Proline related genes expression and physiological changes in indica rice response to water-deficit stress

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Abstract

Water deficit stress is a major abiotic stress causing to reduce crop productivity especially rice. Rice has been reported as drought susceptible, which decline plant growth and development in both seedling and reproductive developmental stages. Induced mutant in rice crop against water deficit stress is a fruitful topic. Proline osmolyte is a candidate metabolite to maintain the osmotic pressure in cellular level of water deficit stressed plants. The key enzymes i.e. P5CS, P5CR and ProDH in proline biosynthesis and degradation are well established. In present study, P5CS, P5CR and ProDH and the final product, proline in rice genotypes at booting stage was investigated when subjected to water-deficit and recovery processes. The expression levels of the P5CS gene in PT1 rice (drought susceptible) and the EE12 mutant line were up-regulated when rice genotypes were exposed to severe water-deficit (7% SWC), whereas P5CR genes in N5G19, IR20 and PT1 were up-regulated by the recovery process to a significant degree (p<0.01). A positive relationship between P5CS expression level and proline content in rice genotypes subjected to water-deficit stress was evidently stated (R² = 0.60). In addition, the expression level of ProDH in rice genotypes was exhibited in the recovery process. Moreover, physiological changes, including maximum quantum yield of PSII (Fv/Fm), water use efficiency (WUE) and net photosynthetic rate (Pn) were significantly reduced when plants were subjected to severe water-deficit stress (7% SWC), leading to retard plant height.

Keywords: rice mutant, net photosynthetic rate, P5CS, P5CR, ProDH, water use efficiency

Abbreviations: Fv/Fm, maximum quantum yield of PSII; Pn, net photosynthetic rate; P5C, Δ1-pyrroline-5-carboxylate; P5CDH, Δ1-pyrroline-5-carboxylate dehydrogenase; P5CR, Δ1-pyrroline-5-carboxylate reductase; P5CS, Δ1-pyrroline-5-carboxylate synthetase; ProDH, proline dehydrogenase; SWC, soil water content; WUE, water use efficiency

Introduction

Water-deficit in plants is one of the most important abiotic stresses, reducing crop productivity over more than 1.2 billion ha, especially in arid and semi-arid regions (Chaves and Oliveira, 2004; Passioura, 2007). In higher plants, proline is a candidate biochemical solute, being well known as a stress indicator, especially of water-deficit stress (Yoshiba et al., 1997). The proline biosynthesis pathway in plants has been well established via glutamate intermediate, using P5CS (Δ1-pyrroline-5-carboxylate synthetase) to P5C (Δ1-pyrroline-5-carboxylate), subsequently oxidized to the final product proline by P5CR (Δ1-pyrroline-5-carboxylate reductase). Also, proline degradation has been discovered through ProDH (proline dehydrogenase) from proline to P5C (Δ1-pyrroline-5-carboxylate) and then P5CDH (Δ1-pyrroline-5-carboxylate dehydrogenase) (Hare and Cress, 1997; Kishor et al., 2005; Trovato et al., 2008; Szabados and Savouré, 2009; Verslues and Sharma, 2010). The function of proline in plant cell defence responses to water-deficit stress has been reported, including signal transduction, osmoregulation and antioxidant systems (Dalauney and Verma, 1993; Hare and Cress, 1997; Hare et al., 1999; Kishor et al., 2005; Szabados and Savouré, 2009). In addition, there are many proline transporter proteins (family), which translocate proline from sink (leaf) to source organs (root), for example, OsProT1 (Igrashi et al., 2000) and AtProT2 (Grallath et al., 2005) can be monitored using radioactive labelling (Raymond and Sminoff, 2002). In rice, proline accumulation in root tissues is greater than in leaf tissues of plants exposed to water-deficit stress (Hien et al., 2003). The genetic manipulation approach for proline accumulation in plants has also been explored previously for identification of the water deficit tolerance trait (Zhu et al., 1998; Su and Wu, 2004; Vendruscolo et al., 2007; Verbruggen and Hermans, 2008). The expression levels of mRNAs related to proline biosynthesis, proline content and physiological changes in rice genotypes may be developed further for use as indices for water deficit tolerance screening in rice breeding programs. Rice, a major carbohydrate crop, is a basal food, especially in Asian countries, feeding more than 3 billion people and providing 50-80% of their daily calorie intake (Khush, 2005). In arid and semi-arid areas, water-deficit is well known as a serious problem in crop production, especially during the plant’s reproductive stage, when they are particularly susceptible (Fukai et al., 1999; Pantuwan et al., 2002; Bouman et al., 2006). In the previous reports, N5G19 is a positive check of water deficit tolerance, whereas IR20 is a negative check (Pantuwan et al., 2002; Uyprasert et al., 2004; Kumar et al., 2006). However, the metabolic flux of proline in the booting stage of indica rice, including mutant lines, in response to water-deficit stress is still to be discovered. The aim of this investigation was to evaluate the expression of proline related genes and proline...
accumulation in the booting stage of different rice cultivars grown under water-deficit conditions and during recovery.

