

# Grain yield and aroma quality of upland rice (var. pare wangi) under various types and periods of drought stress

I G.B. ADWITA ARSA<sup>1\*</sup>, ARIFFIN<sup>2</sup>, NURUL AINI<sup>2</sup>, H.J.D. LALEL<sup>1</sup>

<sup>1</sup>Faculty of Agriculture, Universitas Nusa Cendana, Jl. Adisucipto, Penfui, Kupang 85148, East Nusa Tenggara, Indonesia. Tel./Fax. +62-380-881085, \*email: igbadwitaarsa@yahoo.com

<sup>2</sup>Faculty of Agriculture, Universitas Brawijaya, Jl. Veteran, Malang 65145, East Java, Indonesia

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**Abstract.** Arsa IGBA, Ariffin, Aini N, Lalel HJD. 2017. Grain yield and aroma quality of upland rice (var. pare wangi) under various types and periods of drought stress. *Trop Drylands 1*: 17-23. Rice with unique aroma is often preferable and this trait can be achieved through exposing rice plants to drought and salinity stresses. Yet, particular rice varieties might respond differently to such stresses. The objective of this study was to evaluate the effect of type and period of drought stress on grain yield, physiological characters and aroma quality of aromatic Pare Wangi upland rice variety. Two-factor experiment was conducted in a greenhouse employing a Completely Randomized Design with three replications. The first factor was type of drought stress and the second factor was plant growth stage at which drought stress period was started. Observed data included yield components and grain yield pot<sup>-1</sup>, physiological characters, and aroma quality. Observed data were subjected to analysis of variance, HSD post hoc test, and simple correlation analysis. The research results indicated that the salinity stress effect of 2.0 g NaCl kg soil<sup>-1</sup> caused a higher percentage of unfilled grain as compared to that of other types of stresses. When the salinity stress was started at the booting stage, the chlorophyll content of the rice plants was lower than that of other stresses. Soil moisture stress of 75% FC that was started at booting stage produced the highest 2AP content and aroma quality. Proline content was not significantly correlated with 2AP content but it showed a positive correlation with grain yield pot<sup>-1</sup>.

**Keywords:** Upland rice, drought stress, aroma, proline, chlorophyll

**Abbreviations:** FC: Field Capacity, DAS: days after seeding, 2AP: 2-Acetyl-1-Pyrroline, HSD: Honestly Significant Difference

## INTRODUCTION

Crops are generally cultivated under optimum environmental conditions with minimal environmental stresses. Nonetheless, environmental stress is sometimes needed to obtain the desired traits. For example, it was reported that drought stress, to a certain extent, is useful to increase rice aroma quality of aromatic rice (Buttery et al. 1983; Yoshihashi 2005). This is the case for the aromatic Pare Wangi upland rice variety, a superior local upland rice variety that is now widely cultivated by farmers in South West Sumba District, East Nusa Tenggara Province, Indonesia. The local growers prefer Pare Wangi variety due to its taste and scent aroma, in addition to the higher selling price of the aromatic rice as compared to the non-aromatic ones.

The mechanism of the appearance of aroma in rice in relation to drought stress can be explained as follow. Drought stress results in the increase of various osmotic compounds (Bianco et al. 2000), one of which from nitrogen group is proline amino acid (Mohammadkhani and Heidari 2008). In non-aromatic rice, proline will change into glutamate acid whilst in the aromatic rice, biosynthesis process will produce 2-Acetyl-1-Pyrroline or 2AP (Kibria et al. 2008; Fitzgerald et al. 2010). The difference in biosynthesis is associated with a gene mutation in chromosome number 8, exon 7 that is responsible for the

missing of aldehyde-betaine-enzyme activity (BADH<sub>2</sub>) (Bradbury 2009).

