PUTERI AFIQAH ABDUL WAHAB AND AZIZ AHMAD^{*}

School of Fundamental Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

*Corresponding author: aaziz@umt.edu.my

Abstract: Salinity is one of the major constraints in the rice production worldwide. Rice plants have moderate tolerance towards salinity. Salinity changes cell membrane permeability and fatty acid compositions by releasing the free fatty acids. Nonetheless, the effect of exogenous fatty acid such as arachidonic acid (AA) on rice grown on saline soil is yet unknown. The objective of the current study is to determine the effect of AA on the morphological traits and free fatty acids of rice plant grown under saline conditions. Rice plants grown on saline soil (EC=12 ds/m) were treated with 50 μ M AA on day 45 after transplant. Leaves and panicles were sampled after two weeks of treatment and analysed for fatty acid profile using GC-MS. The morphological traits were observed at the maturity stage. Results showed that AA treatment improved the grain fill-in of the saline stress rice and reduced the accumulation of free fatty acids in the cell. The AA treatment also increased the linoleic acid (18:2), linolenic acid (18:3) in panicles and, dihomo- γ -linolenic acid (20:3) and nervonic acid (24:1) in leaves. The finding suggests that exogenous AA regulates salinity stress in rice by reducing the accumulation of free fatty acids.

Keywords: arachidonic acid, panicle, spikelet, polyunsaturated fatty acid, Oryza sativa

Introduction

Climate change has a big impact on soil performance and functions (Dasgupta et al., 2015), which affects the agriculture sectors. Salinization (Hou et al., 2016) and accumulation of salt in soil (Reddy & Crohn, 2014) are among abiotic factors that contribute to crop production and yield. Saline soil is soil that has an electrolyte concentration (EC) of 4 decisiemens per meter (dS/m) or higher (Amacher et al., 2000). Salinity inhibits plant growth and productivity through osmotic stress and ion toxicity (Tarakcioglu & Inal, 2002). Higher soluble salt in the soil will change the soil pH, nutrient imbalance, reduce water infiltration, and cause accumulation of toxic element in the plant (Tarakcioglu & Inal, 2002; Sacała et al., 2011).

Rice (Oryza sativa L.) is one of the most important cereal crops and a leading staple food in the world (Reddy et al., 2017). Generally, rice is sensitive to pH changes (Huang et al., 2017) but moderately tolerant to salinity (Aguilar et al., 2017). Under high salinity condition, rice plant exhibits leaf chlorosis, stunted growth, failure of spikelet development and seedling establishment and reduction in gain yield (Khan et al., 1997; Ali et al., 2004). When the EC of the soil exceeds 6 dS/m, the grain yield decrease is about 60% to 100% (Kibria et al., 2017). Nonetheless, rice sensitivity towards salinity varies during growth phases (Tadesse et al., 2017). At the vegetative and early reproductive phase, saline effects the panicle initiation and tillering (Zeng et al., 2001; Balkan et al., 2015; Aguilar et al., 2017). Rice is relatively salt tolerant during ripening phase (Khan et al., 1997; Zeng et al.,

2001) and differs among cultivars (Tadesse *et al.*, 2017).

Plant cell contains the plasma membrane comprising bilayer phospholipids that protects the intercellular component of a cell and control the movement of materials across the cell (Roualdes & Rouessac, 2017). Thus, plasma membrane in root cell is an important organ that directly exposed to saline conditions is (Mansour, 2013). In the presence of excessive salt, the plasma membrane will alter its permeability, membrane lipid compositions and the activity of membrane bound enzyme (Salama et al., 2007). As plant experience stresses such as wounding or increasing amount of soluble salt, the plasma membrane releases the FFA (Conconi et al., 1996; Scholz et al., 2015) Endogenous FFAs released into the cell is converted to jasmonates, a lipid-derived hormone that includes jasmonic acid, methyl ester jasmonic acid and amino acid conjugates that involve specific gene activation and a series of enzymatic reactions (Lyons et al., 2013; Yuan & Zhang, 2015). The α-linolenic is the precursor for jasmonates acid biosynthesis catalysed by lipoxygenase enzyme to form the 13(S)-hydroperoxy linolenic acid and converted into several intermediates via oxidation process (Schaller et al., 2004; Lyons et al., 2013; Yuan & Zhang, 2015). In plants including rice, jasmonates play a major role in plant development such as seed germination, root growth, reproduction and senescence and plant defence (Browse, 2009; Fonseca et al., 2009). Jasmonates control the transition of spikelet meristem to flower meristem (Cai et al., 2014) of rice. Spikelet is important for the formation of floral organ determination and grain yield of rice (Tanaka et al., 2014).

