RESEARCH ARTICLE

Alterations in fatty acid composition due to cold exposure at the vegetative stage in rice

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ABSTRACT

Rice is a tropical plant, so cold temperature may be detrimental to its development, depending on the genotype and environmental conditions. Degree of lipid unsaturation has been related to cold tolerance due to its effect on membrane stability. So, the aim of this study was to characterize the fatty acid composition and its alterations due to cold temperature in rice genotypes of diversified origin. Forty-four rice genotypes at the V_4 stage were submitted to two temperature conditions: 10°C and 28°C for two days and after this they had their leaves collected for lipid extraction and quantification. Control plants were allowed to regrow until presenting four leaves fully expanded and then were subjected to 10°C for ten days for cold tolerance evaluation. Plant survival was measured seven days after recovery at 28°C and the genotypes were grouped in three cold tolerance classes: tolerant, intermediate and sensitive. These classes differed for total saturated and unsaturated fatty acids only under the cold temperature treatment. Further analysis of the more abundant fatty acids: linoleic, linolenic and palmitic, showed that the two last ones differed between tolerant and sensitive genotypes. Linolenic acid increased after cold exposure in cold tolerant genotypes while palmitic acid decreased, and an opposite behavior was found in the cold sensitive genotypes. These evidences indicate that these fatty acids are potential molecular markers useful for breeding programs as well as for future basic studies on cold tolerance in rice.

Keywords: *Oryza sativa* L., lipid, linolenic acid, cold tolerance, membrane stability, plant survival.

INTRODUCTION

Rice (*Oryza sativa)* is a tropical cereal cultivated and consumed worldwide. In Brazil, the main rice grower is Rio Grande do Sul (RS) state, that accounts for 70% of the total grain produced in the country. Temperature may oscillate considerably during the rice growing season within this region and affect crop development unfavourably. Anticipation of sowing time has made cold temperature problem at the initial stages of development an important issue that may affect establishment of adequate crop stands and cause chlorosis and plant death. Temperature is an abiotic stress factor to which knowledge of the physiological basis can be useful to define new strategies of selection for cold tolerant genotypes.

According to Wang et al., (2006) temperature variation is one of the biggest environmental stresses to which plants may be submitted. Many species of temperate origin may develop tolerance when exposed to temperature change. This process is known as thermal adaptation that is associated to biochemical and physiological responses caused mainly by alterations in lipidic fluidity of membranes (Hur et al., 2004). Cold acclimation involves altered gene expression that affects membrane composition and accumulation of compatible solutes (Uemura et al., 2006). This is possible through the action of specific enzymes which are capable of altering the level of lipidic unsaturation of membranes. Therefore, fatty acid composition of the lipids that constitute the plant cell membranes is being studied as a key factor for cold sensitivity (Ito and Simpson, 1996).

Lipids of plant cell membranes are characterized by a high content of polyunsaturated fatty acids (Wang et al., 2006) that consist of carboxylic acids with hydrocarbonated chain with a length between 4 and 36 Carbons (C_4 to C_{36}). In some this chain is totally saturated (do not contain double bonds) and non-ramificated; in others the chain contains one or more double bonds. The physical proprieties of fatty acids and of the substances that contain them are mainly determined by the length and degree of unsaturation of this hidrocarbonated chain. This non-polar chain is responsible by the low solubility of fatty acids in water. Under temperature of 25ºC, C_{12} to C_{14} fatty acids have a cerous consistency while unsaturated fatty acids of the same Carbon length are liquid oily.

Studies on the changes in fatty acid composition and its association with cold tolerance in rice are rare, and the great majority of them are related to the types and percentages of fatty acids found in the cultivars (Juliano, 1994; Wu et al., 2000; Kitta et al., 2005; Bravi et al., 2006; Deepa et al., 2008). However, cold tolerance improvement via tissue culture was related to alterations in fatty acid composition, with an increase in fatty acid unsaturation (Bertin et al., 1998).

In order to better understand the behaviour of this trait among rice genotypes with different cold temperature reactions, a study of the fatty acid composition of young rice leaves was proposed. The aim of this study was to verify if cold temperature exposure can alter the unsaturation degree and the fatty acid composition of rice leaves and to determine if these alterations are related to cold tolerance.

MATERIAL AND METHODS

Genetic Material: Plant cultivation and cold tolerance tests were performed in the greenhouse and controlled temperature room of the Rice Experimental Station of the Instituto Rio Grandense do Arroz (IRGA), Cachoeirinha, RS. Extraction and quantification of lipids and fatty acid analysis were performed at the Laboratory of the Food Technology Department of the Santa Maria Federal University (UFSM), in Santa Maria, RS.

