Exogenous Arachidonic Acid and Methyl Jasmonate Induced-Changes on the Free Fatty Acid Profile of Rice (*Oryza sativa* L.) Grown on Saline Soil

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ABSTRACT

Rice growth and productivity are restricted by the uncontrollable abiotic factors such as salinity and drought at various developmental stages. Plants respond to abiotic stress by modifying fatty acid profile and turnover to sequester carbon energies and phospholipid. Therefore, acyl lipid metabolism is crucial throughout the plant life cycle and in response to abiotic or biotic stresses. Nonetheless, a very limited report on the effects of exogenous fatty acids on lipid profile in rice grown under salinity conditions. Therefore, the objective of the study was to determine the effects of foliar application of arachidonic acid (AA) and methyl jasmonate (MeJA) on the fatty acid profile of rice planted on saline soil. AA and MeJA were separately applied on foliar at with or without 50 µM two weeks prior to panicle initiation. The leaves and panicle were analysed for free fatty acid profile using gas chromatography mass-spectrophotometry. Results showed that the levels for at least 25 fatty acids were altered by the exogenous fatty acid treatments in both leaves and panicle. Alpha-linolenic acid was significantly increased in panicle by both AA and MeJA treatments. This finding suggested that α-linolenic acid may play a major role in regulating the panicle development during the salinity stress. In addition, the AA and MeJA treatments reduced the formation of cytosolic free fatty acids in both leaves and panicle of rice plants under saline stress.

Keywords: α-linolenic acid, gas chromatography, rice panicle, free fatty acids

INTRODUCTION

Soil salinity is becoming a global threat to plant growth and crops yield including rice. *Oryza sativa* L. or rice is a staple food for over two billion people worldwide, and the demand for rice consumption has increased yearly as the population increases (Reddy et al., 2017). Nonetheless, yield and productivity of rice are severely affected by salinity as it is one of the most salt-sensitive grain crops (Kumar and Khare, 2016). Rice exhibits various levels of salt tolerance at different growth and developmental stages (Tarakcioglu and Inal 2002; Aguilar et al., 2017); germination phase being relatively salt tolerant, but most sensitive at the reproductive stage which has resulted in reduced grain yield (Khan et al., 1997). Rice cultivated at electrical conductivity (EC) of 3 dS/m (moderate salinity) displayed 12% yield drop (Huang et al., 2017; Reddy et al., 2017). At salinity level above the threshold, rice fails to produce flower or spikelets, florets become sterile and number of unfilled grains increases (Chai et al., 2014; Yuan and Zhang, 2015).

Plant response to soil salinity is a natural, random and complex event. Excess salt exposure usually causes osmotic stress, ionic imbalance and oxidative stress. Osmotic stress is triggered by the low water potential due to low stomatal conductance, which results in an excessive generation of reactive oxygen species (ROS), reduction in cellular electron transport rate and accumulation of sodium and chloride (Khare et al., 2015; Nur Fatin et al., 2017). ROS activity can cause oxidative damage to lipid membrane, pigments, proteins and nucleic acids. Glycerolipids, the fatty acids (FAs) diester and glycerol are the major components of the membrane architecture. The FA moieties either saturated or unsaturated are essential for membrane constituent, growth and development of plants. Each membrane within the plant is unique in its acyl and lipid composition, where the two polyunsaturated fatty acids, 18:2 and 18:3, represent about 70% of total FAs in higher plant species (Mei et al., 2015). Changes in the membrane fatty acid profile are considered to be vital in defense mechanism against fungal infection and osmotic stress (Mansour, 2013) as well as their role in lipid homeostasis, plant performance and lipid trafficking in photosynthetic cells (Li et al., 2016). During pathogen infections, free fatty acids (FFAs) such as palmitic acid, linoleic acid and α-linolenic acid are released by the plasma membrane (Conconi et al., 1996) and subsequently converted into jasmonic acid, a lipid-derived hormone (Yuan and Zhang, 2015). In rice plants, jasmonic acid regulates the transition of spikelet meristem to flower meristem by activating the transcription of *EG1* and *EG2* genes, which subsequently activates other proteins (Chai et al., 2014) under normal soil condition. To date, the cold-induced change of the FA unsaturation level is well documented, where higher unsaturation level is required to be freeze-tolerance to keep correct membrane fluidity. However, information on the FA profile during salinity stress and effects of exogenous FAs remain limited and unclear, and thus, largely open for investigation.

