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ORIGINAL PAPER

Characterization of cold-induced changes in the fatty acids profile of rice seedlings

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Abstract Rice, a staple food for more than one half of the world's population, is one of the most cold-sensitive cereals. Breeding programs aimed at increasing rice production are expected to reduce cold-imposed grain losses. Several reports have demonstrated that cold induce differential effects on the fatty acids profile of membranes in chilling-sensitive and chilling-tolerant plants. In this work, we evaluated changes in fatty acid (FA) composition as a potential screening tool to evaluate chilling sensitivity of rice accessions. Cold exposure led to the preferential accumulation of the polyunsaturated linolenic and linoleic FAs and reduction of palmitic and stearic FAs, besides showing increased lignoceric acid content in roots of the variety. Similarly, roots of cold-exposed line Quila 66304 also presented preferential accumulation of linolenic and linoleic FAs and reduction of palmitic and stearic FAs. Cold exposure also led to enhanced levels of palmitic acid in shoots of Amaroo and, in a smaller extent, in shoots of Quila 66304. Linolenic acid was reduced in the shoots of both Amaroo and Quila 66304, while oleic acid content was reduced in shoots of Amaroo and slightly increased in shoots of Quila 66304. Double-bond index analysis indicated that 18 carbons FAs DBI for roots might be a good screening tool for cold response in rice. Results in this report demonstrate that cold-induced changes in FA profile represent a useful screening tool for early identification of differences in cold acclimation potential among rice accessions.

Keywords Cold sensitivity · Double-bond index · *Oryza sativa* · Polyunsaturated fatty acid

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Introduction

Low temperature is the major growth-limiting factor for warm-season plants (Lee et al. 2005). One of the most important, adverse, effects of low temperature on plants is altered functional properties of cellular membranes in leaves and/or roots. Cold, at the cellular and molecular level, can severely impact membrane fluidity, which also affects metabolic rate and protein turnover (reviewed by Lee et al. 2005). Previous studies have shown that cold induce differential effects on the lipid composition of membranes in chilling-sensitive and chilling-tolerant plants (Gerloff et al. 1966; Thompson 1992; Palta et al. 1993; Lee et al. 2005).

Rice is the staple food for more than half of the world's population (Neeraja et al. 2009), and breeding programs are expected to help to increase rice production to feed an ever-growing population. Rice is a cold-sensitive crop and of 130 million hectares of rice land, nearly one-tenth of earth's arable land, about 10 % is subjected to low temperature (Wu and Garg 2003; Sipaseuth et al. 2007). Decrease in temperature to critical levels can limit seed germination, damage plantlets during vegetative growth (Sipaseuth et al. 2007), and inhibit pollen viability during flowering, thus, affecting grain production (Imin et al. 2004). Cold-sensitivity in rice is not easy to overcome since the critical low temperatures vary according to the plant developmental stage and genetic make up. Chilling stress is a major limiting factor for rice production in temperate areas and at high altitude in the tropics (Zhang et al. 2012). Increasing yield in such areas requires knowledge of varietal chilling sensitivity, which in turn can only be accurately determined by screening under experimental conditions that can reveal the diversity of genotype responses to chilling temperatures (Bertin et al. 1996).

The term cold hardiness has been used to represent in a broad sense, the ability of a plant to adapt to and withstand freezing temperatures. When seen together, the mechanisms associated with this ability are quite diverse ranging from properties that exist at a structural (whole-plant) level to adaptations at the cellular level, including specific metabolites, proteins and changes in membrane structure Gusta and Wisniewski, 2013). Varieties in different plant species differ in their ability to achieve high levels of hardiness. A better understanding of cold-resistance mechanisms is obviously expected to aid the improvement and maintenance of crops in regions affected by chilling temperatures during part of their life cycle (Gerloff et al. 1966). A wide range of cellular responses are known to occur when plants are exposed to a variety of environmental stresses, including cold, and it is recognized for many decades that lipids are important in cold-resistance mechanisms (Sinnott 1918). It is well established that cells tend to respond to disturbing environmental factors by changing membrane lipid composition, and such changes are thought to restore the optimal physical properties (Thompson 1992). In fact, in rice, the early use of electrolyte leakage measurements for varietal screening for chilling tolerance demonstrated a significant negative correlation between leakage from leaf tissue and survival of the whole plant, which corroborates that physical and biochemical processes in membranes are of major importance in determining differential chilling sensitivity among rice varieties (Bertin et al. 1996). A good germination rate is important to guarantee the fast establishment and uniform crop stand. Slow and non-uniform germination under cold temperature usually results in irregular emergence and low plant population density (Krishnasamy and Seshu 1989; Souza 1990). Since management practices usually are not effective on minimizing these cold-induced problems, genetic breeding for cold tolerance, at the plantlet stage, is expected to contribute to a better crop establishment in early sowings.

