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SOLUBLE CARBOHYDRATES OF DEVELOPING LUPIN SEEDS

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Key Word Index—*Lupinus* species; Leguminosae; lupins; carbohydrates; raffinose; galactosylsucrose; development; distribution.

Abstract—The sequence of formation of monosaccharides and raffinose series oligosaccharides in the developing seeds of two lupin species (*Lupinus albus* cv Ultra and *Lupinus angustifolius* cv Unicrop) has been determined. Sucrose and glucose were the only sugars detected immediately after anthesis and the deposition of the higher members of the series did not commence until the onset of the 'drying' phase of seed ripening. The distribution of these components in the seed coats and cotyledons of developing *L. albus* seeds have also been examined. Cotyledons were nearly devoid of glucose throughout the development process. In fully ripened seeds stachyose was the predominant sugar, both in the cotyledons and seed coats. Verbascose was the last oligomer to be deposited in the seeds. In dry seeds the soluble sugars in seed coats accounted for *ca* 7% of the total amount present.

INTRODUCTION

Galactosyl-sucrose oligosaccharides have been reported to be an integral component of a variety of mature, dry and dormant seeds [1–3]. These are known to accumulate in many leguminous species, particularly in the developing seeds [4]. Korytnyx and Metyler [5] hypothesized a stepwise formation of stachyose and raffinose from sucrose in developing *Phaseolus lunatus* seeds. Gould and Greenshields [6] reported changes in the galactose containing oligosaccharides, di- and monosaccharides in the ripening seeds of *Phaseolus vulgaris*. These and other studies on *Vicia Faba* and other legume seeds [7] suggested that raffinose series oligosaccharides do not appear in the tissue until the onset of ripening. Similar studies have been conducted on soybeans using labelled $^{14}CO_2$, suggesting their incorporation of label into maltose took place during initial seed development and appeared in raffinose and stachyose during the later stages of seed maturation [8]. A similar sequence was also followed in soybeans by Amuti and Pollard [9].

Cultivated lupin varieties have been reported to contain an extremely high level of raffinose series oligosaccharides (22% on a dry wt basis) compared to many other food legumes [3, 10]. Being similar to soybeans in chemical constitution [10, 11] lupins may prove to be an excellent source of cheap plant protein of comparable importance with soybeans and other food legumes. In order to improve the nutritional quality of food legumes, a reduction in the levels of oligosaccharides by breeding has been recommended [12, 13]. The present study has, therefore, been conducted to investigate a detailed sequence of formation of these oligomers in two *Lupinus* species of different physiological maturity and to collect information for the elucidation of a possible marker for their oligosaccharide improvement through breeding.

RESULTS AND DISCUSSION

Seed formation commenced immediately after anthesis. An initiation of seed maturation was indicated by a decline in the fr. wts and a simultaneous increase in the dry wts which then remained constant until maturity (Fig. 1). There was a rapid breakdown of chlorophyll after the green phase of seed development and it disappeared completely during the grain filling process. An identical trend was observed in both the species examined. The mono-, di- and oligosaccharide fractions were extracted from the developing seeds at weekly intervals. The endogenous activity of α-galactosidase and invertase was suppressed by macerating the seeds directly in hot aqueous ethanol. The extracts thus prepared were examined by PC and TLC. The extracts of fully mature, dry seeds indicated that the oligosaccharide fraction was composed of the raffinose series oligosaccharides and that the disaccharide fraction was all sucrose, except for a trace of melibiose. The reducing monosaccharides present in extracts during the early stages of seed development were glucose, fructose and galactose with trace amounts of arabinose and xylose detected during the yellowing phase of seed maturation.

A portion of the extracts was also chromatographed on Bio-Gel P-2. The application of gel filtration techniques for the separation of homologous galactose oligomers and other saccharides have been described by various workers [14, 15]. Using this medium (P-2, −400 mesh), Derbyshire and Henry [16] resolved fructans from onions of DPs 3–9. The column prepared for the separation of lupin extracts had a void volume (V_e) of 90 ml and a hold up volume (V_t) of 180 ml. A standard mixture of stachyose (DPs 4), raffinose (DPs 3), sucrose (DPs 2) and glucose (DP 1) gave a complete separation with an elution volume of 140 ml (DPs 4), 152 ml (DPs 3), 168 ml (DPs 2) and 180 ml (DP 1). When a lupin extract was chromatographed, the pattern obtained (Fig. 2) indicated a peak eluted at 130 ml. The fractions containing this peak were pooled together, concentrated by rotary evaporation below 40° and re-run on the same column. It was eluted at the same volume (128 ml) as a sharp single peak. This was calculated and reported as verbascose (DPs 5). Qualitative analysis of samples on PC and TLC confirmed the

