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VARIATION IN LOW MOLECULAR WEIGHT CARBOHYDRATE COMPOSITION OF *PHASEOLUS VULGARIS* SEEDS

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Key Word Index—*Phaseolus vulgaris*; Phaseoleae; Leguminosae; common bean; chemotaxonomy; low M_r carbohydrates.

Abstract—Variations in the low M_r carbohydrate composition have been observed in wild forms of common bean (*Phaseolus vulgaris*) seed. In four of 23 samples, verbascose content in the seeds was quite high and the ratio verbascose–stachyose was more than 1.0. This type of carbohydrate composition was named type A, and has a much higher verbascose content and lower galactinol and stachyose content than the rest of the wild forms, named type B. Although the total and individual carbohydrate content, with the exception of the verbascose content, of the cultivated forms of the common bean were higher than those of wild forms, the carbohydrate composition of the cultivated forms was essentially similar to type B of the wild forms. This carbohydrate composition was considered to be basic to the species.

INTRODUCTION

Food legumes have been domesticated from their wild forms or wild relatives in the past [1, 2], but there are many differences between wild and cultivated forms, e.g., in plant habit and seed weight. However, differences in chemical composition between cultivated and wild forms has not yet been clarified to a significant extent. The research described in the present paper represents a part of an ongoing study aimed at characterizing the low M_r carbohydrates in leguminous seeds and clarifying the chemotaxonomic relationship among leguminous species. During the course of the study, variations in the low M_r carbohydrate composition have been observed in the wild forms of common bean (*Phaseolus vulgaris* L.) seed.

RESULTS AND DISCUSSION

Variations in low molecular weight carbohydrate composition

myo-Inositol, sucrose, raffinose, stachyose and verbascose were identified in all the common bean samples. These carbohydrates are known to be ubiquitous in legume seeds. Galactinol (1L-1-*O*-α-D-galactopyranosyl-*myo*-inositol) and digalactosylglycerol [*O*-α-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl-(1 → 1)-glycerol] were tentatively identified by GC RR_t [3]. In four of the 23 wild samples of the common bean, the verbascose content in the seeds was rather high and the ratio verbascose–stachyose in the seeds was more than 1.0. These samples could not be distinguished from the rest on seed colour, seed weight, or vegetative and floral morphology. This verbascose-rich type of the low MW carbohydrate composition was named type A which, as regards the common bean, has not been reported before. The verbascose content is much higher and the galactinol and stachyose contents are lower than the rest of the wild forms, named type B (Table 1). Type B was considered to be an 'ordinary' type, since the common bean, *P. vulgaris*, is known to lack verbascose [4]. Although the *myo*-inositol, sucrose, raffinose and stachyose contents of the cultivated forms are significantly different from those of the type B wild form, their carbohydrate patterns are essentially similar to them (Table 1).

In order to find out the chemotaxonomic relationship between the cultivated and wild forms of the common bean, the carbohydrate composition data obtained was subjected to principal component analysis [5]. The ordination produced is presented in Fig. 1. Axis Z_1 of the ordination represents the first principal component, which accounts for 35% of the total variation and is positively correlated with the verbascose content and negatively correlated with the galactinol, digalactosylglycerol and stachyose contents. The Z_2 axis shows the second principal component accounting for 24% of total variation and being negatively correlated with the *myo*-inositol and raffinose contents. Samples having a high level of verbascose (sample nos. 1–4) appeared to the right in the plot. Seeds which were rich in galactinol, digalactosylglycerol and stachyose appeared to the left. This ordination suggested that the carbohydrate composition of the cultivated forms is of type B, so that they are related to type B and not to type A wild forms.