Several techniques have been employed to evaluate chilling sensitivity in plants. The most broadly used consists in direct exposure to chilling followed by visual damage assessment. However, some subjectivity is quite often associated with visual rating systems (Bertin et al. 1996). Thus, the development of quantitative assays, especially when allowing the detection of chilling injury prior to visible symptoms, is highly desirable. In this report, we describe cold-induced changes in the FAs profile of two rice accessions which vary for cold tolerance at the seedling stage.

Materials and methods

Plant material and growth conditions under low-temperature exposure

Two rice (*Oryza sativa* L.) accessions were used in this study: Amaroo and Quila 66304. The variety Amaroo is a *japonica* variety originated from Australia, considered to present an above-average cold tolerance, when compared to other Australian varieties (Williams and Wensing 1998). It is a high yielding and high-quality variety, widely used in rice industry. Because Amaroo is a photoperiod-sensitive variety, it can be used to generate varieties capable of escaping critical cold-stress periods (Farrell et al. 2003). Accession Quila 66304 is a cold-tolerant *japonica* line developed in Chile, derived from the cross Cesariot/Oro (Alvarado et al. 1993). Quila 66304 has been used as source of cold tolerance to generate segregating populations in several breeding programs.

Seeds of Amaroo and Quila 66304 were allowed to germinate and develop for 6 days in Petri dishes (100 mm in diameter and 15 mm in height) containing two layers of Whatman n° 1 filter paper plus 7 ml water, at room temperature (25 °C). 6 days after imbibition, seedlings selected for uniformity were transferred to a growth chamber and kept at 14 °C for 72 h under a photoperiod of 12/12 (light/dark) h provided by cool-white fluorescent tubes giving a photosynthetic photon flux density of 200 µmol m^{-2} s^{-1} at the seedlings level. After that, seedlings were kept at room temperature for 3 days, when the average seedling lengths were recorded and tissues harvested for total fatty acids (FAs) profile analysis. Seedlings not exposed to low-temperature treatment were kept at room temperature for 6 days. A completely

randomized design, with three replications (20 individual plants per replication), was used for each treatment (temperatures 25 and 14 °C). The entire dataset obtained in the experiment was used for data analysis. After a significant analysis of variance, the differences between means for seedling lengths were analyzed by the Tukey's test ($p = 0.05$).

Analysis of the total FAs profile

Shoots and roots from the Amaroo and Quila 66304 accessions were submitted to FAs analysis, following conversion of the FAs to FA methyl esters (FAMEs). FAMEs were prepared by heating freeze-dried samples (5 mg) in 3 % HCl in MeOH for 6 h, at 80 °C. After neutralization (Ag_2CO_3), FAMEs were extracted twice with *n*-hexane (1 ml). The extract was analyzed by gas chromatography–mass spectrometry (GC–MS) using a Varian Saturn 2000R gas chromatograph equipped with an DB-225 capillary column (30 × 0.25 mm i.d.), programmed from 50 to 220 °C (40 °C min^{-1}), then kept constant for 30 min. FAMEs were identified by their typical electron impact MS spectra and retention times (*R*t), shown by comparison with standards (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), and quantified according to their relative peak areas (Sassaki et al. 2001).

An estimation of the lipid total unsaturation level, the double-bond index (DBI), for C18 FAs was calculated from the mol % values derived from the GC–MS data, according to the equation: DBI = 1 × (% 18:1) + 2 × (% 18:2) + 3 × (% 18:3)/100, as described by Skoczowski et al. (1994).