AA is a polyunsaturated fatty acid and abundantly present in plasma membrane as a phospholipids. Previous study by Tian *et al.* (2014) reported that AA was released from the membrane cell as a response to stresses or during pathogen attacks (Garcia-Pineda & Lozoya-Gloria, 1999). The phospholipase A2 enzyme catalysed the linoleic acid and α linolenic acid in the cell membrane into free AA (Adam *et al.*, 2008). The accumulation of AA triggers the biosynthesis and accumulation of phtyoalexin, chitinase, lignin and ethylene production (Küpper *et al.*, 2009). To date, the effect of exogenous AA on rice growth under saline conditions remains unknown. Therefore, the objective of this study is to determine the effect of exogenous AA on the morphological traits and endogenous FA composition of *Oryza sativa* L. grown under saline conditions. It was hypothesized that the exogenous AA improve the morphological traits and alter the accumulation of free fatty acid profile in rice plants.

Materials and Methods Study Site

Rice plants were planted and grown under shade in the greenhouse at Universiti Malaysia Terengganu (UMT), Kuala Nerus, Terengganu from September 2017 to January 2018. Biochemical analysis was carried out at Institute of Marine Biotechnology, UMT.

Seedling Preparation and Arachidonic Acid Treatment

Treatment was carried out using the saline soil taken from Kemasin, Bachok Kelantan and filled up in a trough. The soil EC was measured using EC meter and adjusted to a range of 10 to 12 dS/m with sea water obtained from sea near to UMT beach. For seedling preparation, the soil mixture consisting of top soil, river sand and organic matter at ratio of 3:2:1 was The salinity-tolerant genotypes prepared. namely SS1-41 [CSR28] was obtained from the International Rice Research Institute (IRRI), Los Banos and Laguna, Philippines. One hundred seeds were soaked in tap water overnight. After soaking, the seeds were kept moist and in warm temperature (35 °C) to allow germination prior to being transferred to the soil. The water level was maintained in a range of 1-3 cm height. NPK green fertilizer was applied on day 14 at 9 grams/trough. On day 20, the healthy plantlets were transplanted into saline soil in a trough. A randomized block design was used with three replicates (three

troughs; 15 plants per trough), where the plants were sown in three rows with five plants for each row. The water level was maintained in a range of 4-5 cm height. Fertilizer was applied on day 40, 18 grams of NPK green and 3 grams of urea per trough. AA was prepared in 0.05 % (v/v) of Tween 20 at concentration of 50 μ M prior to application on rice foliar. On day 45, 12 mL of 50 μ M AA solutions was sprayed on foliar of six treatment plants, while the control plants were sprayed with 12 mL of 0.05 % (v/v) Tween 20. On day 60, 19 grams of fertilizer (NPK blue) was given to each trough; fertilizer regime was based on recommendation by MARDI.

Sampling and Morphological Traits Analysis

On day 55, leaves and panicles were taken from each trough for both control and treatment plants. Six leaves and panicles were sampled; a total of 36 samples were collected and used for oil extraction and fatty acids analysis. The leftover plants were used for morphological measurement (Table 1). The morphological traits were measured between day 70 (middle of reproductive stage) and day 120 (ripening stage).



