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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 NSG19, IR20 and PT1 were up-regulated by the recovery process to a significant degree ($p \leq 0.01$). A positive relationship between *P5CS* expression level and proline content in rice genotypes subjected to water-deficit stress was evidently stated ($R^2 = 0.60$). 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 (F_v/F_m), water use efficiency (WUE) and net photosynthetic rate (P_n) 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: F_v/F_m_maximum quantum yield of PSII; P_n_net photosynthetic rate; P5C_Δ^1-pyrroline-5-carboxylate; P5CDH_Δ^1-pyrroline-5-carboxylate dehydrogenase; P5CR_Δ^1-pyrroline-5-carboxylate reductase; P5CS_Δ^1-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 semiarid 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 (Δ^1-pyrroline-5-carboxylate synthetase) to P5C (Δ^1-pyrroline-5-carboxylate), subsequently oxidized to the final product proline by P5CR (Δ^1-pyrroline-5-carboxylate reductase). Also, proline degradation has been discovered through ProDH (proline dehydrogenase) from proline to P5C (Δ^1-pyrroline-5-carboxylate) and then P5CDH (Δ^1-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 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, OsProT (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 semiarid 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, NSG19 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 (KDML 105), Pathumthani 1 (PT1) rice cultivar, tolerant (NSG 19) and sensitive (IR 20) cultivars. Expression level of *P5CS* in three mutants and wild type (KDML105), with well watered plants was very low. In mild water deficit conditions (25% SWC), *P5CS* mRNA in the mutant lines, KDML105 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, KDML105 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, KDML105, 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, KDML105, 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 ($R^2 = 0.60$) 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; $R^2 = 0.07$) as well as the regulation of *ProDH* gene was unconnected to proline content (Fig. 3C; $R^2 = 0.28$).

Photosynthetic abilities and growth characters

Maximum quantum yield of PSII (F_v/F_m), water use efficiency (WUE) and net photosynthetic rate (P_n) in the flag leaf tissues were measured as physiological characters. In well watered plants, the F_v/F_m, WUE and P_n in flag leaf tissues were elevated when compared with those in water deficit stressed plants. The F_v/F_m 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 severe water-deficit (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 F_v/F_m and P_n was found (Fig. 4). P_n in NSG19 (19.54% reduction) and KDML105 (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 P_n 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 NSG19, 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 susceptible Ca/H 148 (Parida et al. 2008). In addition, the expression levels of *P5CS* and *P5CR* genes in drought tolerant A1 safflower were better than those in the 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 KDML105 (Thai jasmine rice), are exhibited significantly in response to NaCl salt stress (Somboonwatthanaku et al., 2010). In Andean potatoes, 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 (Schafleitner 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 (Sziderics 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

Table 1. Photoperiod sensitive, originated cultivars and drought tolerant abilities of rice genotypes.

Rice genotypes	Photoperiod sensitive	Aroma flavor	Originated genotypes	Drought tolerance
AA11	+	+	KDML105 mutant	UK
EE12	+	+	KDML105 mutant	UK
FF17	+	+	KDML105 mutant	UK
NSG19	+	-	Inbred line	DT
KDML105	+	+	Natural selection	MDT
IR20	-	-	Inbred line	DS
PT1	-	-	Inbred line	DS

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

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 (Schafleitner et al., 2007; Hien et al., 2003).

Materials and methods

Plant materials

M_4 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 = 1.19%) in 50% shading (acclimatisation) light intensity and grown on for 2 weeks. The pots were arranged on plastic trays (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