Forty-four rice genotypes (Table 1) belonging to the Germplasm Bank of the Rio Grande do Sul Rice Research Institute (IRGA) were evaluated as to lipid composition and cold tolerance at the vegetative stage. The genotypes were chosen due to their different origins and belong to the two rice subspecies, indica and japonica. An accession of *Oryza rufipogun* was also evaluated (Table 1).

Table 1. Origin and subspecies of the 44 rice genotypes evaluated as to lipid composition and cold tolerance at the vegetative stage.

Genotype	Origin	Subspecies	Genotype	Origin	Subspecies
Akitakomachi	Japan	japonica	Oryzica 1	Colombia	indica
Norin 21	Japan	japonica	Oryzica Llanos 5	Colombia	indica
Yunlen 19	China	japonica	Cica 8	Colombia	indica
Yunlen 2	China	japonica	Metica 1	Colombia	indica
Alan	USA	japonica	BR-IRGA 409	Brazil	indica
Bluebelle	USA	japonica	BR-IRGA 410	Brazil	indica
Caloro	USA	japonica	IRGA 416	Brazil	indica
Dawn	USA	japonica	IRGA 417	Brazil	indica
Lemont	USA	japonica	IRGA 420	Brazil	indica
Mercury	USA	japonica	IRGA 422CL	Brazil	indica
New Rex	USA	japonica	IRGA 423	Brazil	indica
Frances	USA	japonica	IRGA 424	Brazil	indica
Rizabela 2	Hungary	japonica	IRGA 2852-20-4-3-3V	Brazil	indica
Diamante	Chile	japonica	EPAGRI 108	Brazil	indica
Carnaroli	Italy	japonica	EPAGRI 109	Brazil	indica
Amaroo	Australia	japonica	Supremo 1	Brazil	indica
Inia Tacuari	Uruguay	japonica	Maravilha	Brazil	japonica
L 2825 CA	Uruguay	japonica	EEA 406 (Mutant)	Brazil	japonica
El Paso L 144	Uruguay	indica	BRS BOJURU	Brazil	japonica
Inia Olimar	Uruguay	indica	Pusa Basmati-1	India	indica
IR60	Philippines	indica	Jasmine	India	indica
IR50	Philippines	indica	*Oryza rufipogon*	Asia	

Experiment Conduction and Lipid Extraction: Seeds of the 44 rice genotypes were sown in trays (59 x 39 x 6 cm) filled with soil. Five genotypes were sown per tray, with four rows (36 cm long) per genotype being a replication. This number of rows per replication was determined in a previous study as the necessary to obtain a minimum of 2 grams of leaf dry matter for lipid extraction, what corresponded to approximately 80 plants (20 plants per row).

The experiment was conducted in a complete randomized block design with four replications for each of the temperature treatments: cold (10°C) and control (28°C), amounting 72 trays. After sowing, the trays were kept in a greenhouse (28°C) and when the plants reached the V_4 stage (Counce et al., 2000), half of the trays (four replications) were taken to a controlled temperature room for the cold treatment (10ºC) for two days. The other 36 trays corresponding to the other four replications were kept at the greenhouse, as a control treatment. After two days of cold treatment, all the plants (cold treatment and control) had all their leaves collected for lipid extraction. This consisted in cutting the base of the plants and putting all the leaves of a same replication in a plastic bag, which was kept in an isopor box filled with ice.

After grinding the leaves, lipid extraction was made according to Bligh and Dyer (1959) methodology. Fatty acid composition was determined by Gas Cromatography, with lipids being saponified and metanolized with KOH solution and then sterefied and metanolized with H_2SO_4 solution (Hartman and Lago, 1973). Methyl-esters fatty acids were analyzed with a Gas Cromatographer (Agilent Technologies - HP 6890) adjusted with a capilar column DB-23 (60m x 0.25mm x 0.25μm) with flame ionization detector. Injection and detection temperature was around 250°C and conduction was made by Nitrogen gas. Padronization of methyl-esters fatty acids and the subsequent retention times were used for fatty acid identification. Fatty acids were expressed as the percentage of total fatty acids contained in the pattern.

Cold Tolerance Evaluation: For cold tolerance evaluation, the original control plants were allowed to regrow at the greenhouse until they presented four completely developed leaves. At this stage, they were taken to the controlled

temperature room set at 10°C and stayed there for ten days. After cold treatment, the plants were taken back to the greenhouse and, after seven days of recovery at 28°C, the genotypes were evaluated as to the percentage of plant survival.

