PERGAMON

Phytochemistry 51 (1999) 499–505

PHYTOCHEMISTRY

Galactinol in the leaves of the resurrection plant *Boea hygroscopica*

Franca Marinone Albini[a,*], Carla Murelli[a], Paola Vita Finzi[a], Monica Ferrarotti[a], Barbara Cantoni[a], Serenella Puliga[b], Concetta Vazzana[b]

[a]*Dipartimento di Chimica Organica, Viale Taramelli 10, 27100 Pavia, Italy*
[b]*Dipartimento di Scienze Agronomiche e Gestione del Territorio Agro-Forestale, P. le Cascine 18, 50144 Firenze, Italy*

Received 19 August 1998; received in revised form 5 January 1999; accepted 12 January 1999

Abstract

The content of low molecular weight substances was analysed in leaf samples of the resurrection plant *Boea hygroscopica* F. Muell. submitted to dehydration. Drying treatment caused a variation in the carbohydrate pool, with a decrease of all sugars except sucrose which notably increased, becoming the prevalent one in dried leaves. Rehydration almost restored the pre-treatment sugar composition. Along with more common sugars galactinol and some higher oligosaccharides of the raffinose family were detected. Their structures were assigned by NMR and GC–MS analyses after acetylation. To our knowledge, this is the first finding in resurrection plants of significant amounts of the galactosyl donor galactinol and of higher galactosyl oligosaccharides, which may have a role in restoring the pre-drying functions upon rehydration.

Keywords: *Boea hygroscopica*; Gesneriaceae; Resurrection plants; Drought tolerance; Sugar analysis; Galactinol; Raffinose family oligosaccharides

1. Introduction

Resurrection plants are a unique example among angiosperms for their ability to survive during drought, when protoplastic desiccation can leave less than 2% relative water content in the leaves (Ingram, & Bartels, 1996 and references therein cited). The resurrection plants are considered an intriguing model for drought tolerance studies, carried on through integrated research strategies combining genetic, biochemical and molecular techniques (Gaff, 1997; Oliver, & Bewley, 1997). In previous studies on the resurrection plants *Craterostigma plantagineum* Hochst (Bianchi, Gamba, Murelli, Salamini, & Bartels, 1991), *Myrothamnus flabellifolia* Welw. (Bianchi et al., 1993), *Sporobolus stapfianus* Gandoger (Marinone Albini et al., 1994; Vazzana et al., 1994; Murelli et al., 1996), we reported the low-molecular weight substance content in the extracts of fresh, dried and rehydrated leaves, showing that the sugar variation due to the dehydration was quite significant. In all cases monosaccharides were the prevailing sugars in fresh or rehydrated leaves, whereas sucrose accumulated during desiccation, according to a trend already reported (Kaiser, Gaff, & Outlow, 1985). *Boea hygroscopica* F. Muell is a resurrection plant indigenous to Australia, belonging to the family of Gesneriaceae (e.g. African violets). For this plant it has been demonstrated that the moisture content of the dried leaf is critical to desiccation tolerance in detached leaves (Bochicchio et al., 1998). Our preliminary results on *Boea* (Bianchi, Murelli, Bochicchio, & Vazzana, 1991) were related to the metabolite variation in detached leaves submitted to dehydration and rehydration. In the present report we describe the content of aqueous extracts of leaves of *Boea* submitted as whole plants to the same treatments. Since no information is known about the com-

* Corresponding author.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00031-X

Table 1
Composition of soluble carbohydrates (mg g^{-1} lyophilized weight and percentage) in aqueous extracts of *Boea* leaves excised from fresh, dehydrated and rehydrated plants. $^{*}P < 0.05$, $^{**}P < 0.01$

