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Effects of dehydration and rehydration on the polar lipid and fatty acid composition of *Ramonda* species

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Endemic Balkan species *Ramonda serbica* and *Ramonda nathaliae* (Gesneriaceae) are rare resurrection flowering plants, known to withstand repeated cycles of desiccation–rehydration in their natural habitat. Analysis of their leaf lipids and fatty acids revealed a rather small amount of total lipids (15.8 mg/g dry weight in *R. serbica* and 19.5 in *R. nathaliae*) and galactolipids, particularly the monogalactosyl-diacylglycerol, considerably poorer in linolenic acid in comparison to other flowering plants (55% for *R. nathaliae* and 64% for *R. serbica*). Severe desiccation leads to a drastic loss in total lipids (76% in *R. serbica* and 71.5% in *R. nathaliae*), especially in monogalactosyl-diacylglycerol, but the recovery is extremely rapid and thorough upon the rewetting. A shift towards the more saturated oleic and linoleic acids in galactolipids occurs at a different time and to a different degree in the two species, and it is not evenly repaired. *Ramonda serbica*, known to be somewhat less resistant, shows a wider range of lipid changes.

Key words: lipid, fatty acids, desiccation-tolerant plants, *Ramonda serbica*, *Ramonda nathaliae*.

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Deux espèces endémiques du Balkan, *Ramonda serbica* et *Ramonda nathaliae* (Gesnériacées) appartiennent au groupe très restreint des plantes à fleurs reviviscentes. Elles sont capables de supporter, dans les conditions naturelles, des cycles répétés de dessication suivie de réhydratation. L'analyse de leurs lipides foliaires révèle une teneur relativement faible en lipides (15,8 mg par g de matière sèche chez *R. serbica* et 19,5 chez *R. nathaliae*) et un pourcentage d'acide linolénique étonnamment bas des galactolipides, en particulier du monogalactosyl-diacylglycérol, comparé aux autres Angiospermes (55% chez *R. nathaliae* et 64% chez *R. serbica*). Une déshydratation sévère induit une perte importante des lipides totaux (perte de 71,5% chez *R. nathaliae* et de 76% chez *R. serbica*), surtout du monogalactosyl-diacylglycérol. Cependant à la réhydratation, la reprise est extrêmement rapide et totale. La sécheresse provoque une diminution du degré d'insaturation des acides gras des galactolipides, et à la réhydratation, la reprise est seulement partielle. *Ramonda serbica*, connue pour être moins résistante que *R. nathaliae*, présente des variations de plus grande amplitude au cours de la déshydratation.

Mots clés : lipide, acides gras, plantes reviviscentes, *Ramonda serbica*, *Ramonda nathaliae*.

Introduction

The discovery of *Ramonda serbica* Panc. (Pancic 1874) (Fig. 1) and *Ramonda nathaliae* Panc. et Petrov. (Petrovic 1885) (Fig. 2), the endemic relics of the Balkan Peninsula, was met with great interest among the botanists of the time, since those *Ramonda* species are both typical relics and at the same time, rare European representatives of the tropic-subtropic family, the Gesneriaceae. These resurrection flowering plants belong to the tertiary mountainous flora, and the glacial age may have been the period when the climatic changes forced them towards the lower regions of the Balkans, where they are found nowadays. Their survival in the present day habitat is largely dependent on drought resistance that they achieve through complete desiccation, and *Ramonda* species are known to enter this state several times during their lifetime. In the process, the plants undergo complete desiccation (Fig. 3), suspending their metabolism but maintaining the ability to resume full biological activities upon rehydration.

Water deficit could easily be expected to affect cell membranes profoundly through structural changes and possible breakdown. An efficient repair and full reconstitution of membrane integrity during rehydration would be a prerequisite to cell survival.

