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Thylakoidal membrane lipid composition and its reflex in protoplasmic drought tolerance and photosynthetic capacity of almond tree

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SUMMARY – Fourteen (five Portuguese and nine foreign) varieties from a collection of *Prunus amygdalus,* Batsch, grown in Quinta do Valongo (DRATM, Mirandela, Portugal) were studied in what concerns photosynthetic capacity (A_{max}), protoplasmic tolerance evaluated through electrolyte leakage (I%) under osmotic stress and thylakoids lipid composition. Significant differences were found as regards some parameters, namely A_{max}, I% and total lipid content. Of the six varieties, which presented the highest A_{max} values (≥45 μmol/m^2.s), four were Portuguese. The case of 'José Dias' was particularly interesting, a promising variety that presented an enhanced membrane tolerance to dehydration (lower I%) associated to higher lipid contents, namely galactolipids. Such features may be related to better photosynthetic performance and reflect a higher degree of adaptation.

Key words: Lipids, membrane leakage, osmotic stress, photosynthesis, *Prunus amygdalus*.

RESUME – *"Composition lipidique de la membrane des thylakoïdes et influence sur la tolérance protoplasmique à la sécheresse et sur la capacité photosynthétique de l'amandier". Quatorze variétés (cinq portugaises et neuf étrangères) appartenant à une collection de* Prunus amygdalus *Batsch, cultivées à Quinta do Valongo (DRATM, Mirandela, Portugal) ont été étudiées en ce qui concerne la capacité photosynthétique (A_{max}), la tolérance protoplasmique évaluée à travers la fuite d'électrolytes (I%) en conditions de stress osmotique et la composition lipidique des thylakoïdes. Des différences importantes ont été trouvées notamment pour l'A_{max}, I% et le contenu en lipides totaux. Des six variétés qui présentaient les valeurs les plus hautes de A_{max} (≥45 μmol/m^2.s), quatre étaient portugaises. Le cas de la variété 'José Dias' semble particulièrement intéressant car elle présente une bonne tolérance membranaire à la déshydratation (valeurs réduites de I%) et plus de lipides (surtout galactolipides). Ces caractéristiques peuvent être en rapport avec de bonnes performances au niveau de la photosynthèse et indiquer une meilleure adaptation à l'environnement.*

Mots-clés : *Lipides, dommage membranaire, stress osmotique, photosynthèse,* Prunus amygdalus*.*

Introduction

Higher plants have evolved a number of physiological adaptations that allow them to survive, continue growing, and even produce acceptable yields under water-limiting conditions (Castel and Fereres, 1982). Photosynthesis is one of the primary processes responsible for productivity. Chloroplast membranes are tightly related to photosynthetic performance as light reactions (light harvesting and electron transport) are located in thylakoids (Ristic *et al.*, 1992), which represent 60-80% of the total cellular membranes in mesophyll (Webb and Green, 1991). Membrane lipids of higher plants may play a role in adaptation ability to adverse environments (Kuiper, 1985).

Almond tree is considered a drought-tolerant species, being commonly grown in marginal areas of scarce water supply or even in desert regions of Mediterranean coast (Torrecillas *et al.*, 1988). Intraspecific differences concerning photosynthetic rates, thylakoid lipids and membrane capacity to tolerate osmotic stress may be the result of different adaptations occurred during populations establishment. The knowledge of such physiological and biochemical features may be useful for breeding purposes, and may underlie distinct behaviours as regards drought tolerance.

The aim of this work was to compare almond tree varieties growing in the field in what concerns photosynthetic capacity and dehydration tolerance, evaluated through electrolyte leakage following PEG treatment. As changes in these parameters may be tightly related to thylakoid membrane lipid composition, the latter was also analysed.

