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# Glycinebetaine alleviates water deficit stress in *indica* rice using proline accumulation, photosynthetic efficiencies, growth performances and yield attributes

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## Abstract

We investigated the role of GlyBet (100 mM) applied exogenously in alleviating water-deficit induced stress in *indica* rice cv. Pathumthani 1 (PT1). Exogenous GlyBet increased proline concentration in the leaf tissues of water deficit stressed plants depend on a degree of soil water content (SWC), especially at severe water deficit (25% SWC). Foliar GlyBet (100 mM) significantly enhanced chlorophyll a ($Chl_a$) and total chlorophyll (TC) content in the leaf tissues of water stressed plants (25% SWC) and stabilized total carotenoids ($C_{x+c}$) in 25 and 36% SWC better than those in controlled plant. A positive correlation was observed between $Chl_a$ and maximum quantum yield of PSII ($F_v/F_m$), and TC and photon yield of PSII ($\Phi_{PSII}$). In contrast to control plants, where $F_v/F_m$ and $\Phi_{PSII}$ and net photosynthetic rate ($P_n$) declined, Glybet pre-treated plants maintained a high level of $F_v/F_m$, $\Phi_{PSII}$ and $P_n$ even under 25% SWC. Even at the harvest stage, plant height and yield traits such as panicle length and weight, fertility percentage and one-hundred grain weight of GlyBet pre-treated plants were better than those in the control plants when exposed to water stress. The study concludes that exogenous application of GlyBet significantly alleviates water-deficit in *indica* rice and maintains grain yield.

**Keywords:** Net photosynthetic rate, photosynthetic pigments, soil water content, water deficit alleviation, yield traits.
**Abbreviations:** $Chl_a$_chlorophyll a; $Chl_b$_chlorophyll b; TC_total chlorophyll; CRBD_Completely Randomized Design; DAS_day after sowing; DW_dry weight; EC_electro-conductivity; FP_fertility percentage; FW_fresh weight; GlyBet_glycinebetaine; LSD_Least significant difference; $F_v/F_m$_maximum quantum yield of PSII; $P_n$_net-photosynthetic rate; HGW_one-hundred grain weight; PL_panicle length; PW_panicle weight; PT1_Pathumthani 1; PEPC_phosphoenol pyruvate carboxylase; PH_plant height; $\Phi_{PSII}$_photon yield of PSII; Rubisco_ribulose-1,5-bisphosphate carboxylase/oxygenase; $C_{x+c}$_total carotenoids; SWC_soil water content.

## Introduction

Rice is the major source of carbohydrate to millions of people world over, particularly in Asia (Peng et al., 2009). To achieve food security for increasing population in this region, there is an urgent need to increase the rice productivity. The problem has further aggravated due to global warming that has limited the availability of fresh water for rice crop, particularly under rain-fed conditions (Laborte et al., 2012). To overcome this problem of water-deficit, researchers' world over are screening cultivars for drought tolerance and discovering drought tolerant genotypes, along with yield traits, for future rice breeding programs (Guan et al., 2010).

Drought is one of the most important abiotic stresses that severely reduce crop productivity. Glycine betaine (GlyBet) amino acid has been found to act as osmoprotectant and improve the growth and development of plants exposed to a variety of abiotic stresses including drought, temperature and salinity. Previous studies have demonstrated that biochemical, physiological and morphological changes occur in plants in response to water deficit (Basu et al., 2010; Cha-um et al., 2010).