Polar lipid changes could be anticipated in the process, and the subject has been receiving considerable attention. Fatty acid composition of desiccation tolerant mosses was found to change during water stress as well as the activity of several enzymes involved in lipid metabolism; two different enzymatic pathways were proposed to explain tissue defence against the uncontrolled loss of unsaturated fatty acids (Dhindsa and Matowe 1981; Stewart and Bewley 1982). In higher plants, water deficit leads to a decline in polyunsaturated fatty acid content of leaves, particularly in the glycolipid fractions (Chetal et al. 1981; Pham Thi et al. 1982; Wilson et al. 1987; Monteiro de Paula et al. 1990). It also results in a profound overall drop in MGDG, the major leaf glycolipid (Ferrari-Iliou et al. 1984), owing to an increased lipolytic activity (El-Hafid et al. 1989) and to the inhibition of precursor uptake and desaturation activity (Pham Thi et al. 1985, 1987).

ABBREVIATIONS: DGDG, digalactosyl-diacylglycerol; DPG, diphosphatidylglycerol; FAC, fatty acid composition; GL, galactolipids; MGDG, monogalactosyl-diacylglycerol; NL, neutral lipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, phospholipids.

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FIG. 1. A well-hydrated plant of *Ramonda nathaliae* in its natural habitat.

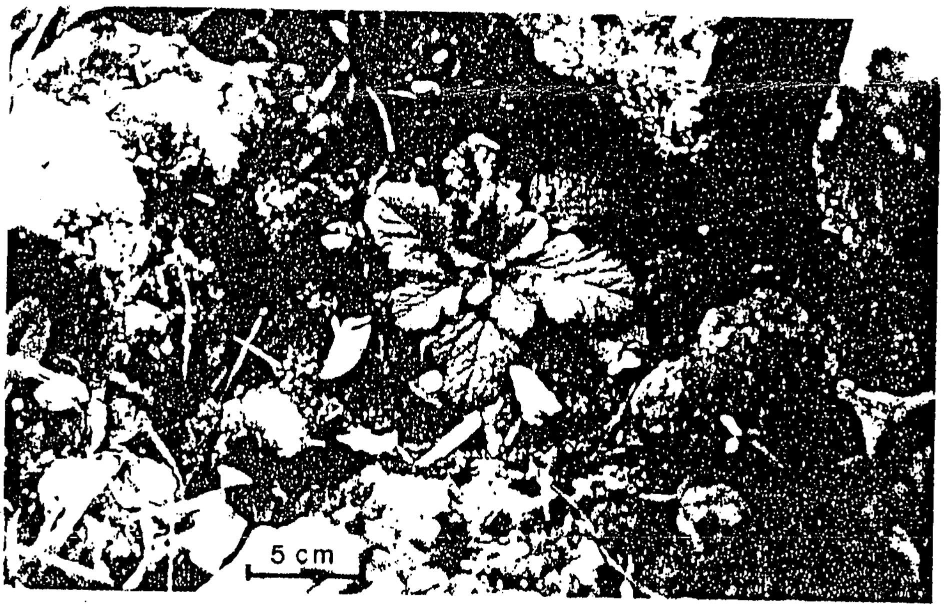

FIG. 2. A well-hydrated plant of *Ramonda serbica* in its natural habitat.

To date knowledge is lacking on the lipid content and composition of the two *Ramonda* species, and the present study was undertaken to examine them as they appear in restricted water supply conditions, as well as during desiccation and recovery.

Materials and methods

Plant material and drought treatment

Whole plants (including soil surrounding the roots) of *R. serbica* and *R. nathaliae* were collected from their natural habitat in the south eastern regions of Serbia, Yugoslavia. They were transferred to the laboratory and put into flat pots where some plants were kept well hydrated at all times (control plants) while the others were subjected to drought by withholding water. Relative humidity of the air was kept at 30%. The drought treatment lasted 7 days. The severely desiccated plants were then rehydrated by spraying distilled water on the leaves and by rewatering the soil.

