change on rice production in Africa and causes of simulated yield changes. *Glob. Chang. Biol.* **2017**, *24*, 1029–1045. [[CrossRef](#)] [[PubMed](#)]
12. Prabnakorn, S.; Maskey, S.; Suryadi, F.X.; de Fraiture, C. Rice yield in response to climate trends and drought index in the Mun River Basin, Thailand. *Sci. Total Environ.* **2018**, *621*, 108–119. [[CrossRef](#)]

13. Bandara, J.S.; Cai, Y. The impact of climate change on food crop productivity, food prices and food security in South Asia. *Econ. Anal. Pol.* **2014**, *44*, 451–465. [\[CrossRef\]](#)
14. Li, T.; Angeles, O.; Radanielson, A.; Marcaida, M.; Manalo, E. Drought stress impacts of climate change on rainfed rice in South Asia. *Clim. Chang.* **2015**, *133*, 709–720. [\[CrossRef\]](#)
15. Hayashi, K.; Llorca, L.; Rustini, S.; Setyanto, P.; Zaini, Z. Reducing vulnerability of rainfed agriculture through seasonal climate predictions: A case study on the rainfed rice production in Southeast Asia. *Agric. Syst.* **2018**, *162*, 66–76. [\[CrossRef\]](#)
16. Shao, H.; Chen, X.-Y.; Chu, L.-Y.; Zhao, X.-N.; Wu, G.; Yuan, Y.-B.; Zhao, C.-X.; Hu, Z.-M. Investigation on the relationship of proline with wheat anti-drought under soil water deficits. *Colloids Surf. B Biointerfaces* **2006**, *53*, 113–119.
17. Zhang, L.; Li, S.; Liang, Z. Differential plant growth and osmotic effects of two maize (*Zea mays* L.) cultivars to exogenous glycinebetaine application under drought stress. *Plant Growth Regul.* **2009**, *58*, 297–305.
18. Ahanger, M.A.; Tyagi, S.R.; Wani, M.R.; Ahmad, P. Drought tolerance: Role of organic osmolytes, growth regulators, and mineral nutrients. In *Physiological Mechanisms and Adaptation Strategies in Plants under Changing Environment*; Ahmad, P., Wani, M.R., Eds.; Springer: New York, NY, USA, 2014; pp. 25–55.
19. Ahmad, P.; Hameed, A.; Abd-Allah, E.F.; Sheikh, S.A.; Wani, M.R.; Rasool, S.; Jamsheed, S.; Kumar, A. Biochemical and molecular approaches for drought tolerance in plants. In *Physiological Mechanisms and Adaptation Strategies in Plants under Changing Environment*; Ahmad, P., Wani, M.R., Eds.; Springer: New York, NY, USA, 2014; pp. 1–29.
20. Ashraf, M.; Foolad, M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **2007**, *59*, 206–216. [\[CrossRef\]](#)
21. Kurepin, L.; Ivanov, A.G.; Zaman, M.; Pharis, R.P.; Allakhverdiev, S.I.; Hurry, V.; Hüner, N.P.A. Stress-related hormones and glycinebetaine interplay in protection of photosynthesis under abiotic stress conditions. *Photosynth. Res.* **2015**, *126*, 221–235. [\[CrossRef\]](#)
22. Rathinasabapathi, R.; Gage, D.A.; Mackill, D.J.; Hanson, A.W. Cultivated and wild rice do not accumulate glycinebetaine due to deficiencies in two biosynthetic steps. *Crop Sci.* **1993**, *33*, 534–538. [\[CrossRef\]](#)
23. Kishitani, S.; Takanami, T.; Suzuki, M.; Oikawa, M.; Yokoi, S.; Ishitani, M.; Alvarez-Nakase, A.M.; Takabe, T. Compatibility of glycinebetaine in rice plants: Evaluation using transgenic rice plants with a gene for peroxisomal betaine aldehyde dehydrogenase from barley. *Plant Cell Environ.* **2000**, *23*, 107–114. [\[CrossRef\]](#)
24. Shirasawa, K.; Takabe, T.; Takabe, T.; Kishitani, S. Accumulation of glycinebetaine in rice plants that overexpress choline monooxygenase from spinach and evaluation of their tolerance to abiotic stress. *Ann. Bot.* **2006**, *98*, 565–571. [\[CrossRef\]](#)
25. Soren, K.R.; Ali, K.; Tyagi, V.; Tyagi, A. Recent advances in molecular breeding of drought tolerance in rice (*Oryza sativa* L.). *Indian J. Biotechnol.* **2010**, *9*, 233–251.