Materials and methods

Plant material

In this study six years old plants of *Prunus amygdalus,* Batsch, grafted on a peach-almond hybrid (GF-677), were used. Fourteen varieties were chosen, from which five were Portuguese ('José Dias', JD; 'Duro d' Estrada', DE; 'Duro Italiano', DI; 'Casanova' CN; and 'Verdeal', V) and the remaining were foreign ('Moncayo', MO; 'Tuono', T; 'Guara', GU; 'Ferraduel', FL; 'Ferrastar', FR; 'Ferragnès', FS; 'Marcona', MA; 'Nonpareil', NP; 'Garrigues', GA). Trees belong to a collection settled in Quinta do Valongo (DRTAD) located in Mirandela (Northeast of Portugal at 41°31' N latitude and 7°12' W longitude). Rainfall was about 600 mm per year, and maximum air temperature around 39°C during summer months.

Photosynthetic capacity

Photosynthetic capacity (A_{max}) was determined in a leaf disc oxygen electrode LD2/2 (Hansatech, Kings Lynn, UK) under saturating light (*ca*. 1500 μmol/m^2.s) and CO_2 (*ca*. 7%, obtained through a carbonate/bicarbonate buffer) to completely overcome the stomatal resistance, according to preliminary experiments.

Electrolyte leakage

Fifteen leaf discs (0.8 cm^2) per sample were cut out from leaves, rinsed three times with deionized water, and floated for 5 h on deionized water (control), or on a 30% (w/v) polyethylene glycol (PEG) MW 600 solution (osmotic potential: -1.13 MPa). After washing, the discs were allowed to float on deionized water for 24 h (rehydration), at room temperature. During this period membrane leakage was monitored with a Crison 522 conductimeter (Crison Instruments, Spain). An injury index (I%) was calculated according to the formula I% = [1-(T-S/T- C)]x100 (Scherbakova and Kacperska-Palacz, 1980), where S and C represent the electrolytes released by stressed and control samples, respectively, and T the total electrolyte content measured after heating the control samples in their effusate at a temperature of 90ºC for 2 h.

Lipid analysis

Fresh leaf samples (10 g) were homogenized in a buffer medium and centrifuged for thylakoidal membranes isolation following the method of Droppa *et al*. (1987). The chloroplastic lipid fraction was extracted from the resulting pellet using a mixture of chloroform/methanol/water (1/1/1, by vol.) according to Allen *et al*. (1966). Fatty acids were saponified and methylated with BF_3 using heptadecanoic acid (C17:0) as internal standard, according to Metcalfe *et al.* (1966), and analysed by gas liquid chromatography (UNICAM 610 gas chromatograph, fused silica capillary column DB-Wax, 0.25 mm I.D., 0.25 μm film thickness, 30 m long), using heptadecanoic acid (C17:0) as an internal standard. Lipid classes were separated by thin layer chromatography according to Lepage (1967). After spraying with primuline and visualisation under UV, the bands corresponding to galactolipids and phospholipids were scraped off and quantified spectrofotometrically according to Yemn and Willis (1954) and Peach and Tracy (1956), respectively.

Statistical analysis

Results were statistically analysed by the Statistix program version 7.

Results and discussion

In what concerns A_{max} (Fig. 1), varieties could be grouped in three main ranges. 'Ferrastar', 'Duro d'Estrada', 'Nonpareil', 'José Dias', 'Verdeal' and 'Duro Italiano' presented the highest rates (≥45 μmol/m^2.s), while 'Tuono', 'Ferraduel', 'Marcona', 'Guara', 'Moncayo' and 'Garrigues' were in the lowest range (≤35μmol/m^2.s). The remaining varieties presented intermediate values.

