BRIEF COMMUNICATION

Water stress induced changes in the leaf lipid composition of four grapevine genotypes with different drought tolerance

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Abstract

To dissect differences in both lipid accumulation and composition and the role of these modifications during drought stress, four grapevine cultivars exhibiting differential tolerance to drought were subjected to water shortage. Tolerant cultivars, Kahli Kerkennah and Cardinal, exhibited higher leaf water potential (Ψ_w), and lower lipid peroxidation compared to the sensitive cultivars Guelb Sardouk and Superior Seedless during stress. Total lipid amounts increased during stress only in the leaves of the tolerant cultivars. Drought induced increases in the ratios digalactosyl-diacylglycerol/monogalactosyldiacylglycerol and phosphatidylcholine/phoshatidylethanolamine of almost all the drought stressed cultivars. Moreover, the overall analysis of the composition of fatty acids revealed that a linolenic acid was prevalent in grapevine and the unsaturation level of lipids increased under water stress in all the cultivars. Specific adjustments in the lipid composition during stress could compromise stress tolerance.

Additional key words: glycolipids, leaf water potential, peroxidation, phospholipids, unsaturation, *Vitis vinifera*.

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Drought is one of the most severe constrains to crop production, and plants that undergo water deficit manifest a wide range of behaviours, ranging from high sensitivity to tolerance. Grapevine (*Vitis vinifera* L.) is among the species which often suffer water deficit, mainly due to the low rainfall, as well as the high evapotranspiration in its cultivation areas. What is more interesting is that different vine cultivars exhibit high variability in their tolerance against drought stress. Daily course of leaf water potential ($\Psi_{leaf.}$) can be an efficient indicator of water stress, as it includes the rate of transpiration, the soil water availability as well as the internal hydraulic properties of the plant (*e.g.* Choné *et al.* 2001, Grzesiak *et al.* 2006).

Cell membranes are of the first receptors of stress and they can protect the cell through modifications affecting both stress perception and rigidity of the cell structure. Quantitative changes in the membrane lipids, such as unsaturation level of phospholipids and glycolipids, could affect membrane fluidity (Quartacci *et al.* 2002). Moreover, the overall stability of the membrane depends on the fine orchestration of the balance of phosphatidylcholine/phoshatidylethanolamine (PC/PE) and digalactosyldiacylglycerol/monogalactosyldiacyl-glycerol (DGDG/MGDG), the latter affecting mainly the photosynthetic apparatus. In addition to that, cell membranes are threatened from reactive oxygen species (ROS) produced *via* the metabolism of the cell, and/or as a result of stress (Vassilev *et al.* 2004, Pospíšilová *et al.* 2005, Koca *et al.* 2006). Cell membranes, after being

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attacked by ROS, produce lipid peroxides that can be used as a stress indicator.

Taken together the available data, it can be assumed that both qualitative and quantitative changes in the lipid composition may compromise the response against drought (Fazeli *et al.* 2007). In this respect, we tried to correlate the differential tolerance to drought stress of four *Vitis vinifera* cultivars with water deficit induced changes in their leaf water potential and lipid peroxidation.

Four *Vitis vinifera* L. cultivars, the Tunisian local cvs. Kahli Kerkennah and Guelb Sardouk and the imported cvs. Cardinal and Superior Seedless were cultivated in pots in a controlled greenhouse with mean day temperature 24 °C, mean relative humidity 65 %, photoperiod 16 h and irradiance 25 W m^{-2}. Vines were grown for three months under sufficient water supply, and subsequently were divided into two groups: the first was regularly watered, whereas irrigation was suspended in the second set for three weeks. Leaf water potential (Ψ_{leaf}) was periodically measured at midday between 11:00 and 13:00 in adult fully expanded leaves by a *PMS 100* pressure chamber (*PMS*, Albany, USA; Scholander *et al.* 1964). Lipid peroxidation was determined by estimating the concentration of the malondialdehyde (MDA) produced by the thiobarbituric acid reaction. MDA was determined by a colour reaction with thiobarbituric acid (Fazeli *et al.* 2007). Absorbance was measured at 532 nm and values were corrected by substracting the absorbance at 600 nm. Lipids were extracted according to the method of Folch modified by Bligh and Dyer (1959) in a solvent mixture chloroform/methanol (2:1, v/v). Afterwards, the homogenate was centrifuged at 3 000 *g* for 15 min, and the chloroformic phase (including lipids) was evaporated at 40 °C under vacuum using a rotary evaporator. The residue was immediately re-dissolved in 2 cm^3 toluene/ethanol conservation mixture (4/1, v/v). Phospholipids (PL), glycolipids (GL) and neutral lipids (NL) were separated and extracted by thin layer chromatography (TLC) on silica gel plates (*Merck,* Darmstadt, Germany) using the solvent mixture chloroform/acetone/methanol/acetic acid/water (50/20/10/10/5, v/v) (Lepage 1967). Fatty acids were analyzed according to the method of Metcalfe *et al.* (1966) with a *Hewlett-Packard 4890D* (Albany, USA) gas chromatographer equipped with a 30 cm × 0.25 mm × 0.25 μm thickness film capillary column (*HP-Innowax*; *Agilent Technologies,* Wilmington, USA) coupled to a flame ionisation detector (column temperature 210 °C). The injector and detector were maintained at 230 °C and 250 °C, respectively. Nitrogen was the carrier gas (1 cm^3 min^{-1}). One way variance analysis (*ANOVA-MANOVA*) and Duncan's test were used for the statistical analysis.