Results

Proline-related gene expression and proline content

P5CS, P5CR and ProDH mRNA expression levels in the flag leaf of rice genotypes under conditions of well watering (control), 25% SWC (mild water deficit), 7% SWC (severe water deficit) and recovery (56% SWC or full irrigation) were investigated in three mutant lines, AA11, EE12 and FF17, wild type (KDMI 105), Pathumthani 1 (PT1) rice cultivar, tolerant (NSG 19) and sensitive (IR 20) cultivars. Expression level of P5CS in three mutants and wild type (KDMI 105), with well watered plants was very low. In mild water deficit conditions (25% SWC), P5CS mRNA in the mutant lines, KDMI 105 and PT1 was up-regulated. Similarly, mRNA expression was found in the flag leaf tissues of rice genotypes subjected to severe water-deficit stress (7% SWC), dropping during the recovery stage, especially in EE12 and PT1 (Fig. 1A). In the case of P5CR, expression levels in rice genotypes grown under well watering were near to the ground. An increase expression level in mild water-deficit was detected in AA11, FF17, KDMI 105 and PT1. In addition, P5CR in the flag leaf tissues of rice genotypes was up-regulated in plants subjected to severe water-deficit and also in the recovery process, excluding EE12 (down-regulation) and FF17 (absence) (Fig. 1B). In contrast, the expression of ProDH mRNA in this study was very low (<1.0 relative expression level), particularly in the PT1 cultivar (Fig. 1C). In the recovery stage, ProDH expression levels in the rice genotypes, AA11, EE12, NSG19, KDMI 105, IR20 and PT1 were up-regulated (Fig. 1C). The accumulation of the final product, proline, in the flag leaf tissues was also evaluated. In well watered plants, proline content was lower than 2 μmol g⁻¹ FW in all rice genotypes. It was accumulated relative to the degree of water-deficit stress (25% and 7% SWC), decreasing in the recovery stage, for example, in EE12, NSG19, KDMI 105, IR20 and PT1 genotypes (Fig. 2). In contrast, the proline content in the mutant lines, AA11 and FF17 was inversely related to water-deficit stress and recovery. For proline biosynthesis, the regulation of P5CS gene in rice genotypes was positively correlated with proline content (R² = 0.60) in plants exposed to water-deficit stress (Fig. 3A). On the other hand, the regulation of P5CR in rice genotypes was unrelated to proline content (Fig. 3B; R² = 0.07) as well as the regulation of ProDH gene was unconnected to proline content (Fig. 3C; R² = 0.28).

