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PINITOL AND MYO-INOSITOL ACCUMULATION IN WATER-STRESSED SEEDLINGS OF MARITIME PINE

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Abstract—Seedlings of two maritime pine populations from France ('Landes') and Morocco ('Tamjoute') were subjected to water stress. Aerial parts and roots of the seedlings were analysed for D-pinitol (3-*O*-methyl-D-inositol) and myo-inositol In well-watered plants, pinitol concentration was two to three times higher in the Moroccan plants than in the French plants Pinitol accumulated to the same relative degree in water-stressed seedlings of the two populations. Myo-inositol concentration seemed only to increase in roots of the Moroccan plants The physiological function of pinitol is discussed

INTRODUCTION

One possible adaptative response of plants with respect to drought is the accumulation of intracellular solutes. The identification of such components and their increase in water-limiting conditions could be of potential use in selection of drought adapted varieties These solutes are found to vary according to species, but consist of two groups.inorganic ions (K^+, Cl^-, Na^+, SO_4^{2-} . . .) and organic components (malate, proline, betaine, polyols .) [1, 2]. But accumulation with water stress is not sufficient to prove the adaptative value of the solute The increase could be the result of metabolic perturbations or accumulation of assimilates because of slower growth [3, 4]. Thus selection for varieties of cereals rich in proline has produced ambiguous results [5, 6].

During the last 10 years, some authors, e.g. Wyn Jones *et al.* [7], have emphasized the importance of intracellular localisation of solutes. Interest has focused on the cytoplasmic compartment in which perturbations have a direct effect on the metabolic function of the plant Borowitzka *et al.* [8] used the term 'compatible solutes' to describe the organic, non-toxic components that accumulate in the cytoplasm in response to low water potentials. Polyols seem to be suitable as compatible solutes according to Sacher and Staples [9] since they have no carboxyl or amine group that can interfere with other cellular components Ahmad *et al* [10] found that sorbitol had no effect on the activity of some cytoplasmic enzymes extracted from a halophyte *Plantago maritima* However there is no evidence in the literature as to intracellular localization.

This report describes the effect of water stress on the levels of pinitol and myo-inositol in seedlings of maritime pine. Comparison is made between two populations: 'Landes' (France) drought sensitive, and 'Tamjoute' (Marocco), adapted to drought [11-13]. In a previous study [14], measurement of water and osmotic potentials showed the occurrence of an osmotic adjustment in roots of water-stressed seedlings of maritime pine. The osmoregulation seemed to be more accentuated in the Moroccan plants.

D-Pinitol is one of the most abundant cyclitols in plants [15] and is a major component in the Pinaceae, Leguminosae and Caryophyllaceae. Its biosynthetic pathway has been elucidated in gymnosperms and leguminous plants [16] and includes myo-inositol as a precursor. The physiological role of pinitol is as yet unknown, but seems to be related to adaptation of plants to drastic osmotic conditions: pinitol is one of the most important constituents in numerous species of mangroves [17], and large amounts have been reported in leaves of the desert shrub jojoba (*Simmondsia chinensis*) [18]; recent work has provided evidence of its accumulation as a response to water deficit [19, 20], or after application of a salt stress [21, 22] Gorham [22] suggested that the accumulation was not the result of metabolic perturbations because no simultaneous increase in proline, apparently an indicator of such injuries, has been noted. Ford [20] showed that pinitol level in water-stressed leaves of *Cajanus cajan* did not continue to rise with more severe water deficits

RESULTS

Effect of water deficit on pinitol and meso-inositol content

Pinitol. The range of pinitol concentrations in well-watered seedlings (Fig. 1, for ψ msec = -0.03 MPa) was from 0.5 to 2 mg/g dry weight for the cotyledons and primary needles, and twice to three times lower for the hypocotyls and roots. Comparing the two populations, whatever the part of the plant considered, the values were two to three times higher for the seedlings from Tamjoute than for the seedlings from Landes.

In the cotyledons primary needles and roots of the plants subjected to the water-deficit treatments, pinitol increased with the same relative rate for the two populations of maritime pine + 100% in the aerial parts and

