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Water-deficit tolerant classification in mutant lines of indica rice

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ABSTRACT: Water shortage is a major abiotic stress for crop production worldwide, limiting the productivity of crop species, especially in dry-land agricultural areas. This investigation aimed to classify the water-deficit tolerance in mutant rice (*Oryza sativa* L. spp. *indica*) genotypes during the reproductive stage. Proline content in the flag leaf of mutant lines increased when plants were subjected to water deficit. Relative water content (RWC) in the flag leaf of different mutant lines dropped in relation to water deficit stress. A decrease RWC was positively related to chlorophyll a degradation. Chlorophyll a , chlorophyll b , total chlorophyll , total carotenoids , maximum quantum yield of PSII , stomatal conductance , transpiration rate and water use efficiency in mutant lines grown under water deficit conditions declined in comparison to the well-watered, leading to a reduction in net-photosynthetic rate. In addition, when exposed to water deficit, panicle traits, including panicle length and fertile grains were dropped. The biochemical and physiological data were subjected to classify the water deficit tolerance. NSG19 (positive control) and DD14 were identified as water deficit tolerant, and AA11, AA12, AA16, BB13, BB16, CC12, CC15, EE12, FF15, FF17, G11 and IR20 (negative control) as water deficit sensitive, using Ward's method.

Keywords: ward cluster analysis, drought tolerance, fertile grain, photosynthetic pigments, water relation

Introduction

Water shortage is a major abiotic stress for crop production worldwide, limiting the productivity of crop species, especially in dry-land agricultural areas (> 1.2 billion hectares) (Chaves and Oliveira, 2004; Kijne, 2006; Passioura, 2007). Rice crop (*Oryza sativa* L.) improvement including conventional breeding program (Jongdee et al., 2006; Venuprasad et al., 2007; Kumar et al., 2008; Ndijiondjop et al., 2010; Guan et al., 2010) and mutant lines (Koh et al., 2007; Jiang et al., 2007; Cairns et al., 2009; Thang et al., 2010) is one of the strategies for water-deficit tolerant trait. Chemical and irradiation techniques are conventionally approached to induce mutation in a large population of mutant lines and pyramiding drought tolerant trait (Cairns et al., 2009; Thang et al., 2010). Plant biochemical changes, such as enhanced accumulation of stress metabolites and increased antioxidant enzymes are evidently expressed in plant responses to water deficit stress. Moreover, physiological characters include reduced relative water content, pigment degradation, decreased stomatal conductance, reduced internal CO_2 concentration, net photosynthetic rate (P_n) reduction and growth inhibition prior to plant death have been well published (Chaves and Oliveira, 2004; Reddy et al., 2004; Cattivelli et al., 2008).

Plant biochemical and physiological changes in crop species in response to water deficit stress have been implemented as criteria for screening water-deficit tolerance in plant breeding programs (Ashraf and Foolad, 2007; Ashraf, 2010). Yield traits are the most important criteria for water-deficit tolerance screening (Fukai et al., 1999; Yang et al., 2001; Pantuwan et al., 2002; Kumar et al., 2008). Some previous studies suggested that multivariate cluster analysis is practically required for water-deficit classification in rice breeding programs (Cabuslay et al., 2002; Cha-um et al., 2010). In Asian countries, rice plays a key role as major carbohydrate crop. It is a basal food, feeding more than 3 billion people and providing 50-80 % of their daily calorie intake (Khush, 2005). In rainfed paddy fields, water shortage has been well known as being a serious issue, especially in the reproductive stage, during which plants are particularly sensitive, leading to low crop yield (Fukai et al., 1999; Pantuwan et al., 2002; Bouman et al., 2006). In the genetic resources, NSG19 is a positive check of water-deficit tolerance, which is utilized in drought tolerance screening, whereas IR20 is a negative check (Mitchell et al., 1998; Wade et al., 1999; Pantuwan et al., 2002; Uyprasert et al., 2004; Kumar et al., 2006). This investigation aimed to classify the water-deficit tolerance in mutant rice genotypes during the reproductive stage.