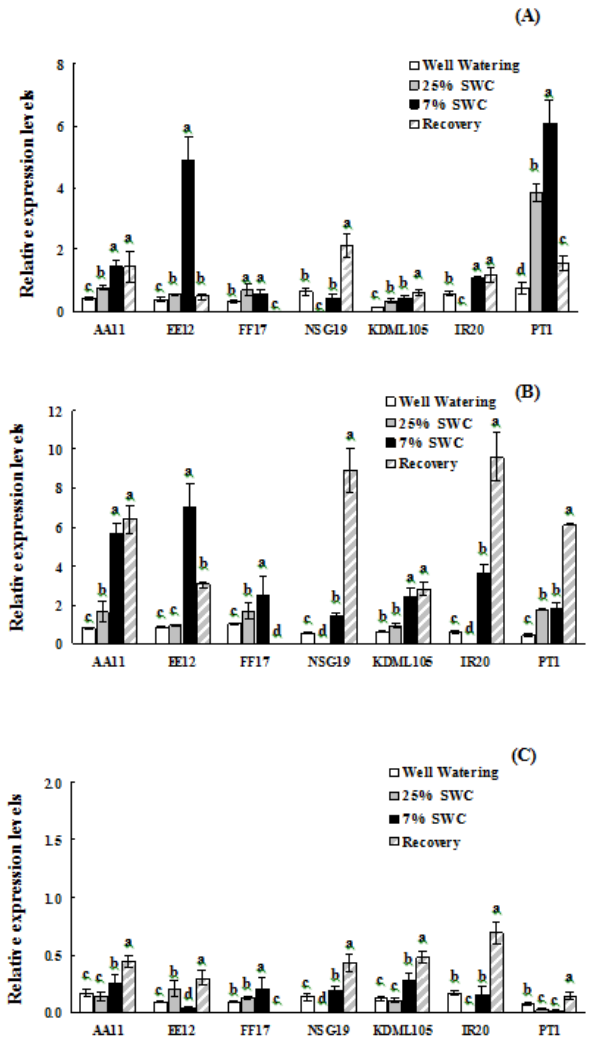

Fig 1. Relative expression levels of *P5CS* (A), *P5CR* (B) and *ProDH* mRNAs (C) in rice genotypes in the booting stage when exposed to water-deficit stress and during recovery. Error bars represented by ±SE. Different letters in each rice line show significant difference at $p \leq 0.01$ by Least Significant Difference (LSD).

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 2. Sequence of primers for real-time RT PCR.

Name	Accession Number	Forward primer (5'→3')	Reverse primer (5'→3')
P5CS	D49714.1	TGGCAATTCGAAGTGGTAAT	AGCAAATCTGCGATCTCATC
P5CR	NM_001051928.1	TGGTCTGGTCATCGAAGATT	TCAGCTGTCCAAACTTTTCC
ProDH	NM_001071853.1	ATTGCTCTCGTCTTCCTCCT	ATGACTCGATCGCTTCACTC
Ubiquitin	D12629	TTGTCCTGCGCCTCCGT	GGCATAGGTATAATGAAGTCCAATGC

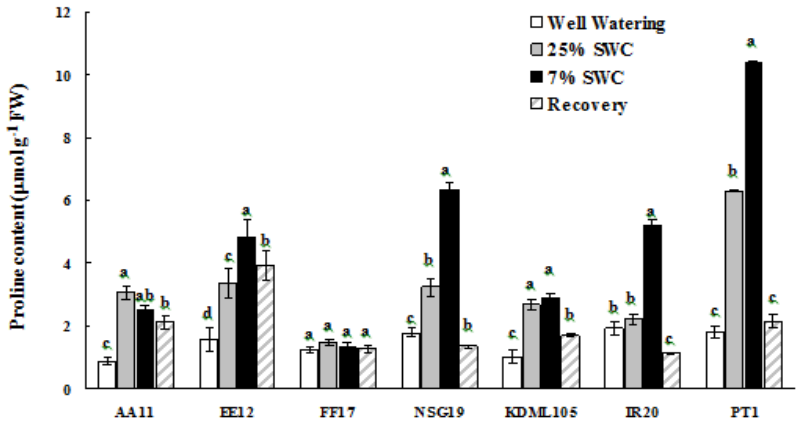

Fig 2. Proline content of rice genotypes in the booting stage when exposed to water-deficit stress and during recovery. Error bars represented by ±SE. Different letters in each rice line show significant difference at $p \leq 0.01$ by Least Significant Difference (LSD).

by 58°C for 30 s, according to the manufacturer's protocol (Kapa SYBR Fast qPCR Kit, Kapa Biosystems Technologies). The amplification efficiency of the reaction was calculated from the component data using LinRegPCR (Ramakers et al., 2003). Gene expression was calculated in relation to the level of ubiquitin gene expression using the delta CT method.