Lipid and fatty acid analyses were made on the following plants: (*i*) control plants (C): water potential (ψ) = -0.5 to -0.6 MPa, WC = 80%; (*ii*) moderately stressed plants (S1, 4 days dehydration): ψ = -1.9 to -2.0 MPa, WC = 53%; (*iii*) severely stressed plants (S2, 7 days dehydration): ψ = unmeasurable (less than -6 MPa), WC = 11%; (*iv*) plants rehydrated for 2 days (R1): ψ = -0.6 to -0.7 MPa, WC = 77.5%; and (*v*) plants rehydrated for 6 days (R2): ψ = -0.5 to -0.6 MPa, WC = 82%.

Water potentials (MPa) were measured in a pressure chamber (Scholander et al. 1964). The water content (WC) is the percentage of water on a fresh weight basis and equals the fresh weight minus the dry weight divided by the fresh weight and multiplied by 100

FIG. 3. Desiccated plants of *Ramonda* in natural habitat.

Lipid analysis

The leaves were fixed in boiling water and lipids were extracted in chloroform – methanol (Allen and Good 1971). Lipids were separated by thin layer chromatography (Lepage 1967). Fatty acids from the total lipid extracts and from the lipid classes were quantified by gas – liquid chromatography, using heptadecanoic acid as internal standard (for details of the methods, see Pham Thi et al. 1985).

All results are means of three replicates ± SD.

Results

Total leaf lipid content

The polar lipid content of leaves was found to be in the range of 15 to 20 mg/g DW for both *Ramonda* species (Fig. 4), a rather low value compared with that usually found in other flowering plants (30 to 50 mg/g DW) (see for example Pham Thi et al. 1990). Progressive desiccation resulted in a steady and steep reduction, so that in severely stressed plants it was well below the half of the initial values. This profound drop showed an extremely rapid and thorough recovery upon rewatering, and in 2 days the lipid content was regained, rising even somewhat over the original values. This pattern was similar in both species.

Fatty acid composition of total leaf lipids

The analysis showed the lipids to consist of palmitic (C 16 : 0), palmitoyl (C 16 : 1), stearic (C 18 : 0), oleic (C 18 : 1), linoleic (C 18 : 2), and linolenic (C 18 : 3) acids (Table 1), as found in the majority of higher plants, particularly those who belong to the so-called 18 : 3 group (Jamieson and Reid 1971). It is noteworthy that the main component is linoleic acid, which accounted for about 50% of the total fatty acid content, whereas linolenic acid represented about 30% in contrast with other flowering plants in which linolenic acid is the most abundant fatty acid in the green leaves (see Benson 1964).

In *R. nathaliae*, desiccation led to little changes in the relative distribution of the main fatty acids, except a slight increase in C 18 : 3 percentage under moderate stress, paralleled to some extent by a decrease in C 18 : 2. In *R. serbica*, in severely stressed leaves, the C 18 : 3 percentage decreased significantly, while that of C 18 : 2 increased.

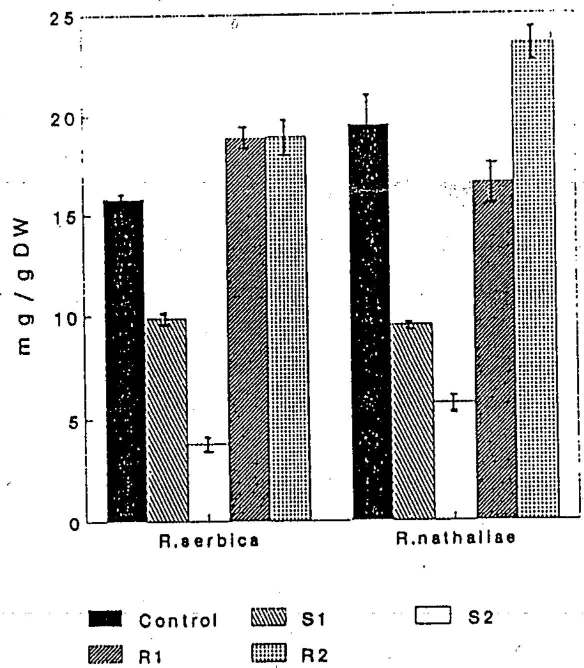

FIG. 4. Effects of dehydration and rehydration on the total lipid content of *Ramonda* leaves (in mg/g DW). Control, well-hydrated plants; S1, moderately stressed plants; S2, severely stressed plants; R1, plants rehydrated for 2 days; R2, plants rehydrated for 6 days. For more details, see Materials and methods.

