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EFFECTIVENESS OF OSMOPROTECTANTS IN IMPROVING AROMA QUALITY AND YIELD OF PARE WANGI UPLAND RICE VARIETY GROWN ON TWO DIFFERENT SOIL TYPES IN EAST NUSA TENGGARA

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ABSTRACT

This study aimed to evaluate the effectiveness of exogenous osmoprotectant application in increasing the stability of aroma quality and grain yield of Pare Wangi on different soil types. A two factors greenhouse experiment was designed according to Split Plot design with three replications. The first factor was soil types of specific and target location. The second factor was application of exogenous osmoprotectants, i.e. without osmoprotectant, 10 mM proline, 20 mM proline, 10 mM sorbitol, 20 mM sorbitol, 10 mM sucrose and 20 mM sucrose. Observed data included soil physical and chemical properties, rice vegetative and reproductive growth and physiological characters, and rice aroma quality. Collected data were subjected to analysis of variance, followed by an Honestly Significant Difference (HSD) post hoc and a simple correlation tests. Results indicated that grain yield per pot was higher on soil from target location than on soil from specific location, but concentration of proline and 2AP, and the aroma scores were in the opposite direction. Besides more effective in increasing grain yield, the osmoprotectants proline and sucrose, each at 10 mM, were also better in maintaining rice aroma quality compared tosorbitol osmoprotectants, and aroma score showed a significantly positive correlation with 2AP concentration.

Keywords: 2AP; aroma; osmoprotectant; upland rice

INTRODUCTION

The current demand for rice in the province of East Nusa Tenggara (ENT) has not been fully met by local production, which necessitates import of rice from other regions in Indonesia to fulfill the demand. Increasing productivity of the upland rice variety “Pare Wangi” under the existing production area/location is one of the strategic efforts undertaken to increase rice production in the province. One of the base technologies that need to be developed to support the efforts is the one that can improve the productivity of the rice crop while maintaining the quality of the rice aroma under drought conditions, especially for those occuring during the flowering and grain filling stages. One technology that needs to be studied is the use of exogenous osmoprotectants-compounds that can maintain or increase osmotic pressure in leaf cells or tissues.

The use of exogenous osmoprotectant has been reported to increase plant’s tolerance to drought, because it can maintain tissue water potential and antioxidant systems to adjust the integrity of the cell membrane and the rate of photosynthesis (Farooq, Basra, Wahid, Ahmad, & Saleem, 2009). Osmo-protectants that have been studied and proven to increase yield of Basmati 2000 rice cultivar on soil at moisture content of 50% field capacity are Glycine-betaine (GB) and Salicylic Acid (SA). Application of GB in the range of 0-200 mM was also effective for increasing percentage of filled grains and grain weight per plant of rice under 150 mM NaCl stress (Cha-Um & Kirdmanee, 2010). GB application at a concentration of 50 mM was reported to result in the highest percentage of filled grain and grain yield per plant compared to other treatments.

Other osmoprotectants that have been studied are *amino acid proline* and *trehalose* (Garcia et al., 1997; Nounjan, Nghia, & Theerakulpisut, 2012). A 10 mM concentration of

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both types of osmo-protectants applied to aromatic rice seedlings grown at 100 mM NaCl salinity can reduce the ratio of Na^+/K^+ and improve plant's recovery. The recovery mechanism occurs through increased osmotic pressure in leaf cells or tissues, in adjustment to the osmotic pressure of the root tissue, allowing the water absorbed by roots to be translocated to the top of the plant (Nounjan, Nghia, & Theerakulpisut, 2012). As a result, the metabolic processes of plants provided with exogenous osmoprotectant are better than the control plants. In addition to its fuction as an osmoprotectant, proline is also suspected to be a precursor of 2AP compound, i.e. the main compound resulting in scent aroma in aromatic rice (Kibria, Islam, & Begum, 2008). The use of exogenous proline in this study is very relevant with the goal of increasing grain yield and aroma quality of Pare Wangi upland rice variety. Other compounds that were also reported to be effective as osmoprotectants are sorbitol and sucrose (Bianco, Rieger, & Sung, 2000). Application of the osmo-protectant through leaves increases the number and rate of organic acid secretion by plant roots, thereby improving the ability of roots to absorb mineral nutrients and stimulating plant cell division.