The increase of drought stress may be caused by a reduction in water supply from plant roots and excessive transpiration rate during the life cycle of the plant (Farooq et al. 2008, 2009). The reduction in water supply to the plant can be caused by a decrease in soil moisture and sometimes induced by an increase in soil salinity (Levitt 1980). For instance, Yoshihashi et al. (2004) reported the trial of Khao Dawk Mali 105 aromatic rice variety that found the 2AP content of rice planted on the sites with clay soil texture was lower (388 ppb) than that planted on sandy soil texture (518-528 ppb). Roychoudhury (2008) reported a significant increase of rice aroma quality in Gobindobhog variety that was subjected to salinity stresses compared to the control or without salinity treatment, and aroma quality of Gobindobhog variety was also higher than that of the non-aromatic rice variety.

In addition to drought stress, either because of the soil water deficit or the increase in soil salinity, the increase of 2AP content of aromatic rice plants may also be caused by soil nutrient factors such as Zn content (Jin-xia et al. 2009), P content (Rohilla et al. 2000), N content (Yang et al. 2012), and organic matter content (Champagne 2008; Islam and Sikder 2011). In general, it is reported that the balance of macro and micronutrients most significantly affects the increase of aroma quality of aromatic rice (Ram et al. 2013).

The mechanisms of interaction between drought stress and nutrient balance factor in increasing the 2AP content of rice are not well understood until now. The negative correlation between aroma quality and productivity (Arsa et al. 2011; Yang et al. 2012) is possibly associated with the interaction of the above factors, implying that the increase in aroma quality is at the expense of the decrease in productivity. The lower level of drought stress will cause the amount of proline formed to be low enough to inhibit the nutrient uptake. On the other hand, high-level drought stress will induce a high amount of proline that may inhibit nutrient uptake. Thus, the amount of proline and the availability of nutrients in low or high-stress levels are not the optimum conditions for 2AP compound production. Therefore, it is necessary to determine the moderate level of drought stress for a maximum 2AP production. This can be done by arranging drought stress treatments; levels of soil moisture and or soil salinity and drought stress periods. Drought stress occurs during the phase of vegetative to harvest will give higher stress than that during the phase of reproductive or flowering to harvest. Differences in the length of drought stress period will affect the physiological processes of plants, which in turn affect the yield components and grain yield (Akram et al. 2013) and influence the aroma quality of rice.

Based on the above description, the objective of the present study was to evaluate the effect of drought stress by arranging the type and the length of drought stress period on grain yield, physiological characters, and aroma quality of the aromatic Pare Wangi upland rice variety.

## MATERIALS AND METHODS

### Experimental design

This study employed a Completely Randomized Design with a 3 x 3 factorial treatment design. The first factor was type of drought stress (K), which consisted of 3 levels, i.e. soil moisture of 75% Field Capacity (FC) without NaCl (k1), soil moisture of 100% FC and 2.0 g NaCl kg soil<sup>-1</sup> (k2), and soil moisture of 75% FC and 1.0 g NaCl kg soil<sup>-1</sup> (k3). The second factor was drought stress period (F), which consisted of 3 levels, i.e. panicle initiation phase (at 60 DAS) (f1), booting phase (at 80 DAS) (f2), and panicle heading phase (at 100 DAS) (f3). A total of 9 treatment combinations were applied, each was 3 replicates. In total, 27 experimental units were included in the present study. Each experiment unit consisted of 2 planting pots (one pot for destructive and the other for non-destructive observations). Variables observed in this study included (i) yield component characters (number of panicles, number of grains (grains panicle<sup>-1</sup>), unfilled grains (%), weight of 100 grains (g), grain yield pot<sup>-1</sup> (g)), (ii) leaf tissue analysis, (iii) physiological characters (chlorophyll content and free proline content), and (iv) rice aroma quality (2AP content and aroma score).

