

FATTY ACID PROFILE OF SALINITY TOLERANT RICE GENOTYPES GROWN ON SALINE SOIL

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ABSTRACT

This paper reports the effect of salinity stress on the fatty acid profile of salinity-tolerant rice grown on saline soil. Three salinity-tolerant rice genotypes obtained from IRRI, namely SS1-40, SS1-41 and SS1-42, were used. The leaves of the control and treatment plants were sampled during the various developmental phases: vegetative, reproductive and ripening. The fatty acid methyl ester (FAME) contents were analysed using gas chromatography techniques. The results revealed that salinity stress significantly influenced the fatty acid content of the three rice genotypes. Palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic acid (18:3) are the fatty acids that may be involved in the regulation of salinity stress. The levels of these fatty acids were higher in plants that planted under salinity stress than in the control during the vegetative and reproductive stages, but lower during the ripening stage. The finding suggested that salinity stress triggers the biosynthesis of fatty acids, such as linolenic acid, responsible for adaptation and growth development of rice plants in response to salinity stress.

Key words: palmitic, stearic, oleic, linoleic, linolenic, salinity stress, rice genotypes

INTRODUCTION

Rice is a staple food for over two billion people (Amirjani, 2010). However, its production can be influenced by the abiotic stresses, an uncontrollable limitation factor worldwide. The agricultural land that is affected by salinity has been annually increasing due to land salinisation as a consequence of an artificial irrigation (Munns and Tester, 2008). A possible and promising solution to this problem is the genetic improvement of rice varieties that are tolerant to salinity. Plants possess various strategy defence mechanisms against salinity stress. Specifically, the cell membrane is the first machinery that plays a critical role in the resistance of plant cells to environmental stresses (Lodhi *et al.*, 2009). Fatty acids are the main components of cell membrane that are responsible for the fluidity of cells in response to stress. In fact, changes in the profile of fatty acids, which are the sole component of membrane lipids, regulate membrane fluidity; thus, they are considered to be vital in the salt

tolerance of plants (Upchurch, 2008). Changes in the membrane lipid or fatty acid composition would alter the membrane permeability, membrane potential and activities of membrane-bound enzymes. The plasma membrane permeability was also an effective selection criterion for salt tolerance in various crops (Mansour, 2013; Munns and Tester, 2008). Marine microalgae grown in saline medium also contain higher levels of polyunsaturated fatty acids, linoleic acid C_{18:2} and linolenic acid C_{18:3} than those grown in fresh water medium (Cha *et al.*, 2011). Furthermore, lipids or fatty acids are also involved in mechanisms that defend against the accumulation or excess production of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), superoxide (O₂⁻¹) and hydroxyl radical (OH·) (Halliwell, 2006). These extremely reactive molecules obstruct the redox system and activate the prime metabolic pathways by directly changing the enzyme activity, DNA structure and membrane properties (Konieczny *et al.*, 2008). High concentrations of H₂O₂ increase oxidative stress by increasing the lipid peroxidation and modification of membrane permeability (Kosova *et al.*, 2013). To

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date, reports on the fatty acid profile in plants under salinity stress have been limited. Therefore, this research aimed to determine the fatty acid contents in salinity-tolerant rice genotypes, namely, SS1-40, SS1-41 and SS1-42, during the vegetative, reproductive and ripening stages.

MATERIALS AND METHODS

Plant Material and Treatment

Three salt-tolerant genotypes, namely SS1-40 [IR 66946-3R-178-1-1(FL 478)], SS1-41 [CSR 28] and SS1-42 [IR 55179-3B-11-3], were obtained from the International Rice Research Institute (IRRI), Los Banos, and Laguna, Philippines. The experimental design was complete randomized, and the experiment was carried out under glasshouse. The selected seeds were germinated and sown for 15 days in normal soil. The saline soil for the salinity treatment was taken from Kemasin, Bachok Kelantan and used to fill a planting trough located in the green house at Universiti Malaysia Terengganu. The salinity level of the soil in the trough was adjusted to EC 8 dS/m with seawater. For subsequent stress treatments, 15-day-old seedlings for each genotype were transferred into the trough in triplicate. The plants were observed daily, and the water level, pH and salinity were monitored weekly. To quantify the fatty acid levels, one healthy leaf from each genotype was harvested during the seedling (15 days after seeding), vegetative (45 days after seeding), reproductive (70 days after seeding) and ripening (90 days after seeding) stages. The required morphological traits were recorded accordingly.