Following rehydration, the relative proportion of C 18 : 3 to C 18 : 2 did not regain its normal values, even after 6 days.

Lipid composition

In Figs. 5 and 6 the main lipid classes of *Ramonda* leaves are represented in milligrams per gram leaf dry weight. In normal conditions, the galactolipid MGDG appeared as the major lipid class. In both species it was greatly reduced in severe desiccation but nearly fully recovered early during rehydration. While having roughly the same general pattern of lipid

TABLE 1. Effect of dehydration and rehydration on the fatty acid composition of total lipids in *Ramonda* leaves

Treatment*	Fatty acid (weight as % of total lipids)					
	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3
			Ramonda nathaliae			
C	11.0±0.4	2.1	1.9	2.4	50.2±2.4	32.3±2.5
S1	8.9±0.9	1.9	2.1	3.4	47.4±2.5	36.2±1.8
S2	11.2±0.5	1.2	0.9	1.7	51.2±1.9	33.6±2.0
R1	11.3±1.3	3.0	2.7	4.3	53.1±1.7	25.7±0.8
R2	12.4±0.8	2.1	1.7	4.8	49.3±1.5	29.7±1.1
			Ramonda serbica			
C	11.5±0.7	1.9	1.9	2.8	48.4±2.3	33.4±3.3
S1	11.4±1.5	3.9	2.1	4.2	46.1±3.2	32.3±2.8
S2	11.9±0.9	1.2	1.6	4.6	54.0±2.9	26.7±3.3
R1	10.8±2.1	2.1	1.2	3.1	53.6±1.0	29.2±0.9
R2	12.3±1.7	2.6	2.1	5.6	52.3±2.6	25.2±1.6

*C, control, well-hydrated plants; S1, plants submitted to moderate water stress; S2, plants submitted to severe water stress; R1, plants rehydrated for 2 days; S2, plants rehydrated for 6 days.

class distribution, the two *Ramonda* species showed certain differences during water stress.

In *R. serbica* (Fig. 5), the drop in MGDG content was extremely severe before the very rapid and nearly complete recovery upon rewetting. The same trend in changes, but to a much lesser extent, was observed in DGDG and in PC. In contrast, PE, other phospholipids (including PI, PG, and DPG), and neutral lipids (NL) were much less touched by desiccation. In fact, their relative amount rose remarkably during desiccation to triple and double that of the initial levels (23.7% of total lipids in S2 leaves instead of 7% in C for PE, 20.4% instead of 8.8% for the other phospholipids, and 21.1% instead of 12.7% for NL).

In *R. nathaliae* (Fig. 6), the drop in MGDG content under drought stress was also severe, but much less than in *R. serbica*. On the other hand, the decrease in PE and NL was more important. Consequently, in desiccated leaves, the relative distribution of lipid classes is closer to that in control leaves, suggesting a greater stability of the membranes in *R. nathaliae* than in *R. serbica*. On rehydration, the situation rapidly became normal.

Fatty acid composition of lipid classes

The fatty acid composition of the four main lipid classes (MGDG, DGDG, PC, and PE) is presented in Table 2 (*R. serbica*) and Table 3 (*R. nathaliae*).