Components	T_0		T_2		T_3		T_{10}		T_{11}		T_{17}		T_{25}		T_{res}	
	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%
Fructose	14.3±2.1	7.0±0.07	20.1±0.14	8.8±0.01**	10.5±0.71	5.3±0.35*	17.5±5.4	8.6±1.9	19.3±0.78	9.1±1.3	11.3±1.7	4.5±0.69	6.1±0.78*	1.9±0.07**	28.7±6.5	10.5±0.71*
Glucose	18.5±0.99	9.2±1.8	24.5±6.1	10.8±2.8	16.6±0.07	8.3±0.07	28.7±7.6	14.2±2.5	42.8±0.21**	20.3±3.7	16.4±1.7	6.5±0.7	8.8±3.1*	3.0±1.5*	34.1±7.4	12.5±0.71
Alditols	3.8±0.84	1.8±0.16	2.11±0.13	0.93±0.06*	1.3±0.42	0.65±0.21*	tr	tr	tr	tr	tr	tr			tr	tr
myo-Inositol	4.6±0.28	2.3±0.45	4.23±0.25	1.87±0.10	1.3±0.43*	0.65±0.21*	1.05±0.2*	0.45±0.10*	tr	tr	tr	tr			tr	tr
Sucrose	27.6±5.0	13.4±0.71	32.7±0.49	14.3±0.35	22.5±2.3	11.2±1.1	76.7±12.2*	38.2±2.6**	93.6±25.4*	43.3±4.2**	162.7±6.4**	64.5±2.3**	280.3±51.3*	91.0±3.1**	116.2±28.3*	43.3±4.2**
Cellobiose	1.9±0.40	0.92±0.08	tr	tr	0.8±0.01	0.4±0.01	tr	tr	tr	tr	tr	tr			tr	tr
Gentiobiose	1.9±0.42	0.91±0.07	2.02±0.01	0.89±0.01	0.8±0.12	0.4±0.07	2±0.14	1.0±0.01	tr	tr	tr	tr			tr	tr
Galactinol	43±2.1	21.1±1.8	75.8±13.0	33.1±5.4	56.8±2.1*	28.3±0.99*	26.2±6.9	13.3±4.6	8.4±1.3**	4.03±1.3**	6.5±2.1**	2.6±0.85**	3.2±0.21**	1.03±0.08**	27.2±4.5*	10.0±0.01*
Raffinose	37.5±4.3	18.1±2.4	28.8±6.4	12.6±2.8	31.2±2.9	15.6±1.5	22.3±1.4*	11.2±1.7*	12.7±0.46*	6.04±1.3*	12.6±0.1*	5.0±0.02*	7.4±1.1**	2.47±0.75*	31.1±3.2	11.5±0.71
Melezitose	3±2.0	1.41±0.78	2.02±0.01	0.89±0.01	tr	tr	tr	tr	tr	tr	tr	tr			tr	tr
Maltotriose	1.9±0.40	0.91±0.07	tr	tr	1±0.01	0.5±0.01	tr	tr	tr	tr	tr	tr			tr	tr
Stachyose	20.5±2.1	10.0±0.28	21±1.4	9.2±0.57	21.7±1.6	10.8±0.85	11.5±1.1*	5.8±1.0*	17.7±6.5	8.2±1.6	22.7±3.7	9.0±1.4	tr	tr	19.9±2.4	7.5±2.1
Pentasaccharide	20.5±0.71	10.1±1.7	11±1.4*	4.8±0.57	34.4±2.9*	17.1±1.4*	13.5±0.92	6.8±1.1	19.9±7.0	9.2±1.6	13.9±1.8*	5.5±0.71	tr	tr	12.1±0.07**	4.5±0.71*
Hexasaccharide	6.1±4.0	2.8±1.6	4.5±0.71	1.97±0.33	2±0.57	1±0.28	tr	tr	tr	tr	6.3±1.7	2.5±0.71			2.4±0.1	1.0±0.01
Total	204.9±27.4		228.7±2.0		200.7±0.07		200.25±18.2		214.2±37.8		252.3±1.13		307.2±50.0		271.5±44.1	

position of low molecular weight substances in samples at different desiccation times, we examined a closer timing of sampling for the new experiment, in order to correlate in a more accurate way the leaf content with the RWC variation.

2. Results and discussion

Fresh leaves of *B. hygroscopica* were excised from well-watered plants, grown in a glass-house. Some whole plants were then dehydrated for 2, 3, 10, 11, 17 and 25 days and some of the 25 days dried plants were rehydrated for 4 days. During the experiment, the leaves were detached from the stressed plants at each sampling date. Chloroform treatment and methanol–water extraction of all leaf samples, following the described procedure (Marinone Albini et al., 1994), gave epicuticular waxes and aqueous and organic extracts as separated fractions. Table 1 shows the content of the sugars and related compounds occurring in aqueous extracts, analysed by GC as trimethylsilyl derivatives. Known compounds were identified both by coinjection with the corresponding authentic samples and by GC–MS comparison. The reported data appeared qualitatively and quantitatively different from those previously reported for some desiccation-tolerant and -sensitive angiosperms (Kaiser et al., 1985; Bianchi, Murelli, Bochicchio, & Vazzana, 1991; Bianchi et al., 1991; Bianchi et al., 1993; Marinone Albini et al., 1994; Vazzana et al., 1994; Murelli et al., 1996; Ghasempour, Gaff, Williams, & Gianello, 1998). In particular, besides fructose, glucose, sucrose and raffinose usually occurring in plant tissues, we observed in *Boea* leaves the intriguing presence at the same time of fair amounts of galactinol and of some higher oligosaccharides of the raffinose family, unprecedented for the resurrection plants.