Fatty acid Extraction and Quantification

At harvest, the oil from the leaf was extracted and analysed according to Cha *et al.* (2011). The sample was digested in 10 mL of HCl (conc.) for 30 min and subsequently extracted twice in 25 mL of n-hexane. The n-hexane residue was vaporised using a double boiling technique at 80°C until a constant weight was obtained. The total lipid content was quantified by weight after complete drying. The oil was then esterified as described by Cha *et al.* (2011). The fatty acid composition in the methyl ester (FAME) was determined by capillary GC on a SP-2560, 100 m x 0.25 mm x 0.20 µm capillary column (Supelco) that was installed on a Hewlett Packard 5890 gas chromatograph equipped with a Hewlett Packard 3396 Series II integrator and a 7673 controller, a flame ionization detector and split injection (Agilent Technologies Inc., Santa Clara, CA). The initial oven temperature was set to 140°C, held for 5 min, subsequently increased to 240°C at a rate of 4°C min⁻¹ and then held for 20 min. Helium was used as the carrier gas at a flow rate of 0.5

mL·min⁻¹, and the column head pressure was 280 kPa. Both the injector and the detector temperatures were set to 260°C. The split ratio was 30:1. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards.

Statistical analysis

The data were subjected to a normality test and statistically analysed using multi-variance, and the significant differences were identified with a post hoc Tukey's honestly significant difference (HSD) test at $p < 0.05$ using a Statistical Package (SPSS).

RESULTS AND DISCUSSION

Morphology Traits

The salinity stress was found to alter the morphology traits of the three genotypes (Table 1). Salinity stress reduced the length of leaves, panicles and height of SS1-40 and SS1-42 plants but increased these parameters in SS1-41. However, the width of leaves did not significantly differ ($P > 0.05$). Salinity stress also affected the leaf angle, type of panicles and leaf senescence, but this effect was genotype-specific. Interestingly, variety SS1-42 exhibited earlier and faster leaf senescence than the control plant. Early senescence is a good trait for rice; however, its mechanism remains unclear (Liu *et al.*, 2008).

Total FAME and Fatty Acid Content

Table 2 shows the FAME content in SS1-40, SS1-41 and SS1-42 during the vegetative, reproductive and ripening stages. Salinity stress significantly increased ($p < 0.05$) the FAME content in SS1-40 during the reproductive and ripening stages. In SS1-41 and SS1-42, the value for FAME decreased. The major fatty acid groups identified in these rice varieties were palmitic acid C_{16:0}, stearic acid C_{18:0}, oleic acid C_{18:1}, linoleic acid C_{18:2} and linolenic acid C_{18:3}. Salinity stress significantly enhanced ($p < 0.05$) the contents of these fatty acids during the vegetative and reproductive stages. However, their contents decreased and were lower than those of the control during the ripening stage (Fig. 1 and 2). During the vegetative stage, linolenic acid was the main fatty acid in rice leaves under salinity stress. The levels of this fatty acid were 0.4-, 2.0- and 4.3-fold higher than in the control for SS1-40, SS1-41 and SS1-42, respectively. The results suggested that polyunsaturated fatty acids, particularly α -linolenic acid, might play a role in the defence mechanism against salinity stress during plant development. Plants respond to biotic stress by remodelling membrane fluidity and by releasing α -linolenic (18:3) acid from membrane lipids. The adjustment of membrane fluidity maintains an

Table 1. The morphology traits of rice genotypes under control and salinity treatment. Values with same small capital letter in the same role were not significantly different ($p>0.05$). Data are means \pm Sd, (n=3)