### Preparation of media, planting, and fertilization

Planting media used in the present study was soil taken from the area where the Pare Wangi upland rice variety has

always been cultivated. The soil was first cleaned from plants debris and sieved with a 2.0 mm sieve size. Then, 7 kg of the sun-dried soil was filled into each planting pot. Each pot was then labeled and arranged in a greenhouse following the employed treatment design. Before planting, each pot was watered following the drought stress treatments with the amount of water given into the pot following the method of Wargadiputra and Harran (1983). Planting was done using five seeds per pot, and three healthy seedlings were retained two weeks after germination. The plants were maintained until harvest. Fertilizers were applied at planting time with, respectively, 250 kg Urea ha<sup>-1</sup> (1.0 g Urea pot<sup>-1</sup>), 100 kg SP36 ha<sup>-1</sup> (0.4 g SP36 pot<sup>-1</sup>) and 200 kg KCl ha<sup>-1</sup> (0.8 g KCl pot<sup>-1</sup>). Weed was manually controlled throughout the experiment, while the pests were controlled by insecticide (Demolish 18 EC) spray once a week during flowering and grain filling stages.

### Treatment application

The initial soil moisture for each pot was maintained at 100% FC (w/w), whereas drought stress treatments were given in a growth stage of the plant according to the assigned treatment. The addition of water to meet the drought stress treatment was given by gravimetric method. The NaCl was given according to the assigned treatment by dissolving the NaCl in water prior to its application to the growing media.

### Harvesting and grain sampling

Harvesting was conducted when the panicles turned yellow and hardened. Harvest was done on the whole grain yields in non-destructive pots. The grain yields were then observed for yield components and grain yield per pot. Sufficient amount of grain was then used to test both rice 2AP content and organoleptic assay.

### Leaf tissue analysis

Leaf tissue analysis was carried out at flowering stage (110 DAS). Two upper leaves (the second and the third leaf below the flag leaf) of each plant in destructive pot units were taken then were bulked for all replicates. A total of 9 composite leaf samples were produced which were then used to determine the leaf tissue contents of N, P, K, Na, and Zn by using Atomic Absorption Spectrophotometer (Perkin-Elmer 3110; J&W Scientific, Folsom, CA, USA) following the standard method of AOAC (2000).

### Measurement of chlorophyll content

Chlorophyll content was measured as follows: 2 g leaf sample was crushed with a mortar, and then added with 10 ml of 80% acetone. After that, the filtrate was poured through a funnel by a filter paper into a glass flask. The filtered filtrate was taken as many as 1 mL, and then diluted to a volume of 10 mL. This filtrate absorbance was measured by using a spectrophotometer. Total chlorophyll content was then calculated with the standard formula according to Arnon (1949), i.e. the sum of chlorophyll a and chlorophyll b. This procedure was applied to all leaf samples used to measure total chlorophyll content. The

formula for calculation of chlorophyll-a ( $\text{mg L}^{-1}$ ) was=  $12.70 \times \text{OD663} - 2.69 \times \text{OD645}$ , while the chlorophyll-b ( $\text{mg L}^{-1}$ ) was=  $22.9 \times \text{OD645} - 4.68 \times \text{OD663}$ . The unit was then converted into  $\text{mg g}^{-1}$  fresh weight.

#### Level of free proline

The level of free proline (KPB) was determined following the procedure of Bates et al. (1973). A total of refined 0.5 g leaf sample was added with 10 mL of 3% (v/v) sulfosalicylic and then stirred. The filtrate was then filtered through a filter paper (Whatman No.40). Two mL of the filtrate were taken and reacted with 2 mL of acidic ninhydrin and 2 mL of glacial acetic acid. This process was carried out in a test tube at  $100^\circ\text{C}$  for 1 hour and was then terminated by immersing the reaction tube in cold water (liquid ice). Proline extracts were obtained by adding 4 mL of toluene to the filtrate mixture for 15-20 seconds, and then stirred with a stirrer (stirrer test tube) and kept at room temperature to allow separation of toluene and water phases. Toluene phase absorbance was measured with a spectrophotometer at a wavelength of 520 nm (toluene was used as a blank). Total KPB was calculated by regression curve generated using the standard solutions.