Photosynthetic abilities and growth characters

Maximum quantum yield of PSII (Fv/Fm), water use efficiency (WUE) and net photosynthetic rate (Pn) in the flag leaf tissues were measured as physiological characters. In well watered plants, the Fv/Fm, WUE and Pn in flag leaf tissues were elevated when compared with those in water deficit stressed plants. The Fv/Fm in IR20 and PT1 was significantly reduced in plants subjected to severe water-deficit (25.88% and 30.29%, respectively), increasing again during recovery (Table 2). WUE in FF17 was maintained to a significant degree when exposed to 25% SWC (32.72% reduction), while WUE in other lines of rice decreased significantly (>45% reduction), then improved in the recovery process (Table 1). A positive correlation between Fv/Fm and Pn was found (Fig. 4). Pn in NSG19 (19.54% reduction) and KDMI 105 (29.3% reduction) was maintained to a considerable degree when compared to IR20 (64.028% reduction) and PT1 (79.44% reduction) (Table 1). As well as, the Pn reduction in three mutant lines was ranged from 46.17% to 57.48%. Moreover, plant height (PH) in all rice lines was consistently reduced prior to recovery in plants subjected to severe water-deficit, except in the case of NSG 19, in which PH was maintained (Table 2).

Discussion

In the present study, P5CS expression level in the PT1-drought sensitive cultivar increased relative to the degree of water-deficit stress, leading to enriched proline in the flag leaf tissue. Contrastingly, P5CR and ProDH expression levels in PT1 were unchanged when compared to other cultivars, except in the recovery process. In the Taichung Native 1 rice cultivar, P5CR and ProDH activities decreased in plants subjected to PEG-induced water-deficit (Hsu et al., 2003). The mRNA expression of P5CS1 and P5CS2 in three rice cultivars, differing in drought and salt tolerance, has been well established. In the case of the drought tolerance trait, two isoforms of P5CS genes in the drought tolerant DR2 cultivar are regulated in seedlings by mannitol-induced water-deficit for 48 h. In the leaf tissues, relative expression levels of P5CS1 and P5CS2 genes in DR2 grown under water-deficit stress are lower than in CR203 (drought susceptible), relating to proline content (Hien et al., 2003). In contrast, the activity of P5CS and proline content in drought-tolerant rice cultivars, N-22 and CR 143-2-2, showed a greater improvement than in drought susceptible Panidhan and Pusa-169 (Choudhary et al., 2005). In cotton genotypes, the expression levels of P5CS and P5CR genes in drought tolerant Ca/H 680 were superior to those in drought sensitive cultivar Nira (Thippeswamy et al., 2010). Expression levels of P5CS (2 isoforms; OsP5CS1 and OsP5CS2) and P5CR mRNAs, as well as proline accumulation in callus and suspension culture of KDMI 105 (Thai jasmine rice), are exhibited significantly in response to NaCl salt stress (Somboonwathanakul et al., 2010). In Andean potatoe, the expression levels of P5CS in drought tolerant cultivars (Sullu, SA2563, Perricholi and Puka Pishgush) are superior to those in drought susceptible cultivars (Cceccorani and Leona), whereas ProDH expression is opposed (Schalleitner et al., 2007). Proline was accumulated in the leaf tissues depending on the degree of water-deficit stress, especially in drought susceptible IR20 and PT1. Similarly, proline content in the leaf tissues of CR203 (drought tolerant) is enriched to a greater degree than in DR2 (drought susceptible) (Hien et al., 2003). In pepper plants, the expression levels of P5CS and P5CR in the leaf tissues dropped significantly in plants subjected to water-deficit stress and proline was accumulated only in the root tissues (Sziederics et al., 2010). In Arabis stelleri, P5CS, P5CR, ProDH expression levels in response to mannitol-induced water-deficit stress, as well as proline accumulation, were represented similar to the present study, (Jung et al., 2010). Also, the expression levels of these genes were up-regulated in Brassica napus when exposed to polyethylene glycol (PEG)-induced water-deficit (Xue et al., 2008). In EE12 mutant lines of rice, the expression levels of P5CS and P5CR were exhibited by extreme water-deficit stress (7% SWC). The expression level of P5CS1 in mutant Arabidopsis (ggt1-1; glutamate:glyoxylate transferase1-1) was down-regulated, whereas ProDH gene was up-regulated when subjected to PEG-induced water-deficit. The expression patterns in mutant genotypes differ from the wild type (Verslues et al., 2007). Also, proline content in Arabidopsis mutants lines, p5cs1-1, p5cs1-2, p5cs1-3 and p5cs1-4 grown under 20% SWC was accumulated to a lower level than that.
in wild type (Székely et al., 2008). The electron transport rate of
PSII photosynthetic abilities in the drought tolerant
genotype of cotton, Ca/H 680, grown under drought stress,
was maintained better than that in drought susceptible Ca/H
148 (Parida et al., 2008). A reduction of WUE has previously
been used as an effective index for water-deficit tolerance
screening in rice genotypes (Casbuslay et al., 2002), cowpea
(Anyia and Herzog, 2004) and mulberry (Guha et al., 2010).
The WUE, F_{v}/F_{m} and P_{n} parameters in water-deficit tolerant
genotypes were stabilised when subjected to water-deficit
conditions, especially in rice (Casbuslay et al., 2002; Cha-
um et al., 2010), leading to maintenance of plant growth, as
indicated by factors including plant height, plant fresh weight
and plant dry weight (Schafleiter et al., 2007; Hien et al.,
2003).