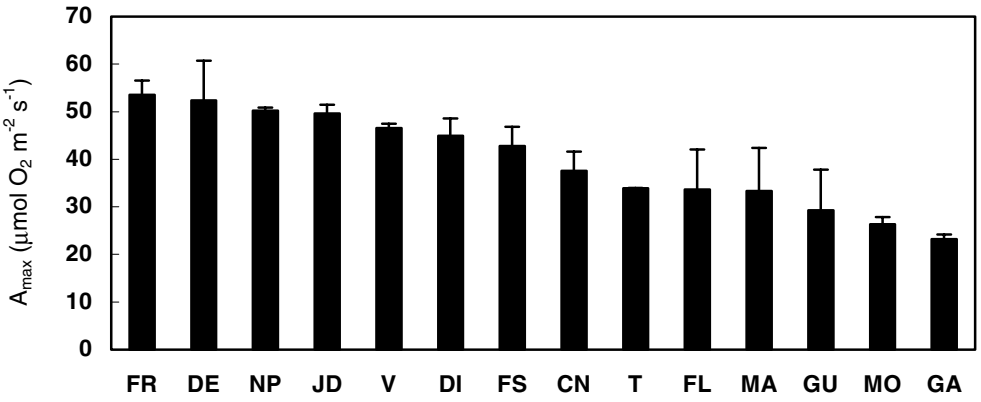

Fig. 1. Photosynthetic capacity (A_{max}) of fourteen *Prunus amygdalus* varieties grown under rainfed conditions in the Northeast of Portugal (Mirandela, Quinta do Valongo). Results are means of three measurements ± SE.

As regards the injury index (I%) under PEG-induced dehydration, 'Tuono' and 'Verdeal' showed the highest membrane damage (highest I% values), being significantly more affected ($P<0.05$) than 'Moncayo', 'Marcona', 'Guara', 'Ferragnès', 'Duro d'Estrada' and 'José Dias' (Fig. 2).

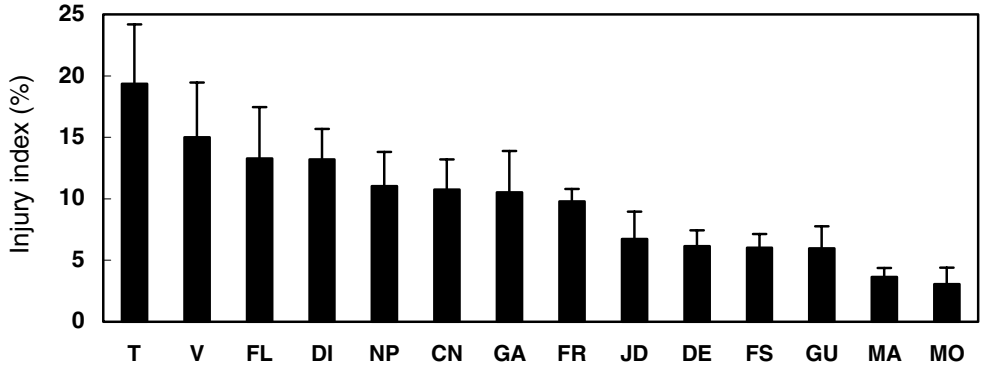

Fig. 2. Injury index (I%) of fourteen varieties of *Prunus amygdalus* after a 5 h dehydration of leaf discs with PEG 600 (-1.13 MPa). Trees were grown under rainfed conditions in the Northeast of Portugal. Results are means of three measurements ± SE.

'José Dias', 'Ferrastar' and 'Ferragnès' presented the highest total fatty acids (TFA) values (7.5±1.7, 6.9±0.3 and 5.9±0.3 mg/mg Chl, respectively), in opposition to 'Tuono' (Fig. 3), which showed the lowest values (2.5±0.1 mg/mg Chl). Such a reduced lipid amount reveals a low membrane content, and may be responsible for lower photosynthetic performance and increased membrane damage under osmotic stress in 'Tuono'. On the contrary the Portuguese variety 'José

Dias', which showed high photosynthetic activity as well as abundant membrane systems, was moderately affected by osmotic stress. However a correlation between lipid amount, A_{max} and membrane damage under PEG-induced dehydration could not be established among the fourteen varieties.