Materials and Methods

M_4-seeds of twelve mutant rice cultivars, MT 4-01 (code AA11), MT 4-02 (code AA12), MT 4-03 (code AA16), MT 4-04 (code BB13), MT 4-05 (code BB16), MT 4-06 (code CC12), MT 4-07 (code CC15), MT 4-08 (code DD14), MT 4-09 (code EE12), MT 4-10 (code FF15), MT 4-11 (code FF17) and MT 4-12 (code G11), derived from γ-irradiation and ethyl methane sulfonate (EMS) mutagens of jasmine rice (*Oryza sativa* L. ssp. *indica* cv. KDML 105) 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 (acclimatization) 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. Acclimatized plants were transferred directly to water-flooded pots (15 cm in diameter × 30 cm in height) containing clay soil. The experiment site was located at Klong Luang, Pathumthani, Thailand (Latitude 14°01′12″ N and Longitude 100°31′12″ E) and conducted between Aug. and Nov. 2010. In the booting stage [85 days after sowing (DAS)], soil water content (SWC) was adjusted to 56 % (WW; full irrigation or well-watered) and 7 % (WD; 14 days withholding irrigation or water-deficit). The 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). Relative water content (RWC), proline content in the leaf blade, photosynthetic pigments, chlorophyll fluorescence, net-photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (g_s) in flag leaf and panicle traits in rice plants were measured.

Relative water content (RWC) was calculated according to Bonnet et al. (2000). This parameter was calculated from fresh weight (FW) dry weight (DW) and turgid weight (TW) following the equation; RWC (%) = [(FW-DW)/(TW-DW)] × 100.

Proline in the root and leaf tissues was extracted and analyzed 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.

Chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total chlorophyll (TC) content, were analyzed following the methods of Shabala et al. (1998) and total carotenoid (C_{x+c}) concentrations were assayed according to Lichtenthaler (1987). One hundred milligrams of leaf material was collected and placed in a 25 mL glass vial along with 10 mL 95.5 % 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 using a UV-visible spectrophotometer at 662 nm and 644 nm wavelengths. The C_{x+c} concentration was also measured by spectrophotometer (HACH DR/4000; Model 48000, HACH Company, Loveland, Colorado, USA) at 470 nm. A solution of 95.5 % acetone was used as a blank.

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 735 nm). 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}), stomatal conductance (g_s; mmol H_2O 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 equation: WUE (%) = [P_n/E] × 100. Panicle length, fertile grains, sterile grains, total grains and one-hundred seed weight per panicle in the well-watered (WW) or water deficit (WD) were measured. Yield loss, RWC and WUE abilities, proline accumulation, P_n reduction, F_v/F_m diminution, and pigment degradation were calculated following Cha-um et al. (2009).

The experiment was arranged as 12 × 2 factorials in Completely Randomized Block Design (CRBD) with eight replicates (n = 8). The mean values obtained were compared using Duncan's New Multiple Range Test (DMRT) and analyzed with SPSS software. Yield loss, RWC and WUE abilities, proline accumulation, P_n reduction, F_v/F_m diminution, and pigment degradation in the flag leaves of rice grown under severe water deficit stress were assessed in order to classify cultivars as either tolerant or sensitive using Ward's method of Hierarchical cluster analysis in SPSS software.

Results and Discussion

Proline content in flag leaf of water-deficit stressed plant was increased ($p \leq 0.01$) and higher than that in controlled plant for 1.3-3.5 folds. For example, proline in AA11 and CC15 grown under water deficit stress was accumulated for 3.5 and 3.4 folds, respectively when compared with those in well-water condition (Figure 1). Relative water content (RWC) in the flag leaf of mutant rice lines dropped in plants exposed to water deficit stress (Table 1). The reduction of RWC in AA16 and CC15 lines of mutant rice was 15.4 % and 14.6 % when subjected to water deficit stress. A positive correlation between RWC and chlorophyll content was demonstrated (Figure 2).

Photosynthetic pigments, chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll a (TC) and total

Table 1 – Relative water content (RWC), chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}) content in mutant rice genotypes grown under well-watered (WW) and water deficit stress (WD) for 14 days.