Materials and methods

Plant materials

Ma seeds of three mutant rice cultivars, MT 4-01 (code AA11), MT 4-09 (code EE12) and MT 4-11 (code FF17),
derived from γ-irradiation and ethyl methane sulfonate
(EMS) mutagens of jasmine rice (Oryza sativa L. ssp. indica
cv. KDML 105) (Theerawitaya et al., 2011; Cha-um et al.,
2012), along with seeds of jasmine rice (KDML105),
Pathumthani 1 (PT1), Nam Sa Gui 19 (NSG19; positive
control) and IR20 (negative control) (Table 1), were
germinated and transplanted to pots containing clay soil
(EC = 2.687 dS m⁻¹; pH = 5.5; organic matter = 10.36%; total
nitrogen = 0.17%; total phosphorus = 0.07%; total potassium
= 2.687 dS m⁻¹) and IR20 (negative control) (Table 1), were
germinated and transplanted to pots containing clay soil
(30 × 45 cm). Water irrigation was supplied using a
moisture spray. Acclimatised plants were transferred directly
to water-flooded pots (15 cm in diameter × 30 cm in height)
containing clay soil.

Water deficit treatments

The experiment site was located at the Thailand Science
Park, Pathumthani, Thailand (Latitude 14°01'12"N,
Longitude 100°31'12"E) and conducted between August and
November 2010. In the booting stage [85 days after sowing
(DAS)], soil water content (SWC) was adjusted to 56% (well
irrigation or control), 25% (7 days withholding irrigation or
mild water-deficit), 7% (SD: 14 days withholding irrigation
or severe water deficit) and recovery (re-watering). 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 related gene expression

Leaf tissues were frozen in liquid nitrogen and ground, then,
mRNA was extracted using the modified cetyl trimethyl
ammonium bromide (CTAB)-based extraction method
(Chang et al., 1993). For cDNA synthesis, 1 µg of total RNA,
treated with RQ1 RNase-free DNase (Promega, Madison,
WI), was reverse-transcribed with a First Strand cDNA
Synthesis kit (SuperScript II strand synthesis system;
Invitrogen, Carlsbad, CA) according to the manufacturer’s
instructions. Quantitative RT-PCR (qRT-PCR) reactions
were carried out on a 7500 Real-Time PCR System (Applied
Biosystems) with primers as described in Table 1.