Therefore, the objective of this study was to determine the effects of exogenous arachidonic acid (AA) and methyl jasmonate (MeJA) on the free fatty acid profile of rice panicle grown under saline condition. The findings of this study would provide information on the contribution of fatty acids in regulating salinity stress.

MATERIALS AND METHODS

In this study, the salt-tolerant rice genotype, namely SS1-41 [CSR28], was obtained from the International Rice Research Institute (IRRI), Los Banos and Laguna, Philippine. The material has been screened for salinity tolerant in IRRI and Universiti Malaysia Terengganu (UMT) (Ma et al., 2018). The experimental design used was a completely randomised design and the experiment was carried out under glass house at UMT. Seedlings were grown on normal soil for 15 days and transplanted onto saline soil, EC=12 dS/m adjusted with seawater. Three replicates (n=3) were used with 15 seedlings per trough. The AA and MeJA treatments were following the method previously described by Chai et al. (2014). Two weeks prior to panicle initiation (approximately 40 days after planting), 2 mL of 50 μM of AA or MeJA prepared in 0.05% (v/v) Tween-20 was sprayed on the foliar of treated plants; this period of time would allow the biochemical events from the leaves to the panicle to occur. Control plants were sprayed with distilled water. The fertiliser was applied as recommended by MARDI. Panicle and leaves were sampled after early panicle initiated (day 55 to 60) and analysed for the fatty acid profile. Total oil extraction and fatty acid esterification were carried out as previously described (Aziz et al., 2015). Fatty acid methyl esters (FAMEs) were chromatographed on gas chromatography mass-spectrophotometry (GC-MS) and the metabolites were identified by comparison with the FAME-library in the Institute of Marine Biotechnology, UMT.

RESULTS AND DISCUSSION

Our results showed that AA or MeJA treatments had reduced the accumulation of most of the cytosolic free fatty acids (FFAs) in both leaves and panicle of rice planted under saline conditions (Tables 1 and 2). This finding indicated that treatment with exogenous AA or MeJA managed to maintain or reduce the FFAs level in the cytosol, which subsequently diminish the effects of salinity stress (Conconi et al., 1996). The AA treatment had increased the maed acid (20:3) and nervonic acid (24:1) in leaves and the linoleic acid (18:2) and α-linolenic acid (18:3) in panicles, respectively. At the same time, FAs that were decreased were linoleic acid, erucic acid and legnoric acid in leaves, stearic acid and nervonic acid in panicle, and palmitic acid in both leaves and panicles (Table 1). On the other hand, MeJA treatment had increased the eicosapentaenoic acid (20:5) and behenic acid (22:0) in leaves and α-linolenic in panicles. A major reduction was recorded on the palmitic acid, linolenic acid and nervonic acid (NA) in both leaves and panicle (Table 2).

Table 1. Effects of arachidonic acid treatment on fatty acid composition in leaves and panicles