Galactinol, firstly isolated in sugar beets (Brown, & Serro, 1953), was detected later on in 16 families including species of the Bignoniaceae, Buddlejaceae, Ericaceae, Labiatae and Onagraceae (Senser, & Kandler, 1967a). Galactinol is of common occurrence in plants that also contain the raffinose family oligosaccharides (review by Obendorf, 1997), being not a reserve substance but an intermediate in their biosynthesis, as suggested by kinetic experiments of ^{14}C labeling of galactinol, raffinose and stachyose in leaves of *Lamium maculatum* (Senser, & Kandler, 1967b). An already reported (Mayer, & Schmidt, 1997) biosynthetic pathway of formation of higher sucrosyl oligosaccharides is shown in Fig. 1. Galactinol, known as galactosyl donor, provides the new α-D-galactosyl group that couples with the C-6 hydroxyl of the non-reducing α-D-galactose moiety of the minor homologue

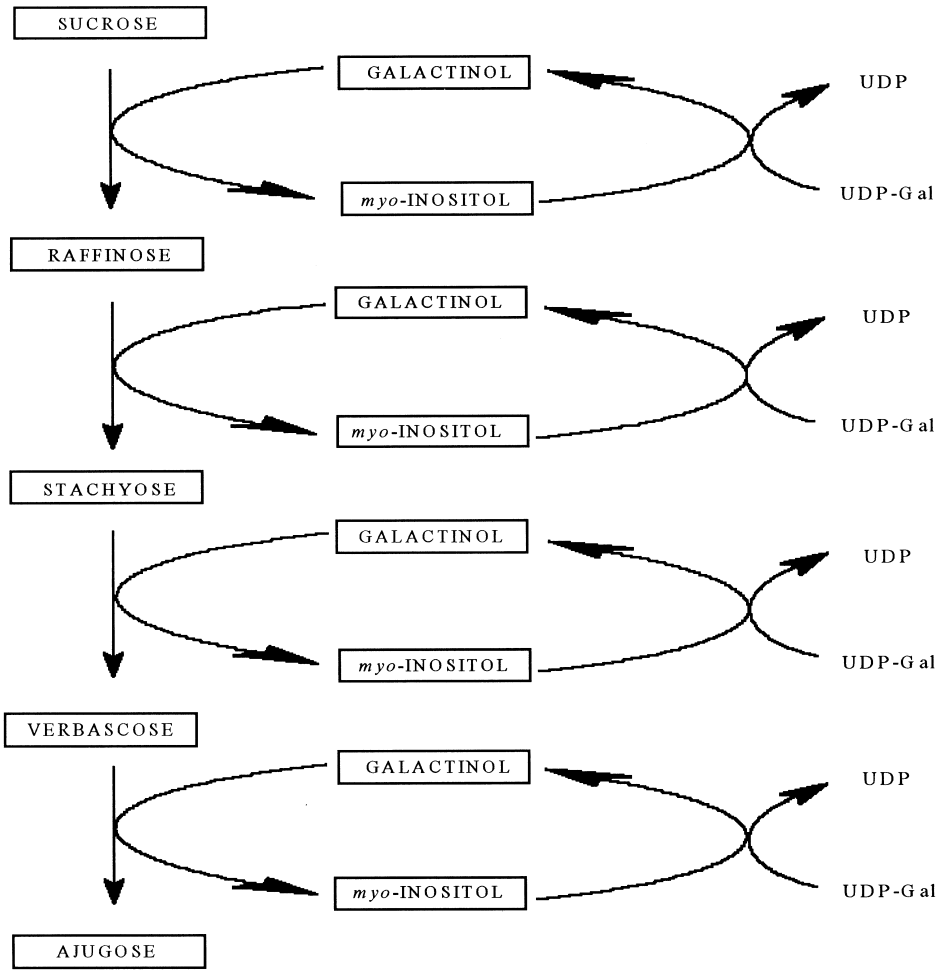
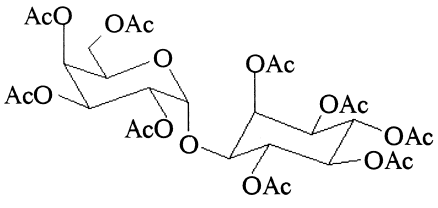
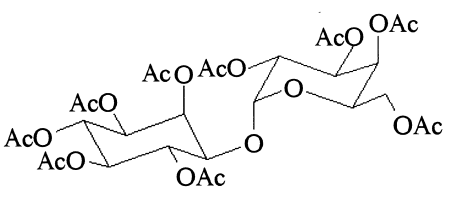