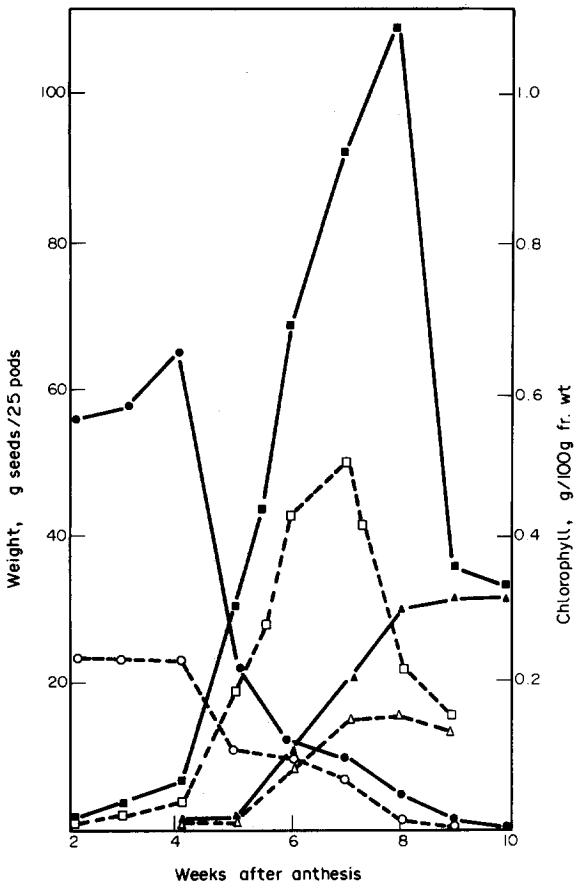

Fig. 1. Changes in chlorophyll, fr. and dry wts of developing lupin seeds (——) Ultra and (– – –) Unicrop. (●, ○) Chlorophyll; (■, □) fr. wt and (▲, △) dry wt.

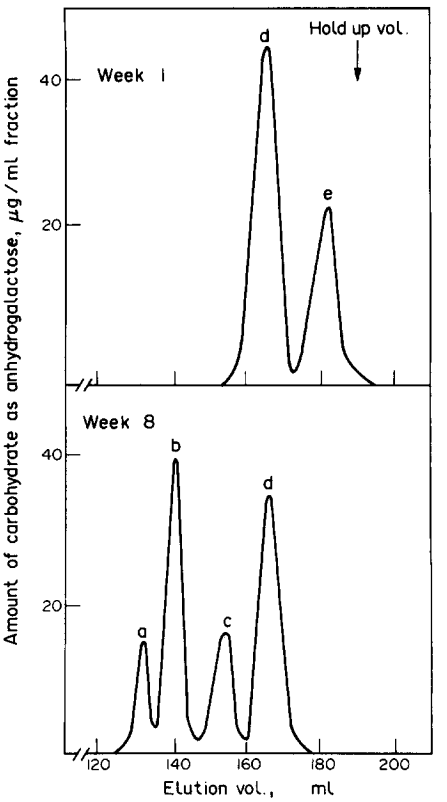

Fig. 2. Gel-filtration pattern (Bio-Gel P-2, minus 400 mesh) patterns of the ethanol soluble extract of *L. albus*. (a) Verbascose; (b) stachyose; (c) raffinose; (d) sucrose and (e) glucose.

presence of an additional sugar when run against a standard mixture of stachyose, raffinose, sucrose, glucose and fructose.

The changes that accompanied seed development in two species are shown in Figs. 3 and 4. There was a steady inflow of soluble carbohydrates into the developing cotyledons. In *L. albus*, a maximum concentration of soluble sugars occurred at the beginning of the drying phase and this was followed by a slight decrease as the seeds reached maturation. In the case of *L. angustifolius* this maximum paralleled the end of the yellowing phase, which then declined rapidly during the drying process (weeks 8 and 9). Also there was a slow depletion of soluble carbohydrates from maximum level to maturity in the case of *L. albus* which accounted for only 3.2%. In comparison, this depletion was greater (44.8%) in the case of *L. angustifolius*.