In response to water deficit/ drought conditions, maintenance of cellular osmotic pressure/osmoregulation is the major mechanism (Serraj and Sinclair, 2002; Thapa et al., 2011). Earlier studies have reported that molecules like glycine betaine, proline, mannitol, sorbitol, and trehalose act as osmoprotectants under water stress and help to maintain plant growth and development (Valliyodan and Nguyen, 2006; Hadiarto and Tran, 2011). Glycine betaine (GlyBet), a member of quaternary ammonium compounds, is an osmolyte that is pre-dominant in higher plants subjected to drought condition (Chaitanya et al., 2009). GlyBet has been reported to protect photosynthetic machinery, stabilize the structure of Rubisco (ribulose-1,5-bisphosphate carboxylase/ oxygenase), and act as oxygen radical scavenger under drought (Mäkelä et al., 2000; Ashraf and Foolad, 2007; Chen and Murata, 2008; Anjum et al., 2012; Rezaei et al., 2012). It has been argued that exogenous GlyBet application could be a promising way to directly maintain and enhance the growth and yield in monocot crops such as rice (Farooq et al., 2008), wheat (Ma et al., 2006) and maize (Anjum et al., 2011). Previous studies have reported that foliar spray of GlyBet significantly improves growth performances of fine grain aromatic rice seedlings subjected to drought stress (Farooq et al., 2008, 2010).

However, studies investigating the effect of GlyBet pretreatment on yield traits of rice plant under drought conditions are lacking. We therefore conducted a study to investigate the potential of foliar GlyBet application to enhance the drought tolerant abilities prior to grain harvesting in drought sensitive rice cultivar.

**Table 1.** Chlorophyll a ($Chl_a$), chlorophyll b ($Chl_b$), total chlorophyll (TC), total carotenoids ($C_{x+c}$), maximum quantum yield of PSII ($F_v/F_m$) and photon yield of PSII ($\Phi_{PSII}$) in rice plants pre-treated with glycine betaine (0 and 100 mM) and subsequently subjected to various soil water contents (SWC) and recovery.

| Glybet (mM) | SWC (%) | $Chl_a$ ($\mu g\ g^{-1}$ FW) | $Chl_b$ ($\mu g\ g^{-1}$FW) | TC ($\mu g\ g^{-1}$FW) | $C_{x+c}$ ($\mu g\ g^{-1}$FW) | $F_v/F_m$ | $\Phi_{PSII}$ |
|---|---|---|---|---|---|---|---|
| 0 | 56 | 97.3 | 55.3 | 152.6 | 3.75 | 0.877 | 0.716 |
|  | 45 | 97.7 | 55.6 | 153.2 | 3.54 | 0.862 | 0.666 |
| 100 | 56 | 99.3 | 56.8 | 156.1 | 3.23 | 0.871 | 0.727 |
|  | 45 | 97.0 | 56.2 | 153.1 | 3.32 | 0.868 | 0.677 |
| *ANOVA* |  | NS | NS | NS | NS | NS | NS |
| 0 | 56 | 93.6 | 56.2 | 149.7 | 3.22a | 0.848 | 0.711 |
|  | 36 | 94.1 | 56.8 | 151.0 | 2.74b | 0.862 | 0.671 |
| 100 | 56 | 94.6 | 58.0 | 152.6 | 3.40a | 0.879 | 0.722 |
|  | 36 | 94.9 | 57.1 | 152.6 | 3.09ab | 0.897 | 0.677 |
| *ANOVA* |  | NS | NS | NS | * | NS | NS |
| 0 | 56 | 88.9a | 55.9 | 147.7a | 3.16b | 0.868a | 0.692a |
|  | 25 | 72.4b | 58.8 | 128.3b | 2.82c | 0.510b | 0.398b |
| 100 | 56 | 82.6ab | 58.2 | 140.8ab | 3.59a | 0.845a | 0.707a |
|  | 25 | 78.3ab | 59.8 | 138.1ab | 3.24ab | 0.809a | 0.654a |
| *ANOVA* |  | * | NS | * | * | ** | ** |
| 0 | 56 | 94.8 | 54.7 | 149.5 | 3.77a | 0.878 | 0.753 |
|  | Recovery | 95.6 | 54.7 | 150.3 | 3.02b | 0.862 | 0.672 |
| 100 | 56 | 98.9 | 55.5 | 154.4 | 3.46ab | 0.868 | 0.728 |
|  | Recovery | 97.0 | 54.7 | 157.7 | 2.95b | 0.880 | 0.767 |
| *ANOVA* |  | NS | NS | NS | * | NS | NS |

Different letters in each column show significant difference at $p \leq 0.05$ (*), $p \leq 0.01$ (**) by Least significant difference (LSD). $^{NS}$ represented non-significant difference.