Rice lines	Water stress	RWC	Chl_a	Chl_b	TC	C_{x+c}
		%	μg g^{-1} FW			
AA11	WW	93.1 a	95.2 a	57.5 a	152.7 a	2.8 b
	WD	50.8 h	22.4 d	21.5 c	43.9 f	1.3 ef
AA12	WW	86.5 bc	89.6 ab	55.0 ab	144.6 b	3.3 ab
	WD	48.3 hi	29.0 d	21.5 c	50.5 f	1.7 de
AA16	WW	90.0 ab	92.7 a	56.2 ab	148.9 ab	2.9 b
	WD	76.2 de	19.7 d	22.7 c	42.4 f	1.2 ef
BB13	WW	90.0 ab	87.1 ab	56.5 ab	143.6 b	2.8 bc
	WD	57.0 g	22.9 d	19.6 c	42.5	1.9 de
BB16	WW	76.7 de	90.7 a	55.8 ab	146.5 ab	2.9 b
	WD	44.3 ij	24.1 d	19.8 c	43.9 f	0.9 g
CC12	WW	86.6 bc	80.2 ab	55.5 ab	135.7 cd	3.0 ab
	WD	40.5 jk	22.9 d	23.5 c	46.4 f	0.8 g
CC15	WW	85.0 bc	81.1 ab	56.7 a	137.8 cd	2.7 bc
	WD	72.6 e	22.1 d	19.8 c	46.9 f	0.7 g
DD14	WW	64.7 f	81.1 ab	53.7 ab	134.9 cd	3.1 ab
	WD	35.7 k	77.2 b	20.6 c	97.8 e	0.7 g
EE12	WW	93.0 a	82.9 ab	52.2 ab	135.1 cd	3.6 a
	WD	51.4 h	21.6 d	18.7 c	40.3 f	0.9 g
FF15	WW	80.9 cd	72.5 b	54.3 ab	126.18 d	2.7 bc
	WD	63.2 f	20.4 d	21.4 c	41.8 f	0.8 g
FF17	WW	74.9 e	50.2 c	51.0 ab	101.2 e	2.3 cd
	WD	53.4 gh	20.5 d	18.5 c	39.0 f	0.9 g
G11	WW	94.3 a	79.9 a	53.7 ab	133.6 d	3.0 ab
	WD	63.5 f	16.0 d	21.8 c	37.8 f	0.8 g

Different letters in each column show difference at $p \leq 0.01$ by Duncan's New Multiple Range Test (DMRT).

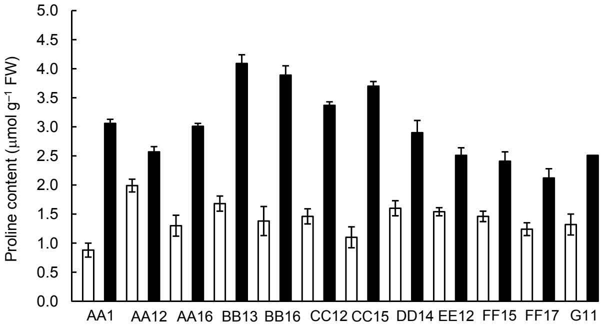

Figure 1 – Proline content in the flag leaf of mutant rice genotypes grown under well-watered (WW; light bar) and water deficit stress (WD; dark bar) for 14 days. Error bars represented by ±SE.

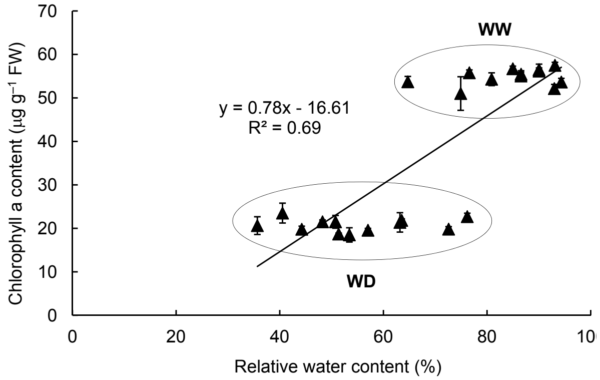

Figure 2 – Chlorophyll a content due to relative water content in mutant rice genotypes grown under well-watered (WW) and water deficit stress (WD) for 14 days. Error bars represented by ±SE.

carotenoids (C_{x+c}) in rice crop were decreased when subjected to water deficit stress (Table 1). In DD14 mutant lines, Chl_a content in water-deficit stressed plants was maintained (Table 1). The degradation percentage of Chl_a (5.1 %) and TC (27.4 %) in DD14 plants subjected to water-deficit was lower than in other rice lines (65.1-71.7 %). Degradation of Chl_a pigments was positively correlated to maximum quantum yield of PSII (F_v/F_m) (Figure 3), leading to reduced net photosynthetic rate (P_n) (Figure 4). Photosynthetic abilities including F_v/F_m, P_n, stomatal conductance (g_s) and transpiration rate (E) drastically decreased when exposed to water deficit conditions (Table 2). In addition, water use efficiency (WUE) was similarly trended, except in AA12, BB13, BB16, CC12 and CC15 was alleviated (16.9, 24.4, 8.3, 27.6 and 11.1 % reduction). Panicle length in rice lines grown under water deficit stress showed no differences, except in CC12 (25.4 % reduction) and FF15 (35.0 % reduction). Number of fertile grains decreased (*$p \leq 0.01$*) when plants subjected to water-deficit conditions whereas number of sterile grains was increased (Table 3). A thousand-grain weight was unchanged when subjected to water deficit except in

Table 2 – Maximum quantum yield of PSII (F_v/F_m), net-photosynthetic rate (P_n) transpiration rate (E), stomatal conductance (g_s) and water use efficiency (WUE) in mutant rice genotypes grown under well-watered (WW) and water deficit stress (WD) for 14 days.