26. Farooq, M.; Basra, S.M.A.; Wahid, A.; Cheema, Z.A.; Cheema, M.A.; Khaliq, A. Physiological role of exogenously applied glycinebetaine to improve drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). *J. Agron. Crop Sci.* **2008**, *194*, 325–333. [\[CrossRef\]](#)
27. Cha-um, S.; Samphumphuang, T.; Kirdmanee, C. Glycinebetaine alleviates water deficit stress in *indica* rice using proline accumulation, photosynthetic efficiencies, growth performances and yield attributes. *Aust. J. Crop Sci.* **2013**, *7*, 213–218.
28. Jalal-ud-Din; Khan, S.U.; Khan, A.; Naveed, S. Effect of exogenously applied kinetin and glycinebetaine on metabolic and yield attributes of rice (*Oryza sativa* L.) under drought stress. *Emir. J. Food Agric.* **2015**, *27*, 75–81. [\[CrossRef\]](#)
29. Mäkelä, P.; Peltonen-Sainio, P.; Jokinen, K.; Pehu, E.; Setälä, H.; Hinkkanen, R.; Somersalo, S. Uptake and translocation of foliar-applied glycinebetaine in crop plants. *Plant Sci.* **1996**, *121*, 221–223. [\[CrossRef\]](#)
30. Agboma, P.C.; Sinclair, T.R.; Jokinen, K.; Peltonen-Sainio, P.; Pehu, E. An evaluation of the effect of exogenous glycinebetaine on the growth and yield of soybean: Timing of application, watering regimes and cultivars. *Field Crop Res.* **1997**, *54*, 51–64. [\[CrossRef\]](#)
31. Xing, W.; Rajashekar, C.B. Alleviation of water stress in beans by exogenous glycine betaine. *Plant Sci.* **1999**, *148*, 185–195. [\[CrossRef\]](#)
32. Cruz, F.J.R.; Castro, G.L.S.; Silva Júnior, D.D.; Festucci-Buselli, R.A.; Pinheiro, H.A. Exogenous glycine betaine modulates ascorbate peroxidase and catalase activities and prevent lipid peroxidation in mild water-stressed *Carapa guianensis* plants. *Photosynthetica* **2013**, *51*, 102–108. [\[CrossRef\]](#)

33. Kurepin, L.V.; Ivanov, A.G.; Zaman, M.; Pharis, R.P.; Hurry, V.; Hüner, N.P.A. Interaction of glycine betaine and plant hormones: Protection of the photosynthetic apparatus during abiotic stress. In *Photosynthesis, Structures, Mechanisms and Applications*; Hou, H.J.M., Najafpour, M.M., Moore, G.F., Allakhverdiev, S.I., Eds.; Springer International Publishing: New York, NY, USA, 2017; pp. 185–202.
34. Wasusun, A.; Wongpiyachon, S.; Songchitsomboon, S.; Sukviwat, W.; Maneenin, P.; Pakkethaiti, S. Rice variety RD43: Medium glycemic index rice for niche market. *Thai Rice Res. J.* **2017**, *8*, 45–53.
35. Suwannaporn, P.; Pitiphunpong, S.; Champangern, S. Classification of rice amylose content by discriminant analysis of physicochemical properties. *Starch* **2007**, *59*, 171–177. [[CrossRef](#)]
36. Pengkumsri, N.; Chaivasut, C.; Saenjum, C.; Sirilun, S.; Peerajan, S.; Suwannalert, P.; Sirisattha, S.; Sivamaruthi, B.S. Physicochemical and antioxidative properties of black, brown and red rice varieties of northern Thailand. *Food Sci. Technol.* **2015**, *35*, 331–338. [[CrossRef](#)]
37. Pongprayoon, W.; Tisarum, R.; Theerawitaya, C.; Cha-um, S. Evaluation and clustering on salt-tolerant ability in rice genotypes (*Oryza sativa* L. subsp. *indica*) using multivariate physiological indices. *Physiol. Mol. Biol. Plant* **2019**. [[CrossRef](#)]
38. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [[CrossRef](#)]
39. Shabala, S.N.; Shabala, S.I.; Martynenko, A.I.; Babourina, O.; Newman, I.A. Salinity effect on bioelectric activity growth, Na⁺ accumulation and chlorophyll fluorescence of maize leaves: A comparative survey and prospects for screening. *Aust. J. Plant Physiol.* **1998**, *25*, 609–616. [[CrossRef](#)]
40. Loggini, B.; Scartazza, A.; Brugnoli, E.; Navari-Izzo, F. Antioxidant defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiol.* **1999**, *119*, 1091–1100. [[CrossRef](#)]
41. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [[CrossRef](#)]
42. Cha-um, S.; Supaibulwatana, K.; Kirdmanee, C. Glycinebetaine accumulation, physiological characterizations and growth efficiency in salt-tolerant and salt-sensitive lines of indica rice (*Oryza sativa* L. ssp. *indica*) in response to salt stress. *J. Agron. Crop Sci.* **2007**, *193*, 157–166.