The reaction cycles were modified as follows: 94°C for 10
min, 40 cycles of 94°C for 3 s followed

<table>
<thead>
<tr>
<th>Rice genotypes</th>
<th>Photoperiod sensitive</th>
<th>Aroma Flavor</th>
<th>Originated genotypes</th>
<th>Drought tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA11</td>
<td>+</td>
<td>+</td>
<td>KDML105 mutant</td>
<td>UK</td>
</tr>
<tr>
<td>EE12</td>
<td>+</td>
<td>+</td>
<td>KDML105 mutant</td>
<td>UK</td>
</tr>
<tr>
<td>FF17</td>
<td>+</td>
<td>+</td>
<td>KDML105 mutant</td>
<td>UK</td>
</tr>
<tr>
<td>NSG19</td>
<td>+</td>
<td>-</td>
<td>Inbred line</td>
<td>DT</td>
</tr>
<tr>
<td>KDML105</td>
<td>+</td>
<td>-</td>
<td>Natural selection</td>
<td>MDT</td>
</tr>
<tr>
<td>IR20</td>
<td>-</td>
<td>-</td>
<td>Inbred line</td>
<td>DS</td>
</tr>
<tr>
<td>PT1</td>
<td>-</td>
<td>-</td>
<td>Inbred line</td>
<td>DS</td>
</tr>
</tbody>
</table>

+ = Positive to target character, - = Negative to target character, UK = Unknown genotypes, DS = Drought sensitive, DT = Drought tolerance, MDT = Moderate drought tolerance

Fig 1. Relative expression levels of P5CS (A), P5CR (B) and ProDH mRNAs (C) in rice genotypes in the booting stage when exposed to water-deficit stress and during recovery. Error bars represented by ±SE. Different letters in each rice line show significant difference at p ≤ 0.01 by Least Significant Difference (LSD).
Table 2. Sequence of primers for real-time RT PCR.

<table>
<thead>
<tr>
<th>Name</th>
<th>Accession Number</th>
<th>Forward primer (5′→3′)</th>
<th>Reverse primer (5′→3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P5CS</td>
<td>D49714.1</td>
<td>TGGCAATTCGAAGTGTAAAT</td>
<td>AGAAATCTGCTACTTCATC</td>
</tr>
<tr>
<td>P5CR</td>
<td>NM_001051928.1</td>
<td>TGGTCTGTCATCGAAAGTT</td>
<td>TCGCTGTTCAACATTTC</td>
</tr>
<tr>
<td>ProDH</td>
<td>NM_001071853.1</td>
<td>AGTGGCTGCTGCTCTCCTTCT</td>
<td>AGTACCTGATGCTCTACCTC</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>D12629</td>
<td>TGTCCTCGCCCTCCCTT</td>
<td>GCCATGTATATAAGTCCTCAATOC</td>
</tr>
</tbody>
</table>

Fig 2. Proline content of rice genotypes in the booting stage when exposed to water-deficit stress and during recovery. Error bars represented by ±SE. Different letters in each rice line show significant difference at $p \leq 0.01$ by Least Significant Difference (LSD).

Proline content assay

Proline in the flag leaf tissues was extracted and analysed according to the method of Bates et al. (1973). Fifty milligrams of fresh material was ground with liquid nitrogen in a mortar. The homogenate powder was mixed with 1 mL aqueous sulfosalicylic acid (3% w/v) and filtered through filter paper (Whatman #1, England). 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 M $\text{H}_3\text{PO}_4$) and filtered through paper (Whatman #1, England). The reaction was terminated by placing the container in an ice bath. The reaction mixture was mixed vigorously with 2 mL toluene. After cooling to 25°C, the chromophore was measured by spectrophotometer (HACH DR/4000; Model 48000, HACH Company, Loveland, Colorado, USA) at 520 nm using L-proline as a standard.