The diversity of grapevine genotypes with respect to their tolerance to water stress seems large; some cultivars exhibit high tolerance as cv. Syrah or drought avoidance as cv. Grenache (Schultz 1996). Water deficit confers loss of cell water content and concomitantly water potential and pressure potential. To exhibit the diffe-rential tolerance of the four genotypes used throughout our experiments we evaluated both Ψ_{leaf} and lipid peroxidation. Regarding control grapevines, no signifi-cant differences were observed under control conditions in Ψ_{leaf} among the four different genotypes, it remained between -0.4 and -0.9 MPa during a period of approximately three weeks (Table 1). On the contrary, water stressed grapevines displayed a gradually declining Ψ_{leaf} reaching values as low as -2 MPa (Table 1). After three weeks of withholding irrigation, of tolerant cvs. Kahli Kerkennah and Cardinal decreased to -1.6 and -1.7 MPa, respectively, Ψ_{leaf} of sensitive cvs. Superior Seedless and Guelb Sardouk decreased to -1.9 and -2 MPa respectively (Table 1). This result is in accordance with previous studies suggesting that short-term drought stress induce a smaller impact on the water potential in the resistant than in the sensitive genotypes (Grzesiak *et al.* 2006).

Lipid peroxides (MDA) accumulated faster in Superior Seedless (the most sensitive genotype) and the degree of

Table 1. Effect of water deficit on leaf water potential (Ψ_{leaf}) [-MPa], MDA content [nmol g^{-1}(f.m.)], and total lipid content [mg g^{-1}(f.m.)] of the two tolerant (Kahli Kerkennah and Cardinal) and the two sensitive (Guelb Sardouk and Superior Seedless) *Vitis vinifera* cultivars. Data are the means of four replicates ± SE. * - significant difference between control and stressed plants at *P* < 0.05 and letters represent statistical difference between stressed cultivars for the water potential values and between controls and stressed for the lipid peroxidation values at *P* < 0.05 according to Duncan's test.

Cultivar		Ψ_{leaf}			MDA			Lipids
		7 d	14 d	24 d	7 d	14 d	24 d	24 d
Kahli	control	0.56 ± 0.20	0.57 ± 0.10	0.65 ± 0.05*	2.10 ±0.02*c	2.10 ±0.25*bc	2.03 ±0.19*c	18.3 ± 1.1*
Kerkennah	stressed	0.73 ± 0.10a	0.92 ± 0.20ab	1.64 ± 0.26c	2.21 ±0.12b	2.70 ±0.17b	4.23 ±0.3a	21.7 ± 3.6
Cardinal	control	0.45 ± 0.08*	0.39 ± 0.09*	0.74 ± 0.10*	1.65 ±0.008*c	2.00 ±0.19*bc	2.50 ±0.13*bc	25.0 ± 0.5
	stressed	0.54 ± 0.17a	1.15 ± 0.30bc	1.77 ± 0.15d	1.66 ±0.12c	2.40 ±0.16ab	4.35 ±0.31a	26.6 ± 5.4
Guelb	control	0.44 ± 0.11*	0.49 ± 0.15*	0.65 ± 0.11*	1.40 ±0.1*b	1.76 ±0.1*b	2.48 ±0.16*bc	13.1 ± 1.1
Sardouk	stressed	0.73 ± 0.08a	1.19 ± 0.34b	1.90 ± 0.15c	2.73 ±0.11ab	2.40 ±0.03ab	4.80 ±0.15a	11.8 ± 0.6
Superior	control	0.53 ± 0.07*	0.53 ± 0.14*	0.57 ± 0.09*	1.38 ±0.16*d	2.02 ±0.36*c	2.70 ±0.07*b	16.4 ± 3.6*
Seedless	stressed	0.75 ± 0.15a	1.18 ± 0.26ab	2.14 ± 0.55c	2.56bc	3.20b	4.60a	12.2 ± 1.1