## Results

### *Water deficit enhanced proline accumulation*

Proline concentration increased significantly in water stressed plants compared to that in the control plants with well watering conditions. The increase was in relation to degree of soil water content (SWC), peaked at 25 % SWC, and proline concentration declined during recovery process (Fig. 1A). At 36 % and 25 % SWC treatment proline accumulation in 100 mM exogenous GlyBet pre-treated plants was 1.53 and 2.18 folds, respectively, of that in plants without GlyBet pretreatment (Fig. 1A).

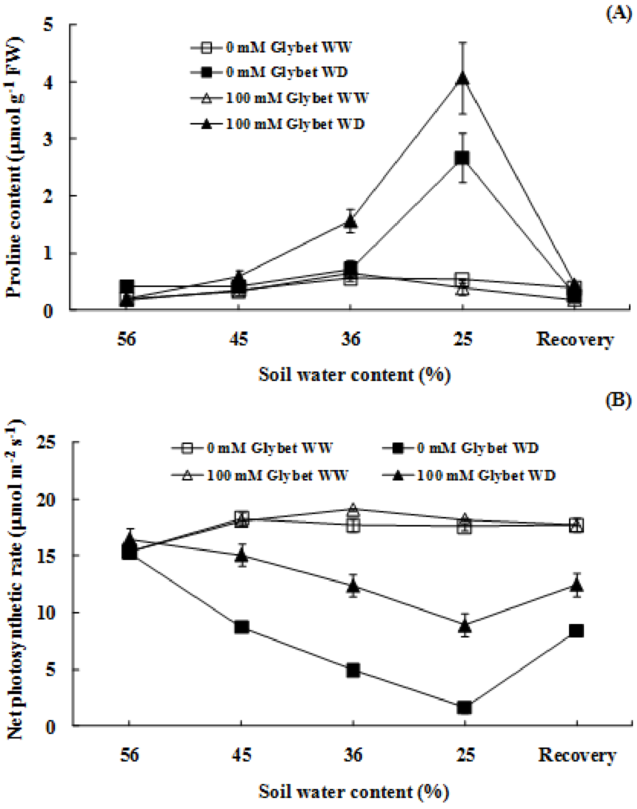


**Fig 1.** Proline content (A) and net photosynthetic rate (B) in rice plants pre-treated with glycine betaine (0 and 100 mM) and subsequently subjected to various soil water contents (SWC) and recovery.

### *Exogenous GlyBet enhanced photosynthetic ability under water deficit stress*

Net photosynthetic rate ($P_n$) was significantly declined when plants were subjected to water deficit stress at 45 %, 36 % and 25 % SWC, respectively. In contrast, the $P_n$ was elevated in water-deficit stressed plants with 100 mM GlyBet pretreatment (Fig. 1B). Amount of chlorophyll a ($Chl_a$) and total chlorophyll (TC) in the leaf tissues of rice plants under water stress without GlyBet pretreatment was significantly lesser when plants exposed to 25% SWC, (Table 1). Upon watering of water stressed plants (25% SWC), the degradation of $Chl_a$ and TC declined by 18.56 and 13.13 %, respectively. In contrast, the $Chl_a$ and TC in 100 mM GlyBet pre-treated plants were maintained (5.01 and 1.92% degradation, respectively). In contrast, there was no change in the amount of $Chl_b$ under all treatments (Table 1). Total carotenoids concentration in the leaf tissues was very sensitive to water stress, and it declined significantly when exposed to mild water deficit (36% SWC) in plants without GlyBet pretreatment where ~ 15% degradation was noticed.