Photosynthetic abilities

Chlorophyll fluorescence emission from the adaxial surface on the leaf was measured using 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). A leaf, adapted to dark conditions for 30 min using leaf-clips, was initially exposed to the modulated measuring beam of far-red light (LED source with typical peak at wavelength 735nm). Original ($F_o$) and maximum ($F_m$) fluorescence yields were measured under weak modulated red light ($<0.5\mu\text{mol} \text{ m}^{-2} \text{s}^{-1}$) with 1.6 s pulses of saturating light ($>6.8\mu\text{mol} \text{ m}^{-2} \text{s}^{-1}$ PAR) and calculated using FMS software for Windows®. The variable fluorescence yield ($F_v$) was calculated by the equation of $F_m – F_o$. The ratio of variable to maximum fluorescence ($F_v/F_m$) was calculated as maximum quantum yield of PSII photochemistry. Net photosynthetic rate ($\text{P}_n$; $\mu\text{mol} \text{ m}^{-2} \text{s}^{-1}$), transpiration rate ($E$; mmol m$^{-2}$ s$^{-1}$) and water use efficiency (WUE; %) were measured using a Portable Photosynthesis System (Model LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA) with an Infra-red Gas Analyser following Cha-un et al. (2007). WUE was calculated according to the equation: WUE (%) = $[\text{P}_n/E] \times 100$.

Experiment design

The experiment was arranged as Completely Randomized Block Design (CRBD) with six replicates (n=6). The mean values obtained were compared using Least Significant Difference (LSD) and analyzed with SPSS software.

Conclusion

Proline biosynthesis genes, including $P5CS$ and $P5CR$, in rice lines were regulated by water-deficit stress, especially severe water-deficit stress (7% SWC). Moreover, the regulation of $ProDH$ gene in all rice lines was found to be evident in the recovery step, having a function in proline degradation, and relating to low proline content. Physiological and growth performance in three mutant lines, NSG19 (water-deficit tolerant) and KDML105 were better than those in IR20...
Table 3. Maximum quantum yield of PSII \((F_v/F_m)\), water use efficiency (WUE), net photosynthetic rate \((P_n)\) and plant height (PH) of rice genotypes in the booting stage, when exposed to water-deficit stress and during recovery.