43. Harinasut, P.; Tsutsui, K.; Takabe, T.; Nomura, M.; Takabe, T.; Kishitani, S. Exogenous glycinebetaine accumulation and increased salt tolerance in rice seedlings. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 366–368. [[CrossRef](#)]
44. Demiral, T.; Türkan, I. Exogenous glycinebetaine affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. *Environ. Exp. Bot.* **2006**, *56*, 72–79. [[CrossRef](#)]
45. Cha-um, S.; Kirdmanee, C. Effect of glycinebetaine on proline, water use and photosynthetic efficiencies, and growth of rice seedlings under salt stress. *Turk. J. Agric. For.* **2010**, *34*, 517–527.
46. El-Hendawy, S.E.; Kotab, M.A.; Al-Suhaibani, N.A.; Schmidhalter, U. Optimal coupling combinations between the irrigation rate and glycinebetaine levels for improving yield and water use efficiency of drip-irrigation maize grown under arid conditions. *Agric. Water Manag.* **2014**, *140*, 69–78. [[CrossRef](#)]
47. Zhao, X.X.; Ma, Q.Q.; Liang, C.; Fang, Y.; Wang, Y.Q.; Wang, W. Effect of glycinebetaine on function of thylakoid membranes in wheat flag leaves under drought stress. *Biol. Plant.* **2007**, *51*, 584–588. [[CrossRef](#)]
48. Ma, Q.Q.; Wang, W.; Li, Y.H.; Li, D.Q.; Zou, Q. Alleviation of photoinhibition in drought-stressed wheat (*Triticumaestivum*) by foliar-applied glycinebetaine. *J. Plant Physiol.* **2006**, *163*, 165–175. [[CrossRef](#)]
49. Gupta, N.; Thind, S.K.; Bains, N.S. Glycine betaine application modifies biochemical attributes of osmotic adjustment in drought stressed wheat. *Plant Growth Regul.* **2014**, *72*, 221–228. [[CrossRef](#)]
50. Ma, X.L.; Wang, Y.J.; Xie, S.L.; Wang, C.; Wang, W. Glycinebetaine application ameliorates negative effects of drought stress in tobacco. *Russ. J. Plant Physiol.* **2007**, *54*, 472–479. [[CrossRef](#)]
51. Iqbal, N.; Ashraf, Y.; Ashraf, M. Modulation of endogenous levels of some key organic metabolites by exogenous application of glycine betaine in drought stressed plants of sunflower (*Helianthus annuus* L.). *Plant Growth Regul.* **2011**, *63*, 7–12. [[CrossRef](#)]
52. Mahouachi, J.; Argamasilla, R.; Gómez-Cadenas, A. Influence of exogenous glycine betaine and abscisic acid on papaya in responses to water-deficit stress. *J. Plant Growth Regul.* **2012**, *31*, 1–10. [[CrossRef](#)]
53. Hussain, M.; Malik, M.A.; Farooq, M.; Ashraf, M.Y.; Cheema, M.A. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agron. Crop Sci.* **2008**, *194*, 193–199. [[CrossRef](#)]
54. Osman, H.S. Enhancing antioxidant-yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline. *Ann. Agric. Sci.* **2015**, *60*, 389–402. [[CrossRef](#)]

55. Anjum, S.A.; Farooq, M.; Wang, L.C.; Xue, L.L.; Wang, S.G.; Wang, L.; Zhang, S.; Chen, M. Gas exchange and chlorophyll synthesis of maize cultivars are enhanced by exogenously-applied glycinebetaine under drought conditions. *Plant Soil Environ.* **2011**, *57*, 326–331. [[CrossRef](#)]
56. Rezaei, M.A.; Jokar, I.; Ghorbanli, M.; Kaviani, B.; Kharabian-Masouleh, A. Morpho-physiological improving effects of exogenous glycine betaine on tomato (*Lycopersicum esculentum* Mill.) cv. PS under drought stress conditions. *Plant Omic* **2012**, *5*, 79–86.
57. Gupta, N.; Thind, S.K. Improving photosynthetic performance of bread wheat under field drought stress by foliar applied glycine betaine. *J. Agric. Sci. Technol.* **2015**, *17*, 75–86.
58. Cha-um, S.; Supaibulwatana, K.; Kirdmanee, C. 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. *J. Agron. Crop Sci.* **2006**, *192*, 25–36. [[CrossRef](#)]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).