Morphology traits	SS1-40		SS1-41		SS1-42	
	Control	Salinity Treatment	Control	Salinity Treatment	Control	Salinity Treatment
Flag-leaf length	38.5 \pm 0.5 ^a cm	25.5 \pm 0.5 ^d cm	16.3 \pm 2.3 ^e cm	33.6 \pm 1.0 ^c cm	35.5 \pm 0.5 ^b cm	28.6 \pm 2.2 ^d cm
Flag-leaf width	1.3 \pm 0.2 ^a cm	1.4 \pm 0.2 ^a cm	1.1 \pm 0.1 ^a cm	1.3 \pm 0.2 ^a cm	1.4 \pm 0.2 ^a cm	1.3 \pm 0.2 ^a cm
Leaf length	58.0 \pm 2.0 ^a cm	25.6 \pm 2.2 ^e cm	13.5 \pm 0.5 ^f cm	52.5 \pm 0.5 ^b cm	37.0 \pm 0.8 ^d cm	38.9 \pm 0.2 ^c cm
Leaf width	1.0 \pm 0.3 ^a cm	0.8 \pm 0.1 ^a cm	1.0 \pm 0.08 ^a cm	1.2 \pm 0.3 ^a cm	1.0 \pm 0.3 ^a cm	1.0 \pm 0.3 ^a cm
Panicle length	20.8 \pm 1.0 ^a cm	17.4 \pm 0.6 ^a cm	19.3 \pm 2.3 ^a cm	22.0 \pm 2.0 ^a cm	18.5 \pm 0.5 ^a cm	18.1 \pm 0.1 ^a cm
Seedling height	78.3 \pm 2.3 ^d cm	65.3 \pm 2.3 ^f cm	87.8 \pm 1.0 ^b cm	93.0 \pm 3.0 ^a cm	84.0 \pm 0.8 ^c cm	73.2 \pm 0.5 ^e cm
Leaf angle	Erect	Erect	Erect	Erect	Erect	Droopy
Panicle type	Compact	Compact	Compact	Inter-mediate	Inter-mediate	Compact
Leaf senescence	Slow	Late and slow	Slow	Slow	Inter-mediate	Early and fast
Flag-leaf angle	Erect	droopy	Erect	Erect	Erect	Erect

Table 2. The Fatty Acid Methyl Ester (FAME) per sample (mg/mg) of rice genotypes under control and salinity treatment. Values with same small capital letter in the same role were not significantly different ($p>0.05$). Data are means \pm Sd, (n=3)

	FAME/sample (mg/mg)					
	SS1-40		SS1-41		SS1-42	
	Control	Salinity Treatment	Control	Salinity Treatment	Control	Salinity Treatment
Vegetative	0.62 \pm 0.18 ^a	0.61 \pm 0.07 ^a	0.62 \pm 0.28 ^a	0.79 \pm 0.19 ^a	0.62 \pm 0.18 ^a	0.79 \pm 0.17 ^a
Reproductive	1.21 \pm 0.53 ^a	0.66 \pm 0.04 ^b	1.21 \pm 0.34 ^a	0.65 \pm 0.04 ^b	1.21 \pm 0.43 ^a	1.40 \pm 0.64 ^a
Ripening	2.21 \pm 0.70 ^b	2.73 \pm 0.47 ^b	3.90 \pm 0.70 ^a	2.29 \pm 0.47 ^b	2.42 \pm 0.70 ^b	1.71 \pm 0.47 ^b

environment suitable for the function of critical integral proteins during stress (Upchurch, 2008), which allows the passage of ions and nonelectrolytes through transport proteins (i.e. channels, carriers) as well as the lipid phase of the membrane. Changes in nonelectrolyte permeability and electrolyte leakage under saline conditions indicated modifications in the proteins and lipid matrix of the plasma membrane in salt-sensitive cultivars, whereas the effect was minimal in salt-tolerant ones under saline conditions (Mansour, 2013). Although salinity stress affects all stages of the growth and development of a rice plant, the responses to salinity varied by growth stage, concentration and the duration of coverage to salt (Joseph *et al.*, 2010). The vegetative stage is considered the most crucial stage in rice development occurs as the seedlings try to adapt to a new environment. Cell membranes are the major sites for controlling active and passive solute fluxes and they are important for regulation the ion uptake in plants (Munns and Tester, 2008). Mineral imbalances in saline environments often affect the structure and chemical composition of the lipid bilayer membrane and may thereby affect the selective ability of the membrane to transport

solute and ions inwards. The membranes may also become leaky to the solutes they contain (Lodhi *et al.*, 2009).

During the reproductive stage, palmitic acid was the most abundant fatty acid in the SS1-41 and SS1-42 varieties. In SS1-40, the content of linolenic acid remained high during this growth stage. The reproductive or early stage is a complex phenomenon that involves many physiological and biochemical changes that lead to the activation of the embryo. The results suggest that the polyunsaturated fatty acids might be hydrolysed into saturated fatty acids, such as palmitic acid (Fig. 1 and 2).