Rice lines	Water stress	F_v/F_m	P_n	E	g_s	WUE
			μmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	mmol H_2O m^{-2} s^{-1}	%
AA11	WW	0.91 a	8.16 cd	2.64 a	0.074 a	33.0 a
	WD	0.78 b	3.47 gh	0.41 k	0.013 f	12.2 d
AA12	WW	0.89 a	8.41 bc	1.22 gh	0.036 cd	17.6 c
	WD	0.79 b	4.44 f	0.77 ij	0.017 ef	14.5 c
AA16	WW	0.89 a	6.01 e	2.20 b	0.036 cd	35.6 a
	WD	0.78 b	4.20f g	0.43 jk	0.014 f	9.7 d
BB13	WW	0.89 a	6.49 e	1.79 cd	0.030 cd	27.8 b
	WD	0.78 b	2.88 h	0.59 ij	0.015 ef	21.0 bc
BB16	WW	0.89 a	8.86 ab	1.39 fg	0.044 bc	15.7 c
	WD	0.78 b	2.95 h	0.42 k	0.014 f	14.4 c
CC12	WW	0.89 a	7.94 cd	1.48 ef	0.039 bc	18.7 c
	WD	0.79 b	4.61 f	0.63 ij	0.024 de	13.6 c
CC15	WW	0.88 a	8.45 bc	1.75 cd	0.042 bc	20.7 bc
	WD	0.77 b	4.89 f	0.90 hi	0.023 de	18.4 c
DD14	WW	0.90 a	6.30 e	2.13 bc	0.035 cd	33.8 a
	WD	0.79 b	3.52 gh	0.62 ij	0.014 f	18.2 c
EE12	WW	0.90 a	7.45 d	1.56 ef	0.042 bc	21.0 bc
	WD	0.77 b	4.41 f	0.43 jk	0.014 f	9.9 d
FF15	WW	0.91 a	6.61 e	1.91 bc	0.029	28.9 b
	WD	0.79 b	4.52 f	0.26 k	0.015 ef	5.8 e
FF17	WW	0.88 a	8.38 bc	1.75 de	0.053 b	20.9 bc
	WD	0.77 b	4.41 f	0.36 k	0.012 f	8.1 d
G11	WW	0.90 a	9.51 a	1.76 de	0.034 cd	18.5 c
	WD	0.77 b	4.69 f	0.40 k	0.013 f	8.6 d

Different letters in each column show difference at $p \leq 0.01$ by Duncan's New Multiple Range Test (DMRT).

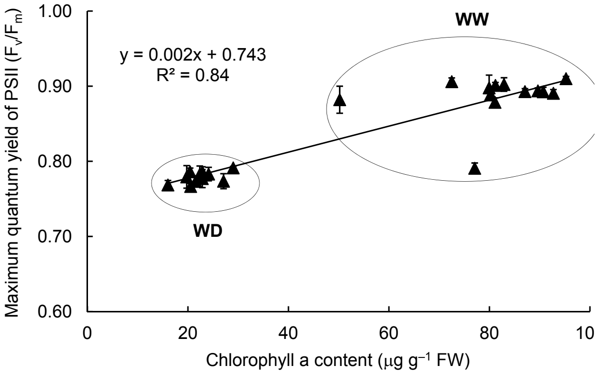

Figure 3 –Maximum quantum yield of PSII (F_v/F_m) due to chlorophyll a content in mutant rice genotypes grown under well-watered (WW) and water deficit stress (WD) for 14 days. Error bars represented by ±SE.