Linolenic acid (18 : 3) was found to be the main fatty acid component in MGDG, but in a lower percentage, i.e., 55–65% compared with 80–90% in the typical flowering plants (Douce and Joyard 1980). Water stress decreased its content; it reached the lowest point during severe desiccation in *R. serbica* leaves (Table 2) and in early rehydration in *R. nathaliae* leaves (Table 3). Again, the drop was much more pronounced in *R. serbica* (from 64.4 to 29.7%), where the percentage also remained low into the rehydration period. The loss of linolenic acid during water stress was closely accompanied by the rise in linoleic acid (18 : 2) levels.

Linolenic and linoleic acids were nearly equally represented in DGDG composition. The similar shift towards the less unsaturated fatty acid was observed to a various degree in DGDG of both species during water stress.

Concerning the phospholipids PC and PE, the relative distribution of palmitic (C 16 : 0), linoleic (C 18 : 2), and linolenic (C 18 : 3) acids depends on the intensity of the stress as well as on the plant species. Thus, in *R. nathaliae*, the unsaturation of PC and PE increased under stress, owing to an increase in the ratio of C 18 : 3 to C 18 : 2, whereas in *R. serbica* it decreased. Upon rehydration, the fatty acid composition of PC and PE did not return to the control values.

Discussion

Resurrection flowering plants are rare in the existing world vegetation, and nearly all their representatives occur in the southern hemisphere (Gaff 1977, 1981). Endemic species *R. serbica* and *R. nathaliae* are in this respect all the more unusual since they occur in the northern hemisphere. Very similar in appearance, their taxonomic validity was challenged soon after their discovery (Velenovsky 1898; Vandas 1909; Doflein 1921). Soon enough, their distribution and morphological and ecological differences (Kosanin 1939), along with chromosome specificities (Glisic 1924), established them as independent sibling species. More recent investigations on the respective areas of distribution and their climatic and geographic properties (Stevanovic and Stevanovic 1985; Stevanovic et al. 1986*a*, 1987), and particularly on the ecophysiological characters and water balance of both species (Stevanovic 1986), clearly demonstrated that the two species show noticeably different preferences for habitat conditions. Although both are successful resurrection plants, the somewhat more xeromorphic *R. nathaliae* also appears to be more resistant to harsh climate. This slightly different tolerance is maintained even in rare sympatric habitats (Stevanovic et al. 1986*b*) where it is translated into recognizably specific microenvironments.

Grossly changed appearance under desiccation with evident overall plant friability is highly suggestive of potentially serious structural damage. Nevertheless, rewatering brings about a rapid and thorough recovery of full biological potential in both *Ramonda* species. Little general knowledge is available on the composition of structural lipids in resurrection flowering plants. In this regard, lipid analysis of *Ramonda* species brought to light several distinctive features.