Palmitic acid was the most abundant fatty acid during the ripening stage in all genotypes. Salinity stress did not significantly affect the fatty acid content. Surprisingly, few fatty acids, such as arachidic acid C_{20:0}, *cis*-11, 14, 17- eicosatrienoic acid C_{20:3n3}, behenic acid C_{22:0}, *cis*-13, 16- docosadienoic acid C_{22:2} and lignoceric acid C_{24:0} (Data not shown), were found during the reproductive and ripening stages, but this finding did not persist during the vegetative stage. This phenomenon suggests that long chain fatty acids were synthesised at the end of the growth

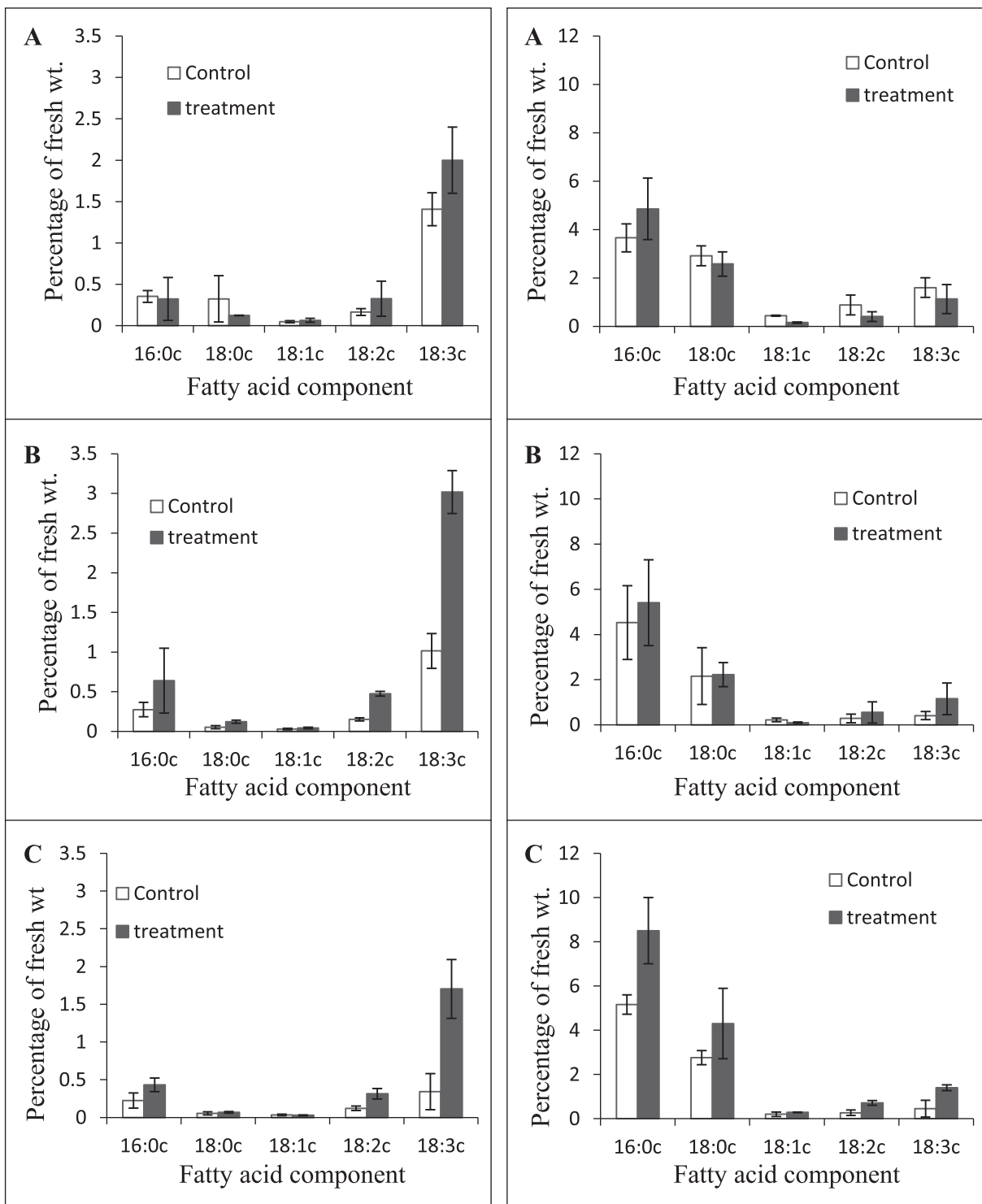


Fig. 1. The fatty acid content in three rice genotypes grown under salinity stress during the vegetative stage (leaf) and reproductive stage (right); genotypes SS1-40 (A), SS1-41 (B) and SS1-42 (C). Bar indicate the standard error (n=3).