The precautionary principle in the use of exogenous osmoprotectant needs to be done. Yuan & Lin (2008) stated that the role of Salicylic Acid (SA) is not always consistent and SA is not always able to solve the problems of abiotic stress. Application of SA at the optimum level is required to avoid its adverse effect. At a low level or vice versa at the excessive level, application of SA may increase the susceptibility of plants to abiotic stresses, including drought stress. Even at the optimum level, SA effect can be specific for each type of plant. For rice crop, Yuan & Lin (2008) also stated that the optimum level of SA application ranges from 0.1-0.5 mM. Therefore treatment of any kind of osmoprotectant should be tested at different levels.

Excessive levels of drought or non drought stress is not beneficial for aromatic rice (Kibria, Islam, & Begum, 2008; Yang et al., 2012). Therefore, a growing environment with moderate level of drought stress has to be set as an appropriate environ-mental condition forgetting optimal crop yield and aroma production of the rice. The same levels of soil moisture for different soil textures can produce different levels of stress on the plants (Saxton & Rawls, 2006). This is likely to be the determining factor for differences in aroma quality and yield between the specific location and the target (expansion) location of Pare Wangi variety (Arsa, Lalel, & Adu-Tae, 2011). Such differences are expected to be solved with the application of exogenous osmoprotectants.

Based on the above description, the purpose of this study was to determine the effectiveness of exogenous osmoprotectant treatments in maintaining the quality of the aroma and increasing yield of Pare Wangi rice variety grown on two different soil types, i.e. those taken from the specific and target locations of the rice cultivation.

MATERIALS AND METHODS

Experimental Design

This study was conducted by using a Split Plot Design with three replications. The treat-ments applied consisted of two factors with 7 x 2 treatment combinations. The main plot factor was soil types (M), which consisted of two types of soil as the growing media from specific (m1) and target (m2) locations. The second factor was application of exogenous osmoprotectant packages with the following treatment levels: without osmoprotectant, 10 mM proline, 20 mM proline, 10 mM sorbitol, 20 mM sorbitol, 10 mM sucrose and 20 mM sucrose. In total, 14 treatment combinations were assigned, with 42 experimental units, which then further sub-divided into 84 planting pots, one group of 42 pots for destructive and the other group of 42 pots for non-destructive observations.

Variables observed in this study consisted of (a) soil physical properties (texture, field capacity and permanent wilting point moisture contents), and chemical properties of the soil (organic Carbon (C-organic), nitrogen (N), phos-phorus (P), potassium (K), natrium (Na), Zinc (Zn) and electric conductivity (EC)) and of the leaf tissue; (b) vegetative and reproductive growth properties of rice plant (plant height, leaf area, tiller number, days to flowering, panicle number, number of grains per panicle, percentage of unfilled grains, weight of 100 grains, and grain yield per pot); (c) physiological properties (total chlorophyll, and free proline), and (d) aroma quality of the rice based on 2AP content and organoleptic aroma value.

Determination of Specific and Target Location

The specific location (m1) was Kalembu Kaha village, Sub-District of Loura, and the target location (m2) was Kori village, Sub-District of North Kodi. Both locations are upland rice production centers in South West Sumba Regency, ENT Province, Indonesia. The m1 is the original area where Pare Wangi upland rice variety has usually been cultivated with a strong aroma quality as its uniqueness. Meanwhile, the m2 is the area where Pare Wangi rice is cultivated but with low aroma quality or without aroma flavour at all (Arsa, Lalel, & Adu-Tae, 2011).

Preparation of Media, Planting, Fertilization and Osmoprotectant Application

Planting medium used in the present study consisted mainly of natural soil taken from m1 and m2. The soils were first sun dried for two days then the soils granule was separated by using a 2.0 mm sieve and was put into each planting pot, 7.0 kg each. The prepared planting pots were labeled and arranged in a greenhouse following the treatment design employed. Before planting, each pot was watered until 100% (w/w) of soil field capacity level,with approximately 773 mL and 812 mL of water given into the pot containing media from location m1 and m2, respectively.