Fatty Acids	Leaves (peak area)			Panicle (peak area)		
	C	T	Δ TL-CL	C	T	ΔTP-CP
C4:0; Butyric acid	0.75	0.12	-0.63	0.03	0.04	0.01
C6:0; Caproic acid	0.02	-	-0.02	0.03	-	-0.03
C12:0; Lauric acid	0.06	0.05	-0.01	0.07	0.06	-0.03
C14:0; Myristic acid	0.12	0.07	-0.05	0.10	0.06	-0.04
C14:1; Tetradecenoic acid	0.02	0.09	0.07	0.07	0.15	0.08
C15:0; Pentadecylic acid	0.23	0.09	-0.14	0.15	0.10	-0.05
C15:1; Pentadecenoic acid	0.87	0.40	-0.47	0.41	0.36	-0.05
C16:0; Palmitic acid	3.30	1.37	-1.93	2.30	0.06	-2.24
C17:0; Margaric acid	0.44	0.19	-0.25	0.13	0.02	-0.11
C17:1; Heptadecenoic acid	0.11	-	-0.11	0.05	0.15	0.10
C18:0; Stearic acid	0.27	0.29	0.02	0.75	0.18	-0.57
C18:1; Oleic acid	0.28	0.21	-0.07	0.12	0.16	-0.04
C18:2; Linoleic acid	1.21	0.11	-1.10	0.44	1.03	0.59
C18:3; α-linolenic acid	0.33	0.16	-0.17	0.28	2.58	2.30
C20:0; Arachidic acid	0.19	0.09	-0.10	0.13	0.08	-0.05
C20:1; Gondoic acid	0.01	0.16	0.15	0.30	0.10	-0.20
C20:2; Eicosadienoic acid	0.14	0.01	-0.13	-	0.01	0.01
C20:3; Maed acid	0.79	2.02	1.23	0.53	0.61	0.08
C20:5; Eicosapentaenoic acid	0.06	0.12	0.06	0.07	0.04	-0.03
C22:0; Behenic acid	0.06	0.22	0.16	0.14	0.18	0.04
C22:1; Erucic acid	1.12	0.07	-1.05	0.08	0.20	0.12
C22:2; Docosapentainoic acid	0.02	0.04	0.02	0.02	0.11	0.09
C23:0; Trycosylic acid	0.13	0.36	0.23	0.03	0.19	0.16
C24:0; Legnoceric aci	1.91	0.17	-1.74	0.30	0.21	-0.09
C24:1; Nervonic acid	2.57	6.38	3.81	3.62	2.26	-1.36

Note: C = control, T = treatment, values are based on the peak areas, Δ is the differences between C and T.

Our previous study (Aziz et al., 2015; Ma et al., 2018) showed that under salinity stress the linoleic acid and α-linolenic acid were increased in rice leaves during the reproduction stage. This indicated that the AA and MeJA treatments had succeeded to overcome the salinity stress by reducing the accumulation of free linoleic acid and α-linolenic in the cytosol. The results also showed that the level of unsaturation fluctuated in the plant organs, which resulted from the exogenous fatty acid treatments. Physiologically, fatty acid desaturation is crucial for maintaining the cellular function, membrane fluidity and plant viability under stress (Mei et al., 2015). However, how these desaturases operate and are regulated remain open for study. Results also showed less accumulation of palmitic acid in leaves after the AA or MeJA treatment which might be due to the palmitic acid being transported to panicle or elongated to stearic acid (18C) and finally converted to unsaturated FAs. Palmitic acid is the substrate for the formation of stearic acid. Our results showed that α-linolenic acid was highly increased in the panicle of treated plants (Tables 1 and 2). The main species of FAs in higher plants are 16C and 18C, which represent about 30 and 70% of total FAs (Mei et al., 2015) with various saturation levels from 16:0 and 18:0 to 16:3 and 18:3, respectively. These observations suggested that the final PUFA levels depend on the rate of cell division and the desaturase, which are responsible for the formation of 18:2 and 18:3 on phospholipids in fast-growing cells during panicle development. Furthermore, α-linolenic acid is the primary substrate for the octadecanoid pathway in the jasmonic acid biosynthesis. Jasmonic acid is a phytohormone that regulates the inflorescence morphogenesis and successful formation of floral organs (spikelets) in rice (Chai et al., 2014) and sorghum (Jiao et al., 2018), where fertile flowers determine grain production (Yuan and Zhang, 2015).