Fig. 1. The role of galactinol in the formation of higher raffinose family oligosaccharides.

in the series. Indirect evidence for the site of stachyose biosynthesis was obtained by determining the occurrence and distribution of stachyose, raffinose and galactinol in *Cucumis melo* cv Ranjadew (Holthaus, & Schmitz, 1991). Studies on enzyme activities for the synthesis of these sugars and their distribution in different plant organs led to the conclusion that stachyose was synthesized mainly in mature leaves. We also detected significant amounts of galactinol, along with raffinose and stachyose, in *C. melo* mature leaves (Ferrarotti, 1996). Fair amounts of raffinose were reported in fresh, desiccated and rehydrated leaves of the resurrection plant *S. stapfianus* (Marinone Albini et al., 1994). Raffinose and smaller amount of the galactosyl donor galactinol were identified in two European genera of the resurrection plants *Ramonda* and *Haberlea* (Müller, Sprenger, Bortlik, Boller, & Wiemken, 1997).



1



2

The structure of galactinol has been assigned on spectroscopic basis to the compound isolated from the crude acetylation mixture of aqueous extracts of both *C. melo* (Ferrarotti, 1996) and *Boea* leaves (see Section 3). The ^{1}H-NMR data quite agree with the structure of 2,3,4,5,6-penta-*O*-acetyl-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1-1)-L-*myo*-inositol **1**. The spectrum of the peracetylated sample (Table 2) showed the presence of a doublet at δ 5.15, due to the α-anomeric proton ($J_{1',2'}=3.5$ Hz). Galactose H-4′ was a typical close double doublet at δ 5.45 ($J_{3',4'}=3$ Hz; $J_{4',5'}=1$ Hz). The unique equatorial *myo*-inositol proton H-2 appeared as a deshielded triplet at δ 5.62 ($J=2.5$ Hz), coupled with H-1, dd at δ 3.91 and H-3, dd at δ 4.97. All the other protons showed *trans* diaxial couplings ($J=10$ Hz). ^{1}H-NMR resonances were assigned by standard methods that rely on correlation through chemical bonds (COSY). To our knowledge, no spectral data were available in literature for the peracetylated galactinol. Mayer and Schmidt (1997) describe the ^{1}H-NMR spectrum of the acetylated α-(1-1)-connected D-*myo*-inositol diastereoisomer of **1**, which is called isogalactinol (**2**), along with the ^{1}H- and ^{13}C-NMR spectra of the OH-unsubstituted galactinol. The ^{13}C-NMR spectrum of galactinol had been already reported (Schweizer, & Horman, 1981). To get availability of galactinol in more consistent amounts, we carried out an independent synthesis, so obtaining both the peracetylated galactinol and isogalactinol, along with the corresponding β-anomers (Marinone Albini, Murelli, & Piccinini, 1998). ^{1}H-NMR spectral data of the two α-linked galactopyranosyl-*myo*-inositols were in one case fully consistent with those reported for isogalactinol (Mayer, & Schmidt, 1997) and in the other case superimposable with the data of the natural galactinol. The oligosaccharides reported in Table 1 were identified as penta- and hexasaccharide on the basis of their GC retention time, by comparison with those of maltopentaose and maltohexaose samples (Fluka). The structure of the pentasaccharide, present in all leaf samples except that of 25 days dried leaves, was determined according to GC–MS data. Its fragmentation patterns as persilylated derivative closely corresponded to those already reported for the minor homologues stachyose and raffinose. The most relevant ions are reported in Section 3. The peaks at *m/z* 451 and 437 present in the mass spectra of all the tri-, tetra- and pentasaccharide are particularly significant. The ion at *m/z* 451 can be related to the fragment of the terminal pyranosydic galactose (Radford, & DeJongh, 1972), as well as the deriving ion at *m/z* 361, due to the loss of silanol from C-6. As previously reported (Curtius, Völlmin, & Müller, 1968; Radford, & DeJongh, 1972), the ion at *m/z* 437 is diagnostic for the presence of a fructofuranoside moiety. This ion can derive from the molecular ion (*m/z* 540) after loss of CH_2OTMSi in the case of the isolated fructose, as well as from the fragment of the anomerically uncoupled fructose (*m/z* 467) after loss of CH_2O and TMSi migration in a more complex molecule. The ion *m/z* 437 is always present in the mass spectra of the sucrose and of the raffinose family oligosaccharides. Thus, we suggest that the fructose containing pentasaccharide can be identified as verbascose. In persilylated sugars the peaks at *m/z* 204 and 217 were related respectively to pyranosidic and furanosidic ring structures (Radford, & DeJongh, 1972). The presence of both the peaks, already observed in the MS spectra of sucrosyl oligosaccharides, provides a further justification for this structure assignment to the pentasaccharide[1]. Because of the limits of the mass spectrometer used, which is equipped with a GC apparatus, we could not analyse the persilylated hexasaccharide, with a higher molecular weight, in the same way of the verbascose. We decided to determine through DCI (desorption or direct chemical ionization) the molecular ions of acetylated galactosyl oligosaccharides, using ammonia as reagent gas. The acetylation is a sugar derivatization alternative to the silylation, that provides more stable compounds. Since we could not obtain all the sugars as pure compounds (see Section 3), we analysed a crude mixture of peracetylated oligosaccharides, obtaining the following molecular ions (M^+):