A positive relationship between $Chl_a$ and maximum quantum yield of PSII ($F_v/F_m$) (Fig.2A), TC and photon yield of PSII ($\Phi_{PSII}$) (Fig. 2B) was observed. The $F_v/F_m$ and $\Phi_{PSII}$ were stabilized in 100 mM GlyBet pretreated plants under water

**Table 2.** Plant height (PH), panicle length (PL), panicle weight (PW), fertility percentage (FP) and one-hundred grain weight (HGW) in rice plants pre-treated with glycine betaine (0 and 100 mM) and subsequently subjected to water stress.

| Glybet (mM) | Treatment | PH (cm) | PL (cm) | PW (g) | FP (%) | HGW (g) |
|---|---|---|---|---|---|---|
| 0 | WW | 94.2c | 25.2a | 1.42b | 78.5a | 1.39c |
| | WD | 83.4d | 17.3c | 0.49c | 32.5c | 0.61d |
| 100 | WW | 102.7a | 26.1a | 2.42a | 78.8a | 2.41a |
| | WD | 98.5b | 22.3b | 1.63b | 73.1b | 1.77b |
| *ANOVA* | | ** | ** | ** | ** | ** |

Different letters in each column show significant difference at *p ≤0.01* (**) by Least significant difference (LSD).

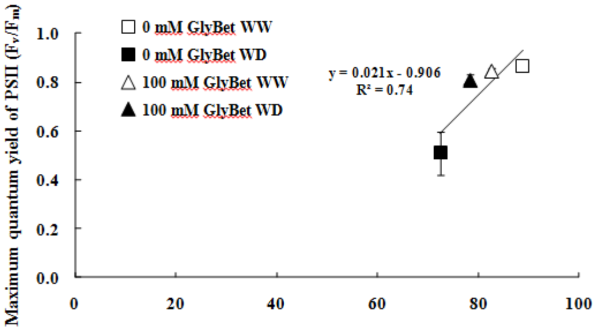
stress; however, these were declined by ~41% and 43%, respectively, in water-deficit stressed plants without GlyBet pretreatment (Table 1). We observed an improvement in $P_n$ in GlyBet pre-treated plants, whereas a significant decline in $P_n$ was observed in plants without GlyBet pretreatment (Fig. 3A). Enhanced $P_n$ under GlyBet pretreatment correlated well with greater yield / productivity as indicated by increased / greater panicle weight (Fig. 3B). Further, exogenous GlyBet treatment improves the morphological characters under severe water deficit (Table 2 and Fig. 4).

### *GlyBet improves yield traits under water deficit stress*

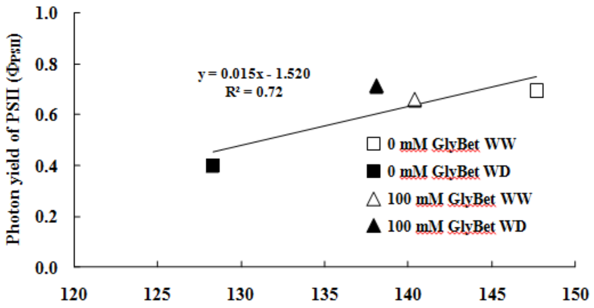
Exogenous GlyBet recuperated the rice plants from water stress as indicated by greater plant height (PH) in GlyBet pretreated plants relative to without GlyBet pretreatment. PH was inhibited by 4.09% in GlyBet pre-treated plants compared to 9.74% reduction in untreated plants, when subjected to water stress (Table 2). Similarly, panicle length (PL) and panicle weight (PW) declined significantly in plants without GlyBet pretreatment and > 31 and 65% reduction, respectively, was observed. The fertility percentage (FP) and one-hundred grain weight also declined (significant at *P≤0.01*) by 58.6 and 56.2%, respectively, in plants without GlyBet treatment. However, the yield traits of rice crop were improved by exogenous application of 100 mM GlyBet before exposure to water stress condition.