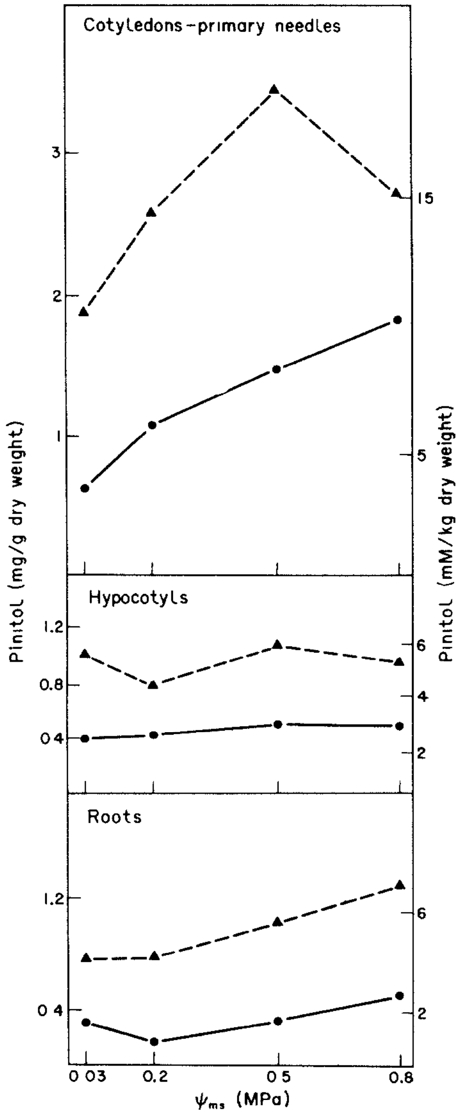

Fig 1 Effect of water stress on D-pinitol concentrations in different parts of three-weeks old seedlings of the two provenances of maritime pine (mg/g dry wt and mM/kg dry wt) Provenances Landes (● — ●), Tamjoute (▲ --- ▲) ψ_{ms} water-potential of the mineral solution

+ 20 to 40% in the roots for the treatment of ψms = −0 5 MPa (Fig. 1)

Myo-inositol. Higher values were found for this compound compare to pinitol especially in the upper part of the seedling (Fig. 2) The French plants were richer than the Moroccan plants for the cotyledons primary needles, but poorer for the roots With increasing water deficits, an accumulation of myo-inositol occurred only in roots of the population 'Tamjoute'

In Figs 1 and 2, the evolution of the two compounds was also expressed in mM/kg dry weight The values agree with the view that myoinositol is the precursor of pinitol except for roots of the Moroccan provenance, an increase in pinitol is related to an equivalent decrease in myo-inositol

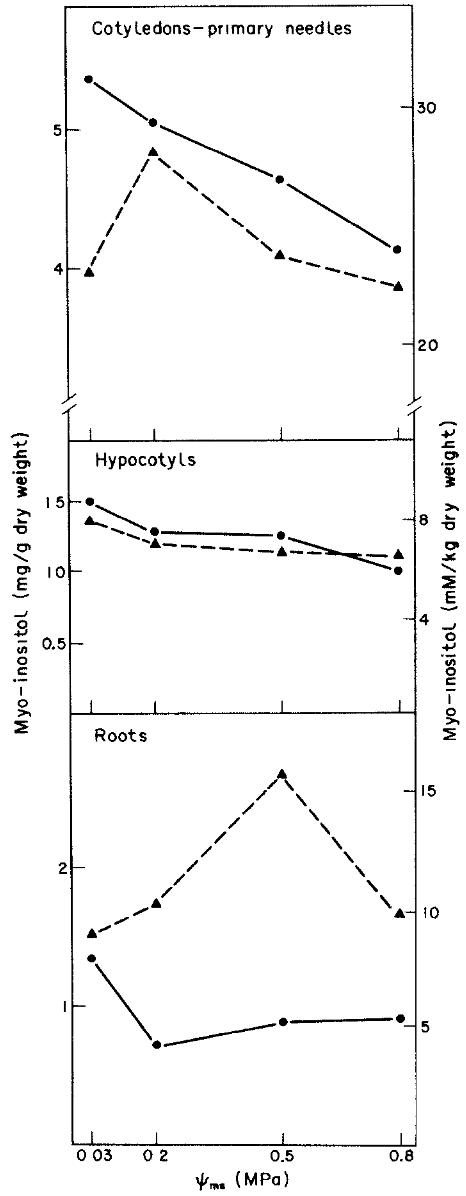

Fig 2 Effect of water stress on myo-inositol concentrations in different parts of three-weeks old seedlings of the two provenances of maritime pine (mg/g dry wt and mM/kg dry wt) Provenances Landes (● — ●), Tamjoute (▲ --- ▲) ψ_{ms} water-potential of the mineral solution

To estimate the osmoregulatory role of pinitol, the data were also related to water content of tissue (mM/l, Table 1) The osmotic contribution appeared to be low on a whole cell basis However it becomes significant if a cytoplasmic localization of the polyol is assumed (Table 1) For the calculation, the cytoplasmic volume is considered to represent 5% of total water content of tissue [23, 24] Previous cytological observations on roots [25] showed no cell plasmolysis until an applied water stress of −0.5 MPa for more severe stress, peripheral cells began to be damaged, but no effect on root growth was noted

Concentrations of pinitol and myo-inositol in the apex (3–4 mm length) and rest of the roots (*Table* 2).