Results

Effect of low temperature on shoot and root elongation of Amaroo and Quila 66304

In a preliminary experiment carried out to characterize the effects of cold on shoot and root elongation of Amaroo and Quila 66304, we grew 6-day-old rice seedlings for 3 days at 14 °C. After the cold treatment, root elongation of Amaroo did not significantly ($p = 0.05$) differ from root elongation of Quila 66304 (Table 1). However, Quila 66304 presented shoot elongation significantly higher than Amaroo.

Table 1 Effect of low temperature on root and shoot elongation of Amaroo and Quila 66304

Accession	Shoot average length (mm)	Root average length (mm)
Amaroo	50.1[b]	76.3[a]
Quila 66304	61.3[a]	75.0[a]

Based on the Tukey's test, average length of roots followed by the same letters do not differ at $p = 0.05$

Fatty acid composition of shoots and roots of rice

A total of 13 different FAs were identified in Amaroo plantlets, while in Quila 66304 the total number of identified FAs was 11 (Table 2). Amaroo plantlets not exposed to cold (25 °C; control) presented 13 and 8 different FAs in shoots and roots, respectively (Table 2). Quila 66304 plantlets not exposed to cold presented eight different FAs in shoots and 11 FAs in roots (Table 2).

Stearic (18:0), palmitoleic (16:1) and especially palmitic (16:0) acid were the major FAs found in shoots of both rice accessions used in this study, comprising 80.8 and 93.2 % of the total FAs content in shoots of plantlets of Amaroo and Quila 66304 grown at 25 °C, respectively (Table 2). The major FAs found in roots of Amaroo and Quila 66304 plantlets grown at 25 °C were 16:0 and 18:0 FAs, comprising 78.2 and 83.1 % of the total FAs content, respectively. Interestingly, the amount of 18:0 FA in non-cold-exposed shoots of both, Amaroo and Quila 66304 varieties was exactly the same.

The most significant difference in the FAs profile between plantlets of accessions Amaroo and Quila 66304 not exposed to cold is the amount of 16:0 and oleic (18:1) FAs, respectively, higher and lower in shoots of Amaroo, when compared to shoots of Quila 66304. Regarding the presence of 16:1 FA in Amaroo, the FA was restricted to shoots, regardless roots were exposed or not to cold. However, for Quila 66304, the 16:1 FA was present in both, shoots and roots, though shoots presented a much higher amount of this FA (Table 2). Similarly to what was found for Amaroo, roots from Quila 66304 presented an about twofold higher the amount of the 18:0 FA, compared to shoots.

Effect of cold exposure on the fatty acid composition of shoots and roots of Amaroo

For shoots of Amaroo, exposure to cold treatment resulted in enhanced content of 16:0 and behenic (22:0) FAs, reduced content of 16:1, 18:1, linoleic (18:2), linolenic (18:3) and lignoceric (24:0), and virtually no change in heptadecanoic (17:0) and 18:0 FA content (Table 2). For roots, cold exposure resulted in increased content of 18:1, 18:3, and especially 18:2 and 24:0 FAs. Cold treatment also led to reduced content of 16:0, 17:0, and 18:0 FAs, and virtually no change in arachidic (20:0) acid content.

Table 2 Fatty acid composition (mol %) of shoots and roots of the Amaroo and Quila 66304 grown at 25 °C (control) or 14 °C (cold treatment)