RESULTS

The rice genotypes presented a wide variation in relation to the percentage of survival after cold treatment. The survival rates varied from zero to 100% and, based on this feature, the genotypes were classified into one of the following classes of cold susceptibility: highly tolerant (100% survival rate), tolerant (between 70 and 99.9% survival rate), intermediate (between 30 and 69.9% survival rate), sensitive (between 0.1 and 29.9% survival rate) and highly sensitive (0% survival rate) (Table 2).

Japonica genotypes presented the highest levels of tolerance (Figures 1a and 1b), varying from intermediate to highly tolerant (Table 2), while indica genotypes behaved as sensitive or highly sensitive (Figures 1c and 1d, Table 2). The *Oryza rufipogun* genotype evaluated behaved as cold sensitive (Table 2).

Figure 1. Cold tolerant genotypes in the control treatment in the greenhouse (a) and after ten days of cold (10°C) treatment (b); and cold sensitive genotypes in the control treatment in the greenhouse (c) and after ten days of cold (10°C) treatment (d).

Table 2. Cold reaction (10 days at 10°C) in the vegetative stage (V_4) of 44 rice genotypes evaluated after seven days of recovery in the greenhouse (28°C) by the percentage of plant survival.

Genotype	Subspecies	Cold reaction	Genotype	Subspecies	Cold reaction
Amaroo	japonica	HT[a]	El Paso L 144	indica	S
BRS Bojuru	japonica	HT	Inia Olimar	indica	S
Akitakomachi	japonica	T	IR60	indica	S
Norin 21	japonica	T	Cica 8	indica	S
Yunlen 19	japonica	T	IR50	indica	HS
Yunlen 2	japonica	T	Pusa Basmati-1	indica	HS
Alan	japonica	T	Jasmine	indica	HS
Caloro	japonica	T	Oryzica 1	indica	HS
New Rex	japonica	T	Oryzica Llanos 5	indica	HS
Rizabela	japonica	T	BR-IRGA 409	indica	HS
Diamante	japonica	T	BR-IRGA 410	indica	HS
Frances	japonica	T	IRGA 416	indica	HS
Maravilha	japonica	I	IRGA 417	indica	HS
Bluebelle	japonica	I	IRGA 420	indica	HS
Dawn	japonica	I	IRGA 422CL	indica	HS
Lemont	japonica	I	IRGA 423	indica	HS
Mercury	japonica	I	IRGA 424	indica	HS
Carnaroli	japonica	I	IRGA 2852-20-4-3-3V	indica	HS
Inia Tacuari	japonica	I	EPAGRI 108	indica	HS
L 2825 CA	japonica	I	EPAGRI 109	indica	HS
EEA 406	japonica	I	Supremo 1	indica	HS
			Metica 1	indica	HS
			Oryza rufipogon		HS

[a] HT= Highly Tolerant; T = Tolerant; I = Intermediate; S = Sensitive; HS = Highly Sensitive.

Total fatty acid contents obtained in the rice genotypes were divided into saturated and unsaturated fractions, by summing the means of each type of saturated and unsaturated fatty acids obtained in the four replications of the two temperature treatments (cold and control). The results are presented in Table 3 in which the values of total saturated and unsaturated fatty acids were shown for each class of cold susceptibility. For this, tolerant and highly tolerant genotypes were grouped as a "tolerant" class, the sensitive and highly sensitive genotypes were grouped as a "sensitive" class. The values presented are the means of saturated and unsaturated fatty acids of all genotypes grouped in that class of cold susceptibility (Table 3).

There is a higher percentage of unsaturated fatty acids in relation to saturated ones in all rice genotypes, with the first corresponding to around 70% of total fatty acids and the saturated ones corresponding to around 30% of this total. This is expected once rice is a vegetable and so lipid unsaturation is higher to saturation. Lipid composition at normal temperature for the species growth (28°C) was not significantly different among the three different classes of cold susceptibility, both

for the unsaturated as well as for the saturated fatty acids (Table 3). After two days of cold exposure (10°C) at the V_4 stage, however, it was observed a rise in the concentration of saturated fatty acids in the cold sensitive genotypes, while in the tolerant and intermediate genotypes this was not altered. Unsaturated fatty acids behaved the opposite, lowering their concentration in the sensitive and intermediate classes and rising in the tolerant genotypes after cold exposure. Although these alterations were not significant in plants grown at 28°C, they led to a significant difference in the cold susceptibility in response to the cold treatment (10°C). Thus, the non-significant differences among the cold reaction classes at 28°C became significant at 10°C, with the sensitive rice genotypes presenting higher percentages of saturated fatty acids and lower of unsaturated fatty acids than the tolerant genotypes under the cold treatment (Table 3).