Hexasaccharide	$C_{76}H_{102}O_{51}$	1830.5
Pentasaccharide	$C_{64}H_{86}O_{43}$	1542.5
Stachyose	$C_{52}H_{70}O_{35}$	1254.4
Raffinose	$C_{40}H_{54}O_{27}$	966.3

Table 2
^{1}H-NMR data of peracetylated galactinol, **1**. The chemical shifts are in δ values (ppm) from TMS. *J* (Hz): 1′,2′ = 3.5; 2′,3′ = 11; 3′,4′ = 3; 4′,5′ = 1; 1,2 = 2.5; 1,6 = 10; 2,3 = 2.5; 3,4 = 10; 4,5 = 10; 5,6 = 10

Galactose protons	δ_H	*myo*-Inositol protons	δ_H
H-1′	5.15 d	H-1	3.91 dd
H-2′	5.23 dd	H-2	5.62 t
H-3′	5.11 dd	H-3	4.97 dd
H-4′	5.45 dd	H-4	5.51 t
H-5′	4.26 m	H-5	5.09 t
H-6′α	4.26 m	H-6	5.51 t
H-6′β	3.98 m		
Acetyls	2.21; 2.13; 2.09(×2); 2.05; 2.02; 2.01; 2.00; 1.96		

[1] We thank the reviewers and Editor for the kind information on the availability of verbascose from Megazyme (Australia).

These values fully agree with the calculated values, so confirming the presence of the expected oligosaccharides, namely a hexa-, penta-, tetra- and trisaccharide, respectively. We tentatively assigned to the hexasaccharide the structure of ajugose as higher homologue in the raffinose family after raffinose, stachyose and verbascose. As reviewed in Dey (1990), verbascose, firstly isolated in 1910 from the roots of *Verbascum thapsiforme*, occurs in most leguminous plants. Its formation from galactinol and stachyose was reported as enzymatic synthesis from the seeds of *Vicia faba* (Dey, 1990). Ajugose was firstly detected in the roots of *Ajuga nipponensis* in 1942. Its biosynthesis involves galactinol as the D-galactosyl donor and verbascose as the acceptor. Kandler and Hopf (1982) reported that the enzyme preparations from *Pisum sativum* and *Vicia sativa* were able to catalyse the synthesis of this hexasaccharide. Independent of galactinol, higher oligomers in leaves may result from an acid transferase vacuolar enzyme, as found by Backmann et al. (Bachmann, Matile, & Keller, 1994; Bachmann, & Keller, 1995) in leaves of *Ajuga reptans*.