Fatty acids	Fatty acids[a] (mol %)								Main mass spectral fragments (m/z)
	Amaroo shoots 14 °C	Amaroo shoots 25 °C	Amaroo roots 14 °C	Amaroo roots 25 °C	Quila shoots 14 °C	QuilaShoots 25 °C	Quila Roots 14 °C	Quila Roots 25 °C	
C8:0	tr	0.5	–	tr	tr	tr	tr	tr	202, 158, 117, 99, 74, 55, 43, 41
C10:0	–	tr	–	–	–	–	–	–	230, 199, 166, 157, 125, 98, 74, 55, 41
C14:0	–	0.9			–	–	–	–	242, 199, 143, 129, 74, 55, 43, 41
C16:0	60.8	47.4	18.0	39.8	62.2	60.6	34.7	45.6	270, 227, 185, 143, 74, 55, 43, 41
C16:1	5.4	12.5	–	–	4.5	11.7	tr	1.9	254, 236, 237, 192, 97, 69, 55, 41
C17:0	4.1	3.9	3.8	6.8	3.0	5.0	0.8	5.2	284, 253, 241, 143, 129, 87, 74, 41
C18:0	20.7	20.9	12.6	38.4	26.5	20.9	9.5	37.5	298, 255, 199, 143, 129, 74, 55, 41
C18:1	tr	6.2	12.5	10.7	0.7	tr	9.6	6.4	296, 265, 222, 180, 74, 69, 55, 41
C18:2	–	1.4	21.2	4.0	–	–	32.2	3.3	294, 263, 220, 178, 95, 81, 74, 67, 41
C18:3	–	1.5	7.0	tr	–	1.7	9.8	tr	292, 195, 194, 150, 107, 93, 79, 67
C20:0	tr	tr	0.1	0.1	2.8	tr	tr	tr	326, 263, 199, 171, 143, 95, 74, 41
C22:0	8.8	2.7	–	–	tr	–	tr	tr	354, 311, 255, 199, 143, 87, 74, 41
C24:0	–	1.4	24.8	–	tr	–	3.4	tr	382, 339, 283, 255, 199, 143, 74, 41

[a] FA methyl esters obtained after methanolysis and analyzed by GC–MS (column DB-225), tr. less than 0.1 %

Effect of cold exposure on the fatty acid composition of shoots and roots of Quila 66304

For shoots of Quila 66304, exposure to cold treatment led to an enhancement in the content of 18:0, 18:1, and 20:0 FAs, a reduction in the content of 16:1, 17:0, and 18:3 FAs, and virtually no change in the content of 16:0 FA (Table 2). For roots, exposure to cold resulted in enhanced content of 18:1, 18:2, 18:3, and 24:0 FAs, and reduced content of 16:0, 16:1, 17:0, and 18:0 FAs.

Effect of the cold exposure on the double-bond index (DBI)

An estimation of the lipid total unsaturation level, the ratio of unsaturated (18:1, 18:2, and 18:3) to saturated (18:0) × 100, was calculated for shoots and roots of Amaroo and Quila 66304.

The 18 carbons FA double-bond index (DBI) for shoots of Amaroo decreased from 13.5 to 0 after the cold treatment (Table 3), while for shoots of Quila 66304, the DBI decreased 7.3-fold. For roots, the DBI increased 8.0-fold in Quila 66304 and 4.1-fold in Amaroo.

Table 3 Effect of cold exposure on the DBI, for shoots and roots of Amaroo and Quila 66304 rice accessions

Sample variation	DBI[a] (%)
Amaroo shoots −14 °C	0.0
Amaroo shoots −25 °C	13.5
Amaroo roots −14 °C	75.9
Amaroo roots −25 °C	18.7
Quila 66304 shoots −14 °C	0.7
Quila 66304 shoots −25 °C	5.1
Quila 66304 roots −14 °C	103.4
Quila 66304 roots −25 °C	13.0

[a] Calculated as described by Skoczowski et al. (1994) by mol % values from the GC–MS analysis

Discussion

In this study, we have found that 18:0, 16:1, and 16:0 FAs comprised 80.8 and 93.2 % of the total FAs content of Amaroo and Quila 66304 shoots, respectively. In addition, 16:0 and 18:0 FAs were the major FAs in roots, comprising 78.2 and 83.1 % of the total FAs content of Amaroo and Quila 66304, respectively. In a study involving six different cultivars, 18:3 FAs was found to be, by far, the major FAs in the total lipids fraction of leaves of rice (Bertin et al. 1996). When seen together, these data clearly show that FAs composition of rice accessions change significantly according to the genotype. Thus, studies aimed at using FAs composition to access inherited cold tolerance should be preceded by full characterization of the FAs profile instead of looking for specific FA(s).

Analysis of the FAs profile of lipid extract previously demonstrated that 18:2, 18:1, and 16:0 FAs were the major FAs in rice seeds (Lee et al. 1965). Later, Zhou et al. (2003) confirmed that these FAs were the major FAs in both, brown and milled rice seeds, comprising more than 90 % of the total FAs content. More recently, analysis of the FAs profile of various rice genotypes showed that 18:2, 18:3, and 16:0 FAs were the major FAs in rice leaves (Da Cruz et al. 2010). Previously reported data indicated that 18:2 and 16:0 FAs are the non-organ-specific prevalent FAs in rice, suggesting that these two FAs are highly conserved toward structural and/or storage function in rice. However, this was not the case for the two rice varieties used in this work, i.e., 18:2 FA was not a prevalent FA in Amaroo or Quila 66304. Since the work carried out by Da Cruz and co-workers (2010) used various rice genotypes, from different origins, the reason(s) why 18:2 is not a major FA in Amaroo and Quila 66304 remain to be investigated.