Figure 1: Rice plants on Day 7 (A), the position of treatment and control rice plants on saline soil in the trough (B)

Oil Extraction

Samples were oven-dried at 60 °C and measured for dry weight. Oil extraction was carried out based on methods by Cha et al. (2011). The dried samples were powdered and mixed with HCl_{conc} at ratio 0.1: 2 (g: mL), then incubated in boiling water bath for 15 min. Then, hexane (2 mL) was added into the mixture and vigorously vortexed. The extraction was repeated twice with 2 mL of hexane. The upper layer of the mixture was transferred into an empty rotary-evaporator flask, incubated in a water bath at 60 °C that had been previously fixed with rotary evaporatory system. The pressure of suction was set to 335 mbar. The oil obtained in the flask was transferred into a vial and dried overnight in oven at 70 °C until a constant weight was obtained.

Fatty Acid Methyl Esterification

Fatty acid esterification to methyl esters followed the methods used by Cha et al. (2011). Oil (50 mg) was transferred to condenser flask, and four pieces of boiling chips and 4 mL of 0.5 methanolic NaOH were added. Then, % Lieberg condensers were attached to the flask and the mixture was heated on a heating mantle for 3 min. While the heating process took place, 5 mL of 20% boron-trifluoride methanol (BF3-MeOH) and 2 mL of n-heptane were added into the mixture respectively. The flask was removed from heating mantle and the Lieberg Condensor after the vapours had been condensed. Fifteen ml of saturated sodium chloride was added and mixed well. The mixture was transferred into a 50 mL test tube and the upper layer of the mixture was shifted

into 5 mL bijou bottle containing sodium sulphate anhydrous. The FAME obtained was filtered using syringe filter (nylon 0.4 μ m) and ready for gas chromatography analysis.

Mass Spectrometry-Gas Chromatography (GC-MS)

FAME analyzed was using gas chromatography equipped with 0.25-mm of ionization detector, Rtx5MS-30m capillary column and single 150 psi EPC split-splitless injection ports. Helium was used as carrier gas at a constant flow rate of 1 mL/minute. The temperature of injector was set to 300 °C. The oven temperature was set to 100 °C for 10 minutes for isothermal heating and was increased to 300°C for 20 minutes at a rate of 10 °C per minute. Final oven temperature was held for 10 minutes. The individual fatty acid compositions were identified by comparison between retention times of FAME library from the MS-GC.

Statistical Analysis

Data were statistically normalized and analyzed using the multi-variance analysis. The

significant different of mean was identified with a *t*-test, p = 0.05.

Results and Discussion *Morphology Traits*

Table 1 shows the morphological traits of rice plants treated with exogenous AA and control. The leaf length of control plants were significantly (p < 0.05) 1.14-fold higher than treatment plants. A similar trend was observed on the plant height, which were 1.12-fold higher than treatment plants. On the other hand, the tiller number per plant, panicle length and spikelet number per panicle did not significantly differ between treatment and control (Table 1). The spikelet number was in range of 68 to 96 per panicle. Interestingly, the number of filled grain per panicle was significantly higher (p < 0.05) in treatment, which was 3.23-fold higher compared to the control.

Table 1: The morphology traits of rice treated with arachidonic acid and control. Values with same small capital letter in the same role did not significantly different. Data are mean \pm sd (n=3), p \leq 0.05 significant different using *t*-*test*.

Morphological traits	Control	AA	<i>p</i> value
		Treatment	(t-test)
Leaf length (cm)	75.8 ± 1.2^{a}	66.1 ± 4.1^{b}	0.0452
Plant height (cm)	80.7 ± 1.4^{a}	71.6 ± 3.4^{b}	0.0315
Tillers number/plant	8.6 ± 0.5^{a}	$7.6\pm0.5^{\mathrm{a}}$	0.1012
Panicle length (cm)	17.9 ± 0.6^{a}	$19.3\pm1.5^{\rm a}$	0.2609
Spikelet number/panicle	79 ± 11^{a}	82 ± 14^{a}	0.7651
Filled grain/Panicle	12 ± 3^{b}	42 ± 12^{a}	0.0443
Percentage of filled grain/panicle (%)	15.4 ± 2.5^{b}	45.7 ± 1.9^{a}	0.0001