<table>
<thead>
<tr>
<th>Rice genotypes</th>
<th>Water stress</th>
<th>(F_v/F_m)</th>
<th>WUE ((\mu\text{mol CO}_2/\mu\text{mol }\text{H}_2\text{O}))</th>
<th>(P_n) ((\mu\text{mol }\text{CO}_2/\text{m}^2\text{ s}^{-1}))</th>
<th>PH (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA11</td>
<td>Well watering</td>
<td>0.910a</td>
<td>12.20a</td>
<td>8.16b</td>
<td>72.25a</td>
</tr>
<tr>
<td></td>
<td>25% SWC</td>
<td>0.785c</td>
<td>6.53b</td>
<td>5.17c</td>
<td>72.75a</td>
</tr>
<tr>
<td></td>
<td>7% SWC</td>
<td>0.783c</td>
<td>2.42c</td>
<td>3.47d</td>
<td>69.75b</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>0.802b</td>
<td>12.8a</td>
<td>10.26a</td>
<td>65.13b</td>
</tr>
<tr>
<td>EE12</td>
<td>Well watering</td>
<td>0.900a</td>
<td>9.90a</td>
<td>7.45b</td>
<td>65.33a</td>
</tr>
<tr>
<td></td>
<td>25% SWC</td>
<td>0.777b</td>
<td>6.71b</td>
<td>5.64c</td>
<td>68.50a</td>
</tr>
<tr>
<td></td>
<td>7% SWC</td>
<td>0.773b</td>
<td>3.38c</td>
<td>4.01c</td>
<td>59.88b</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>0.786b</td>
<td>9.91a</td>
<td>10.58a</td>
<td>60.50b</td>
</tr>
<tr>
<td>FF17</td>
<td>Well watering</td>
<td>0.880a</td>
<td>8.10a</td>
<td>8.38a</td>
<td>64.75b</td>
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<td></td>
<td>25% SWC</td>
<td>0.811b</td>
<td>5.47b</td>
<td>6.13c</td>
<td>61.50a</td>
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<td></td>
<td>7% SWC</td>
<td>0.767c</td>
<td>5.45b</td>
<td>4.41d</td>
<td>55.75b</td>
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<tr>
<td></td>
<td>Recovery</td>
<td>0.817b</td>
<td>8.07a</td>
<td>7.36b</td>
<td>54.00b</td>
</tr>
<tr>
<td>NSG19</td>
<td>Well watering</td>
<td>0.815a</td>
<td>18.78a</td>
<td>8.29a</td>
<td>71.13a</td>
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<tr>
<td></td>
<td>25% SWC</td>
<td>0.772b</td>
<td>7.07c</td>
<td>7.76a</td>
<td>68.00a</td>
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<tr>
<td></td>
<td>7% SWC</td>
<td>0.762b</td>
<td>3.91d</td>
<td>6.67b</td>
<td>68.25a</td>
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<tr>
<td></td>
<td>Recovery</td>
<td>0.810a</td>
<td>14.56b</td>
<td>7.86a</td>
<td>70.50a</td>
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<tr>
<td>KDML105</td>
<td>Well watering</td>
<td>0.814a</td>
<td>8.13a</td>
<td>8.07a</td>
<td>81.13a</td>
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<tr>
<td></td>
<td>25% SWC</td>
<td>0.802a</td>
<td>5.45b</td>
<td>6.91b</td>
<td>80.75a</td>
</tr>
<tr>
<td></td>
<td>7% SWC</td>
<td>0.797a</td>
<td>2.37c</td>
<td>5.70c</td>
<td>71.55b</td>
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<tr>
<td></td>
<td>Recovery</td>
<td>0.812a</td>
<td>9.98a</td>
<td>7.79a</td>
<td>69.20b</td>
</tr>
<tr>
<td>IR20</td>
<td>Well watering</td>
<td>0.800a</td>
<td>7.04a</td>
<td>6.83a</td>
<td>48.75a</td>
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<tr>
<td></td>
<td>25% SWC</td>
<td>0.756b</td>
<td>3.18b</td>
<td>5.84b</td>
<td>52.50a</td>
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<tr>
<td></td>
<td>7% SWC</td>
<td>0.593c</td>
<td>0.67c</td>
<td>2.44c</td>
<td>40.00b</td>
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<tr>
<td></td>
<td>Recovery</td>
<td>0.818a</td>
<td>8.34a</td>
<td>5.40b</td>
<td>36.00c</td>
</tr>
<tr>
<td>PT1</td>
<td>Well watering</td>
<td>0.832a</td>
<td>7.02a</td>
<td>7.15a</td>
<td>108.67a</td>
</tr>
<tr>
<td></td>
<td>25% SWC</td>
<td>0.758b</td>
<td>3.64b</td>
<td>5.50b</td>
<td>108.75a</td>
</tr>
<tr>
<td></td>
<td>7% SWC</td>
<td>0.580c</td>
<td>0.43c</td>
<td>1.47d</td>
<td>93.00b</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>0.808ab</td>
<td>2.81b</td>
<td>3.85c</td>
<td>86.67c</td>
</tr>
</tbody>
</table>

Different letters in each rice line show significant difference at \(p \leq 0.01\) by Least Significant Difference (LSD).

Fig 3. Relationships between \(P5CS\) expression and proline content (A) and \(P5CR\) expression and proline content (B) of rice genotypes in the booting stage exposed to water-deficit stress and relationship between \(ProDH\) expression and proline content (C). Error bars represented by ±SE.
(water deficit susceptible) and PT1. Also, the mutant lines AA11, EE12 and FF17 should be classified as moderate water-deficit tolerant as KDML105, according to physiological characters and growth performances.

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Fig 4. Relationship between maximum quantum yield of PSII (F/Fo) and net photosynthetic rate (Pn) of rice genotypes in the booting stage exposed to water-deficit stress. Error bars represented by ±SE.

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