In this study, roots of Amaroo and Quila 66304 presented an amount about twofold higher of 18:0 FA, when compared to shoots. However, this difference in 18:0 FA content was not found for other rice varieties such as IR64 (Dubey et al. 2010). The reason(s) for this differential 18:0 FA content of Amaroo and Quila 66304, compared to other rice varieties is not clear and requires further investigation.

Effect of the cold exposure on the fatty acid profile of roots

Analysis of the FAs profile of cold-exposed and non-exposed (control) Amaroo and Quila 66304 plantlets were carried out to develop a quantitative assay that allowed early evaluation of chilling sensitivity, especially for rice-breeding programs. The FA profile of both Amaroo and Quila 66304 roots changed during hardening mainly due to the preferential accumulation of the polyunsaturated 18:3 and 18:2 FAs, reduction of 16:0 and 18:0 FAs, and increased 24:0, especially in Amaroo. Together, 18:3 and 18:2 FAs, increased from 4.0 to 28.2 % of the FA present in the Amaroo roots during hardening, while for the Quila 66304 roots, this increase was more significant, rising from 3.3 to 42.0 %. The 18:2 FA content of the roots increased remarkably after the cold hardness. 18:2 FA content increased 330 and 776 %, respectively, for the Amaroo and Quila 66304 varieties. The most common visible symptom of chilling injury in plantlets is water loss, which often results in severe wilting (Herner 1990). This water loss has been attributed to changes in membrane permeability, especially the plasmalemma, which allows water and solutes to leak out into the intercellular space, particularly in chilling sensitive plants (Lyons 1973). During low-temperature acclimation of higher plants, polyunsaturated FAs have been found to increase in many plant species (reviewed in De Palma et al. 2008). This adaptive mechanism is considered to compensate low-temperature-related loss of membrane integrity, by reducing the lateral packing order of acyl chains within the membrane interior (Hazel 1997). Thus, the raise in the number of unsaturated double-bonds driven by the enhanced 18:3 FA content found in this study is expected to contribute toward changing the FAs profile of the cellular lipids and consequently to the hardening process for both varieties. However, the larger increases in the 18:2 FA content found for Amaroo and Quila 66304 indicate that the deposition of significant amounts of 18:2 FA in roots of both rice varieties may play a more important role in the hardening process, fulfilling a requirement to increase the amount of unsaturation in the plant lipid, as it has been pointed out for decades (Lyons et al. 1964; Gerloff et al. 1966). However, the larger increase in 18:2 FA content found for the Quila 66304 line suggests that this accession might more effectively acclimate to cold, when compared to Amaroo.

In this work, exposure to cold also resulted in enhancement in 24:0 FA content, a rare saturated FA, in roots of Quila 66304, and especially in roots of Amaroo. Changes in 24:0 FA content have been previously reported to be related to environmental stress. In hibernating mammals, such as hamsters (Carneheim et al. 1989) and marmots (Ruf and Arnold 2008), it is well established that 24:0 FA accumulates in fat during entry into hibernation. Conversely, in non-hibernating animals, such as rats, exposure to cold has been shown to lead to reduced 24:0 FA content (Ogawa et al. 1992). In grape rootstocks, 24:0 FA content has been found to be negatively correlated to the ability of the rootstocks to accumulate chloride in leaves (Kuiper 1968). Although we are not aware of other reports relating changes in 24:0 FA content to cold exposure in plants, data from saline stress experiments (Kuiper

1968) and data from this experiment indicate that the changes in 24:0 FA content in roots might be potentially related to cold stress in plants.

