

Chill-induced changes in fatty acid composition of tonoplast vesicles from hypocotyls of *Vigna unguiculata* (L.) Walp.

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ABSTRACT

The present study was undertaken to investigate the changes induced by chilling on fatty acid composition of tonoplast vesicles from hypocotyls of *Vigna unguiculata* (L.) Walp. The 7 day-old control seedlings were grown at 25°C while treated seedlings were submitted to low temperatures (10°C and 4°C) for 4 days after 3 days germination. The chilling stress resulted in a differential inhibition of plant growth at 10°C and 4°C. Following chilling at 10°C and 4°C, the rate of unsaturated to saturated fatty acids increased under chilling stress. Our results suggest that the increase of unsaturated fatty acids content as well as the higher rate unsaturated/saturate fatty acids might be used by *V. unguiculata* plants as an adaptation mechanism likely to maintain the vacuolar membrane fluidity under low temperatures.

Key words: chilling, cowpea, lipid, fatty acids, membrane, vacuole.

RESUMO

O presente trabalho analisa as mudanças na composição de ácidos graxos de vesículas de tonoplasto de hipocótilos de *Vigna unguiculata* (L.) Walp induzida por estresse causado por baixas temperaturas. Plântulas em condição controle desenvolveram-se por 7 dias à temperatura de 25°C e as plântulas tratadas foram submetidas a 10 ou 4°C por 4 dias, 3 dias após a germinação a 25°C. O frio (10 ou 4°C) induziu um efeito inibitório no desenvolvimento das plântulas e esse efeito foi mais pronunciado a 4°C. Além disso, o grau de insaturação dos ácidos graxos nas vesículas de tonoplasto aumentou sob o efeito do frio, o que pode indicar um aumento da permeabilidade das vesículas da membrana vacuolar. Nossos resultados sugerem que plantas de *V. unguiculata* aumentaram o seu conteúdo de ácidos graxos insaturados bem como a razão entre ácidos graxos insaturados/saturados, como mecanismo de adaptação ao frio numa tentativa de manter a fluidez da membrana vacuolar.

Palavras-chave: ácidos graxos, caupi, frio, lipídios, membrana, vacúolo

INTRODUCTION

Low temperature is an important environmental factor that influences the growth, development, survival and distribution of plants (Levitt, 1980; Renaut et al., 2004; Lee et al., 2009). Most plants from temperate regions can cold-acclimate in nonfreezing temperatures (Guy, 1990). However, plants adapted to tropical climates are usually more sensitive to chilling and this sensibility could vary widely (Yoshida et al. 1993; Nogueira et al., 2003). One of the most pronounced effect of chilling in plants is the alterations in the cellular membranes (Kasamo et al., 2000; Larkindale and Huang, 2004; Lee et al., 2005). Lipids are recognized as major chemical component of biological membranes playing an important role in cellular activities throughout the permeability, fluidity, and membrane-bound enzyme activities (Quinn and Williams, 1978; Yoshida and Uemura, 1986). Indeed, these diverse functions depend of the membrane stability and its fatty acids composition. Few reports address the effect of chilling on membranes from organelles such as vacuolar membrane (tonoplast) (Kasamo et al. 2000; Yoshida et al. 1993). In this context, we propose to investigate chill-induced changes on the fatty acid composition of tonoplast vesicles isolated from hypocotyls and their effect on development of *Vigna unguiculata* (L.) Walp.

MATERIAL AND METHODS

Seeds of *V. unguiculata* (L.) Walp cultivar Vita 3 (Seeds Bank of Universidade Federal do Ceará) were previously imbibed in distilled water for 1 h and germinated in filter paper, in darkness at 25°C (control). Three-day-old-seedlings were exposed to 10°C and 4°C for four days. For evaluation the growth effect by chilling the three-day-old-seedlings were submitted also for 3 days at 10°C and placed back at 25°C for 1 day. The fresh weight of different parts of seedlings was measured using analytic balance. At the end of treatment (7 days) the hypocotyls from plants were harvested for the isolation of tonoplast-enriched vesicles (Mariaux et al. 1997 modified by Otoch et al. 2001).

The total lipids from tonoplast vesicles were extracted in chloroform:methanol: water (1:1:1) according Bligh and Dyer (1959) with some modifications (Monteiro de Paula

et al., 1990). The fatty acids were quantified by gas liquid chromatography, as previously described (Monteiro de Paula et al., 1990). The lipid bands were scraped off, saponified and fatty acids were methylated with boron trifluoride. Heptadecanoic acid (C17:0) was used as an internal standard for gas chromatography analysis.

RESULTS AND DISCUSSION

The exposure of 7-day-old seedlings of *V. unguiculata* (L.) Walp to chilling stress (10 and 4°C) resulted in drastic impairment of growth (Table 1). It was observed that inhibition of seedling development was proportionally affected by low temperatures. The roots fresh weight was inhibited by chilling *c.a* 34% at 10°C and 45% at 4°C but this inhibition was overcome when seedlings returned to control temperature (25°C), resulting in growth recover. Hypocotyls were also inhibited by chilling with greater inhibition observed at 4°C (~62%), twice than that at 10°C (36.4%). The epycotyl was revealed as the most sensitive organ to chilling, showing a decrease in fresh weight by 40% at 10°C and 67% at 4°C. It should be noted that when seedlings were transferred back to ambient conditions (25°C), the damage promoted by chilling to epocotyls was nearly irreversible, contrary to hypocotyls (Table 1).