## Discussion

Proline accumulation is corroborated by earlier findings that exogenous application of GlyBet induces proline accumulation in monocots (Farooq et al., 2008, 2010; LinXin et al., 2009). Foliar supply of GlyBet (100 mM) has been demonstrated to enhance proline accumulation in Super Basmati rice seedling under water stress (50% field capacity) by 1.63 folds of that under well irrigation (100% field capacity) (Farooq et al., 2008). These workers further reported that proline concentration in Super Basmati rice seedling with exogenous supply of GlyBet (150 mM) was a function level of water stress and their interactions (Farooq et al., 2010). Likewise, a parallel increase in proline concentration was observed in two maize cultivars ($S_9$ and $S_{911}$) at all developmental stages (seedling, elongation, heading and maturity) in response to GlyBet pretreatment (50 mg $L^{-1}$) under drought stress condition (LiXin et al., 2009). These observations suggest that proline enrichment in GlyBet pretreated plants play a key role as osmoregualtion defense mechanism under drought stress. However, these observations are in sharp contrast to findings in dicotyledonous plants such as tobacco (Ma et al., 2007) and papaya (Mahouachi et al., 2012) where proline concentration in 80 mM and 50 mM Glybet pretreated plants was lesser than in untreated plants. Further, exogenous GlyBet application at reproductive developmental stage is more effective for proline accumulation than that at vegetative stage of water deficit stressed sunflower (Iqbal et al., 2011).



(A)
Chlorophyll a content (μg $g^{-1}$ FW)



(B)
Total chlorophyll content (μg $g^{-1}$ FW)

**Fig 2.** Relationships between chlorophyll a content ($Chl_a$) and maximum quantum yield of PSII ($F_v/F_m$) (A), total chlorophyll content (TC) and photon yield of PSII ($\Phi_{PSII}$) (B) in rice plants pre-treated with glycine betaine (0 and 100 mM) and subsequently subjected to 25% soil water contents (SWC).

The observations made in the present study are supported by earlier findings that GlyBet (50-100 mM) significantly improves chlorophyll concentration in the water deficit leaf tissues (Mäkelä et al., 2000; Ma et al., 2007; Zhao et al., 2007). Likewise, 100 mM GlyBet supplied exogenously has been demonstrated to maintain $Chl_a$, $Chl_b$ and TC in drought stressed leaves (35% field capacity) of maize cultivar ND95 (Anjum et al., 2011). In contrast, no change was observed in the $C_{x+c}$ concentration in two maize cultivars, Dongdan-60 and ND-95, upon GlyBet treatment under drought conditions (Anjum et al., 2011). Nevertheless, a significant decline in photosynthetic pigments of water stressed plant without Glybet application is likely to directly affect the light harvesting and electron transport system in photosytem II, which is indicated by chlorophyll fluorescence parameters.

Earlier, a higher $F_v/F_0$ in drought stressed bean treated with 10 mM GlyBet (soil drench) was observed in comparison to those without Glybet treatment (Xing and Rajashekar, 1999). Similarly, an increased $F_m$ and $F_v/F_m$ has been reported in two wheat genotypes, HF9703 and SN215953, pretreated with 100 mM Glybet when submitted to decreased RWC (Ma et al., 2006). Further, $\Phi_{PSII}$ has been reported to improve significantly upon foliar supply of 80 mM GlyBet in two tobacco varieties, DHJ5210 and ZY100, (Ma et al., 2007). Previous findings have reported that enrichment of photosynthetic pigments and chlorophyll fluorescence in GlyBet pretreated plants directly enhances $P_n$ in the water stress (Ma et al., 2007; Anjum et al., 2011). Earlier, 100-150 mM GlyBet pretreatment has been reported maintain $CO_2$ assimilation rate and $P_n$ in rice plants subsequently exposed to drought stress parallel to that in the control (well watering) (Farooq et al., 2008). Under water stress, GlyBet has been suggested to play a function role as an osmolyte and prevent the chloroplast organelles, chlorophyll pigments, Rubisco/PEPC enzymes and light harvesting protein complexes (Mäkelä et al., 2000; Zhao et al., 2007). The maintenance of photosynthetic ability by exogenous application of GlyBet further results in enhanced overall growth performance and productivity under drought condition.