Reduction in the monounsaturated 16:1 FA was the most significant common feature of shoots of both, Amaroo and Quila 66304, after cold exposure. The 16:1 FA content was reduced at a similar extent (about twofold) in shoots of both, Amaroo and Quila 66304, after the cold exposure. Working with a chilling-tolerant rice cultivar and four cultivars from high altitudes, Bertin et al. (1998) also observed a decrease in the 16:1 FA content in leaves of all of the rice cultivars, after cold exposure. Thus, the reduction in 16:1 FA in leaves/shoots seems to be a widespread event during cold acclimation in rice.

Effect of the cold exposure on the fatty acid profile of shoots

Analysis of FAs profile demonstrated an increase of 16:0 FA for shoots of the Amaroo after cold exposure. For shoots of Quila 66304, the 16:0 FA content remained essentially unchanged after the cold treatment. Reduction in 16:0 FA has been previously related to cold exposure in other plant species such as in the freezing-tolerant, cold-acclimating wild potato species (*Solanum commersonii*) (Palta et al. 1993). Nevertheless, reduction in 16:0 FA has also been found for the freezing-sensitive, non-acclimating cultivated species (*Solanum tuberosum*), after cold exposure (Palta et al. 1993). 18:3 FA was reduced in shoots of both varieties used in this study, after exposure to cold. 18:3 FA has been previously reported to be reduced in plasma membrane lipids of leaflets of *Solanum commersonii*, but increased in *Solanum tuberosum*, after cold exposure (Palta et al. 1993). Somewhat surprisingly, 18:3 FA has been shown to be increased, after cold exposure, in plasma membrane lipids of leaves of cold-tolerant rice genotypes, while 16:0 FA decreased. An opposite response was found for cold-sensitive rice genotypes (Da Cruz et al. 2010). The results from Da Cruz and co-workers led those authors to propose 18:3 and 16:0 FAs as potential screening parameters to differentiate rice genotypes regarding cold tolerance. Although results from these three reports are conflicting, the use of 18:3 and 16:0 FAs content, in organs other than leaves, as cold-tolerance screening parameters for rice cannot be ruled out at this moment, since they could be a consequence of genotype-related differences in molecular mechanisms involved in cold acclimation.

18:1 FA content was reduced in Amaroo shoots after cold exposure. Conversely, this FA was slightly increased in Quila 66304 shoots after exposure to cold. For winter wheat (*Triticum aestivum*), reduction in 18:1 FA content was also observed in membranes of leaves, after cold exposure (Janda et al. 2007). However, a former study showed that the 18:1 FA was reduced in leaves of both, *Solanum tuberosum* and *S. commersonii*, after cold exposure (Palta et al. 1993). Thus, the variation in 18:1 FA after cold treatment by itself does not seem to be a good candidate to differentiate rice genotypes regarding cold tolerance.

Cold treatment more effectively reduced the amount of saturated FAs and enhanced the amount of unsaturated in roots of both, Amaroo and Quila 66304 plantlets, when compared to shoots. Moreover, cold-induced enhancement of hydraulic resistance is thought to result in water imbalance in shoots of chilling-sensitive plants (Lee et al. 2004). Thus, since enhanced unsaturation of lipids in membranes increases water fluidity (Lee et al. 2005), and water flow across the root cylinder is a major parameter affecting root hydraulics (Lee et al. 2005), the cold-induced enhancement of unsaturation of lipids observed in roots of both rice accessions in this work likely facilitate their acclimation to cold.

Effect of the cold exposure on the double-bond index (DBI)

Changes in lipid metabolism are known to be related to cold hardness for quite some time (Gerloff et al. 1966). In an early work, Lyons et al. (1964) obtained functional mitochondria from various plant species differing in their sensitivity to chilling injury. They observed that the mitochondria from chilling-resistant plants were more flexible and presented a higher amount of unsaturated FAs, compared to mitochondria from chilling-sensitive plants. DBI is a measure of the average number of double-bonds per FA molecule and, consequently, an indicator of the degree of unsaturation of FAs. In the Lyons and co-workers' study, the highest DBI were shown by chilling-resistant plants, while the chilling-sensitive species presented the lowest values. Then, Lyons and co-workers suggested that metabolic injury caused in chilling-sensitive's tissues may have been caused by the inability of relatively inflexible mitochondria to work properly at low temperature. Artificial manipulation of FAs unsaturation in mutants or transgenic plants has provided further support for a role of FAs unsaturation in temperature stress response and resistance (reviewed in Nishida and Murata 1996 and Iba 2002). As a matter of fact, because membrane fluidity generally increases with increasing amounts of unsaturated lipid, a gradual saturation in DBI has been pointed out for quite some time to possibly contribute to chilling-tolerance (Lee et al. 2005).