Hereditability of the variation

Table 2 shows that the composition of seeds of the progeny is similar to that of the parental seeds indicating that it is inherited. Since *P. vulgaris* is well-known as a predominantly self-fertilizing species [1], this carbohydrate character would have been transmitted from their

Table 1. Low M_r carbohydrate composition in seeds of wild and cultivated forms of *Phaseolus vulgaris*

CIAT's sample no.	Low M_r carbohydrate content, (g/100 g dry matter)						
	myo-Inositol	Sucrose	Galactinol	Digalactosyl-glycerol	Raffinose	Stachyose	Verbascose
Wild forms—type A							
1 G02771	0.04	1.31	0.02	0.01	0.29	1.26	2.49
2 G07479	0.02	1.95	0.02	0.01	0.35	1.27	2.70
3 G09980	0.06	2.06	0.02	0.01	0.23	0.88	2.02
4 G09995	0.02	2.15	0.01	0.01	0.45	1.40	1.47
Mean	0.035	1.868	0.018	0.010	0.330	1.203	2.170
s.d.	0.019	0.381	0.005	0.000	0.094	0.224	0.546
Wild forms—type B							
5 G00996	0.02	2.78	0.05	0.02	0.29	3.51	0.07
6 G06386	0.05	1.53	0.05	0.01	0.26	3.47	0.11
7 G06388	0.01	3.25	0.04	0.02	0.22	3.44	0.10
8 G07225	0.01	2.62	0.02	0.01	0.30	2.76	0.12
9 G07469	0.03	3.14	0.06	0.02	0.27	3.44	0.04
10 G09981	0.10	2.12	0.05	0.02	0.43	3.05	0.03
11 G09982	0.05	3.68	0.06	0.02	0.17	2.98	0.06
12 G09983	0.06	3.08	0.04	0.02	0.24	2.76	0.07
13 G09984	0.01	2.83	0.03	0.01	0.24	2.32	0.04
14 G09986	0.13	2.39	0.06	0.01	0.42	2.23	0.21
15 G09987	0.09	1.58	0.05	0.01	0.44	3.70	0.09
16 G09988	0.01	3.24	0.02	0.03	0.37	3.55	0.08
17 G09989	0.09	2.40	0.05	0.01	0.47	3.57	0.02
18 G09991	0.03	1.95	0.05	0.02	0.32	3.50	0.02
19 G09992	0.04	1.79	0.06	0.02	0.34	3.48	0.02
20 G09996	0.04	1.85	0.03	0.01	0.28	3.29	0.15
21 G09997	0.02	1.48	0.04	0.01	0.32	3.95	0.14
22 G09998	0.04	1.44	0.05	0.01	0.34	3.50	0.19
23 G10000	0.05	2.26	0.03	0.01	0.38	2.40	0.67
Mean	0.046	2.390	0.044	0.015	0.321	3.205	0.117
1s.d.	0.034	0.692	0.013	0.006	0.082	0.496	0.145
Cultivated forms (n = 103)							
Mean	0.076	3.258	0.055	0.018	0.432	3.722	0.096
s.d.	0.027	0.561	0.024	0.005	0.160	0.642	0.187
Difference between type A and type B	n.s.	n.s.	**	n.s.	n.s.	**	**
Difference between type A of wild forms and cultivated forms	**	**	**	**	n.s.	**	**
Difference between type B of wild forms and cultivated forms	**	**	n.s.	n.s.	**	**	n.s.

n.s., Not significant; **significant at the 1% level.

ancestors. The wild form of *P. vulgaris* and/or *P. aborigineus* Burk. is assumed to be the wild ancestor(s) of the cultivated form but the taxonomic relationship between them has not yet been completely clarified [1, 2]. The results of this study indicate that the carbohydrate compositions of the majority of the cultivated forms closely resemble the type B composition of the wild forms and suggests that the cultivated forms are derived from type B wild forms.

EXPERIMENTAL

Plant materials. Mature seeds (126 samples, including 23 wild forms) were provided by Centro Internacional de Agricultura Tropical (CIAT, Cali, Columbia), where the world collection of the common bean is maintained. Cultivated forms were randomly selected from CIAT's germplasm collection. All wild forms available were obtained from there. Six wild forms of *P. vulgaris* were sown at an experimental field for upland crops at the Okinawa branch of the Tropical Agriculture Research Center (TARC), Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan. Mature seeds of five wild forms were harvested. The seed samples obtained were stored below −18° until used.

Chemicals. Commercial *myo*-inositol, sucrose and raffinose were used without further purification. Stachyose and verbascose were isolated from the seeds of adzuki bean [*Vigna angularis* (Willd.) Ohwi et Ohashi] and pea (*Pisum sativum* L.), respectively.