Table 3. Means of percentage of saturated and of unsaturated fatty acids of the three classes of cold reaction obtained after evaluation of 44 rice genotypes at the vegetative stage under two temperature treatments.

Cold reaction	Saturated fatty acids			Unsaturated fatty acids		
	28(°C)	10(°C)	Variation	28(°C)	10(°C)	Variation
Tolerant	A 28.7 a	A 28.6 a	-0.1	A 72.0 a	A 72.6 a	0.6
Intermediate	A 29.1 a	A 29.2 ab	0.1	A 72.0 a	A 70.5 a	-1.5
Sensitive	A 29.3 a	A 31.8 b	2.5	A 71.1 a	A 68.7 b	-2.4

Lowercase letters compare classes of cold reaction in each temperature treatment.

Uppercase letters compare temperature treatments in each class of cold reaction.

Means followed by the same letter do not differ by the least square means test ($\alpha = 0.05$)

In order to better understand the changes in lipid composition, it was decided to study the three more abundant fatty acids. Linoleic acid, linolenic acid and palmitic acid together corresponded to about 82 to 92% of total fatty acids extracted in the rice leaves in the present study, so they were further analyzed to verify the influence of the temperature treatments on their relative percentages and how they behaved in the three classes of cold reaction. The two first ones are unsaturated fatty acids consisting of double and triple bonds, respectively, while palmitic acid is a saturated fatty acid. By keeping the genotypes grouped according to their cold reaction, a factorial analysis of variance was conducted to verify the influence of each of the factors (cold reaction and temperature) and their interaction on the three fatty acids mentioned (Table 4).

Temperature treatment and the interaction between cold reaction and temperature influenced significantly linoleic acid content. On the other hand, linolenic acid and palmitic acid were significantly affected by cold susceptibility and the interaction cold susceptibility x temperature stress (Table 4). In other words, cold susceptibility was explained by the alterations in the content of linolenic acid and palmitic acid, while temperature treatment influenced the linoleic acid content. The significant interactions between cold reaction and temperature treatment for all the three fatty acid contents (Table 4) showed clearly that the genotypes fatty acid content varied according to both their cold reaction and temperature.

Table 4. Variance analysis for the content of three fatty acids of 44 rice genotypes grouped in classes of cold reaction and evaluated under two temperature regimes at the vegetative stage.

Source of variation	Degree of freedom	Mean Square		
		Linoleic acid	Linolenic acid	Palmitic acid
Cold reaction	2	1.45	93.63**	15.79**
Temperature	1	23.00**	2.55	0.52
Cold reaction x temperature	2	7.21**	30.66**	31.59**
Error	82	0.77	7.03	2.10
Coefficient of variation		5.74	5.49	6.29

** Significant at 1% level (F test).

According to the significance of the factors and their interaction in the analysis of variance, means comparison for linoleic acid were done among the treatments in each class of cold susceptibility (Table 5) and for linolenic acid and palmitic acid the comparisons were made among classes of cold reaction in each temperature treatment (Tables 6 and 7). In the case of linoleic acid content, sensitive genotypes did not present significant alterations in response to temperature treatment, while both intermediate and tolerant genotypes presented significantly higher contents at the control temperature (Table 5). Linolenic acid content did not vary among cold responsive classes at the control temperature, but upon cold treatment it was significantly higher for tolerant and intermediate genotypes (Table 6).

In the case of palmitic acid, the only saturated fatty acid, the only significant difference at the control treatment was between intermediate and sensitive genotypes, with the first ones presenting slightly higher content than sensitive ones. However, under cold treatment, both tolerant and intermediate genotypes presented significantly lower levels of palmitic acid content than the sensitive ones (Table 7).

Table 5. Means comparison between temperature treatments for linoleic acid content in each class of cold susceptibility.

Temperature	Class of cold susceptibility		
	Sensitive	Intermediate	Tolerant
Control (28°C)	15.3	16.4 *	15.8 *
Cold (10°C)	15.2	14.7	14.2

* Significant difference between temperature treatments according to Dunnett's test ($\alpha = 0.05$).

Table 6. Means comparison among classes of cold reaction for linolenic acid content in each temperature treatment.