Planting was conducted in December 2014 using five seeds per pot. Two weeks after germination, 3 healthy seedlings per pot were kept for the remaining of the experiment.

Fertilizers were applied at planting with, respectively, 250 kg ha^{-1} Urea (1.0 g Urea pot^{-1}), 100 kg ha^{-1} SP36 (0.4 g SP-36 pot^{-1}) and 200 kg ha^{-1} KCl (0.8 g KCl pot^{-1}). Weed was physically controlled throughout the experiment, while pest was controlled by insecticide (Demolish 18 EC) application once a week during flowering and grain filling stage. Rice was harvested after more than 90% of grains per panicle had turned yellow and hardened.

A solution of 10 mM and 20 mM of proline, sorbitol and sucrose were prepared by separately dissolving 1.15 g and 2.30 g proline, 1.82 mL and 3.64 mL sorbitol, and 3.42 g and 6.84 g sucrose, respectively into 1.0 L of tap water. The osmo-protectant solutions were sprayed three times at 10 day intervals, starting at 85 DAS (days after seeding), i.e. after applying water stress of 75% field capacity at 80 DAS.

Leaf Tissue Analysis

Leaf tissue analysis was performed at floweringstage (110 DAS). Two upper leaves (the second and third leaf below the flag leaf) of each plant in destructive pot units were taken then were bulked for all replicates. A total of 14 composite leaf samples were produced which were used to determine N, P, K and Na leaf tissue using Atomic Absorption Spectrophotometer (Perkin Elmer 3110; J & W Scientific, Folsom, CA, USA) following the standard method of AOAC (2000).

Measurement of Chlorophyll

Measurement of chlorophyll content was done by crushing 0.5 g of leaves samples with a mortar, and then added with 10 ml of 80% acetone. The homogenate was filtered into an Erlenmeyer flaskthrough a funnel by using filter paper (Whatman No. 40). One mL of filtrate was then transferred into volumetric flask and was diluted to a final volume of 10 mL. Absorbance of the diluted filtrate was measured with a spectrophotometer at 645 nm and 663 nm wavelength. Total chlorophyll (chlorophyll-a and chlorophyll-b) content was calculated with the standard formula according to Arnon (1949). The formula for calculation of chlorophyll-a (mg L^{-1})= 12.70 x OD663 - 2.69 x OD645, while the chlorophyll-b (mg L^{-1})= 22.9 x OD645 - 4.68 x OD663. The unit was then converted into mg g^{-1} fresh weight.

The Content of Free Proline

Levels of free proline (FP) was determined following the procedure of Bates, Waldren, & Teare (1973). A total of 0.5 g of leaf sample was refined using a mortar and the refined leaf sample was added with 10 mL of sulfosalisilat 3%, and was stirred. The homogenate was filtered by using filter paper (Whatman No. 40). Two mL of filtrate was 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°C for 1 hour.The process was terminated by immersing the reaction tube in cold water. Proline extracts was obtained by adding 4 mL of toluene to the filtrate mixture for 15-20 seconds, then was stirred and then kept at room temperature to allow separation of toluene and water phases. Toluene phase absorbance was measured by using a spectrophotometer at a wavelength of 520 nm (toluene used as a blank). Total FP was calculated based on the regression curve generated using standard solutions.

Measurement of 2AP Content and Aroma of Rice

The content of 2AP in rice wasmeasured using composite samples of rice grain of each treatment following method of Lalel, Singh, & Tan, 2003) and Wongpornchai, Dumri, Jongkaewwattana, & Siri (2004). The volatile compounds were extracted using headspace solid phase micro extraction (HS-SPME) technique with the 100 µm polydimethyl siloxan SPME manual device (Supelco Co., Bellefonte, PA, USA). Separation and quantification of 2AP compound was achieved using GC-MS (Hewlett Packard 5890 series, USA) equiped with a DB5MS capillary column (50 m x 0.2 mm id., 0.33 µm film thickness; J & W Scientific, Folsom, CA, USA). Organoleptic test was conducted by ten panelists who have been trained to assess the aroma of rice following modified method of Itani, Tamaki, Hayata, Fushimi, & Hashizume (2004). The rice was cooked in a test tube for 15 minute and kept warm at 40ºC. Each sample was smelled by the panelists and scored between 0-4 (0= no aroma, 1= faint aroma, 2= indicated aroma, 3= strong aroma, 4= very strong aroma). The scores of each sample were then averaged.