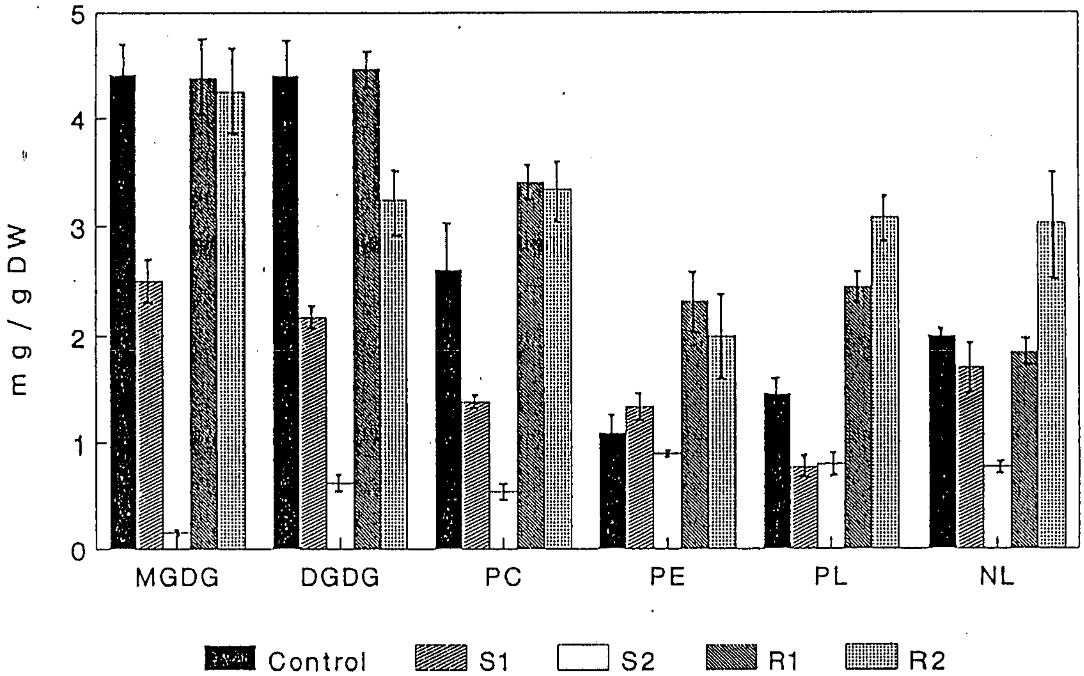

FIG. 5. Effects of dehydration and rehydration on the lipid content of *Ramonda serbica* leaves (in mg/g DW). MGDG, monogalactosyl-diacylglycerol; DGDG, digalactosyl-diacylglycerol; PC, phosphatidyl-choline; PE, phosphatidyl-ethanolamine; PL, other phospholipids; NL, neutral lipids. Other abbreviations as in Fig. 4.

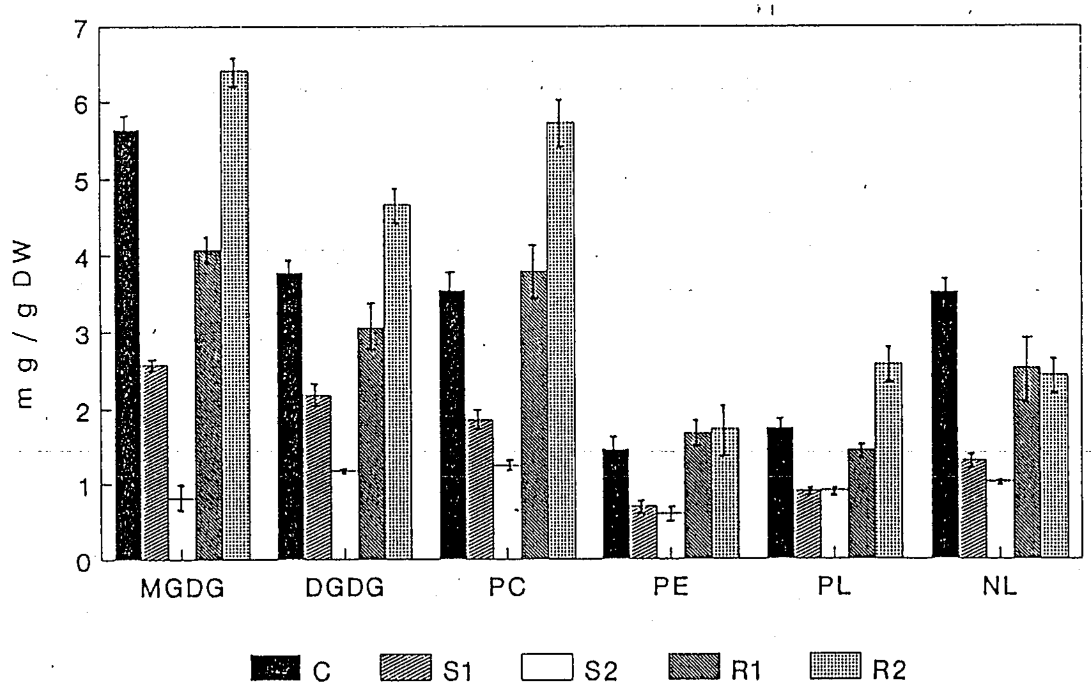

FIG. 6. Effects of dehydration and rehydration on the lipid content of *Ramonda nathaliae* leaves. Abbreviations as in Fig. 5.