#### Measurement of 2AP content and aroma score

The rice 2AP content was measured using composite samples of each treatment following the method of Lalel et al. (2003) and Wongpornchai et al. (2004). The volatile compounds were extracted using headspace solid-phase micro-extraction (HS-SPME) technique with the 100  $\mu\text{m}$  poly dimethyl siloxane SPME manual device (Supelco Co., Bellefonte, PA, USA). Separation and quantification of the 2AP compound were achieved using GC-MS (Hewlett Packard 5890 series, USA) equipped with a DB5MS capillary column (50 m x 0.2 mm id., 0.33  $\mu\text{m}$  film thickness; J & W Scientific, Folsom, CA, USA). Total 2AP was calculated using external standard (calibration curve). The organoleptic assay was conducted by ten trained panelists to assess the rice aroma score following the modified method of Lestari et al. (2011). Rice was cooked in a test tube for 15 minutes and kept warm at  $40^\circ\text{C}$ . Rice aroma quality was determined by scoring in a range of 0-4 (no aroma to very strong aroma).

#### Statistical analysis

The observed data were subjected to analysis of variance (ANOVA) following a Completely Randomized Design approach. An HSD post hoc test at 5% significance level was then conducted to compare the treatment means. Simple correlation analysis was also performed to examine the correlation between variables.

## RESULTS AND DISCUSSION

#### Yield components and grain yield

Our research results revealed that type of drought stress treatment caused no significant effect on the number of

panicles, number of grains, weight of 100 grains and grain yield per pot but the treatment significantly affected the percentage of unfilled grains. The drought stress induced by soil moisture of 100% FC and 2.0 g NaCl  $\text{kg soil}^{-1}$  (k2 treatment) caused a significantly higher unfilled grain percentage than that of the other treatments. Furthermore, the effect of drought stress period started at booting phase (at 80 DAS) (f2 treatment) caused no significant effect on the observed variables except the 100-grain weight. Drought stress initiated at booting phase (f2) produced a significantly lower 100-grain weight than that started at panicle initiation phase (f1) and panicle heading phase (f3). Grain yield per pot did not differ among the treatments of drought stress period (Table 1).

#### Chlorophyll and proline content

Drought stress type induced by soil moisture of 75% Field Capacity (FC) without NaCl (k1) and soil moisture of 75% FC and 1.0 g NaCl  $\text{kg soil}^{-1}$  (k3) caused no significant effect on chlorophyll content, on the other hand, drought stress induced by soil moisture of 100% FC and 2.0 g NaCl  $\text{kg soil}^{-1}$  (k2) started at booting phase (f2) produced a lower chlorophyll content as compared to that started at panicle initiation phase (f1) or panicle heading phase (f3) (Table 2). Furthermore, k1 treatment produced higher proline content than that of either k2 or k3 treatments, respectively. Drought stress type started at f1 stage also produced higher proline content as compared to that started at f2 and f3 (Table 2).

**Table 1.** Yield components and yield of Pare Wangi upland rice variety under various types and periods of drought stress

Treatment (s) <sup>1)</sup>	Yield components and yield <sup>2)</sup>				
	NP	NG	UG	W100	GY
Drought stress type					
k1	11.11a	144.00a	22.64b	2.29a	29.01a <sup>3)</sup>
k2	12.56a	162.56a	32.82a	2.31a	28.01a
k3	12.00a	165.67a	23.46b	2.35a	28.35a
HSD 0.05	2.29	34.33	7.10	0.17	6.06
Drought stress period					
f1	12.11a	155.00a	28.16a	2.42a	29.21a
f2	11.22a	159.67a	24.46a	2.23b	27.84a
f3	12.33a	155.56a	26.30a	2.30ab	28.31a
HSD 0.05	2.29	34.33	7.10	0.17	6.06