From the data reported in Table 1, galactinol (21% of the total amount of soluble carbohydrates), raffinose (18%) and sucrose (13%) were the prevailing sugars in fresh leaves, followed by stachyose and verbascose (10%), glucose (9%), fructose (7%) and ajugose (3%). Alditols (xylitol, ribitol and mannitol), *myo*-inositol, cellobiose, gentiobiose, melezitose and maltotriose were also present in minor amounts. When *Boea* plants were submitted to desiccation, dynamics of the carbohydrate pool was not dissimilar from findings for other drought-tolerant plants, showing a progressive decreasing (2 and 3% in 25 days dried samples) of the concentration of fructose and glucose with the concomitant increase of sucrose (91%). The only reported exception to the hexose decreasing was *C. plantagineum* (Bianchi et al., 1991), where sucrose accumulated at the detriment of an uncommon 2-octulose. Its structure (D-glycero-D-ido-2-octulose) was confirmed subsequently (Howarth, Pozzi, Vlahov, & Bartels, 1996). Desiccation led also to a significant decrease to 1 and 2%, respectively, of galactinol and raffinose with stachyose and higher oligosaccharides present only in trace amounts in dried state. Rehydration almost restored the sugar composition of unstressed leaves. Reduction of the oligosaccharides during drying could suggest that they may not be involved in this phase. There are several ways in which sugars have been proposed to act in conferring tolerance to orthodox seeds as well as to vegetative tissues, as reviewed in Vertucci, and Farrant (1995). Kaiser et al. (1985) reported that the relevant presence of sucrose is always related to extreme dry conditions in plants. As suggested by recent literature (Hoekstra et al., 1997), protection of membranes during dehydration results from a multicompound interaction, where disaccharides play an important role preventing fusion events and contributing to formation of the glassy state. In *Boea* leaves during the water stress sucrose content increased quickly (Table 1), inversely to the RWC values (see Section 3.2). This increase seemed to occur mainly at the expense of fructose and glucose. Our analyses have also shown the presence of galactinol and galactosyl oligosaccharides along with raffinose. Determined at relatively high levels in hydrated leaves, these sugars decreased during the drying treatments. A similar trend was reported (Müller et al., 1997) for the raffinose and galactinol content in leaf samples of the resurrection plants *Ramonda* and *Haberlea*. Present in fair amounts in fresh leaves, these compounds decreased upon water loss. As a conclusion, we have shown that also *Boea hygroscopica*, a resurrection plant of the Gesneriaceae, accumulates sucrose as a protectant against dehydration as well as other resurrection plants previously analysed (Bianchi et al., 1991, 1993; Marinone Albini et al., 1994). The presence in fresh leaves of raffinose and of higher sucrosyl oligosaccharides, along with their galactosyl donor galactinol, might pre-adapt *Boea* to the protective effect of sucrose, according to the suggestions reported by Müller (1997) for *Ramonda* and *Haberlea*. Recently a specific role for oligosaccharides in desiccation tolerance has been questioned (Bochicchio, Vernieri, Puliga, Murelli, & Vazzana, 1997; Lin, Yen, & Chien, 1998). A function not linked to desiccation tolerance might be hypothesized for the raffinose series oligosaccharides in *Boea hygroscopica*: in fact these compounds could be implicated in phloem loading, as suggested by Haritatos, Keller, & Turgeon (1996) in leaves of *Cucumis melo*.

3. Experimental

3.1. General

^{1}H-NMR 300 MHz, in $CDCl_3$, using TMS as int. standard. COSY NMR experiments were performed using the Bruker software. EIMS: 70 eV. DCI: ammonia. Analytical TLC: silica gel (Merck, Kieselgel 60 F_{254}, 0.25 mm); spots were visualized by spraying with a 3% solution of $K_2Cr_2O_7$ in H_2SO_4:H_2O (1:1), followed by heating at 120°C for 10 min; CC: silica gel (Merck, Kieselgel 60, 0.040–0.063 mm); solvent system: (A) $CHCl_3$–EtOH (98:2); (B) *n*-hexane–CH_3COCH_3 (3:2).

3.2. Plant material

The experiment was carried out on *Boea hygroscopica* whole plants. Plant growing conditions were the

same as already reported (Navari-Izzo, Meneguzzo, Loggini, Vazzana, & Sgherri, 1997). Briefly, the plants were grown in well watered pots on leaf mould, in growth chamber at a constant day/night temperature of 27°C, 16-h photoperiod, PFD of 120 μmol m^{-2} s^{-1}, 80–90% relative humidity. Dehydration was imposed by withholding water and the samplings were carried out at a starting time (T_0) and after 2, 3, 10, 11, 17 and 25 days of dehydration, when the RWC values were 94.3, 89.5, 92.5, 91, 77.3, 59 and 26.5%, respectively. The sample T_{res} (resurrection time) was collected 4 days after the starting of rehydration (RWC 80%). RWC was obtained according to Bochicchio et al. (1998). During the experiment, at each sampling time the leaves were excised from five plants. Samples for extraction were selected from the youngest fully expanded leaves, comparable in size and condition in fresh, dehydrated and rehydrated plants. Leaf samples were frozen in liquid N_2, lyophilized and stored at −80°C before analysis.