Statistical Analysis

Data was analyzed using analysis of variance (ANOVA), based on Split Plot design, followed by Honestly Significant Difference (HSD) test at 5% significance level, while the composite data were analyzed using a two-way ANOVA without replication. Simple correlation analysis was also performed to examine the correlation between aroma quality and physiological characters and between each variable.

RESULTS AND DISCUSSION

Physical and Chemical Properties of the Soil

Analysis results of soil physical and chemical properties are presented in Table 1. Differences in chemical properties of soils taken from m1 and m2 were indicated by soil C-organic and N contents, where C-organic (4.57%) and N (0.21%) contents at m1 was higher than those (3.97% C-organic) and (0.13% N) at m2. Furthermore, P, K and Zn contents at m1 were also relatively higher than those at m2. The same situation applies for soil EC, despite the fact that both soil types contained almost the same Na level (2.49 and 2.48 me 100g^{-1}).

Vegetative and Reproductive Growth Characters

ANOVA results indicated that soil types significantly influenced only days to flowering, days to harvest, percentage of unfilled grain, and grain yield per pot (Table 2), in which Pare Wangi rice plants started the anthesis earlier (Table 4) and the percentage of unfilled grains was significantly lower resulting in significantly higher grain yields per pot on soil from the target than those on the specific location (Table 4). These are likely due to differences in physical properties between the two soil types (Table 1), especially soil textures, which can result in differences in the development of the plant root system. The better soil texture for root growth, as well as lower salinity levels on soil from the target location seems to be the determining factor for the differences in days to flowering and other significant agronomic characters.

Table 1. Physical and chemical properties of the soil used in the present study

Types of Analysis	Location	
	Specific (m1)	Target (m2)
Soil texture	Clayey loam	Sandy loam
Field capacity SWC (%)	36.98	28.0
Permanent wilting point SWC (%)	17.64	8.10
C-organic content (%)	4.57	3.97
N (%)	0.21	0.13
P (ppm)	80.25	69.02
K (me.100g^{-1})	1.59	0.82
Na (me.100g^{-1})	2.49	2.48
Zn (ppm)	0.60	0.46
EC (mS)	0.28	0.10

Table 2. Summary of ANOVA results on the plant vegetative and reproductive growth characters

Agronomic Characters	Main effects		Interaction
	Location (m)	**Osmoprotectant (o)**	**m x o**
Plant height at flowering stage (cm)	ns	ns	ns
Maximum number of tillers	ns	ns	ns
Leaf area at flowering stage (cm^2)	ns	ns	ns
Days to Flowering (days)	*	**	ns
Days to harvest (days)	**	**	ns
Grain filling period (day)	ns	*	ns
The number of panicles per pot	ns	**	ns
Total grain (grain $panicle^{-1}$)	ns	**	ns
Unfilled grain percentage (%)	*	*	ns
Weight of 100 grains (g)	ns	ns	ns
Grain weight (g pot^{-1})	**	**	ns

Remarks: ns= non-significant; *,**= significant at p-value ≤ 0.05 and p≤ 0.01, respectively

Table 3. Vegetative and reproductive characters of Pare Wangi upland rice in response to different soil types and exogenous osmoprotectants applied after subjecting the plants to water conditions of 75% field capacity at 80 DAS