Our findings are supported by earlier reports that plant height is significantly improved in Super Basmati rice seedlings (Farooq et al., 2010) and maize cv. Dongdan-60 (Anjum et al., 2011) by GlyBet pre-treatment under water stress, Moreover, the yield traits, grain yield and hundred grain weight in Glybet pretreated plants are considerably improved and better than in those without GlyBet treatment when exposed to drought stress in both monocotyledonous crop species such as wheat (Mahmood et al., 2009) and maize (LiXin et al., 2011) and dicotyledonous species such as bean (Xing and Rajashekar 1999), soybean (Agboma et al., 1997) and sunflower (Hussain et al., 2008). We observed that pre-treatment of GlyBet maintained the morphology and health of the mature plants under 25% SWC. It is quite similar to an earlier report that GlyBet pre-treatment resulted in healthier bean (*Phaseolus vulgaris*) plants, particularly at reproductive stage, under water stress condition (Xing and Rajashekar, 1999). In conclusion, our study demonstrates that exogenous GlyBet pretreatment to rice plants enhanced proline that plays as an important role in defense mechanism, and maintained photosynthetic abilities, growth performances, and yield traits under water stress. Therefore, an exogenous application of GlyBet in rice crop could play an alternative way to improve the productivity when cultivated under water stress.

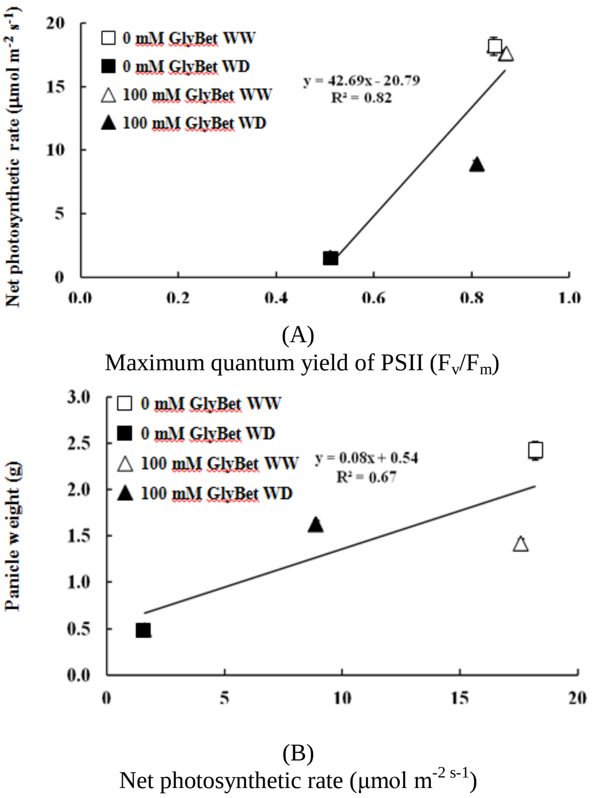


**Fig 3.** Relationships between maximum quantum yield of PSII ($F_v/F_m$) and net photosynthetic rate ($P_n$) (A), $P_n$ and panicle weight (B) in rice plants pre-treated with glycine betaine (0 and 100 mM) and subsequently subjected to 25% soil water contents (SWC).

## Materials and Methods

### *Plant material and treatments*

Seeds of Pathumthani 1 (PT1) rice, a drought sensitive cultivar (Cha-um et al., 2010), provided by Pathumthani Rice Research Center were germinated and transplanted to pots containing clay soil (EC = 2.68 dS $m^{-1}$; pH = 5.5; organic matter = 10.36%; total nitrogen = 0.17%; total phosphorus = 0.07%; total potassium = 1.19%) in 50% shading (acclimatization) light intensity and grown for 2 weeks. The pots were arranged on plastic trays (30 × 45 × 20 cm) under green house conditions. Acclimatized plants were transferred directly to water-flooded pots (ϕ=15 cm, height = 30 cm) containing clay soil (1 kg). The experiment was conducted at the Thailand Science Park, Pathumthani, Thailand (Latitude 14°01'12"N and Longitude 100°31'12"E) during August–November 2010. At the booting stage [85 days after sowing (DAS)], plants were spray-treated with 0 (control) and 100 mM glycine betaine (Greenstrim™, Lallemand, Finland) @ 50 mL per plant, once a day for 2 consecutive days. Then, the soil water content (SWC) was adjusted to 56% (well watering), 45% (1 day withholding irrigation), 36% (3 day withholding irrigation), and 25% (5 days withholding irrigation) and re-watering 3 days prior to grain harvesting. Proline concentrations, photosynthetic pigments, chlorophyll fluorescence, net-photosynthetic rate ($P_n$), in leaf blade and panicle traits in rice plants were determined at the booting stage. In addition, yield attributes were collected measured at the grain harvesting process.