Salinity is one of the crucial factors that limits the rice growth and productivity. In rice, saline stress gives crucial effects on vegetative phase and early reproductive stage. Leaf chlorosis, stunted growth, failure of spikelet development and seedling establishment and reduced grain yield were the symptoms showed by salt-affected rice (Khan *et al.*, 1997; Ali *et al.*, 2004). A recent study showed that when the EC of the soil exceed 6 dS/m, reduction in grain yield from its maximal yield performance decreased about 60% to 100% (Kibria *et al.*, 2017). Our results showed that the measured morphological traits—leaf length, plant height and tiller number—were not improved by the AA treatment. However, the filled grain was improved by the AA treatment. Rice grain filling is associated with grain nitrogen concentration (Wei *et al.*, 2018), which has higher salinity levels of K^+ and NO₃⁻ commonly in low concentrations (Gosh *et al.*, 2016). This result suggests that exogenous AA managed to reduce the salinity stress and permit the grain filling activity.

Fatty acid profile

AA altered the FFA composition in both leaves and panicles of AA treated plants. The results showed that the fatty acid spectrum in both AA treated samples and control contained various types of saturated fatty acids from 4C to 24C and unsaturated fatty acids from 14C to 24C (Table 2). Major fatty acids were significantly increased in the AA treated samples, which were the C20:3 and C24:1 in leaves, C18:2 and C18:3 in panicles (Table 2).

Nervonic acid (C24:1) was the most abundant fatty acid the rice leaves. It was 2.57

mg/mg and 6.38 mg/mg of sample in control and AA treatments, respectively. This result exhibited an increment of C24:1 up to 3.81 mg/g of sample in the leaves of AA treatment. Meanwhile, the C24:1 in panicles was 3.62 mg/mg and 2.26 mg/mg of sample in both control and AA treatments, respectively. This showed a reduction of C24:1 (1.36 mg/g of sample) in the panicles of AA treatment. Results also showed that salinity stress triggered higher accumulation of free FA C24:1 in leaves compared to panicles in the AA treated plants (Figure 2). This suggests that the long chains monounsaturated fatty acid C24:1 may be involved in stress regulation in the leaves during the early reproductive stage. To our knowledge, this is the first report on the presence of C24:1 in rice plants. The FA is a very long chain fatty acid that has been found only on the seed oils of few plants (Huai et al., 2015).

Fatty	Control	Treatment	Δ TL-CL	Control	Treatment	Δ TP-CL
Acid	Leaf	Leaf		Panicle	Panicle	
	(CL)	(TL)		(CP)	(TP)	
C4:0	0.75	0.12	-0.63	0.03	0.04	0.01
C6:0	0.02	-	-0.02	0.03	-	-0.03
C8:0	0.14	0.06	-0.08	0.06	0.05	-0.01
C10:0	0.00	0.02	0.02	0.05	-	-0.05
C12:0	0.06	0.05	-0.01	-	0.06	0.06
C14:0	0.12	0.07	-0.05	-	0.06	-0.06
C14:1	0.02	0.09	0.07	0.07	0.15	0.08
C15:0	0.23	0.09	-0.14	0.15	0.10	-0.05
C15:1	0.87	0.40	-0.47	0.41	0.36	-0.05
C16:0	3.30	1.37	-1.93	2.30	0.06	-2.24
C17:0	0.44	0.19	-0.25	0.13	0.02	-0.11
C17:1	0.11	-	-0.11	-	0.15	0.15
C18:0	0.27	0.29	0.02	0.75	0.18	-0.57
C18:1	0.28	0.21	-0.07	0.12	0.16	-0.04
C18:2	1.21	0.11	-1.10	0.44	1.03	0.59
C18:3	0.33	0.16	-0.17	0.28	2.58	2.30
C20:0	0.19	0.09	-0.10	0.13	0.08	-0.05
C20:1	0.01	0.16	0.15	0.30	0.10	0.70
C20:3	0.79	2.02	1.23	0.53	0.61	0.08
C20:4	0.02	0.06	0.04	0.03	0.07	0.04
C20:5	0.06	0.12	0.06	0.07	0.04	-0.03
C22:0	0.00	0.22	0.22	0.14	0.18	0.04
C22:1	1.12	0.07	-1.05	0.08	0.20	0.12
C22:2	0.02	0.04	0.02	0.02	0.11	0.09
C22:6	0.03	0.12	0.19	0.21	0.10	0.11
C23:0	0.13	0.36	0.23	0.03	0.19	0.16
C24:0	1.91	0.17	-1.74	0.30	0.21	-0.09
C24:1	2.57	6.38	3.81	3.62	2.26	-1.36