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 H_3PO_4) 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 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_0) and maximum (F_m) fluorescence yields were measured under weak modulated red light (<0.5μmol m^{-2} s^{-1}) with 1.6 s pulses of saturating light (>6.8μmol m^{-2} 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_0. The ratio of variable to maximum fluorescence (F_v/F_m) was calculated as maximum quantum yield of PSII photochemistry. Net photosynthetic rate (P_n; μmol m^{-2} 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-um et al. (2007). WUE was calculated according to the equation: WUE (%) = [P_n/E] × 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.

Rice genotypes	Water stress	F_v/F_m	WUE ($\mu mol\ CO_2\ mmol^{-1}\ H_2O$)	P_n ($\mu mol\ m^{-2}\ s^{-1}$)	PH (cm)
AA11	Well watering	0.910a	12.20a	8.16b	72.25a
	25% SWC	0.785c	6.53b	5.17c	72.75a
	7% SWC	0.783c	2.42c	3.47d	69.75b
	Recovery	0.802b	12.8a	10.26a	65.13b
EE12	Well watering	0.900a	9.90a	7.45b	65.33a
	25% SWC	0.777b	6.71b	5.64c	68.50a
	7% SWC	0.773b	3.38c	4.01c	59.88b
	Recovery	0.786b	9.91a	10.58a	60.50b
FF17	Well watering	0.880a	8.10a	8.38a	64.75a
	25% SWC	0.811b	5.47b	6.13c	61.50a
	7% SWC	0.767c	5.45b	4.41d	55.75b
	Recovery	0.817b	8.07a	7.36b	54.00b
NSG19	Well watering	0.815a	18.78a	8.29a	71.13a
	25% SWC	0.772b	7.07c	7.76a	68.00a
	7% SWC	0.762b	3.91d	6.67b	68.25a
	Recovery	0.810a	14.56b	7.86a	70.50a
KDML105	Well watering	0.814a	8.13a	8.07a	81.13a
	25% SWC	0.802a	5.45b	6.91b	80.75a
	7% SWC	0.797a	2.37c	5.70c	71.55b
	Recovery	0.812a	9.98a	7.79a	69.20b
IR20	Well watering	0.800a	7.04a	6.83a	48.75a
	25% SWC	0.756b	3.18b	5.84b	52.50a
	7% SWC	0.593c	0.67c	2.44c	40.00b
	Recovery	0.818a	8.34a	5.40b	36.00c
PT1	Well watering	0.832a	7.02a	7.15a	108.67a
	25% SWC	0.758b	3.64b	5.50b	108.75a
	7% SWC	0.580c	0.43c	1.47d	93.00b
	Recovery	0.808ab	2.81b	3.85c	86.67c