The leaves of *Ramonda* species are rather low in total lipids in comparison to other flowering plants (see for example Pham Thi et al. 1990) and this suggests a low quantity of membrane material. Another unexpected feature is a peculiarly low content of linolenic acid in galactolipids, i.e., 54.7% in MGDG from *R. serbica* and 64.4% in MGDG from *R. nathaliae* instead of 90–95% in other flowering plants (see Benson 1964). Glycolipids containing high percentages of polyunsaturated fatty acids are characteristic of chloroplast membranes (Douce and Joyard 1980).

It is generally accepted that membrane fluidity, i.e., the degree of order and motion in the hydrocarbon core of the lipid bilayer (Stubbs and Smith 1984), depends on the degree of unsaturation of the fatty acids (Stubbs and Smith 1984;

TABLE 2. Effects of dehydration and rehydration on the fatty acid composition of the main lipid classes from *Ramonda serbica* leaves

Lipid and treatment*	Fatty acid (weight as % of total lipids)				
	16 : 0	18 : 0	18 : 1	18 : 2	18: 3
MGDG					
C	1.2	2.6	1.0	30.8±0.3	64.4±1.9
S1	1.4	2.4	2.0	37.4±0.5	56.8±2.1
S2	8.5	0.4	16.0	45.4±1.5	29.7±0.7
R1	1.3	1.6	2.1	48.9±1.0	46.1±0.4
R2	2.6	2.1	2.3	55.7±1.9	36.3±1.7
DGDG					
C	7.1	0.1	1.5	38.8±3.2	52.5±3.0
S1	5.9	0.6	1.1	48.1±1.1	44.2±1.4
S2	6.6	0.2	1.2	54.0±2.3	38.2±2.5
R1	3.4	1.6	0.9	53.1±0.6	41.0±1.7
R2	6.1	1.6	0.9	55.2±0.8	36.2±1.3
PC					
C	18.9±1.3	3.6	2.7	55.8±0.3	19.0±1.2
S1	16.6±1.6	4.2	4.0	61.1±1.9	14.1±0.9
S2	16.6±1.4	10.6	3.4	56.9±1.8	12.5±1.8
R1	15.9±1.3	3.7	6.3	59.1±1.3	15.0±0.4
R2	11.9±1.7	1.3	6.7	64.8±1.9	15.3±0.3
PE					
C	24.3±2.1	3.9	4.1	58.8±3.2	8.9±2.8
S1	25.6±2.5	2.0	7.8	54.9±0.8	9.7±0.2
S2	22.8±1.0	6.1	11.0	52.5±0.4	7.6±1.0
R1	25.1±3.8	4.7	7.4	53.0±3.9	9.8±1.2
R2	20.8±0.7	0.8	9.9	57.2±3.7	11.3±0.5

*See Table 1.

TABLE 3. Effects of dehydration and rehydration on the fatty acid composition of the main lipid classes from *Ramonda nathaliae* leaves