development phase. Upon reaching the ripening stage, the contents of most fatty acids in the leaf increased. No further development occurs during this stage except for the formation of embryos and accumulation of stored carbohydrates. This state is considered stable; the membrane permeability of the leaf during ripening stage is less disturbed,

which increases the accumulation of fatty acids. Improving the varieties by selecting genotypes that overexpress the desaturase gene might be a suitable strategy to increase the biosynthesis of polyunsaturated fatty acids, which regulate salinity stress during the vegetative phase.

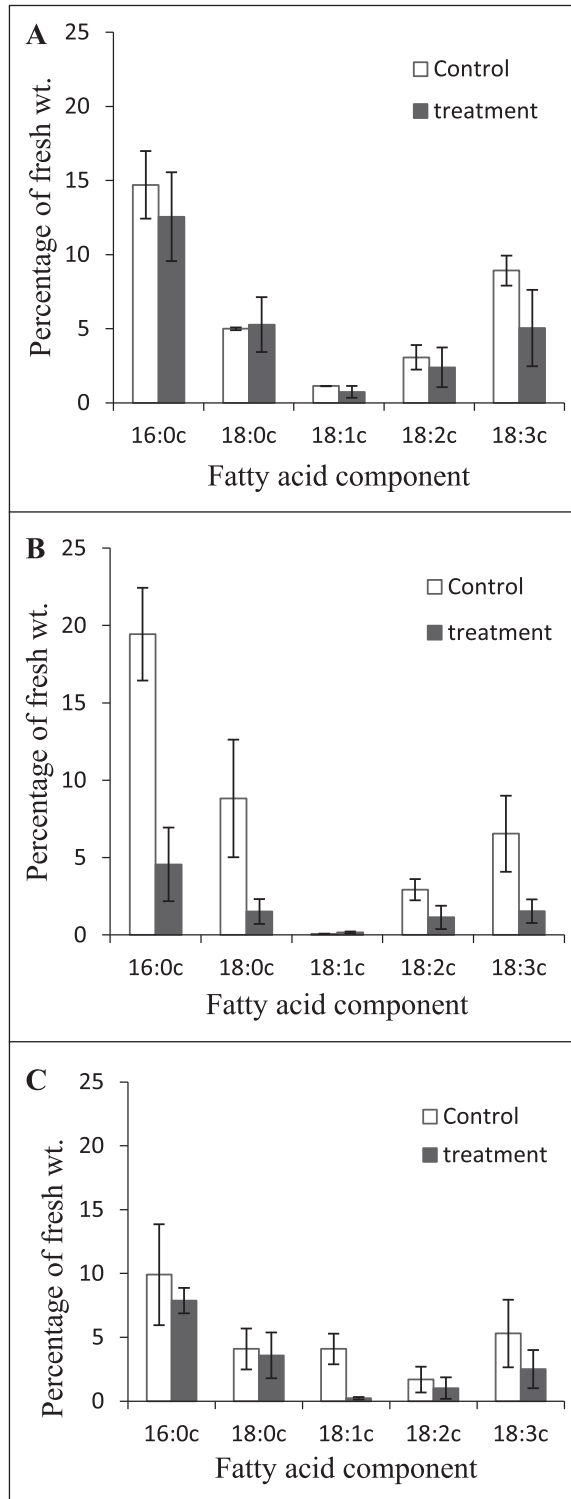


Fig. 2. The fatty acid content in three rice genotypes grown under salinity stress during the ripening stage; variety SS1-40 (A), SS1-41 (B) and SS1-42 (C). Bar indicate the standard error (n=3).

CONCLUSION

The fatty acid composition in rice leaves varied by growth stage. Polyunsaturated fatty acids, particularly linolenic acid, were the major fatty acids found during the vegetative stage. This finding suggests that this important fatty acid may be used as a marker for rice development in salinity stress environments. High levels of the saturated fatty acids palmitic acid and stearic acid were produced during the reproductive and ripening stages.

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