Table 2. Effects of methyl jasmonate on fatty acid composition in leaves and panicle

Fatty Acids	Leaves (peak area)			Panicle (peak area)		
	C	T	Δ TL-CL	C	T	ΔTP-CP
C4:0; Butyric acid	0.75	-	-0.75	0.21	-	-0.21
C6:0; Caproic acid	0.02	-	-0.02	0.03	0.01	0.01
C12:0; Lauric acid	0.06	-	-0.06	0.07	-	-0.07
C14:0; Myristic acid	0.12	-	-0.12	0.10	-	-0.10
C14:1; Tetradecenoic acid	0.02	0.04	0.02	0.04	0.05	0.01
C15:0; Pentadecylic acid	0.23	-	-0.23	0.12	0.11	-0.01
C15:1; Pentadecenoic acid	0.87	0.25	-0.62	0.48	0.51	0.03
C16:0; Palmitic acid	3.30	1.37	**-1.93**	3.64	2.24	**-1.4**
C17:0; Margaric acid	0.44	-	-0.44	0.21	-	-0.21
C17:1; Heptadecenoic acid	0.11	-	-0.11	0.05	-	-0.05
C18:0; Stearic acid	0.27	0.22	-0.05	0.25	0.34	0.09
C18:1; Oleic acid	0.28	0.05	-0.23	0.04	0.09	0.05
C18:2; Linoleic acid	1.21	0.32	-0.89	2.37	0.26	-2.11
C18:3; α-linolenic acid	0.33	0.12	-0.21	0.30	5.29	**4.99**
C20:0; Arachidic acid	0.19	-	-0.19	0.07	0.06	-0.01
C20:1; Gondoic acid	0.01	0.26	0.25	0.40	0.30	0.10
C20:2; Eicosadienoic acid	0.14	0.23	0.09	0.07	0.22	0.15
C20:3; Maed acid	0.79	-	-	0.53	0.40	-0.13
C20:5; Eicosapentaenoic acid	0.02	0.57	**0.55**	0.22	0.96	0.74
C22:0; Behenic acid	0.06	0.84	**0.78**	0.14	0.18	0.04
C22:1; Erucic acid	1.12	0.07	-1.05	0.23	0.20	-0.03
C22:2; Docosapentainoic acid	0.02	0.10	0.10	0.03	0.08	0.08
C23:0; Trycosylic acid	0.13	0.17	0.14	0.45	0.52	0.07
C24:0; Legnoceric aci	1.91	0.17	-1.74	0.16	0.13	-0.03
C24:1; Nervonic acid	4.12	3.25	-0.87	7.12	6.12	-1.00

Note: C = control, T = treatment, values are based on the peak areas, Δ is the differences between C and T.

Our results also showed that salinity stress had induced the release of NA in both leaves and panicle. The exogenous treatment of MeJA had managed to reduce the NA levels in panicle and leaves. However, AA treatment only reduced the NA levels in panicle not in the leaves (Tables 1 and 2). NA is a very long fatty acid involved in the biosynthesis of cortical waxes, seed triacylglycerol and sphingolipid in plants (Haslam and Kunst, 2013). The finding suggested that applied AA might be converted to NA in leaves to allow decomposition of waxes. Transcriptomic analysis on Pokkali (salinity tolerant variety) under salinity stress showed enrichment of genes involved in carotenoid, anthocyanin, wax and shikimate metabolic pathway (Shankar et al., 2016). Nonetheless, further study should be carried out to reveal the AA involvement in triggering the expresseion of related genes and proteins.

CONCLUSIONS

The level of FA desaturation was varied among the rice plant parts. Foliar applications of AA and MeJA induced the accumulation of free fatty acids and desaturation levels of FAs in both leaves and panicle of rice grown in saline soil. FA desaturation might be involved in regulating the salinity stress, where α-linolenic acid was the main fatty acid accumulated in panicle during its development under the salinity stress. This finding suggested that foliar application of AA or MeJA might be a useful approach for rice production in a saline soil region.

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