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

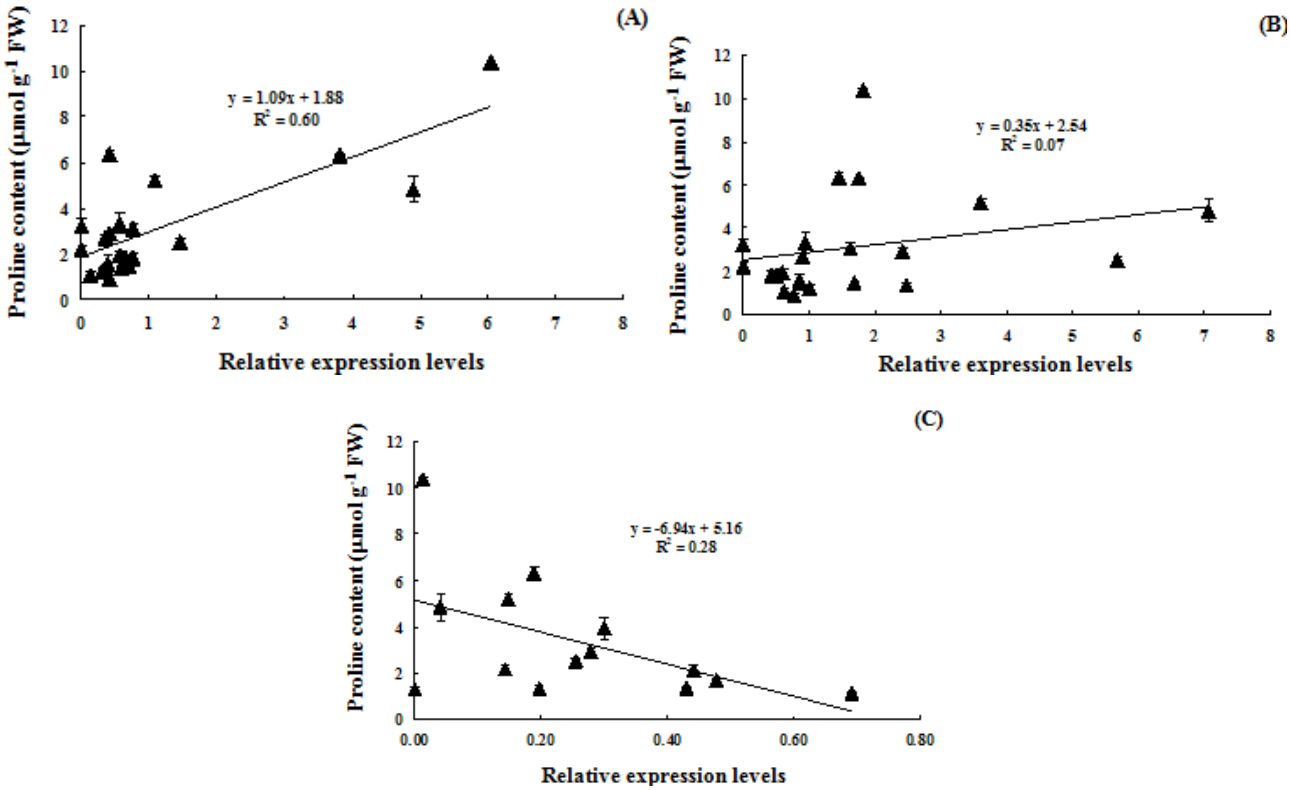

Fig 3. Relationships between *P5CS* expression and proline content (A) and *P5CR* expression and proline content (B) of rice genotypes in the booting stage exposed to water-deficit stress and relationship between *ProDH* expression and proline content (C). Error bars represented by ±SE.

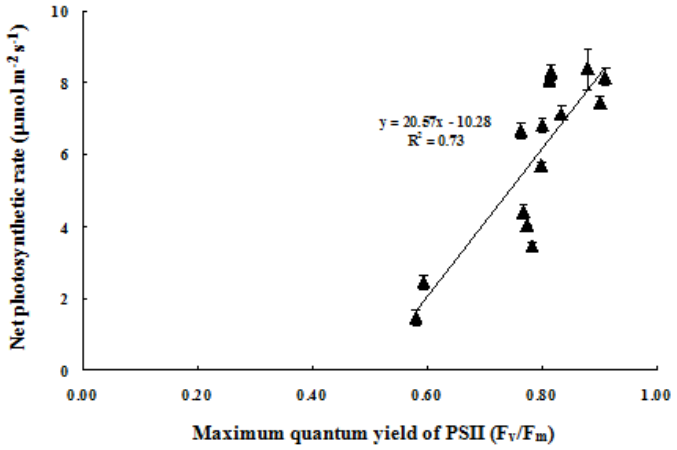

Fig 4. Relationship between maximum quantum yield of PSII (F_v/F_m) and net photosynthetic rate (P_n) of rice genotypes in the booting stage exposed to water-deficit stress. Error bars represented by ±SE.