Glucose and sucrose were the first soluble sugars to appear in the cotyledons, which continued to accumulate until the middle of the yellowing phase. In *L. albus*, the glucose level reached its maximum towards the end of the green phase, steadily decreased during the yellowing phase and almost disappeared on the onset of the drying phase. In *L. angustifolius*, it continued to rise until the middle of the yellowing phase with a rapid decline and complete disappearance towards maturity. Sucrose has been the predominant sugar during the initial phases (green and yellowing phases) in both the species. In *L. angustifolius*, it followed the pattern of total soluble sugars but in the case of *L. albus* it started to decline much earlier (week 7) from maturity.

The deposition of the higher members of the series, raffinose, stachyose and verbascose did not commence until the onset of the drying phase in each of the species. Raffinose and stachyose first appeared in the tissues towards the end of the yellowing phase. A steady increase was observed thereafter and the pattern of their change followed a decrease in the sucrose levels. Both these sugars reached a maximum level as the seeds ripened. Verbascose was the last oligomer to be deposited in the seeds and first appeared in the tissues during the middle of the drying phase and then increased steadily until the seeds were fully matured.

The developing cotyledons of *L. albus* were separated from their seed coats and the distribution of soluble carbohydrates and component saccharides was examined. The analysis was not performed at earlier stages as the seeds were present only as bubbles in the pods, filled with sap. Cotyledon setting did not commence until after the green phase and this marked the initiation of rapid metabolic conversions of glucose and sucrose. At week 5, the greater proportion of the soluble sugars was associated with the seed coats, and sucrose represented the main component, whereas in the cotyledons glucose was present only in trace amounts. Disappearance of glucose was followed by an increase in sucrose both in the seed coats and cotyledons. Other interconversions occurred at

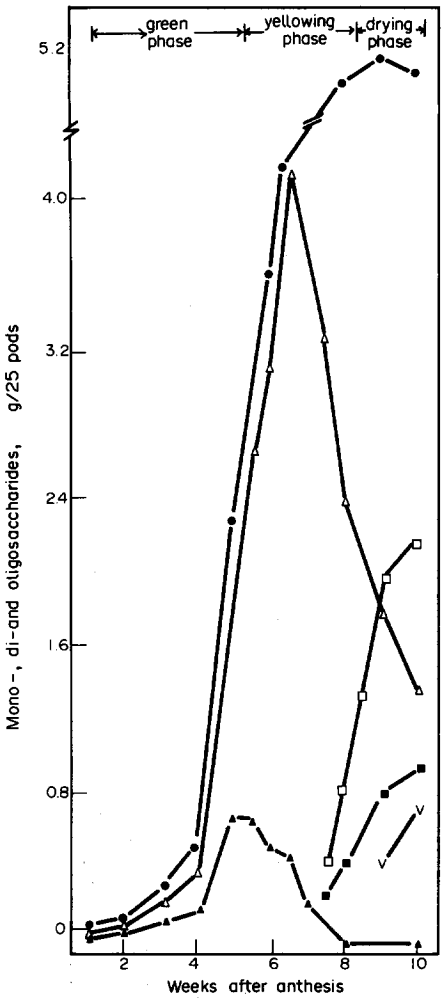

Fig. 3. Pattern of changes in mono-, di- and oligosaccharides of developing *L. albus* seeds. (●) Total; (▲) glucose; (△) sucrose; (□) raffinose; (■) stachyose and (∨) verbascose.

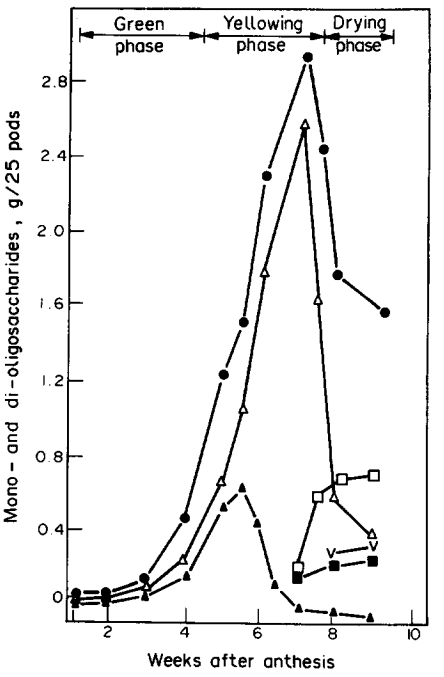

Fig. 4. Pattern of changes in mono-, di- and oligosaccharides of developing *L. angustifolius* seeds. (●) Total; (▲) glucose; (△) sucrose; (□) raffinose; (■) stachyose and (∨) verbascose.

subsequent stages of maturation and at maturity stachyose was the major component present in both the seed parts. At the end of the drying phase when the seeds were fully mature, the soluble sugars in the seed coats accounted for nearly 7% of the total amount present in the whole seed.