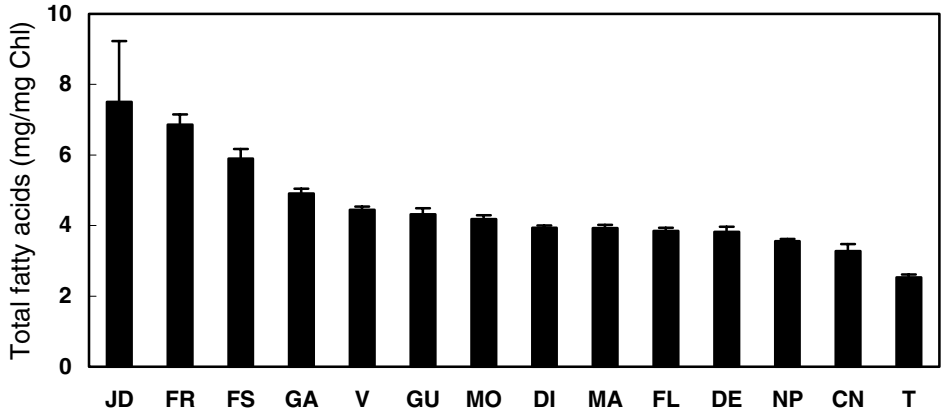

Fig. 3. Total fatty acids (TFA) extracted from thylakoid membranes of fourteen varieties of *Prunus amygdalus* grown under rainfed conditions in the Northeast of Portugal. Results are means of three measurements ± SE.

Regarding galactolipids (Fig. 4) it could be observed that 'José Dias' showed higher monogalactosyl-diacylglycerol (MGDG) and digalactosyl-diacylglycerol (DGDG) contents than 'Tuono', indicating the presence of higher amounts of thylakoids. This is in agreement with the better A_{max} rates found in 'José Dias'.

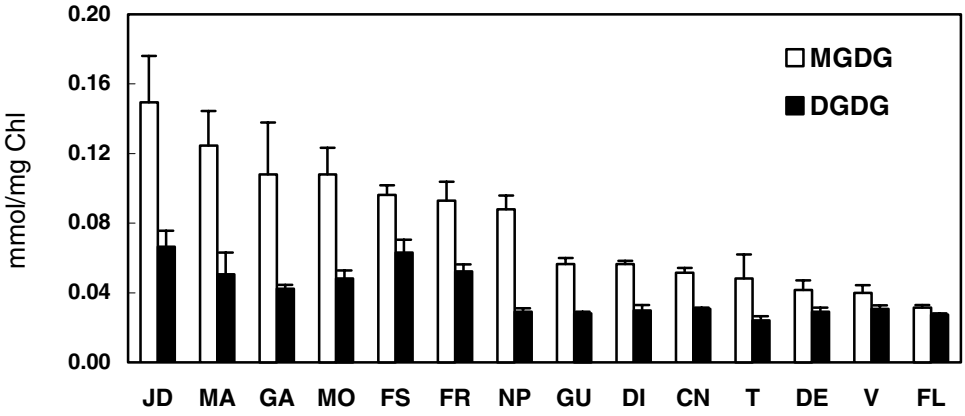

Fig. 4. Galactolipids extracted from thylakoid membranes of fourteen varieties of *Prunus amygdalus* grown under rainfed conditions in the Northeast of Portugal. MGDG, monogalactosyl-diacylglycerol; DGDG, digalactosyl-diacylglycerol. Results are means of three measurements ± SE.

These varieties did not show significant differences in linolenic acid (C18:3) percentage of total fatty acids (Table 1), reflecting similar unsaturation degrees of their membranes expressed by DBI values (*ca*. 5.5). However significant differences were found ($P<0.05$) in the DBI of other varieties, such as the Portuguese 'Verdeal' (*ca*. 6.8) and 'Nonpareil' (*ca*. 3.6).

A high membrane unsaturation may contribute to increase membrane fluidity and functionality

(Raison, 1980), what seems to be the case of 'Verdeal' which presented high A_{max} rates (ca. 46.6 $\mu mol/m^2.s$). However it might also enhance membrane susceptibility to degradation, since polyunsaturated fatty acids are the preferential substrate of peroxidative and hydrolytic enzymes (Sahsah *et al.*, 1998). This might be a hypothesis to explain significant I% values observed in 'Verdeal', reflecting a strong membrane damage and, hence, a low membrane tolerance under PEG-induced dehydration.