Note: <sup>1)</sup> k1: soil moisture of 75% FC + 0 g NaCl; k2: soil moisture of 100% FC + 2.0 g NaCl  $\text{kg soil}^{-1}$ ; k3: soil moisture of 75% FC + 1.0 g NaCl  $\text{kg soil}^{-1}$ ; f1: drought stress initiated at 60 DAS; f2: drought stress initiated at 80 DAS; and f3: drought stress initiated at 100 DAS. <sup>2)</sup> NP: number of panicles  $\text{pot}^{-1}$ , NG: number of grains (grains panicle<sup>-1</sup>), UG: unfilled grains (%), W100: weight of 100 grains (g), GY: grain yield  $\text{pot}^{-1}$  (g). <sup>3)</sup> Numbers followed by the same letter (s) are not significantly different at HSD post hoc test (0.05).

**Table 2.** Chlorophyll and proline content of Pare Wangi upland rice variety under various types and periods of drought stress

Treatment <sup>1)</sup>	Chlorophyll (mg g BS <sup>-1</sup> )			Average	Proline (μmol g BS <sup>-1</sup> )			Average
	f1	f2	f3		f1	f2	f3	
k1	1.29 a	1.37 a	1.15 a	1.27	15.60	14.91	14.21	14.91
	A	AB	A					A <sup>2)</sup>
k2	1.43 a	1.12 b	1.34 ab	1.30	14.88	14.32	14.47	14.56
	A	B	A					AB
k3	1.30 a	1.41 a	1.25 a	1.32	14.38	13.85	14.23	14.15
	A	A	A					B
Average	1.34	1.30	1.25	1.30	14.95 a	14.36 b	14.30 b	14.54

Note: <sup>1)</sup> k1: soil moisture of 75% FC + 0 g NaCl; k2: soil moisture of 100% FC + 2.0 g NaCl kg soil<sup>-1</sup>; k3: soil moisture of 75% FC + 1.0 g NaCl kg soil<sup>-1</sup>; f1: drought stress initiated at 60 DAS; f2: drought stress initiated at 80 DAS; and f3: drought stress initiated at 100 DAS. <sup>2)</sup> Means followed by the same letter (s) are not significantly different at HSD post hoc test (0.05) (small letter shows comparisons in the same row and capital letter shows comparisons in the same column)

### Content of 2AP, aroma score, and leaf tissue nutrient

We found in the present study that the highest 2AP content of rice induced by drought stress type due to soil moisture content of 75% FC + 0 g NaCl (k1) was produced when the stress was applied at booting phase (f2) while drought stress type induced by soil moisture of 100% FC + 2.0 g NaCl kg soil<sup>-1</sup> (k2) and soil moisture of 75% FC + 1.0 g NaCl kg soil<sup>-1</sup> (k3), respectively, produced the highest 2AP content when these treatments were initiated at panicle heading phase (f3). The k1 treatment applied at f2 stage apparently produced the highest 2AP content (3.07 ppb) (Table 3). Aroma scores of rice determined through organoleptic test fell in the range of 1.7 to 2.2. The drought stress type of k1 treatment applied at f2 stage showed the highest rice aroma score and that of k2 treatment applied at panicle initiation phase (f1) showed the lowest one. The range of aroma scores produced by either k1 treatment or k3 treatment was relatively higher than that of k2 treatment. These findings showed that drought stress caused by water deficit or its combination with salinity application was stronger than the salinity application alone (without water deficit) in increasing the rice aroma (Table 3).

The effect of the single treatment drought stress type (K) and drought stress period (F) on the leaf tissue contents of Na, N, P, K, and Zn are presented in Table 4. Na content of leaf tissue treated with k2 (soil moisture of 100% FC + 2.0 g NaCl kg soil<sup>-1</sup>) was higher than that of either k1 (soil moisture of 75% FC + 0 g NaCl) or k3 (soil moisture of 75% FC + 1.0 g NaCl kg soil<sup>-1</sup>) over all periods of drought stress. Effect of k3 treatment tended to increase the leaf tissue Na content as compared to k1 treatment but there was no significant effect of drought stress type and period on leaf tissue contents of N, P, K, and Zn.