Table 2. Effect of water deficit on the composition of total lipids of the two tolerant (Kahli Kerkennah and Cardinal) and the two sensitive (Guelb Sardouk and Superior Seedless) *Vitis vinifera* cultivars. Means ± SD of 3 - 4 independent plant samples. Fatty acids are expressed as percentage of total amount.

Cultivar		$C_{16:0}$	$C_{16:1c}$	$C_{16:1t}$	$C_{16:3}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Kahli	control	28.2 ± 2.2	0.1 ± 0.1	1.2 ± 0.0	1.1 ± 0.7	2.8 ± 0.2	3.4 ± 0.2	14.7 ± 0.1	48.3 ± 3.1
Kerkennah	stressed	22.6 ± 1.9	0.5 ± 0.1	1.4 ± 0.5	0.7 ± 1.0	2.3 ± 0.8	2.6 ± 0.6	11.5 ± 1.4	58.1 ± 3.4
Cardinal	control	24.3 ± 0.3	0.7 ± 0.2	1.6 ± 0.3	1.2 ± 1.1	3.1 ± 1.5	4.5 ± 0.4	14.8 ± 0.8	49.5 ± 0.3
	stressed	20.6 ± 1.7	0.5 ± 0.2	1.1 ± 0.1	0.2 ± 0.0	2.8 ± 0.2	4.4 ± 0.1	16.3 ± 1.5	48.6 ± 1.1
Guelb	control	35.2 ± 2.5	0.8 ± 0.3	1.3 ± 0.7	1.4 ± 0.8	4.5 ± 0.7	4.9 ± 0.8	16.6 ± 1.5	39.1 ± 0.6
Sardouk	stressed	23.0 ± 5.1	0.8 ± 0.2	2.3 ± 0.0	0.2 ± 0.2	2.4 ± 0.5	2.3 ± 0.1	11.4 ± 2.1	57.2 ± 3.5
Superior	control	22.9 ± 2.9	0.7 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	2.9 ± 1.1	3.6 ± 0.3	18.4 ± 0.2	48.9 ± 1.2
Seedless	stressed	20.5 ± 2.7	0.6 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	3.3 ± 0.6	2.3 ± 0.1	13.9 ± 0.2	57.1 ± 1.7

Table 3. Effect of water deficit on the lipid classes of the two tolerant (Kahli Kerkennah and Cardinal) and the two sensitive (Guelb Sardouk and Superior Seedless) *Vitis vinifera* cultivars. * - significant difference between control and stressed plants at $P < 0.05$ according to Duncan's Test.

Cultivar		PC	PE	PG	DGDG	MGDG	PC/PE	DGDG/MGDG
Kahli	control	3.0*	2.5	1.4*	13.1*	9.5	1.20	0.73
Kerkennah	stressed	8.7	2.7	2.4	7.8	12.9	3.21	1.65
Cardinal	control	3.1	2.2	0.1	12.3*	5.9*	1.37	0.48
	stressed	4.4	2.0	0.2	9.8	8.2	2.20	0.84
Guelb	control	4.3	1.9	2.0	9.3	2.2*	2.25	0.24
Sardouk	stressed	4.8	2.2	2.2	8.9	4.5	2.19	0.51
Superior	control	3.6*	2.1	0.8	9.5*	3.1	1.76	0.33
Seedless	stressed	2.4	3.4	1.1	6.1	3.0	0.71	0.50

accumulation at least in the first week correlated well with the susceptibility level of each individual genotype (Table 1). The total amount of fatty acids in the leaves, exhibited a slight increase in the two tolerant cultivars Kahli Kerkennah and Cardinal, remained invariable in Guelb Sardouk and declined in Superior Seedless, but except in the cases of Kahli Kerkennah and Superior Seedless (the most tolerant and sensitive genotypes, respectively), these changes were not statistically significant (Table 2). Further analysis revealed that the main fatty acids in grapevine leaves are linolenic ($C_{18:3}$), followed by palmitic ($C_{16:0}$), linoleic ($C_{18:2}$) and at much lower amounts oleic ($C_{18:1}$), stearic ($C_{18:0}$), palmitolenic ($C_{16:3}$) and palmitoleic ($C_{16:1}$) acids (Table 3). In stressed grapevines, the unsaturation level of the lipids increased in all genotypes, essentially *via* the decrease of the palmitic acid and the concomitant accumulation of the linolenic acid.