Treatment(s)	Vegetative and reproductive characters[1)]					
	PH	**NT**	**LA**	**FD**	**HD**	**GFP**
Soil type (Location)						
Specific	142.86a	18.24a	4468.03a	100.81a	128.71a	27.43a [2)]
Target	140.86a	18.48a	4627.24a	97.67b	126.43b	28.71a
HSD	6.12	1.29	795.73	1.91	1.08	1.67
Osmoprotectant						
Control	140.33a	17.50a	3342.83b	103.17a	129.00a	25.67b
10 mM proline	143.17a	18.33a	4495.47ab	99.00ab	127.00ab	28.00a
20 mM proline	144.00a	18.17a	5120.74a	99.17ab	127.33ab	28.17a
10 mM sorbitol	140.33a	19.50a	4379.17ab	97.67b	126.33b	28.67a
20 mM sorbitol	143.50a	19.83a	5262.03a	97.33b	125.83b	28.50a
10 mM sucrose	136.00a	17.67a	4629.10ab	98.00b	128.00ab	30.00a
20 mM sucrose	145.67a	17.50a	4604.10ab	100.33a	129.50a	27.50a
HSD	14.46	2.90	1627.28	4.24	2.81	3.33

Remarks: [1)] PH= plant height at anthesis (cm), NT= maximum number of tillers, LA= leaf area at anthesis (cm^2), FD= days to flowering (DAS), HD= days to harvest (DAS), GFP= grain filling period (days). [2)] Means followed by the same letter(s) are not significantly different based on the Honestly Significant Difference (HSD) post hoc test (p≤0.05)

Application of exogenous osmoprotectants significantly influenced most agronomic characters of the rice plants, except plant height, maximum number of tillers and weight of 100 grains (Table 2). Total leaf area of rice plants treated with 20 mM proline and 20 mM sorbitol was significantly higher than those of control (Table 3). The positive influence of sorbitol at 10 mM or 20 mM compared to control can be seen on panicle formation. Application of 10 mM sucrose was also significant in increasing grain formation and decreasing the percentage of unfilled grain compared to the control (Table 4).

Plant height and maximum number of tillers of the exogenous osmoprotectant treated plants were not significantly different compared to those of the control treatment (Table 3). This may likely be determined by time of osmo-protectant application that was started on the primordial phase (at 80 DAS) or close to panicle emergence phase at which most of the plant metabolic process are intended for formation of flowers. Application of osmoprotectant at this time aiming at improving the plant photosyntesis process, therefore, caused no significant effect on vegetative characters such as plant height and number of tillers.

Table 4. Yield components of the Pare Wangi upland rice in response to different soil types and types of exogenous osmoprotectants applied after subjecting the plants to water conditions of 75% field capacity at 80 DAS

Treatment(s)	**Yield components[1)]**				
	NP	**NG**	**UG**	**W100**	**GY**
Soil type (Location)					
Specific	15.05a	196.95a	15.00a	2.54a	42.93b [2)]
Target	16.05a	211.05a	12.29b	2.60a	54.33a
HSD	1.65	30.60	2.71	0.12	5.69
Osmoprotectant					
Control	14.50c	167.00b	16.50a	2.55a	36.13b
10 mM proline	15.00bc	223.00ab	13.17ab	2.56a	54.07a
20 mM proline	15.17abc	200.50ab	12.83ab	2.54a	46.79ab
10 mM sorbitol	17.50ab	175.50b	14.50ab	2.68a	49.22ab
20 mM sorbitol	17.83a	207.17ab	13.00ab	2.57a	55.81a
10 mM sucrose	14.00c	244.83a	11.83b	2.62a	51.59a
20 mM sucrose	14.83bc	210.00ab	13.67ab	2.47a	46.79ab
HSD	2.74	60.06	4.18	0.30	13.44

Remarks: [1)] NP= number of panicles per pot, NG= grains per panicle, UG= unfilled grains (%), W100= weight of 100 grains (g), GY= grain yield per pot (g pot^{-1})
[2)] Means followed by the same letter(s) are not significantly different based on the Honestly Significant Difference (HSD) post hoc test ($p \leq 0.05$)

Osmoprotectant treatments, in general, tend to increase leaf area; however, the treatments with 20 mM proline and 20 mM sorbitol significantly increased leaf area when compared with controls (Table 3). This likely occurred through a mechanism that delayed leaf senescence, because lower leaves in the canopy of control plants senesced and dried out faster than those in the rice plants treated with osmoprotectants. This is related to the ability of osmoprotectants in maintaining the water potential of leaf tissue adjusted to the osmotic pressure of the roots, so that water absorbed by roots can be translocated to the top of the plant (Farooq, Basra, Wahid, Ahmad, & Saleem, 2009).