To provide an estimation of the lipid total unsaturation level, we calculated the DBI for 18 carbons FAs from roots and shoots of both rice accessions used in this study. The 18 carbons FAs DBI increased 8.0-fold and 4.1-fold,

respectively, for the roots of the Quila 66304 and Amaroo accessions submitted to the cold treatment. The cold-induced increase in DBI observed in roots of Quila 66304 and Amaroo is largely attributable to increased concentration of 18:3, and especially 18:2 FAs. Similarly to what has been previously reported for various plant species/tissues such as non-hardy and hardy alfalfa root tissues (Gerloff et al. 1966), a preferential accumulation of 18:2 and 18:3 FAs resulted in increased average number of unsaturated bonds in the FAs for both rice accessions used in this study. The cold-induced shift in metabolism of FAs in rice observed in this study is compatible with the concept that exposure to low temperatures causes shifts in lipid metabolism toward more highly unsaturated state (reviewed in Dogras et al. 1977).

The preferential accumulation of relatively large amounts of 18:2 and 18:3 FAs in roots of both varieties after the cold treatment or the conditions resulting from the build up of these FAs may be important for cold hardness and protection of rice cells against cold-induced damage. This cold-induced differential accumulation of FA provides support for the use of cold-induced changes in FAs profile as indicators of cold tolerance in rice. The cold-induced increase in unsaturation found for the rice accessions used in this study indicates increased membrane fluidity, and consequently an ability of these rice accessions, especially Quila 66304, to acclimate to lower temperatures. The largest increase in DBI found for roots of Quila 66304, compared to roots of Amaroo, is consistent with the belief that Amaroo, although presenting above average tolerance to chilling, is more cold-sensitive than Quila 66304. DBI has been used as a screening parameter for enhanced tolerance to high (Liu and Huang 2004) and low (Gerloff et al. 1966) temperature, salt (Elkahoui et al. 2004) and heavy-metal (Poznyak et al. 2002) stress, besides aging (Zabrouskov et al. 2002). Thus, findings from this study indicate that 18 carbons FAs DBI for roots might be a good tool for cold-tolerance screening of rice accessions.

Except for the 18:1 FA, in shoots of Quila 66304, the cold exposure resulted in undetectable or trace amounts of unsaturated 18 carbons FAs in shoots of both accessions of rice used in this study. As a consequence, cold exposure led to reduced 18 carbons FAs DBI for shoots of both, Quila 66304 and Amaroo. When seen together, our data and that of above-mentioned reports obviously show that 18 carbons FAs DBI in shoots is likely not a good tool for screening for cold tolerance of rice accessions.

Conclusions

This study demonstrates that there is a marked shift in the FAs profile between cold-treated and non-treated plantlets of two rice accessions known to differ in their level of tolerance to low-temperature stress. These cold-related shifts in FAs profile were larger in roots than in shoots, in both accessions used in this study, indicating that shifts in FAs profile of roots are likely better indicators of cold tolerance, when compared to shifts in FAs profile of shoots. Furthermore, these changes in FA profiles suggest that membrane structure and function are likely altered by cold exposure.

Cold-induced changes in FAs profile detected in this work, without the need of isolating plasma membranes, represent a useful tool for the early identification of differences in cold-acclimation potential among rice accessions used by breeding programs focused on cold tolerance. Furthermore, this tool also enables the characterization of differences in cold-acclimation potential in plantlets, a plant developmental stage which is sensitive to low temperatures, affecting uniform crop stand and grain production.

The characterization of changes in the FA profile helps to elucidate the mechanism(s) behind the hardening ability of rice accessions in addition to contribution for the establishment of selection parameters for breeding programs focused on cold tolerance.

Author contribution Dr. A. B. Schmidt, M.E. Ferreira and P·H.N. Rangel carried out the cold treatments, collection of morphological data and extraction of the fatty acids samples. G. Sassaki performed the characterization of the fatty acids profile. A.B. Pereira-Netto carried out data analysis and manuscript preparation.

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