### *Soil water content*

Soil water content (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).

### *Proline concentration*

Proline in the fresh leaf tissues was extracted and analyzed according to the method of Bates et al. (1973), using a UV-visible spectrophotometer (model DR/4000, HATCH,

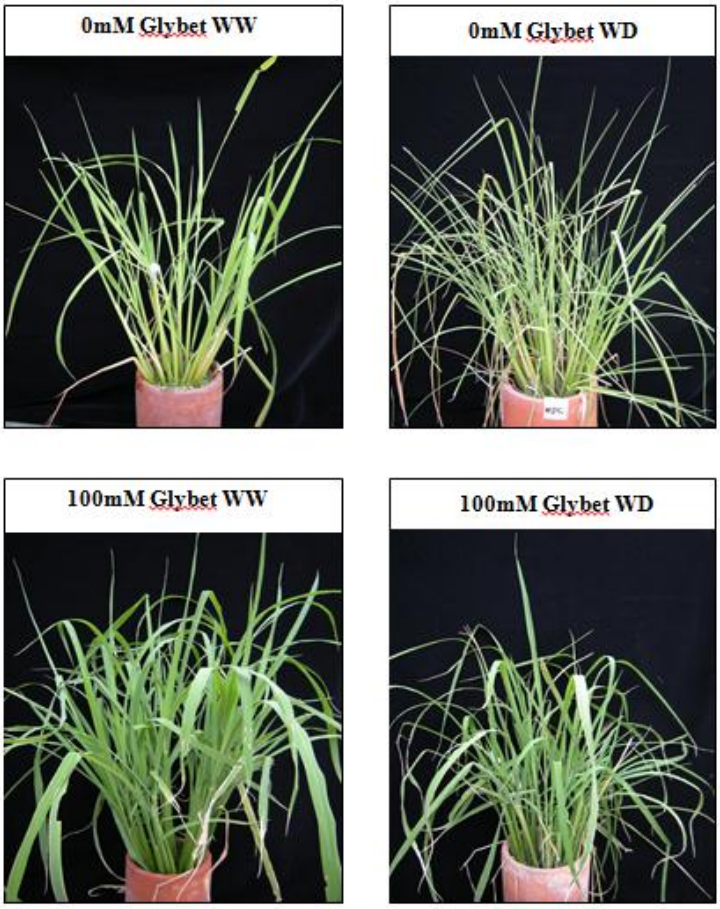


**Fig 4.** Morphological characteristics in rice plants pre-treated with glycine betaine (0 and 100 mM) and subsequently subjected to 25% soil water contents (SWC).

Loveland, Colorado, USA) at 520 nm with L-proline as a standard.

### *Photosynthetic pigments*

Chlorophyll a ($Chl_a$), chlorophyll b ($Chl_b$) and total chlorophyll (TC) in the fresh leaf tissues were analyzed following the methods of Shabala et al. (1998) and total carotenoid ($C_{x+c}$) concentrations were determined according to Lichtenthaler (1987) methods.

### *Photosynthetic efficiency*

Chlorophyll fluorescence emission from the adaxial surface of the leaf was measured using a fluorescence monitoring system (model FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode, as previously described by Loggini et al. (1999).

Net photosynthetic rate ($P_n$; µmol $m^{-2}$ $s^{-1}$) was measured using a Portable Photosynthesis System with an Infra-red Gas Analyzer (Model LI 6400, LI-CORR Inc., Lincoln, Nebraska, USA) following Cha-um et al. (2007).

### *Morphological parameters and yield traits*

Plant height (PH), panicle length (PL), panicle weight (PW), fertility percentage (FP) and one-hundred grain weight (HGW) of rice crop were measured according to Cha-um and Kirdmanee (2010).

### *Experiment design and statistical analysis*

The experiment was arranged as Completely Randomized Design (CRD) with eight independent pot replicates (n = 8) with each pot having 2 plants. The mean values obtained were compared by Least significant difference (LSD) and analyzed using SPSS software.

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