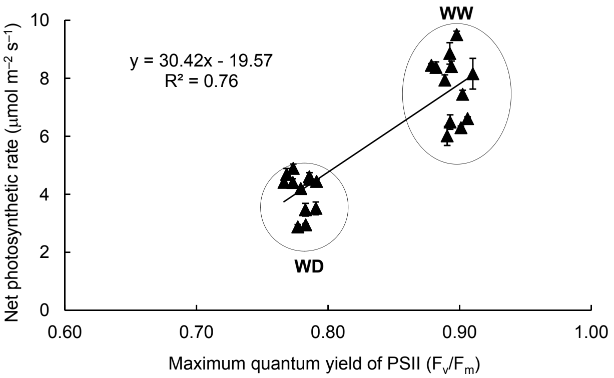

Figure 4 –Net photosynthetic rate (P_n) due to maximum quantum yield of PSII (F_v/F_m) in mutant rice genotypes grown under well-watered (WW) and water deficit stress (WD) for 14 days. Error bars represented by ±SE.

lines, BB13 and FF15. In addition, yield failure, RWC and WUE abilities, proline accumulation, P_n reduction, F_v/F_m diminution, and pigment degradation data were subjected to classify clusters of the group as water deficit tolerant, NSG19, DD14, and water deficit sensitive, AA11, AA12, AA16, BB13, BB16, CC12, CC15, EE12, FF15, FF17, G11 and IR20 using Ward's method (Figure 5).

Proline content in the flag leaf blade increased depending on the reduction of RWC in the leaf tissues. Proline accumulation in the rice crop has been well established as an effective indicator of plant responses to water deficit stress. For example, proline content in PT1 and IR20 (water deficit sensitive) peaked in plants subjected to severe water deficit (7 % SWC) and was higher than in the tolerant cultivars (KDML105 and NSG19) (Cha-um et al., 2010). Similarly, proline in the leaf tissues of drought sensitive, CR203 (3.12 times) is accumulated higher than in drought tolerant cultivars, DR2 (1.41 times) and Cuom (2.06 times), when plants are subjected to water deficit stress (Hien et al., 2003). In contrast,

Table 3 – Yield traits, panicle length (PL), fertile grains (FG), sterile grains (SG) and 1000- grain weight (TGW) in mutant rice genotypes grown under well-watered (WW) and water deficit stress (WD) for 14 days.

Rice lines	Water stress	PL	FG	SG	TGW
		cm			g
AA11	WW	20.88 ab	60.33 cd	18.00 g	27.24 a
	WD	19.33 b	28.33 hi	55.67 c	25.86 ab
AA12	WW	21.13 ab	68.33 bc	17.67 g	27.73 a
	WD	18.00 b	38.67 fg	48.67 c	24.61 ab
AA16	WW	24.38 a	86.00 ab	14.33 g	25.94 ab
	WD	20.33 ab	28.67 hi	82.33 ab	24.90 ab
BB13	WW	24.25 a	90.33 a	24.33 ef	28.23 a
	WD	22.33 a	43.00 ef	90.33 a	22.90 b
BB16	WW	20.63 ab	58.00 cd	19.67 fg	29.98 a
	WD	20.27 ab	27.00 hi	51.67 c	29.15 a
CC12	WW	19.88 ab	53.00 cd	18.33 g	25.83 ab
	WD	14.83 c	23.67 hi	33.67 de	24.14 ab
CC15	WW	19.75 ab	69.00 bc	23.67 ef	23.65 b
	WD	17.00 b	28.33 hi	45.33 cd	21.90 b
DD14	WW	20.00 ab	52.67 cd	18.67 g	27.91 a
	WD	18.33 b	28.00 hi	50.00 c	24.02 ab
EE12	WW	21.00 ab	60.33 cd	24.00 ef	25.39 ab
	WD	18.73 b	28.00 hi	44.33 cd	24.35 ab
FF15	WW	20.50 ab	48.00 ef	15.33 g	23.90 b
	WD	13.33 c	18.67 i	24.67 ef	17.30 c
FF17	WW	20.75 ab	63.33 cd	16.67 g	25.78 ab
	WD	18.67 b	25.00 hi	31.67 ef	23.77 b
G11	WW	20.38 ab	90.33 a	20.00f g	24.52 ab
	WD	18.33 b	31.33 gh	74.67 b	23.43 b

Different letters in each column show difference at $p \leq 0.01$ by Duncan's New Multiple Range Test (DMRT).