(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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References

Anyia AO, Herzog H (2004) Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. Europ J Agron 20:327–339

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Bouman BAM, Humphreys E, Tuong TP, Barker R (2006) Rice and water. Adv Agron 92:187–237

Cabuslay GS, Ito O, Alejar AA (2002) Physiological evaluation of responses of rice (*Oryza sativa* L.) to water-deficit. Plant Sci 163:815–827

Chang S, Puryear J, Cairney J (1993) A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep 11:113–116

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

Cha-um S, Yooyongwech S, Supaibulwatana K (2010) Water-deficit stress in the reproductive stage of four indica rice (*Oryza sativa* L.) genotypes. Pak J Bot 42:3387–3398

Cha-um S, Yooyongwech S, Supaibulwatana K (2012) Water-deficit tolerant classification in mutant lines of indica rice (*Oryza sativa* L. spp. *indica*). Sci Agric 69:135–141

Chaves MM, Oliveira MM (2004) Mechanisms underlying plant resilience to water-deficits: prospects for water-saving agriculture. J Exp Bot 55:2365–2384

Choudhary NL, Sairam RK, Tyagi A (2005) Expression of Δ^1-pyrroline-5-carboxylate synthetase gene during drought in rice (*Oryza sativa* L.). Ind J Biochem Biophys 42:366–370

Coombs J, Hall DO, Long SP, Scurlock JMO (1987) Techniques in Bioproductivity and Photosynthesis. Pergamon Oxford

Dalauney AJ, Verma DPS (1993) Proline biosynthesis and osmoregulation in plants. Plant J 4:215–223

Fukai S, Pantuwan G, Jongdee B, Cooper M (1999) Screening for drought resistance in rainfed lowland rice. Field Crops Res 64:61–74

Grallath S, Weimar T, Meyer A, Gumy C, Suter-Grotemeyer M, Neuhaus JM, Rentsch D (2005) The AtProT family. Compatible solute transporters with similar substrate specificity but differential expression patterns. Plant Physiol 137:117–126

Guha A, Sengupta D, Rasineni GK, Reddy AR (2010) An integrated diagnostic approach to understand drought tolerance in mulberry (*Morus indica* L.). Flora 205:144–151

Hare PD, Cress WA, van Staden J (1999) Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. J Exp Bot 50:413–434

Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul 21:79–102

Hien DT, Jacobs M, Angenon G, Hermans C, Thu TT, Son LV, Roosens NH (2003) Proline accumulation and Δ^1-pyrroline-5-carboxylate synthetase gene properties in three rice cultivars differing in salinity and drought tolerance. Plant Sci 165:1059–1068

Hsu SY, Hsu YT, Kao CH (2003) The effect of polyethylene glycol on proline accumulation in rice leaves. Biol Plant 46:73–78

Igrashi Y, Yoshiba Y, Takeshita T, Nomura S, Otomo J, Yamaguchi-Shinozaki K, Shinozaki K (2000) Molecular cloning and characterization of a cDNA encoding proline transporter in rice. Plant Cell Physiol 41:750–756

Jung Y, Park J, Choi Y, Yang JG, Kim D, Kim BG, Roh K, Lee DH, Auh CK, Lee S (2010) Expression analysis of proline metabolism-related genes from halophyte *Arabis stelleri* under osmotic stress conditions. J Integra Plant Biol 52:891–903

Khush GS (2005) What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol Biol 59:1–6

Kishor PBK, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Curr Sci 88:424–438

Kumar R, Sarawgi AK, Ramos C, Amarante ST, Ismail AM, Wade LJ (2006) Partitioning of dry matter during drought stress in rainfed lowland rice. Field Crops Res 98:1–11

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

Pantuwan G, Fukai S, Cooper M, Rajatasereekul S, O'Toole JC (2002) Yield response of rice (*Oryza sativa* L.) genotypes to drought under rainfed lowlands 2. Selection of drought resistant genotypes. Field Crops Res 73:169–180

Parida AS, Dagaonkar VS, Phalak MS, Aurangabadkar LP (2008) Differential responses of the enzymes involved in proline biosynthesis and degradation in drought tolerant and sensitive cotton genotypes during drought stress and recovery. Acta Physiol Plant 30:619–627