Lipid and treatment*	Fatty acid (weight as % of total lipids)				
	16 : 0	18 : 0	18 : 1	18 : 2	18: 3
MGDG					
C	1.2	1.3	1.1	41.7±0.7	54.7±0.6
S1	1.0	—	1.0	40.1±0.7	57.9±1.6
S2	2.8	—	4.2	42.0±0.8	51.0±1.7
R1	2.2	0.4	3.1	52.8±1.9	41.5±0.6
R2	1.7	1.3	4.9	40.4±1.7	51.7±1.3
DGDG					
C	4.3	3.2	2.0	46.5±0.8	44.0±0.6
S1	5.8	0.6	2.9	47.7±0.6	43.0±0.5
S2	6.8	5.3	2.3	46.2±1.3	39.4±0.3
R1	7.4	4.7	3.7	53.4±1.6	30.8±2.5
R2	5.1	0.4	1.9	50.5±0.9	42.1±1.3
PC					
C	16.6±1.2	2.5	3.1	63.9±0.8	13.9±0.9
S1	14.3±0.4	3.8	3.4	59.7±0.3	18.8±0.7
S2	14.1±0.6	8.9	5.7	52.9±2.5	18.4±0.5
R1	16.9±0.2	3.8	6.0	57.2±2.6	16.1±0.3
R2	16.4±0.8	5.7	6.7	54.5±0.7	16.7±0.5
PE					
C	20.5±1.4	2.0	2.5	66.7±0.7	8.3±0.3
S1	15.2±0.9	2.5	7.8	62.2±1.8	12.3±1.5
S2	20.4±1.7	8.6	8.2	52.9±0.9	9.9±1.6
R1	21.0±3.0	4.4	6.5	57.6±1.9	10.5±1.1
R2	12.0±0.8	6.4	4.9	66.4±2.2	10.3±2.3

*See Table 1.

Bishop et al. 1979). Concerning chloroplast membranes, the efficiency of photosynthesis seems to be largely dependent on their fluidity (Bishop et al. 1982; Ford and Barber 1983). Therefore, the net effect of the observed low linolenic acid content would be a stabilized bilayer and a decreased overall fluidity of the chloroplast membrane and hence its metabolic activity. In resistant membranes, we hypothesize that the price to pay for a greater stability is a reduced efficiency (Pham Thi et al. 1990).

Under water stress, polar lipid content of *Ramonda* leaves decreased, attesting a great loss of membrane; the galactolipid MGDG is the most susceptible to degradative processes induced by drought. A similar phenomenon has been observed in several other plants (Chetal et al. 1981; Ferrari-Iliou et al. 1984; Monteiro de Paula et al. 1990). Finally, the lipid composition of desiccated *Ramonda* leaves is profoundly modified: the ratio of PL to GL increased and the relative proportion of MGDG to DGDG drastically decreased. An increase in the phospholipids relative to galactolipids in leaves indicate a preferential degradation of the chloroplast membranes, and in some stress conditions, at dawn for example, the ratio of MGDG to DGDG decreased as well as the degree of unsaturation of galactolipids, as shown by Öquist (1982).

In vitro, pure lipids spontaneously form bilayers. However, depending on hydration and temperature, they can adopt different configurations (see Seddon 1990). It has been shown that MGDG frequently forms the HexII phase whereas DGDG adopts only lamellar phases. PE has a strong tendency to form the HexII phase, while PC does not form any nonlamellar phases (see Seddon 1990). Even if natural membranes are complex mixtures of several lipid classes, it is not doubtful that variations in their relative proportions induced by drought could influence the physical state of the membrane (Gordon-Kamm and Steponkus 1984; Pearce 1985; Norberg et al. 1990) and hence the activity of membrane proteins (Spector and Yorek 1985).

Though the reduction of *Ramonda* leaf lipids was drastic under dehydration, synthesis upon rewatering is strikingly fast. Similar rapid and extensive changes in phospholipids are observed in drought-tolerant mosses (Stewart and Bewley 1982). Investigations on mosses as well as on other resurrection angiosperms (e.g., *Xerophytica* sp.) report a great stability of the mRNA under desiccation and a rapid synthesis of polyribosomes and proteins on rehydration (Tymms and Gaff 1984; Tucker and Bewley 1976; Dhindsa and Bewley 1978).

The comparison of lipid modifications in the two species is particularly interesting. *Ramonda serbica*, known to prefer drier habitats than *R. nathaliae* (Stevanovic et al. 1987), also shows a greater stability of the membrane lipids and a better capacity to resume on rewatering, particularly regarding fatty acid composition. Clearly, the lipid analysis offers a new insight into the survival strategies of *Ramonda* sp. The observed differences between the two resistant species support previous knowledge of ecological features (Stevanovic 1986) and signal the possibility that specific adaptations might be used to ensure their respective water stress tolerance.

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