3.3. Extraction and isolation

Lyophilized leaf samples of *Boea hygroscopica* were treated as reported (Marinone Albini et al., 1994). To remove the epicuticular waxes, the leaves were previously dipped in $CHCl_3$ for 1 min. Then after drying on blotting-paper, the leaves were homogenized in a blender with MeOH:H_2O (7:3, v/v; tissue/solvent 1:100 w/v). The resulting green slurry was diluted with a similar amount of the same solvent mixture, left overnight at 5°C, filtered and evapd under red. pres. at 35°C to give a solid residue. To remove the lipophylic substances, the residue was partitioned 3 times between water and ethyl acetate. The water soluble sugars were obtained by vacuum evapn as a solid mixture. For qualitative and quantitative GC analyses, samples (20 mg) of aqueous extracts were derivatized with a silylating reagent made up of pyridine:hexamethyldisilane:trimethylchlorsilane in the ratio 2:1:1 (v/v) to get trimethylsilyl ethers (TMSi). The silylated sugars were kept in *iso*-octane for GC and GC–MS analyses, performed as previously reported (Marinone Albini et al., 1994). Analytical data are reported in Table 1. A Student's *t*-test (Godfrey, 1985) was used to compare differences between the concentrations and percentages of sugars at each time with respect to T_0 and to determine statistical significance. Data are presented as means of 5 samples ± standard deviations. Sugar acetylation was carried out on samples (100 mg) of aqueous extracts suspended in a 1:1 $(CH_3CO)_2O$–pyridine soln (8 ml) and left at room temperature for three days. Sugar separation was performed on the acetylated mixtures of fresh (T_0) and partially dried (T_2 and T_3) leaf samples, selected for the significant content of galactinol and higher oligosaccharides. Sugar acetates were chromatographed by CC eluted with solvent system A. Peracetylated fructose, glucose, galactose, alditols, inositol and sucrose, only partially resolved, were eluted in the first fractions from the column and identified either by co-chromatography and by comparison of their GC–MS fragmentation patterns with mass spectra of commercial (Fluka) or synthesized standards. Fractions containing mixtures of galactinol, raffinose and higher sugars were again chromatographed by CC eluted with the solvent system B. Galactinol was obtained as a pure fraction, followed by a fraction containing traces of a second compound, whose ^{1}H-NMR spectrum seemed compatible with the spectrum of a β-linked glycosyl *myo*-inositol. Pure raffinose and stachyose were then isolated, while a mixture of penta- and hexasaccharide was eluted subsequently.

3.4. EIMS

The spectra of the persilylated pentasaccharide and of the minor homologues gave the following fragmentation patterns: verbascose *m*/*z* (rel. int.): 451 (13), 437 (4), 361 (50), 271 (9), 243 (7), 217 (40), 204 (100), 169 (12), 147 (17), 129 (23), 103 (12), 73 (80); stachyose *m*/*z* (rel. int.): 451 (9), 437 (9), 361 (100), 271 (15), 243 (9), 217 (34), 204 (59), 169 (21), 147 (16), 129 (20), 103 (12), 73 (61); raffinose *m*/*z* (rel. int.): 451 (7), 437 (15), 361 (100), 271 (10), 243 (8), 217 (27), 204 (24), 169 (17), 147 (17), 129 (20), 103 (13), 73 (66).

Acknowledgements

We thank Dr. Elisabetta Castellotti for statistical analysis, Professors Lucio Toma and Adriana Bochicchio for helpful discussions, Dr. Mariella Mella for NMR spectra, Professor Giorgio Mellerio, Centro Grandi Strumenti, University of Pavia, for DCI analysis. We thank also the Reviewers for useful suggestions. This research was supported by the Italian Ministry of Agricultural Policies, Project: ''Genetic resistances of agricultural plants to biotic and abiotic stresses''.

References

Bachmann, M., Matile, P., & Keller, F. (1994). *Plant Physiol.*, *105*, 1335.

Bachmann, M., & Keller, F. (1995). *Plant Physiol.*, *109*, 991.

Bianchi, G., Gamba, A., Murelli, C., Salamini, F., & Bartels, D. (1991). *Plant J.*, *1*, 355.

Bianchi, G., Gamba, A., Limiroli, R., Pozzi, N., Elster, R., Salamini, F., & Bartels, D. (1993). *Physiol. Plant.*, *87*, 223.

Bianchi, G., Murelli, C., Bochicchio, A., & Vazzana, C. (1991). *Phytochemistry*, *30*, 461.