The application of exogenous osmoprotectants ultimately increased rice grain yield per pot, and the treatments showing a higher grain yield than control were application of 10 mM proline, 20 mM sorbitol and 10 mM sucrose (Table 4). The higher grain yields were presumably caused by the longer period of filling phase (Table 4), longer panicles and higher grain number, and lower percentage of unfilled grain (Table 4) in the plants treated with these osmo-protectants. Since the interaction effect between the two treatment factors on grain yield per pot was not significant (Table 2), then the effective-ness of the three osmoprotectant treatments can be applied to both types of the soil used as the growing media.

Content of Chlorophyll, Proline, 2AP, Rice Aroma and Leaf Tissue Nutrient

In terms of the main factor effects, soil types significantly influenced levels of proline, 2AP and Na content in leaf tissues. Types of osmoprotectants also significantly influenced chlorophyll, free proline, 2AP and Na contents in leaf tissues (Table 5). However, chlorophyll content was not significantly different between the two soil types. This is likely due to balancing effects of urea fertilization which caused the differences in soil N levels of these two soil types did not affect rates of N absorption from soil. In addition, N requirement of the plant might have been fulfilled by urea fertilization, so that the formation of leaf chlorophyll was not disturbed. This was confirmed by the non-significant difference of leaf tissue N levels between the two soil types (Table 5).

Table 5. Summary of ANOVA results for plant physiological characters and quality of the rice aroma

Physiological characters	Main effects		Interaction
	Location (m)	Osmoprotectant (o)	m x o
Chlorophyll content (mg g^{-1} FW)	ns	**	ns
Proline content (µmol g^{-1} FW)	**	**	ns
2AP Content (ppb)	**	**	-
Aroma Score	ns	ns	-
Na (%)	*	**	-
N (%)	ns	ns	-
P (%)	ns	ns	-
#K (%)	ns	ns	-
Na/K ratio	ns	ns	-

Remarks: ns = non-significant; *,** = significant at p-value ≤ 0.05 and ≤ 0.01 respectively; - = not available because replicated data were not enough for two-way ANOVA; # = content in leaf tissue

Table 6. Effects of soil types and exogenous osmoprotectant on chlorophyll, proline, 2AP contents and aroma score of Pare Wangi upland rice

Treatment (s)	Chlorophyll content (mg g^{-1} FW)	Proline content (µmol g^{-1} FW)	2AP content (ppb)	Aroma score
Soil type (Location)				
Specific	2.89 a	14.42 a	2.33	2.2
Target	3.09 a	13.70 b	1.91	2.1
HSD	0.40	0.11	-	-
Osmoprotectant				
control	2.41 b	17.32 a	2.58	2.7
10 mM proline	3.26 a	15.57 b	2.85	2.4
20 mM proline	2.99 ab	15.09bc	1.63	2.1
10 mM sorbitol	3.18 a	14.39 c	2.00	2.1
20 mM sorbitol	3.15 a	12.93 d	1.35	1.8
10 mM sucrose	3.19 a	12.24 d	2.71	2.2
20 mM sucrose	2.75 ab	10.89 e	1.73	1.9
HSD	0.70	1.03	-	-

Remarks: Numbers followed by the same letter(s) are not significantly different at Honestly Significant Difference (HSD) post hoc test (p≤0.05); - = Not Available because data were analyzed using two-way ANOVA with not enough replication

Chlorophyll contents in leaves of rice plants treated with 10 mM proline, 10 mM and 20 mM sorbitol, and 10 mM sucrose were significantly higher than those of the control plants. Meanwhile chlorophyll content in rice plants treated with 20 mM proline and 20 mM sucrose were not significantly higher than those in control plants (Table 6). This indicates that besides osmo-protectant type, its concentration is also an important factor in improving the effectiveness of osmoprotectant application. Similar phenomenon applies in leaf proline content of the rice plant.