In addition, leaf membranes contain significant amounts of phospholipids (PC, PE and PG) and glyco-lipids (DGDG and MGDG). PC increased significantly in Kahli Kerkennah and Cardinal, remained somewhat unaltered in Guelb Sardouk and decreased in Superior Seedless, while the amounts of PE did not change in both Kahli Kerkennah and Cardinal but increased in the sensitive cultivars. PG and DGDG contents did not show any alterations, while MGDG contents decreased (Table 3). All these modifications resulted to an increase in the DGDG/MGDG ratio in the four cultivars. In contrary, the modifications of the phospholipids composition were more discriminatory between the cultivars since the proportions of the PL classes were modified in the leaves. An increase in the PC/PE ratio was recorded in the tolerant cultivars Kahli and Cardinal (2.6 and 1.6 folds of the controls, respectively), but not in Superior Seedless, in which PC/PE ratio decreased to half. Fatty acid composition of each lipid class revealed that phospho-lipids were rich in palmitic acid. In opposite, the major fatty acid in the MGDG class was the linolenic acid; the composition of DGDG was slightly homogeneous between the $C_{16:1}$ and $C_{18:3}$ (data not shown). Drought induced an increment of the triunsaturated fatty acid $C_{18:3}$ in the different lipid classes, which increased in turn the unsaturation levels; glycolipids, and especially MGDG, were more unsaturated than the phospholipids, except of PG which was the least unsaturated lipid (data not shown).

The membranes alteration occurs generally as the main response of plants to stress (*e.g.* drought: Singh *et al.* 2002, Gigon *et al.* 2004; freezing: Welti *et al.* 2002; salinity: El Kahoui *et al.* 2004). In this report, experimental data revealed that lipid amount decline

occurred in the drought sensitive cultivar Superior Seedless. As reported earlier, these changes are resulting from the lipid hydrolysis *via* the activation of hydrolytic enzymes (Welti *et al.* 2002). Conversely, drought induced an increase in the total lipid content in the tolerant cultivars Kahli and Cardinal; these changes were accompanied by the augmentation of the total fatty acids unsaturation and length. What is known about the structural properties of lipids is that the choline group of the phosphatidylcholine (PC) acquires fluidity to the membrane, whereas the ethanolamine group of the phoshatidylethanolamine (PE) rigidifies the membranes. In the same way, both DGDG and PC are bilayer forming lipids, while MGDG and PE are the two major non lamellar forming lipids and tend to form the hexagonal (H_{II}) phase (Webb *et al.* 1990, Gigon *et al.* 2004). In consequence, the elevated abundance of PC rather than PE in the tolerant cultivars could help to maintain stable and less packed membranes (Zhang *et al.* 1997). In addition, the more noticeable decrease in the amounts of MGDG as well as the increasing unsaturation of this lipid in the tolerant cultivars (Kahli and Cardinal) may be one

of the cellular mechanisms that contribute in maintaining the membrane fluidity under stress (Quartacci *et al.* 1995).

Consequently, the increase of the DGDG/MGDG and PC/PE ratios are signs of stability of the membrane bilayer conformation which can protect membranes from degradation. With the increasing lipid peroxidation confirmed in the stressed leaves, the structural stability of the membranes seems to be the key factor for the protection of the leaves, especially at a pronounced stage of drought stress.

To sum up results obtained, the modifications in the lipid composition of the water stressed grapevine leaves induce numerous modifications within the membrane composition and properties, essentially fluidity and permeability, which influence the whole cellular metabolic processes and control the behaviour of the whole plant, dependently of the intrinsic capacity of each cultivar to resist stress. The differential modification of lipid composition during stress between the sensitive and the tolerant cultivars, suggests that lipid composition, indeed, could have an impact on the overall tolerance of the plant to stress.

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