Bochicchio, A., Vazzana, C., Puliga, S., Alberti, A., Cinganelli, S., & Vernieri, P. (1998). *Plant Growth Regul.*, *24*, 163.

Bochicchio, A., Vernieri, P., Puliga, S., Murelli, C., & Vazzana, C. (1997). In *Basic and applied aspects of seed biology* (pp. 13–22). Kluwer Academic Press.

Brown, R. J., & Serro, R. F. (1953). *J. Am. Chem. Soc.*, *75*, 1040.

Curtius, H. C., Völlmin, J. A., & Müller, M. (1968). *Z. Anal. Chem.*, *243*, 341.

Dey, P. M. (1990). In P. M. Dey, & J. B. Harborne, *Methods in plant biochemistry*, *2* (pp. 189–218). London: Academic Press.

Ferrarotti, M. (1996). Degree thesis, University of Pavia.

Gaff, D. F. (1997). In A. S. Basra, & R. K. Basra, *Mechanisms of environmental stress resistance in plants* (pp. 43–58). The Netherlands: Harwood Academic Publishers.

Ghasempour, H. R., Gaff, D. F., Williams, R. P. W., & Gianello, R. D. (1998). *Plant Growth Regul.*, *24*, 185.

Godfrey, K. (1985). *N. Engl. J. Med.*, *313*, 1450.

Haritatos, E., Keller, F., & Turgeon, R. (1996). *Planta*, *198*, 614.

Hoekstra, F. A., Wolkers, W. F., Buitink, J., Golovina, E. A., Crowe, J. H., & Crowe, L. M. (1997). *Comp. Biochem. Physiol.*, *117A*(3), 335.

Holthaus, U., & Schmitz, K. (1991). *Planta*, *185*, 479.

Howarth, O. W., Pozzi, N., Vlahov, G., & Bartels, D. (1996). *Carbohydr. Res.*, *289*, 137.

Ingram, J., & Bartels, D. (1996). *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, *47*, 377.

Kaiser, K., Gaff, D. F., & Outlow Jr, W. H. (1985). *Naturwissenschaften*, *72*, 608.

Kandler, O., & Hopf, H. (1982). In F. A. Loewus, & W. Tanner, *Encyclopedia of plant physiology. New Series*, *13A* (pp. 348–383). Berlin and New York: Springer.

Lin, T. P., Yen, W. L., & Chien, C. T. (1998). *J. Exp. Botany*, *49*, 1203.

Marinone Albini, F., Murelli, C., Patritti, G., Rovati, M., Zienna, P., & Vita Finzi, P. (1994). *Phytochemistry*, *37*, 137.

Marinone Albini, F., Murelli, C., & Piccinini, D. (1998). Unpublished results.

Mayer, T.G., & Schmidt, R.R. (1997). *Liebigs Ann./Recueil*, 581–859.

Müller, J., Sprenger, N., Bortlik, K., Boller, T., & Wiemken, A. (1997). *Physiol. Plant.*, *100*, 153.

Murelli, C., Adamo, V., Vita Finzi, P., Marinone Albini, F., Bochicchio, A., & Picco, A. M. (1996). *Phytochemistry*, *43*, 741.

Navari-Izzo, F., Meneguzzo, S., Loggini, B., Vazzana, C., & Sgherri, C. L. M. (1997). *Physiol. Plant.*, *99*(1), 23.

Obendorf, R. L. (1997). *Seed Sci. Res.*, *7*, 63.

Oliver, M. J., & Bewley, J. D. (1997). *Horticult. Rev.*, *18*, 171.

Radford, T., & DeJongh, D. C. (1972). In G. R. Waller, *Biochemical application of mass spectrometry* (pp. 313–350). New York: Wiley Interscience.

Schweizer, T. F., & Horman, I. (1981). *Carbohyd. Res.*, *95*, 61.

Senser, M., & Kandler, O. (1967a). *Phytochemistry*, *6*, 1533.

Senser, M., & Kandler, O. (1967b). *Z. Pflanzenphysiol.*, *57*(4), 376.

Vazzana, C., Bochicchio, A., Puliga, S., Vernieri, P., Marinone Albini, F., Murelli, C., Navari-Izzo, F., Quartacci, M.F., & Sgherri, C.L.M. (1994). *Abstracts of International Workshop on Desiccation Tolerance and Sensitivity of Seeds and Vegetative Plant Tissues*, Kruger National Park, South Africa, 17–22 January (p. 44).

Vertucci, C., & Farrant, J. (1995). In J. Kiger, & G. Galili, *Seed development and germination* (pp. 237–271). New York: Marcel Dekker.