With older plants (6-week-old), pinitol concentrations in roots were lower. Nevertheless, the relative variations of the two polyols with water stress in the upper part of roots were found to be the same as in younger seedlings, except there was no increase in myo-inositol in roots of the population 'Tamjoute'.

Pinitol level was lower in the apex than in the rest of the roots while myo-inositol level was maintained. In the water-stressed root tips, the two polyols increased substantially, particularly in the Moroccan seedlings.

DISCUSSION

Pinitol concentrations in maritime pine seedlings were 10 to 20 times lower than those reported in literature for other species (Table 3). However the ratio of aerial-to-root contents was the same (*ca* 2 to 3). In this paper, young seedlings were investigated: samples from older trees would probably give higher values. Myo-inositol in maritime pine reached the same level as in *Honkenya peploides* [21] and in 10 tropical legumes [20] (Table 3)

Table 1. Variation in pinitol concentrations (mM/l) in seedlings of the two provenances of maritime pine submitted to different water deficit treatments

Water deficit treatment (ψ ms in MPa)		−0.03	−0.2	−0.5	−0.8
Pinitol concentration in mM/l					
Cotyledons-primary needles					
Landes	(1)	0.60	1.06	1.64	2.1
		12.0	21.2	32.8	42
Tamjoute	(1)	1.77	2.63	3.88	3.16
	(2)	35.0	52.6	77.6	63.2
Roots					
Landes	(1)	0.14	0.10	0.27	0.45
	(2)	2.8	2.0	5.4	9.0
Tamjoute	(1)	0.36	0.45	0.73	1.05
	(2)	7.2	9.0	14.6	21

(1) Pinitol concentration on a total water content basis of tissue.

(2) Pinitol concentration in cytoplasm assuming that (a) pinitol is only localized in the cytoplasm of cells and (b) cytoplasm volume = 5% total water content of tissue.

Table 2 Pinitol and myo-inositol concentrations (mM/l water content of tissue) in roots of well-watered and water-stressed six-week-old seedlings of the two provenances of maritime pine

	Pinitol (mM/l)				Myo-inositol (mM/l)			
Water deficit -treatment (MPa)	Landes −0.03	Landes −0.5	Tamjoute −0.03	Tamjoute −0.5	Landes −0.03	Landes −0.5	Tamjoute −0.03	Tamjoute −0.5
Remainder (upper) part of roots	0.03	0.06	0.06	0.13	0.25	0.34	0.27	0.30
Root apex	(ε)	0.03	0.01	0.05	0.26	1.8	0.32	4.54

Separated measures were made in apex and remainder part of roots.

Table 3. Comparative values of pinitol and myo-inositol concentrations in different species

References	Species and plant organs		Pinitol mg/g dry weight	Myo-inositol mg/g dry weight
[28]	Different species of alfalfa and clover	leaf	15–30	
	Four species of clover.	leaf	15–30	
		shoot	20–30	
		root	3–5	
[30]	*Trifolium repens*	leaf	25	
	(white clover)	root	10	
	Glycine max	leaf	27	
[11]	*Macroptilium atropurpureum*	leaf	10	
[27]	*Pinus sylvestris*	needles	30	
[14]	10 species of tropical legumes	leaf	2–20	2–6
[12]	*Honkenya peploides*	leaf	14	1.8
Present work	*Pinus pinaster*		1–2	4–5

The better drought-adapted plants from Tamjoute were richer in pinitol, and the degree of relative accumulation of this polyol under water deficit was the same for the two populations Early studies [14] indicated a greater ability of water uptake in drying soil for the Moroccan seedlings The higher level of pinitol could be related to this behaviour. As a myo-inositol increase was also noted in roots, the contribution of the second polyol could also be considered Recent work of Sacher and Staples [9] established a relationship between, myo-inositol increase in leaves and roots of tomato plants submitted to sodium chloride solutions and the genotype adaptation to salt stress

Considering the absolute values of concentrations observed, the osmoregulatory role of the two polyols seems to be only significant if a cytoplasmic localization is assumed. In this case, higher concentrations in root tips could be expected (tissue with a greater percentage of cells without vacuoles) this was only found for myo-inositol in water-stressed roots. Such a comparison is however made between meristematic and differentiated cells. Other solutes can be specific to the growing tissues in another study (unpublished results), the importance of potassium for the osmotic adjustment in apical parts of the seedling has become apparent