Quantitative determination of the low molecular weight carbohydrates. The seeds were finely ground so as to pass through a 0.5 mm sieve. The carbohydrates were determined by the int. standard addition method. The ground samples (100 mg, dry wt)

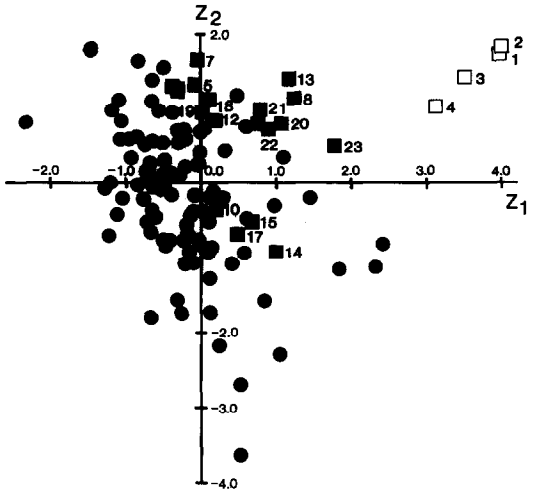

Fig. 1. Ordination plot from principal component analysis of low MW carbohydrate data for 126 *Phaseolus vulgaris* L. samples. For the principal component analysis, see ref. [5]. The diagram shows the position of the samples on the first and second principal component (Z_1 and Z_2) axes, which account for 35% and 24% of the total variation, respectively. Z_1 was positively correlated with the verbascose content and negatively correlated with the galactinol, digalactosylglycerol and stachyose contents. Z_2 was negatively correlated with the *myo*-inositol and raffinose contents. Numbers besides the symbols indicate sample numbers in Table 1. (□) Type A of wild form; (■) type B of wild form; (●) cultivated form.

were extracted with *n*-hexane (1 ml × 3). The defatted sample and phenyl β-D-glucopyranoside (1 mg, as the int. standard) were extracted with 80% EtOH (10 ml) at 100° for 40 min in sealed tubes. The extracts were centrifuged (1150 *g*) and the supernatants (500 μl) dried under N_2. Pyridine (100 μl), HMDS (100 μl) and TFA (10 μl) were added to the residues to prepare the TMSi derivatives. TMSi derivatives were analysed by GC on 0.5 m × 3 mm columns of 2% silicone OV-1 on Chromosorb W HP, 100–120 mesh using temp. programming of 120–340° at 10°/min. The carrier gas (N_2) flow rate was 60 ml/min. Injection port and detector temps were both 350°. Moisture content was determined by heating the ground samples for 2 hr at 135°.

Identification of the carbohydrates. In addition to comparison of RR_t, GC/MS was also used to identify the carbohydrates. GC conditions for recording MS were essentially similar to those for the quantitative determination, but carrier gas (He) flow rate was 10 ml/min. EIMS were obtained with an ionization voltage of 20 eV and an ionization current of 100 μA. Gas heater and ionization chamber temps were 300° and 220°, respectively.

Statistical and multivariate analyses. The computer programs used were GCQD50 for estimation of carbohydrate contents, STATPAC-6 for statistical analysis of data obtained and PCASS for principal component analysis [6].

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Table 2. Low MW carbohydrate composition of seeds of original and progeny of wild forms of *Phaseolus vulgaris*

CIATs sample no.	Low MW carbohydrate content (g/100 g dry matter)						
	myo-Inositol	Sucrose	Galactinol	Digalactosyl-glycerol	Raffinose	Stachyose	Verbascose
Type A							
1 G2771							
Original	0.04	1.31	0.02	0.01	0.29	1.26	2.49
Multiplied	0.08	1.62	0.04	0.01	0.36	1.73	1.81
2 G7479							
Original	0.02	1.95	0.02	0.01	0.35	1.27	2.70
Multiplied	0.08	2.38	0.02	0.01	0.39	1.00	1.23
Type B							
9 G7469							
Original	0.03	3.14	0.06	0.02	0.27	3.44	0.04
Multiplied	0.07	2.20	0.10	0.02	0.23	3.27	0.07
13 G9984							
Original	0.01	2.83	0.03	0.01	0.24	2.32	0.04
Multiplied	0.05	2.24	0.07	0.01	0.18	3.18	0.16
19 G9992							
Original	0.04	1.79	0.06	0.02	0.34	3.48	0.02
Multiplied	0.07	1.59	0.06	0.01	0.20	2.89	0.08

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