Table 1. The effect of chilling on growth development of *Vigna unguiculata* seedlings. Three-day-old-seedlings were transferred to 10 and 4°C during 4d, the three-day-old-seedlings were submitted also to 10°C for 3 days and placed back at 25°C for 1 day. The data represent the average of 90 seedlings for each condition obtained in three independent experiments.

Treatment	Root (g)	Hypocotyl (g)	Epycotyl (g)
25°C (7d, control)	0.298	1.1	0.079
10°C (4d)	0.194	0.7	0.0473
4°C (4d)	0.162	0.42	0.0262
10°C (3d) + return to 25°C (1d)	0.217	0.967	0.0316

The total fatty acid composition of tonoplast vesicles from *V. unguiculata* seedlings in control (25°C) as well as in chill stress conditions (4 and 10°C) was evaluated and revealed the presence of palmitic (16:0), linoleic (18:2), linolenic (18:3) and stearic acid (18:0) (Table 2). In control condition, the prevailing fatty acid was palmitic while stearic

was less representative. The content of saturated fatty acids were decreased by 20% for palmitic acid at both low temperatures and 30 and 44 % for stearic acid at 10°C and 4°C, respectively, than compared to control. Notably, in response to chilling the amount of linoleic acid (18:2) increased 25% at 10°C and 7% at 4°C while the amount of linolenic acid (18:3) were augmented by 27% and 33% at 4°C and 10°C, respectively.

Chilling is an important environmental stress that interferes with the productivity of many important crops. *Vigna unguiculata* (L.) Walp is a tropical culture acclimated to warm temperatures, therefore it is considered a chilling sensitive crop. In fact, the growth development of *V. unguiculata* seedlings submitted to low temperatures was inhibited at 4°C to a more extent than 10°C. Our results are in accordance to those obtained by Darley et al. (1995) with mung bean seedlings (*Vigna radiata* (L.) Wilczek) that revealed that seedlings exposure at 4°C effectively prevented the growth development. Indeed, this effect was not irreversible once growth recover was observed when seedlings exposed to chilling (10°C) were transferred back to ambient warm temperatures (Table 1).

Cold stress triggers drastic changes in plant physiology. The structural and functional stability of membrane is crucial for plant adaptation to temperature stresses, and damage in membrane structure can led to changes in membrane semi permeability as well as changes in some membrane-localized enzymes (Kratsch and Wise, 2000; Lee and Lee, 2000; Vani et al., 2001; Zhang et al., 2001). Therefore we attempted to evaluate tonoplast fatty acid composition from hypocotyls of *V. unguiculata* seedlings under chilling conditions. Our results showed that in control seedlings, the prevailing fatty acid was a palmitic acid (Table 1). In the same way, Yoshida and Uemura (1986) showed that both plasma membrane and tonoplast from mung bean under control condition contain high levels of palmitic acid which result in low degree of unsaturation. The amount of unsaturated fatty acids (18:2, 18:3) from *V. unguiculata* under chilling (10°C and 4°C) was higher than in the control condition (25°C) (Table 2). The enhancement of unsaturated to saturated fatty acids rate in our results can be explained as the vesicles membrane permeability augmentation at low temperatures.

Table 2. Fatty acid composition of tonoplast hypocotyls. The experimental results shown are the average of triplicate measurements. Standard errors of analysis were estimated to be within 3%.

Fatty acid	(%)		
	25°C (control)	10°C	4°C
16:0 (palmitic acid)	41.4	33.2	33
18:0 (stearic acid)	7	4.9	3.9
18:2 (linoleic acid)	27.6	34.5	30
18:3 (linolenic acid)	24	27.4	33

The most common changes in fatty acid composition at low temperatures include an enhancement of unsaturated fatty acids amounts, increased ratio of short chain to long chain fatty acids, and/or alterations in branched chain fatty acid content (Suutari and Laakso, 1994). According to Neidleman (1987) this alteration in fatty acid composition of biological membranes as a common response to chilling is responsible for the maintenance of appropriate membrane fluidity. Our results confirm this idea and demonstrate that cowpea seedlings alter the lipid composition as a part of global adaptive response to chilling stress. These changes could reflect an attempt to maintenance of vacuolar membrane fluidity through the higher unsaturated fatty acid. The vacuole is an organelle that plays important roles in plant physiology, such as storage of metabolites, toxic ions, proteins etc, and is actively involved in membrane transport (Taiz, 1992; Martinoia et al., 2007). The importance of lipids in the membrane structure is well known as well as in modulation of membrane-bound enzymes (Zhang et al., 2006). Also, the lipid environment can play a role in regulation of the vacuolar proton V-ATPase (tonoplast proton pump) (Kasamo et al., 2000; unpublished results). This enzyme is considered as stress marker being responsive to many environmental stresses (Luttge et al., 2001), thus it is likely that the changes in lipid composition detected in present study can be responsible for changes in membrane transport, aside the stability of tonoplast. Future investigations are needed to establish a correlation between the alterations in lipids species and proton pumping mediated by V-ATPase.

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