The precise role of pinitol remains questionable. We considered the possibility of an intracellular localization of pinitol Other authors suggested that polyols accumulated in conducted vessels. Smith and Milburn [26] found that sucrose contributed to osmotic adjustment in the phloem of *Ricinus communis*, in which it is negatively correlated to potassium concentration The stabilization of membrane structures in dry condition by polyols is also assumed [27] These compounds could replace water molecules thanks to their alcohol functions Ford [19] showed for a tropical legume (*Macroptilium atropurpureum*), that the osmotic adjustment occurred because of a change in tissue hydration and not solute accumulation· it is interesting to note that among the chemical constituents investigated, only an increase in pinitol was observed

This physical property is also related to another stress: frost hardiness According to Bieleski [28], an 'hydrated' protein with a polyol could be more tolerant Bieleski mentioned this cryoprotector characteristic to explain seasonal variations of sorbitol in xylem sap [29, 30] and tissues of apple trees [31, 32] sorbitol was more concentrated during winter than during summer, and concentrations increased after hardening treatments Pinitol exhibits similar seasonal fluctuations in needles and bark of gymnosperms [33, 34]. Diamantoglou [33] suggested that polyols serve as winter reserve compound, but Smith and Phillips [35] found a decrease in quantities of glucose, fructose and sucrose in leaves and roots of white clover (*Trifolium repens*) and soya bean (*Glycine max*) placed in darkness, and the return to high initial values after six hr of light, while pinitol concentration was sustained

The present report is one of the first to consider the intraspecific level in the comparison between pinitol accumulation and capacity for drought resistance. For the two populations considered, the level of pinitol concentration in aerial parts is related to the degree of drought adaptation Some further experiments (unpublished data) in seedlings of three other populations seem to confirm this result

EXPERIMENTAL

Plant material Seeds of *Pinus pinaster* (Ait) originating from Landes and Tamjoute were sterilized (30% H_2O_2, 10 to 15 min), before and after a 24 hr water imbibition They were sown on moistened filter paper, and a fungicide (benlate solution, 0 6 g/l) was sprayed every two days As soon as the root reached 1 5 cm length seedlings were grown on aerated mineral solution of Seillac [36] The solution was changed once a week The intensity of light was 30 watt/m^2, with a photoperiod of 14 hr day and 10 hr night, a relative humidity of 45%, 70% and a temperature of 24°, 22° At three-or six-weeks-old, plants were selected for uniformity and submitted to the different treatments Analysis were made on samples of 15, 25, 40 plants (Figs 1 and 2), or 50 plants (Table 2) Data in Figs 1 and 2 are means of two replicates Results are expressed on a dry weight, or a tissue water content basis

Water deficit treatments Water deficit was applied only to roots by inducing an osmotic potential decrease in the mineral solution. Whatever the severity of the applied water deficit, aerial parts of the plants were kept in the constant culture conditions of light, humidity and temperature as indicated above The osmoticum we used was PEG-600 (polyethylene glycol with m_r of 600), after purification on ion exchange resins (Dowex 1 and Dowex 50 W) The PEG-600 was added stepwise to the nutrient solution (−0 1 MPa/12 hr or 24 hr) The different final degrees of water stress were (in MPa) −0 03 (mineral solution without P E G), −0 2, −0 5, −0 8 The plants in different treatments were harvested at the same time and not less than 12 hr after the most severe treatment had reached its final value

Analytical procedures Plant tissues were frozen and freeze-dried before preparation for GC analysis Samples (100 to 800 mg dry weight) were extracted with meOH–$CHCl_3$–H_2O (1 1 1) (21) H_2O–MeOH extracts were then concd, lyophilized and submitted to an oximation in pyridine with hydroxylammonium chloride (6 mg NH_2OH–HCl for *ca* 10 mg sugar) Oximation of sugars increased peak retention time so that they did not interfere with pinitol estimation Carbohydrates were determined as the trimethylsilyl esters (100 µl hexamethyldisilazane and 100 µl trimethylchlorosilane for *ca* 10 mg sugar) The TMSi derivatives were separated in a CP Sil 5 column (25 mm × 0 23 mm) (Chromatograph Intersmat IGC 120 SL) Detector and injector temperatures were 270° and 230° respectively Column oven temp was initially 140° programmed to rise at 2°/min to 270° Carrier gas was N_2 at 16 cm/sec Ribitol was used as the internal standard for quantification (peaks areas integration was performed with an integrator Intersmat ICR-1B) Pinitol and myo-inositol were identified by comparing relative retention times with ribitol, and the identification was occasionally verified by co-chromatography with pure substances, and by mass spectrometry

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