### Correlation between plant characters

Results of correlation analysis between yield components showed that number of grains (grain panicle<sup>-1</sup>) was negatively correlated with number of panicles but there was no correlation between 100-grain weight and number of grains. The correlation analysis also revealed that none of the observed yield components was correlated with grain yield pot<sup>-1</sup> (Table 5). The present study results also showed that N content has no correlation with either each of the

yield components or the grain yield pot<sup>-1</sup>. Similarly, no correlation was observed between Na content of leaf tissue and each of the following characters, respectively: yield components, grain yield pot<sup>-1</sup>, and Na content. Furthermore, chlorophyll content showed only positive correlation with 100-grain weight while proline content apparently has a positive correlation with grain yield pot<sup>-1</sup> but it was not correlated with rice 2AP content (Table 5).

**Table 3.** 2AP and aroma score of Pare Wangi upland rice variety under various types and periods of drought stress

Treatment <sup>1)</sup>	2AP (ppb) +			Aroma Score +		
	f1	f2	f3	f1	f2	f3
k1	2.92	3.07	2.45	2.0	2.2	2.1
k2	2.36	2.01	2.65	1.7	1.8	1.9
k3	2.30	2.33	2.71	2.0	2.0	2.1

Note: <sup>1)</sup> k1: soil moisture of 75% FC + 0 g NaCl; k2: soil moisture of 100% FC + 2.0 g NaCl kg soil<sup>-1</sup>; k3: soil moisture of 75% FC + 1.0 g NaCl kg soil<sup>-1</sup>; f1: drought stress initiated at 60 DAS; f2: drought stress initiated at 80 DAS; and f3: drought stress initiated at 100 DAS; +: data was not subjected ANOVA

**Table 4.** Content of Na, N, P, K and Zn in leaf tissues of Pare Wangi upland rice variety under various types and periods of drought stress

Treatment (s) <sup>1)</sup>	Content in leaf tissues				
	Na (%)	N (%)	P (%)	K (%)	Zn (ppm)
Drought stress type					
k1	0.79	2.19	0.83	1.48	81.16
k2	1.17	2.19	0.87	1.49	80.51
k3	0.91	2.24	0.82	1.55	80.60
Drought stress period					
f1	0.92	2.19	0.82	1.51	80.58
f2	0.95	2.29	0.86	1.48	81.11
f3	1.00	2.14	0.85	1.54	80.57

Note: <sup>1)</sup> k1: soil moisture of 75% FC + 0 g NaCl; k2: soil moisture of 100% FC + 2.0 g NaCl kg soil<sup>-1</sup>; k3: soil moisture of 75% FC + 1.0 g NaCl kg soil<sup>-1</sup>; f1: drought stress initiated at 60 DAS; f2: drought stress initiated at 80 DAS; and f3: drought stress initiated at 100 DAS

**Table 5.** Results of simple correlation analysis between observed variables

Variable	JG	W100	GY	N (%)	Na (%)	Klo	Pro	2AP	Aroma
NP	-0.63 **	0.55 ns	-0.31 ns	-0.54 ns	0.46 ns	0.21 ns	-0.10ns	-0.11ns	-0.29ns
NG		-0.34 ns	0.14 ns	0.46 ns	0.18 ns	-0.18 ns	-0.22ns	-0.36ns	-0.24ns
W100			0.50 ns	-0.02 ns	-0.09 ns	0.70 *	0.36ns	0.30ns	-0.08ns
GY				0.19 ns	-0.56 ns	0.26 ns	0.73*	0.59ns	0.22ns
N (%)					-0.18 ns	0.60 ns	0.03ns	0.23ns	0.12ns
Na (%)						-0.08 ns	-0.35ns	-0.65 *	-0.83 **
Chlo							0.20ns	0.31ns	-0.04 ns
Pro								0.59ns	-0.03 ns
2AP									0.66*