Table 2: Fatty acid composition of leaves and panicles from AA treated and control plants.

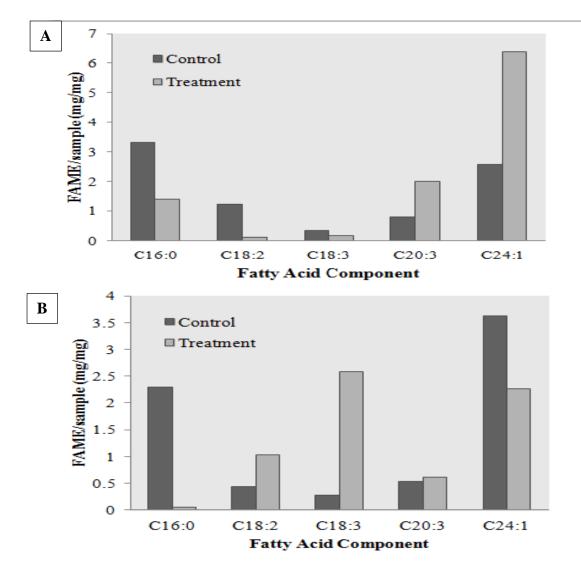


Figure 2: The fatty acid content in FAME sample of leaf (A) and panicle (B) for both control and treatment condition

The dihomo- γ -linolenic acid (20:3) was the second fatty acid that significantly increased in the leaves of AA treatment, which was 1.23 mg/mg sample. Results also showed that five fatty acids were dominant in all samples, which were 16:0, 18:2, 18:3, 20:3 and 24:1 as shown in Figure 2. Meanwhile, the 18:2 and 18:3 were two fatty acids that significantly increased in the panicles of AA treatment. It was 0.59 mg/mm and 2.3 mg/mg of sample, respectively. The current finding showed that exogenous AA controlled the membrane cell from releasing the FFA to the cell. Only two fatty acids were released as FFA in leaves, i.e. C24:1 and C20:3. While in the panicles were the C18:2 and C18:3. The C18:3 released might be used as precursor for the jasmonates biosynthesis (Schaller et al., 2004). The 13 (S)-hydroperoxy linolenic acid can be converted into several other compounds via oxidation process such as 18:2, 18:1 and 16:1 (Conconi et al., 1996; Schaller et al., 2004). Current finding also exhibited that the amount of palmitic acid released under salt stress was inhibited by the exogenous AA in both leaves and panicles (Figure 2). The C16:0 is one of the major constituents released by plant plasma membrane to defend the plant towards injury and stresses. The C16:0 then will be converted into longer fatty acid chain (Adjepong et al., 2017) such as the C24:1. Research has shown that environmental stresses, such as drought,

cold, salt, and heat, can induce changes in fatty acid composition, especially the content of linolenic acid (Aziz *et al.*, 2015; Sui *et al.*, 2018).

Conclusion

The exogenous AA plays an important role in facilitating plant defence mechanism against salinity stress in rice plant. It regulates the release of FFA from plasma membrane into the cell. The exogenous AA also increases the percentage or number of fill-in grain per panicle. Therefore, higher accumulation of polyunsaturated fatty acids such as the α -linolenic acid and dihomo- γ -linolenic acid maybe involved in the regulation of starch transport and storage in rice under salinity stress. Nonetheless, further study is required to confirm the contribution of PUPA in grain filling process.

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