HSD test showed that levels of proline in leaves of rice grown on soil from specific location were significantly higher than those grown on soil from target location. Furthermore, levels of 2AP and aroma scores between these locations showed similar trend with proline levels (Table 6). This may likely to be related to differences in soil texture between the two soil types (Table 1).

Sandy textured soil was reported to have an effect in improving the quality of rice aroma (Yoshihashi, Nguyen, & Kabaki, 2004). In that case, soil texture is probably related to the ability of the soil to maintain soil moisture and levels of drought stress. Nevertheless, the clayey soil of the specific location provided better flavor quality of the rice than those of the sandy loam soil from the target location. The difference in soil water content between field capacity and permanent wilting point for these two soil types was almost similar (Table 1), so that levels of water stress at 75% field capacity was also almost similar between these two soil types. Soil salinity level is likely to be indirect factor causing drought stress on rice plant that is higher at soil from the specific location than soil from the target location. The drought stress triggers an increase in the proline amino acid which then promotes the formation of 2AP compound (Buttery, Ling, Juliano, & Turnbaugh, 1983; Kibria, Islam, & Begum, 2008), resulting in a stronger rice aroma. Effect of salinity in increasing levels of 2AP compound of rice was also reported by Roychoudhury, Basu, Sarkar, & Sengupta (2008).

The effect of osmoprotectants on grain yield per pot (Table 4) was not in line with their influence on the 2AP content and dehusked-rice aroma score. The levels of 2AP and the aroma scores in response to application of proline, sorbitol and sucrose, respectively at a concentration of 20 mM, were even lower than those in the control plants (Table 6). The levels of 2AP showed an increase in rice plants treated with 10 mM proline and 10 mM sucrose compared with those in control plants. This indicated that application of the osmoprotectants proline, sorbitol and sucrose at a concentration of 20 mM more effectively reduced drought stress. This was evident from the reduced levels of leaf tissue proline as a precursor of 2AP compound.

Treatments with proline and sucrose at a concentration of 10 mM also reduced levels of proline in the rice plants, but 2AP levels in the dehusked rice tended to increase as compared with those in control plants. This indicated that other factors may affect the levels of 2AP compounds, presumably the balance of plant nutrients (Roychoudhury, Basu, Sarkar, & Sengupta, 2008). In general, osmoprotectant application through leaves will increase the uptake of water and improve the ability of roots to absorb minerals (Bianco, Rieger, & Sung, 2000), and these would achieve a balance of nutrients required by the rice plants.

Table 7. The effects of soil types and exogenous osmoprotectants on percentage of Na, N, P, K and Na/K ratio in leaf tissues

Treatment (s)	N (%)	P (%)	K (%)	Na (%)	Na/K ratio
Soil type (Location)					
Specific	2.23	0.24	1.74	0.93	0.57
Target	2.37	0.25	1.91	0.89	0.49
Osmoprotectant					
control	2.24	0.23	1.91	0.89	0.47
10 mM proline	2.24	0.25	1.97	0.87	0.44
20 mM proline	2.37	0.26	1.37	0.91	0.67
10 mM sorbitol	2.35	0.25	1.62	0.94	0.65
20 mM sorbitol	2.12	0.24	2.18	0.81	0.37
10 mM sucrose	2.44	0.27	2.19	0.98	0.45
20 mM sucrose	2.36	0.22	1.54	0.97	0.63

Table 8. Results of simple correlation analysis between each plant character

Variable	N (%)	P (%)	K (%)	Na/K	Chlo	Pro	2AP	Aroma
Na (%)	0.339 ns	0.044 ns	-0.329 ns	0.486 ns	-0.263 ns	-0.267 ns	0.365 ns	0.081 ns
N (%)	-	0.569*	-0.267 ns	0.348 ns	0.309 ns	-0.232 ns	-0.032 ns	-0.321 ns
P (%)		-	0.041 ns	0.044 ns	0.311 ns	0.098 ns	0.133 ns	-0.539*
K (%)			-	-0.970**	0.128 ns	-0.088 ns	0.216 ns	0.060 ns
Na/K				-	-0.088 ns	0.005 ns	-0.137 ns	-0.100 ns
Chlo					-	-0.263 ns	-0.182 ns	-0.319 ns
Pro						-	0.402 ns	0.541 *
2AP							-	0.519*