Class of cold reaction	Temperature	
	Control (28°C)	Cold (10°C)
Tolerant	49.1 a	51.3 a
Intermediate	48.8 a	49.4 a
Sensitive	47.7 a	46.1 b

Means followed by the same letter do not differ by the least square means test ($\alpha = 0.05$)

Table 7. Means comparison among classes of cold susceptibility for palmitic acid content in each temperature treatment.

Class of cold susceptibility	Temperature	
	Control (28°C)	Cold (10°C)
Tolerant	22.5 ab	21.8 a
Intermediate	23.5 b	22.3 a
Sensitive	22.3 a	24.8 b

Means followed by the same letter do not differ by the least square means test ($\alpha = 0.05$)

DISCUSSION

The decrease in the plasma membrane fluidity caused by a transition from a liquid-cristallin phase to a gel phase in the cell membranes due to low temperature has been suggested as the primary event of cold damage. This phase transition results in alteration of cell metabolism and leads to damage and death of cold sensitive plants. The membrane content of unsaturated fatty acids is considered to be determinant of the temperature at which the damage may occur (Taiz and Zeiger, 2004).

The present data reveal a clear tendency of reduction in the unsaturated fatty acids and of rise in the saturated fatty acids in cold sensitive rice genotypes exposed to cold at the vegetative stage. This may explain their higher cold sensitivity, considering the role of lipid unsaturation in the maintenance of cell membrane stability (Uemura et al., 2006).

The present study corroborates the hypothesis in which the fatty acid composition and alteration after a cold exposure are different between genotypes with different cold stress susceptibilities. The results demonstrate that all the rice genotypes studied exhibited specific responses to temperature changes in terms of fatty acid composition depending on their cold tolerance. Furthermore, it was possible to identify alterations in specific fatty acids which could be related to the level of susceptibility or tolerance of each genotype characterized.

Tolerant and intermediate genotypes behaved similarly in what refers to fatty acid alterations. Both showed reduction in linoleic acid content upon cold exposure (Table 5), but increase in linolenic acid content (Table 6). Palmitic acid content was reduced after cold exposure in both classes of cold responsive genotypes (Table 7). These results indicate that tolerant and intermediate genotypes reduce lipid saturation and increase lipid unsaturation, which was mainly due to substitution of linoleic acid for linolenic acid. This is in accordance with previous studies in which the crucial role of polyunsaturated fatty acids in cold tolerance has been pointed out with an inhibitor of linonenic acid synthesis (St. John et al., 1979) and also with the *fad* mutants of *Arabidopsis thaliana*, which are defective in the desaturation of membrane lipids (Browse and Somerville, 1991; Somerville and Browse, 1991).

Sensitive genotypes did not alter linoleic acid content due to cold treatment (Table 5), and for the two other fatty

acid contents their behavior was found to be as opposite to that observed for tolerant and intermediate genotypes. They presented lower linolenic acid and higher palmitic acid contents than tolerant and intermediate genotypes under cold treatment (Tables 6 and 7). Thus, in the case of sensitive genotypes, alteration in fatty acid composition was mainly due to substitution of an unsaturated fatty acid for a saturated one, what may even increase their cold susceptibility.

Expression of the rice plastidial omega-3 desaturase genes, OsFAD8, increased at low temperatures and the photosynthetic efficiency and recovery of OsFAD8 knockout mutants were significantly reduced after cold stress as compared to those of wild type plants (Nair et al., 2009). This shows the importance of maintaining adequate levels of unsaturation for plant recovery and survival under cold temperature conditions. In this work, differential alteration in fatty acid composition in response to cold exposure varying among the three classes of cold responsive genotypes represents also an indirect evidence of the differential expression of genes related to lipid unsaturation among rice genotypes with different cold reactions. Linolenic acid and palmitic acid, besides being the most abundant fatty acids extracted in the rice leaves in the present study, seem to be more affected under cold temperature and to exhibit opposite behaviors in cold tolerant and cold sensitive genotypes. Based on these data, linolenic and palmitic acids are proposed to be potential targets to differentiate rice genotypes as to their cold tolerance and as key molecular markers for future studies on cold temperature tolerance in rice.

In this study, japonica genotypes were cold tolerant or behaved as intermediate while all indica genotypes were cold sensitive, as previously postulated (Mackill and Lei, 1997). This is important if we consider that the differences found in fatty acid composition may represent subspecies differentiation and different adaptation mechanisms. Therefore, it may be thought that by altering the fatty acid composition or by selecting genotypes with a more adequate composition under cold temperature we could reduce adaptability under normal temperature. However, transgenic approaches in other crops have showed the importance of raising the content of linolenic acid for improving cold tolerance (Kodama et al., 1994).