In the developing seed between weeks 5 and 8, the cotyledons rest in the middle surrounded by the sap and a very thin film of testa, holding the growing structure in the pod. The sap and the testa contain the reducing sugars, glucose and fructose, but these sugars are not present in

Table 1. Distribution of total soluble carbohydrates and component mono-, di- and oligosaccharides in seed coats and cotyledons of developing *L. albus* seeds

Seed part	Weeks after anthesis	Total soluble carbohydrates* (mg/10 seeds)	Mono-, di- and oligosaccharides (mg/10 seeds)†				
			Glc	Suc	Raff	Stach	Verba
Seed coats	4	458	161.5	295.5	—	—	—
	5	322	147.6	248.6	—	—	—
	6	136	26.4	110.3	—	—	—
	8	85	—	60.8	6.6	18.1	—
	10	36	—	10.1	4.2	18.5	3.0
Cotyledons	4	—	—	—	—	—	—
	5	16	tr‡	18.6	—	—	—
	6	148	tr	123.3	—	—	—
	8	349	tr	239.0	33.0	57.7	—
	10	640	tr	193.0	68.5	318.0	59.1

*Based on anthrone analysis on aqueous extracts.
†Based on anthrone analysis of separated peaks on Bio-Gel P-2.
‡Trace.

the developing cotyledons. These results suggest that there is a sequential formation of raffinose series oligosaccharides in the developing cotyledons and that the higher members of the series are being deposited last. There is no difference in their sequence of formation either in *L. albus* or *L. angustifolius*, the representatives of late and early maturing species, respectively, although there is a slow depletion of total soluble carbohydrates from maximum level to maturity in the late maturing species.

EXPERIMENTAL

Plant material. Lupin cvs Ultra (*L. albus*) and Unicrop (*L. angustifolius*) cultivated in the southern Tablelands of New South Wales were used in the present study. The crops were grown during 1979 and 1980 under normal cultural practices in the exptal plots at the Institute farms. The first flowers that appeared on the lateral branches of the plants were tagged for subsequent sample collections.

Growth properties. These were followed by collecting pod samples in ice-chilled containers at weekly intervals, commencing immediately after anthesis. Seeds were removed from the pods and dried between filter papers before recording wet wts. Dry wts were obtained after drying in an oven at 100° for 24 hr. Chlorophyll content was measured by extraction of a separate batch of seeds with Me_2CO [17].

Carbohydrate content. Estimated using anthrone by the method of ref. [18]. The results were calculated from a standard galactose calibration curve prepared concurrently and reported on an anhydro-galactose basis.

Extraction of soluble carbohydrates. Seeds from 25 pods were separated and homogenized directly in 200 ml hot aq. EtOH (70%). The mixture was immediately boiled for 5 min. On cooling, the soln was filtered through sintered glass and the residue was washed with 50 ml aq. EtOH (70%). At later stages of seed development (drying phase) the residue was re-extracted in a 100 ml portion of 70% EtOH, filtered and washed with EtOH. The EtOH filtrates were then combined. The dry seeds were ground in a Udy cyclone mill (100 mesh sieve) and homogenized in 70% aq. EtOH (250 ml) and the mixture boiled for 5 min. After cooling, the soln was filtered and the residue re-extracted. The combined EtOH filtrates were concd by rotary evaporation (below 40°) to 50 ml and extracted twice with $CHCl_3$ to remove chlorophyll. Final traces of $CHCl_3$ and denatured protein were removed by centrifugation. The aq. soln was then used for the estimation of total soluble carbohydrates and component mono-, di- and oligosaccharides.

Chromatography. The extracts were examined qualitatively by PC, *n*-BuOH–pyridine–H_2O–C_6H_6 (5:3:3:1, upper) was the solvent and *p*-anisidine–HCl or $AgNO_3$ was used for development. Al sheets coated with Si gel 60–Kieselguhr F254 (Merck) were used for TLC and the solvent was *n*-PrOH–EtOH–H_2O (7:1:3). The plates were developed by spraying with 5% H_2SO_4 in EtOH and heating at 100° for 5–10 min.

Separation, estimation and identification on mono-, di- and oligosaccharide fractions. Aliquots of the EtOH extract were also fractionated by gel filtration on polyacrylamide (Bio-Gel P-2, minus 400 mesh). The column fractions collected were assayed with anthrone [18] to distinguish the oligomer peaks. The peaks thus obtained were identified by comparing their elution vols. with those of the standard sugars run under the same conditions.

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