Table 1. Fatty acids composition (mol%) and double bond index (DBI) of thylakoid membrane lipids in fourteen varieties of *Prunus amygdalus*. The determined fatty acids were palmitic (C16:0), *cis* and *trans* hexadecenoic (C16:1*c*, C16:1*t*), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3). Results are means of three measurements ± SE

	mol%								
	<C16:0	C16:0	C16:1*c*	C16:1*t*	C18:0	C18:1	C18:2	C18:3	DBI[†]
FS	2.0	24.4	3.7	1.9	3.2	2.3	11.6	50.8	6.2
S.E.	0.3	1.1	0.3	0.3	0.1	0.1	0.5	0.8	0.3
T	1.9	26.5	2.9	1.2	3.7	2.9	13.5	47.4	5.5
S.E.	0.1	0.6	0.2	0.1	0.1	0.0	0.1	0.5	0.1
GU	1.7	25.0	4.9	1.7	2.6	1.7	8.9	53.5	6.4
S.E.	0.9	0.5	0.5	0.0	0.1	0.0	0.2	0.4	0.2
GA	1.9	25.6	4.1	1.3	4.0	1.9	9.2	52.0	5.8
S.E.	0.4	0.4	0.1	0.0	0.9	0.3	0.0	0.6	0.2
V	1.9	24.0	4.3	1.4	2.2	2.1	11.2	53.0	6.8
S.E.	0.2	0.7	0.2	0.0	0.0	0.1	0.1	0.6	0.2
DE	1.8	27.8	3.6	1.6	2.6	2.3	11.4	48.8	5.5
S.E.	0.3	1.2	0.3	0.1	0.1	0.0	0.2	1.2	0.3
NP	0.6	37.5	2.8	0.0	4.2	2.6	9.1	43.1	3.6
S.E.	13.4	12.0	0.2	0.0	0.2	0.1	0.2	1.0	0.2
DI	1.7	26.7	4.4	0.4	2.6	3.1	8.7	52.5	6.0
S.E.	0.9	1.8	0.3	0.4	0.2	0.2	0.3	2.1	0.7
FL	2.7	24.4	3.9	1.8	2.8	2.1	10.7	51.7	6.2
S.E.	0.3	1.0	0.3	1.2	0.2	0.1	0.3	1.5	0.3
FR	3.0	32.2	5.7	1.6	2.3	1.8	9.0	44.3	4.3
S.E.	0.6	2.2	0.5	0.2	0.2	0.2	0.8	3.0	0.4
CN	9.1	21.6	3.6	0.4	3.8	2.4	9.7	49.4	5.1
S.E.	8.5	10.8	0.3	0.4	0.5	0.2	0.4	1.2	0.4
MO	1.2	36.0	5.0	0.0	2.6	2.0	9.9	43.4	4.0
S.E.	0.6	1.8	0.3	0.0	0.2	0.2	0.3	1.5	0.3
MA	1.6	31.3	1.0	0.0	3.5	3.1	12.3	47.2	4.7
S.E.	0.9	0.8	1.0	0.0	0.2	0.2	0.3	0.8	0.2
JD	1.4	27.4	3.5	0.6	3.0	2.5	14.6	46.9	5.6
S.E.	0.2	1.5	0.2	0.3	0.2	0.1	0.4	1.2	0.3

[†]DBI = [(C16:1c+C16:1t+C18:1+2xC18:2+3xC18:3) / (<C16:0+C16:0+C18:0)].

As concerns *trans*-Δ^3-hexadecenoic acid (C16:1*t*), which is a key fatty acid of thylakoid phosphatidilglycerol (PG), it was observed that 'José Dias' presented a lower percentage than 'Tuono' (Table 1). It has been shown that decreases in C16:1*t* accompanied a shift from oligomeric to monomeric LHCII, what could reflect a mechanism for regulating energy distribution within the photosynthetic apparatus to counteract the potentially deleterious effects of stress-induced photoinhibition (Huner *et al.*, 1989). In this almond tree variety, such a low C16:1*t* percentage could constitute an adaptation feature to the stress conditions.

Conclusions

Among the six varieties presenting the highest A_{max} values, four were Portuguese. However the most well-adapted seems to be 'José Dias', which presented a lower injury index. This may be a good indicator of an enhanced membrane tolerance to dehydration. The high photosynthetic capacity observed in this variety may be related with its highest lipid content, particularly as regards galactolipids, which are main components of thylakoid membranes.

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