Passioura J (2007) The drought environment: physical, biological and agricultural perspectives. J Exp Bot 58:113–117

Ramakers C, Ruijter JM, Deprez RHL, Moorman AFM (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci Letts 339:62–66

Raymond MJ, Smirnoff N (2002) Proline metabolism and transport in maize seedlings at low water potential. Ann Bot 89:813–823

Schafleitner R, Gaudin A, Rosales ROG, Aliaga CAA, Bonierbale M (2007) Proline accumulation and real time PCR expression analysis of genes encoding enzymes of proline metabolism in relation to drought tolerance in Andean potato. Acta Physiol Plant 29:19–26

Somboonwatthanaku I, Dorling S, Leung S, McMaanus MT (2010) Proline biosynthetic gene expression in tissue cultures of rice (*Oryza sativa* L.) in response to saline treatment. Plant Cell Tiss Org Cult 103:369–376

Su J, Wu R (2004) Stress-inducible synthesis of proline in transgenic rice confers faster growth under stress conditions than that with constitutive synthesis. Plant Sci 166:941–948

Szabados L, Savouré A (2009) Proline: a multifunctional amino acid. Trends Plant Sci 15:89–97

Sziderics AH, Oufir M, Trognitz F, Kopecky D, Matušíková I, Hausman JF, Wilhelm E (2010) Organ-specific defense strategies of pepper (*Capsicum annuum* L.) during early phase of water-deficit. Plant Cell Rep 29:295–305

Theerawitaya C, Triwitayakorn K, Kirdmanee C, Smith D and Supaibulwatana K (2011) Genetic variations associated with salt tolerance detected in mutants of KDML105 (*Oryza sativa* L. spp. *indica*) rice. Australian Journal of Crop Science 5:1475–1480

Thippeswamy M, Chandraobulreddy P, Sinilal B, Kumar MS, Sudhakar C (2010) Proline accumulation and the expression of Δ^1-pyrroline-5-carboxylate synthetase in two safflower cultivars. Biol Plant 54:386–390

Trovato M, Mattioli R, Costantino P (2008) Multiple roles of proline in plant stress tolerance and development. Rendiconti Lincei 19:325–346

Uyprasert S, Toojinda T, Udomprasert N, Tragoonrung S, Vanavichit A (2004) Proline accumulation and rooting pattern in rice in response to water-deficit under rainfed lowlands. Sci Asia 30:301–311

Vendruscolo ECG, Schuster I, Pileggi M, Scapim CA, Molinari HBC, Murur CJ, Vieira LGE (2007) Stress-induced synthesis of proline confers tolerance to water-deficit in transgenic wheat. J Plant Physiol 164:1367–1376

Verbruggen N, Hermans C (2008) Proline accumulation in plants: a review. Amino Acids 35:753–759

Verslues PE, Kim YS, Zhu JK (2007) Altered ABA, proline and hydrogen peroxide in an *Arabidopsis* glutamate:glyoxylate aminotransferase mutant. Plant Mol Biol 64:205–217

Verslues PE, Sharma S (2010) Proline metabolism and its implications for plant-environment interaction. Arabidopsis Book 8:1–23

Xue X, Liu A, Hua X (2008) Proline accumulation and transcriptional regulation of proline biosynthesis and degradation in *Brassica napus*. BMB Rep 42:28–34

Yoshiba Y, Kiyosue T, Katagiri T, Ueda H, Mizoguchi T, Yamaguchi-Shinozaki K, Wada K, Harada T, Shinozaki K (1995) Correlation between the induction of a gene for Δ^1-pyrroline-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis thaliana* under osmotic stress. Plant J 7:751–760

Yoshiba Y, Kiyosue T, Nakashima K, Yamaguchi-Shinozaki K, Shinozaki K (1997) Regulation of levels of proline as an osmolyte in plants under water stress. Plant Cell Physiol 38:1095–1102

Zhu B, Su J, Chang M, Verma DPS, Fan YL, Wu R (1998) Overexpression of a Δ^1-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water- and salt-stress in transgenic rice. Plant Sci 139:41–48