Note: ns: not significant; \*,\*\*: significant at  $p\text{-value} \leq 0.05$  and  $p \leq 0.01$ , respectively. NP: number of panicles  $\text{pot}^{-1}$ ; NG: number of grains (grains panicle $^{-1}$ ); W100: weight of 100 grains (g); GY: grain yield  $\text{pot}^{-1}$  (g); Chlo: Chlorophyll content (mgg  $\text{BS}^{-1}$ ); Pro: Prolin content ( $\mu\text{mol g BS}^{-1}$ ); 2AP: 2AP content (ppb), Aroma: Aroma score

## Discussion

Number of unfilled grains under the drought stress type of k2 treatment (2.0 g NaCl kg soil $^{-1}$ ) was higher than that of either k1 treatment (75% FC and 0 g NaCl) or k3 treatment (75% FC and 1.0 g NaCl kg soil $^{-1}$ ) (Table 1). This indicated a higher seed abortion rate under the k2 treatment as compared to either k1 or k3 treatments. The enhancement of Na uptake in k2 treatment (Table 4) showed a tendency of positive correlation with number of panicles but number of panicles has a negative correlation with number of grains, which caused Na content of leaf tissue has a negative correlation with grain yield  $\text{pot}^{-1}$  (Table 5). These findings indicated that k2 treatment affected the photosynthesis process, which hereinafter affected carbohydrate formation and its translocation to the seeds. In the end, lack of carbohydrates caused more seeds not to be fully formed or to become unfilled grains (Jaleel et al. 2009).

Drought stress that was started at f2 stage (80 DAS) caused the plant to produce a lower 100-grain weight compared to that at f1 stage (60 DAS) but it was not significantly different from that at f3 stage (100 DAS) (Table 1). This implies that the supply of carbohydrates from leaves to the seeds was more significantly reduced when the drought stress was started at f2 than at f1. Plants that revived drought stress treatment at f2 stage exhibited leaf chlorosis symptoms and the leaves were then dried out. The symptom was mainly related to the influence of k2f2 treatment that significantly reduced leaf chlorophyll content as compared to k1f2 and k3f2 treatments (Table 2). The decrease in leaf chlorophyll content affected the effectiveness of the photosynthesis process, which in turn reduced the amount of carbohydrates translocated to the seeds.

The tendency of the decrease in leaf chlorophyll content of the plants treated with drought stress types k1 and k3 at the drought stress period f3 (Table 2) was presumably caused by the plant's adjustment mechanisms. At the drought stress treatment period f3, the plants grew without drought stress in the vegetative stage but were then subjected to drought stress in the generative stage, especially close to the flowering stage when the plants' water consumption increased significantly. One of the

plant's adjustment mechanisms in facing such situation was by reducing new chlorophyll formation, and otherwise by increasing chlorophyll molecule degradation. As a result, the decreased leaf chlorophyll content of plants treated with k2 drought stress type at f2 might be related to the increase of leaf tissue Na content (Table 4) triggered by the increase of transpiration process. Visually, this process caused the older thick leaf to be drying out.