Remarks: Chlo= Chlorophyll content (mg g^{-1} FW); Pro= Proline content (µmol g^{-1} FW); 2AP= 2AP content (ppb); Aroma= Aroma score

The markedly lower mean of grain yield per pot of rice grown at soil taken from the specific location than those grown at soil taken from the target location (Table 4) was supported by the fact that the content of Na leaf tissue in plants grown at soil from the specific location is higher than that grown at soil from the target location (Table 7). On the other hand, the influence of soil type on K content of leaf tissue showed the opposite trend. The existence of K elements in leaf tissues has been reported to have an important role in the opening of stomata, so that CO_2 gas can enter the leaf tissue of the rice plants to speed up the process of photosynthesis (Marschner, 1986).

The ability of exogenous osmoprotectants to suppress Na uptake and to reduce Na/K ratio in leaf tissue is an important mechanism in increasing crop yields (Nounjan, Nghia, & Theerakulpisut, 2012). In this study, this mechanism was demonstrated by increased K absorption and reduced Na absorption in the plants treated with 10 mM proline and 20 mM sorbitol (Table 7), which suggests a process of substitution of Na by K, and causing the decrease of Na^+/K^+ ratio. However, effectiveness in reducing the Na^+/K^+ ratio in the leaf tissue was not shown at all other treatments. The treatment with 10 mM sucrose caused an increase in absorption of K as well as Na, but the Na^+/K^+ ratio in this treatment was still lower than that in control plants. Other osmo-protectant treatments, such as 20 mM proline, 10 mM sorbitol, and 20 mM sucrose increased the absorption of Na and N, but reduced K uptake, and the effect was not consistent with the uptake of P (Table 7). These findings are in line with the results reported by Yuan & Lin (2008). They proposed the importance of the correct application concentration of exogenous osmoprotectants in order to obtain their effective effects in improving crop ability to overcome the drought and salinity stresses through adjustment of osmotic pressure and reduction of the Na^+/K^+ ratio.

Correlation between Aroma Quality and Plant Physiological Characters

Correlation analysis results showed that 2AP content was not significantly correlated with Na, N, P, K content and Na/K ratio in leaf tissue (Table 8). This indicated no direct effect of the presence of these elements in the leaf tissue on 2AP content in rice. The existence of these elements in soil was more frequently reported to have association with increased 2AP levels or the strength of husked-rice aroma. The role of Na associated with increased salinity has been widely reported to correlate with increased levels of 2AP (Roychoudhury, Basu, Sarkar, & Sengupta, 2008). A lower soil N generally improves the aroma quality of aromatic rice, while the presence of P has been reported to show significant correlation with the 2AP level of husked rice, in which its concentration increases in husked rice (Rohilla, Singh V., Singh U., Singh R., & Khush, 2000).

Correlation between the levels of 2AP and other characters of the plants has some practical benefits in estimating the strength of aroma of a particular variety (in selection) with an easier and/or more efficient method. In this study, a tendency of positive correlation between levels of proline and 2AP levels is in line with the concept of 2AP biosynthesis. The correlation is likely to be stronger if the level of drought stress and onset of the stress are also considered, as has been reported by other researchers (Gay et al., 2010; Itani, Tamaki, Hayata, Fushimi, & Hashizume, 2004). A significant positive correlation between levels of 2AP and aroma scores has proven that scent aroma of the upland rice is largely determined by the levels of 2AP in the husked rice.

CONCLUSION

Types of soil and osmoprotectant significantly influenced grain yield/pot and aroma quality of upland rice var. Pare Wangi but there was no significant interaction effect between these two factors. Grain yield was higher in rice grown on soil from target location than that grown on soil from specific location, but levels of proline, 2AP and aroma scores indicated the opposite trends. The 2AP levels significantly correlated with aroma scores. Application of the osmoprotectants proline and sucrose at 10 mM was more effective in improving grain yield and maintaining aroma quality compared with other treatments.

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