To our knowledge this is the first report on fatty acid composition of different rice genotypes and its relation with cold tolerance at the vegetative stage. The results reported here show that there is variability in fatty acid composition within the rice species that may be useful for improving cold tolerance in breeding programs. The data highlighted the relevance of studying linolenic and palmitic acids as new targets for biochemical and molecular approaches towards the characterization and control of the cold tolerance mechanism in the rice crop.

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REFERENCES

Bertin, P, Bullens P, Bouharmont J, Kinet J-M (1998) Somaclonal variation and chilling tolerance improvement in rice: changes in fatty acid composition. Plant Growth Regul. 24: 31-41.

Bligh EC, Dyer WJ (1959) A rapid method of total lipid. Extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Bravi E, Pereetti G, Montanari L (2006) Fatty acids by high-performace liquid chromatography and evaporative light-scattering detector. J. Chromatogr. 1134: 210-214.

Browse J, Somerville C (1991) Glycerolipid synthesis: biochemistry and regulation. Annu. Rev. Plant Physiol. Plant Mo1. Biol. 42: 467-506.

Counce PA, Keisling TC, Mitchell AJ (2000) A uniform, objective, and adaptive system for expressing rice development. Crop Sci. 40: 436-443.

Deepa G, Singh V, Naidu, KA (2008) Nutrient composition and physicochemical properties of Indian medicinal rice- Njavara. Food Chem. 106: 165-171.

Hartman L, Lago BCA (1973) Rapid preparation of fatty, methyl esters from lipids. Laboratory Pract. 22: 457-477.

Hur J, Jung K-H, Lee C-H, An G (2004) Stress-inducible *OsP5CS2* gene is essential for salt and cold toleranc in rice. Plant Sci. 167: 417–426.

Ito MK, Simpson KL (1996) The biosynthesis of ω3 fatty acids from 18:2ω in *Artemia* spp. Comp. Biochem. Phys.115: 69-76.

Juliano BO (1994) Polysaccharides, proteins and lipids of rice. In: Juliano BO (ed), Rice Chemistry and Technology, pp.59-174. The American Association of Cereal Chemists, St. Paul, USA.

Kitta K, Ebihara M, Iizuka T, Yoshikawa R, Isshiki K, Kawamoto S (2005) Variations in lipid content and fatty acid composition of major non-glutinous rice cultivars in Jpn. J. Food Compos. Anal. 18: 269–278.

Kodama H, Hamada T, Horiguchi G, Nishimura M, Iba K (1994) Genetic enhancement of cold tolerance by expression of a gene for chloroplast *W-3* fatty acid desaturase in transgenic tobacco. Plant Physiol. 105: 601-605.

Mackill DJ, Lei X (1997) Genetic variation for traits related to temperate adaptation of rice cultivars. Crop Sci. 37: 1340-1346.

Nair PMG, Kang IS, Moon BY, Lee CH (2009) Effects of low temperature stress on rice (*Oryza sativa* L.) plastid omega-3 desaturase gene, OsFAD8 and its functional analysis using T-DNA mutants. Plant Cell Tiss.Org. 98: 87-96.

Somerville C, Browse J (1991) Plant lipids: metabolism, mutants, and membranes. Science 252: 80-87.

St. John JB, Christiansen MN, Ashworth EN, Gentner WA (1979) Effect of BASF 13-338, a substituted pyridazinone, on linolenic acid levels and winterhardiness of cereals. Crop Sci. 19: 65-69.

Taiz L, Zeiger E (2004) Fisiologia Vegetal. 3rd ed. Artmed, Porto Alegre.

Uemura M, Tominaga Y, Nakagawara C, Shigematsu S, Minami A, Kawamura Y (2006) Responses of the plasma membrane to low temperatures. Physiol. Plantarum 126: 81-89.

Wang J, Ming F, Pittman J, HanY, Hu J, Guo B, Shen D (2006) Characterization of a rice (*Oryza sativa* L.) gene encoding a temperature-dependent chloroplast ω-3 fatty acid desaturase. Biochem. Bioph. Res. Co. 340: 1209–1216.

Wu J, Hwang I-T, Hatzios KK (2000) Effects of chloroacetanilide herbicides on membrana fatty acid desaturation and lipid composition in rice, maize, and sorghum. Pestic. Biochem. Phys. 66: 161-169.