Observed rice 2AP content and aroma score presented in Table 3 indicated that the range of 2AP content of plants treated with drought stress type k1 was higher than that of other treatments, either k2 or k3. This might have been caused by the increase of salinity that affected the biosynthesis of proline to become 2AP compound. At the drought stress type k1, it was likely that more proline was used to form 2AP compound as compared to that of k2 and k3. Meanwhile, at the drought stress types k2 and k3, the formation of proline might be more useful as an osmoprotectant than as a precursor of 2AP compound. This was mainly seen in the drought stress types started at f1 and f2 stages, where lower 2AP contents were produced compared to that at f3. On the contrary, the drought stress type k1 started at f3 stage was also likely to reduce proline content (as a precursor of 2AP compound), so that the 2AP content at this treatment was lower than that of k1 started at either f1 or f2 stages (Table 3). Gay et al. (2010) reported that the formation of rice 2AP compound was predicted to occur before heading phase; meanwhile, Itaniet et al. (2004) found that the highest 2AP content was reached at 4-5 weeks after heading phase. Therefore, the different treatments of drought stress levels and starting periods applied in this study presumably determined the observed difference in time (phase) for the highest rice-2AP production we found in the present study.

Grain yield  $\text{pot}^{-1}$  was positively correlated with 100-seed weight, meanwhile, number of panicles plant $^{-1}$  and number of grains panicle $^{-1}$  showed a negative correlation (Table 4), and consequently, these two yield components have no contribution to grain yield  $\text{pot}^{-1}$ . Grain yield  $\text{pot}^{-1}$  was mainly determined by 100-seed weight that was positively correlated with number of panicles. Shahidullah et al. (2009) reported that in aromatic rice, number of panicles was positively correlated with grain yield but its

effect has frequently occurred indirectly through other characters such as 100-seed weight and number of grains panicle<sup>-1</sup>.

There was no correlation between grain yield pot<sup>-1</sup> and leaf tissue N content, which may likely be related to the observed negative correlation between leaf tissue N content and number of panicles, and on the other side, there was a positive correlation between leaf tissue N content and number of grains panicle<sup>-1</sup>. It was also revealed in the present study that there was no correlation between Na content and grain yield pot<sup>-1</sup>. The non-significant positive correlation between leaf tissue Na content and number of panicles, in addition to the negative correlation between number of panicles and number of grains, maybe the causal factor of the non-significant negative correlation between leaf tissue Na content and the grain yield pot<sup>-1</sup> (Table 5).

The observed positive correlation between rice 2AP content and grain yield pot<sup>-1</sup> (Table 5) shows that the increase of rice 2AP content will be followed by the increase of grain yield pot<sup>-1</sup> if the plants were exposed to a moderate level of drought stress after generative phase. This phenomenon was likely to involve endogen proline activity. The significant positive correlation observed between proline and grain yield pot<sup>-1</sup> was also supported by the tendency of a positive correlation between the leaf tissue proline content and the rice 2AP content. Thus, there is a tendency for a positive correlation between rice 2AP content and grain yield pot<sup>-1</sup>. The positive correlation between proline content and rice 2AP content observed in the present study demonstrates that Pare Wangi rice variety has experienced a moderate level of drought stress. In contrast to this finding, previous studies (Kibria et al. 2008; Yang et al. 2012) reported that 2AP content of rice was negatively correlated with grain yield.

Based on the results of the present study, the following conclusions are made: (i) The treatment interaction of type and period of drought stress did not affect the grain yield pot<sup>-1</sup> but affected both leaf tissue chlorophyll content and rice 2AP-compound content. (ii) The main effect of drought stress type separately affected the percentage of unfilled grains, and the drought stress period affected the 100-grain weight. (iii) The percentage of unfilled grains caused by salinity level of 2.0 g NaCl kg soil<sup>-1</sup> was higher than that caused by soil moisture of 75% FC and the combination of the treatments (soil moisture of 75% FC and salinity level of 1.0 g NaCl kg soil<sup>-1</sup>). (iv) Stress treatment of salinity level of 2.0 g NaCl kg soil<sup>-1</sup> started at booting stage (80 DAS) produced lower chlorophyll content as compared to other treatments. (v) Drought stress type of soil moisture of 75% FC started at booting stage produced the highest rice 2AP-compound content and aroma score. (vi) Proline content of leaf tissue has no correlation with rice 2AP-compound content but it has a positive correlation with grain yield pot<sup>-1</sup>.

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