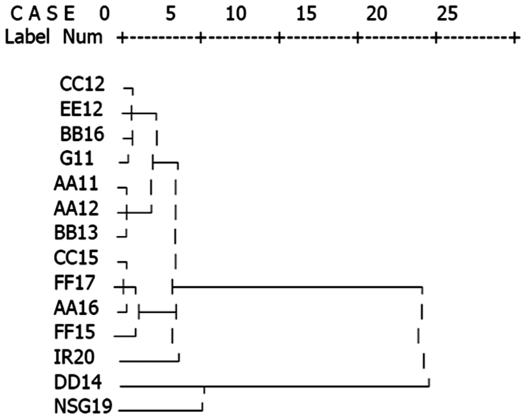

Figure 5 – Ward's dendrogram for mutant rice lines to classify as water-deficit sensitive, AA11, AA12, AA16, BB13, BB16, CC12, CC15, EE12, FF15, FF17, G11 and IR20 (negative control) and water-deficit tolerant, DD14 and NSG19 (positive control), using yield loss, RWC and WUE abilities, proline accumulation, P_n reduction, F_v/F_m diminution, and pigment degradation.

P5CR and *P5CS* genes in proline biosynthesis in drought tolerant (Ca/H680) are up-regulated resulting in enriched proline contents greater than those in drought sensitive (Ca/H148) when exposed to drought stress (Parida et al., 2008). So, proline accumulation in rice crop responses to drought stress is still unclear and unsuitable to play a critical role as good indicator. Relative water content (RWC) in mutant lines of rice had a similar decreasing trend when subjected to water deficit stress. For some rice lines (AA16 and CC15), RWC in the flag leaf tissues was maintained for 84.6 % and 85.4 %, respectively when exposed to water deficit stress. In Gangyon 527, Yixiangyou 9 and Gangyou 188 cultivars of rice, RWC in the leaves decreased depending on a degree of water deficit stress (Wang et al., 2010). In addition, the RWC in drought tolerant Ca/H 680 was maintained at higher levels than that in drought sensitive Ca/H 148 when subjected to drought conditions (Parida et al., 2008). However, only RWC in the leaf tissues is inappropriate to make a decision for the drought tolerance in rice cultivar (Pantuwan et al., 2002; Jongdee et al., 2006).

A reduction of RWC in the flag leaf was positively related to pigment degradation. In the present study, Chl_a and TC degradation percentage in "DD14" mutant lines in water deficit condition was lower than other lines. Similar results are presented in NSG19 and KDML105 grown under water deficit stress (Cha-um et al., 2010). Also, the photosynthetic abilities i.e. chlorophyll fluorescence and net photosynthetic rate (P_n) in drought tolerant genotype (NSG19) are also better than in drought sensitive genotype (IR20). For example, the P_n in the flag leaf of NSG19 was reduced in 19.5 % whereas P_n of IR20 was reduced in 64.3 % (Cha-um et al., 2010). In this investigation, two rice genotypes were chosen as positive (drought tolerance) and negative control (drought sensitive). Moreover, relative transpiration in NSG19 (0.35) and IR20 (0.48) quite differ when plants are subjected to water deficit (PEG-induced -0.5 MPa) for 6 days (Cabuslay et al., 2002).

The normalized transpiration rate (NTR) in three modern rice, BRS Primavera, BRSMG Curinga and BRS Soberana and one traditional rice, Douradão has been investigated as indicator in plant responses to the fraction of transpirable soil water (FTSW), which is identified the *p* factor (at 0.95 NTR) as the adaptability of rice genotypes to drought prone environments (Heinemann et al., 2011). In previous publications, the plant morphological characters i.e. leaf rolling, leaf chlorosis and green leaf area have been well established (Cabuslay et al., 2002; Bernier et al., 2008). Moreover, yield traits, such as grain yield, productivity and grain sterility are the most popular parameters used to identify water deficit tolerance in rice breeding programs (Fukai et al., 1999; Yang et al., 2001; Pantuwan et al., 2002; Kumar et al., 2008; Venuprasad et al., 2008; Wang et al., 2010). Multivariate indices including yield failure, RWC and WUE abilities, proline accumulation, P_n reduction, F_v/F_m diminution, and pigment degradation data have been employed to classify members of water deficit tolerance, NSG19 and DD14. In addition, water-deficit tolerance classification in rice crop using multivariate parameters has been well established (Cubusley et al., 2002; Jongdee et al., 2006; Cha-um et al., 2010).

In conclusion, chlorophyll a, total chlorophyll, total carotenoids and fertile grain traits of mutant rice genotypes "DD14" in response to water deficit stress were superior to those in other cultivars and can be played as multivariate criteria for water-deficit tolerance classification. Mutant rice line "DD14" was identified as water deficit tolerance using Ward's cluster analysis. The water-deficit tolerant line of rice from this investigation should be further verified in the rainfed paddy field.

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