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Genetic Analysis of the Raffinose Family Oligosaccharides in Common Bean

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Abstract. **Common bean (*Phaseolus vulgaris* L.) is a nutritionally complete food, but contains antinutritional compounds that reduce digestibility. One group of compounds includes the raffinose family oligosaccharides (RFOs) (raffinose, stachyose, and verbascose), which are partly responsible for flatulence after beans are eaten. RFOs stabilize cell membranes during seed desiccation and when the seed rehydrates during germination. While low levels of RFOs are desirable nutritionally, high levels may enhance germination and emergence, particularly in cold, wet soils. Eight landraces selected for high and low sucrose, raffinose, and stachyose content, were crossed in a diallel mating design to investigate genetic control of the RFOs. Derivatized soluble sugars were measured using gas–liquid chromatography. Fructose, sucrose, raffinose, and stachyose were detected. In the F_1, fructose varied from 0.1 to 2.5 mg·g^{-1} dry weight (DW), sucrose from 17.2 to 56.5 mg·g^{-1} DW, raffinose from 0.1 to 4.1 mg·g^{-1} DW, and stachyose ranged from 7.6 to 43.7 mg·g^{-1} DW. Griffing's analysis estimates of general combining ability were on average, 16.5 times larger than specific combining ability for all the RFOs, indicating that additive genetic variance was most important. Significant reciprocal differences were detected in the F_1 and F_2, but not in the F_3. RFO accumulation was partially dominant as indicated by Hayman's analysis. Narrow sense heritability averaged over F_2 and F_3 generations for sucrose, raffinose, stachyose, total sugar, and total oligosaccharides were 0.22, 0.54, 0.44, 0.17, and 0.27, respectively. Moderate heritabilities indicate that manipulation of RFO accumulation in this set of bean lines would probably need to be done on a progeny row basis with replication.**

Nutritionally, common bean (*Phaseolus vulgaris*) is high in vitamin B, proteins, folic acid, complex carbohydrates, and soluble fiber. Bean also contains antinutritional compounds such as tannins, hemagglutinins, phytate, trypsin inhibitors, α-amylase inhibitors, indigestible starch, and α-oligosaccharides of the raffinose family (Barampama and Simard, 1993; Furuichi et al., 1988; Sathe and Salunke, 1981). Cooking inactivates hemagglutinins and protein inhibitors but has little effect on other antinutritional factors. Along with indigestible starch, raffinose family oligosaccharides (RFOs) (raffinose, stachyose, and verbascose) may lead to digestive upset. The human digestive track lacks α-galactosidase necessary to break the α-1-6 linkages between the hexose monomers present in the RFOs. Consequently, RFOs pass to the lower intestine where enteric bacteria metabolize them to hydrogen, methane, and carbon dioxide gases, which are expelled as flatus.

RFOs may provide cell membrane stabilization during the process of seed maturation and desiccation and again when the seed rehydrates during germination (Bernal-Lugo and Leopold, 1995; Koster, 1991). In the proper ratio with sucrose, RFOs allow cell membranes to enter a vitreous state that stops diffusion and prevents deleterious reactions from occurring in the cell upon seed desiccation. Through its rigidity and bulk, this glass-like structure also prevents cell membranes from rupturing.

Beans with white seedcoats are particularly susceptible to membrane damage when imbibed in cold, wet soils (Dickson and Boettger, 1976; Dickson and Petzoldt, 1988; Powell et al., 1986). Because certain market classes of dry beans and all commercially important snap beans are white-seeded, breeders need traits to select that would improve germination, particularly under cold stress conditions. While selection for low levels of RFOs might reduce flatulence associated with the consumption of dry bean, selecting for high levels of RFOs could benefit snap beans.

Values for sucrose, raffinose, and stachyose content in bean seeds reported in the literature varied from 15 to 27, 2 to 5, and 18 to 38 mg·g^{-1} dry weight (DW), respectively (Barampama and Simard, 1993; Borejszo and Khan, 1992; Fleming, 1981; Kuo et al., 1988; Ortega-Delgado and Rodriquez-Coquiez, 1979; McPhee and Myers, 1996; Sathe et al., 1984). Most researchers have studied RFOs in United States dry bean market classes, which represent a rather narrow genetic base (McClean et al., 1993). The identity of cultivars representing a market class was unknown in several studies because seed lots were purchased from local markets. Seed purchased from the markets are likely to be a mix of two or more cultivars. Two research groups have identified greater genetic variability in RFOs in non-United States sources of germplasm. Ortega-Delgado and Rodriquez-Coquiez (1979) examined Mexican landraces, while McPhee and Myers (1996) analyzed bean germplasm from the Eastern and Western Hemispheres.

Little is known about inheritance of RFOs in common bean. A genetic analysis of RFO accumulation and development of lines with high and low RFOs is needed to determine the influence of RFOs on digestibility and cell membrane stabilization. Therefore, the following research was conducted to examine gene action, and determine heritabilities for accumulation of RFOs.

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Table 1. Soluble sugar content of eight landraces and cultivars of common bean used in a diallel mating design.

Accession	Market			Sugar content (mg·g^{-1} dry wt)			
no.[z]	class	Origin	Ratio[y]	Sucrose	Raffinose	Stachyose	Total
G01106	Coscorron	Chile	H:H:H	25.9	2.0	22.6	50.5
PUE 40	Black	Mexico	H:H:L	30.0	4.0	10.7	44.7
G15306A	Sugar bean	Zambia	H:L:H	21.1	0.3	23.7	45.1
G13817	Sugar bean	Zambia	H:L:L	31.2	0.4	17.1	48.7
PR 40	Small red	Puerto Rico	L:H:H	12.4	1.7	23.5	37.5
Coscorron	Coscorron	Chile	L:H:L	15.0	3.0	14.9	32.9
G18270	Cacahuate	Mexico	L:L:H	13.1	0.4	24.2	30.2
G18354	Coscorron	Chile	L:L:L	12.7	0.2	16.9	29.8
$LSD_{0.05}$				9.9	1.0	9.2	

[z]For details on source of these lines see McPhee and Myers (1996).
[y]High (H) or low (L) content of sucrose:raffinose:stachyose, respectively.

We chose a diallel design for genetic analysis because RFOs are measured as continuous variables. Diallel analysis as applied to plant genetics has been controversial because of issues surrounding types of design, choice of populations, and interpretation of results (Christie and Shattuck, 1991). Because we were working with a self-pollinated crop and a nonrandom reference population, we have chosen to be conservative in interpretation of our results.

Materials and Methods

Plant materials and experimental design. Seventy-nine bean accessions were screened for soluble sugar content (McPhee and Myers 1996) and each line was classified as having high or low sucrose, raffinose, and stachyose. Review of the literature and our own results indicated that variation for RFOs in North American bean lines was limited, but that greater variation existed in materials with a more diverse genetic background. Therefore, we chose eight landraces to represent as much potential genetic diversity for RFOs as possible (Table 1). See McPhee and Myers (1996) for details on origins and types of bean accessions.

The eight landrace parents were crossed in all possible combinations according to Griffing's (1956) method I diallel design. Crosses were performed in the greenhouse at the University of Idaho, Moscow, during Spring 1993 and again in Spring 1994. Harvested F_1 seeds were planted four per 1.8-L pot, and pots were arranged in a randomized complete block design with four replications in a greenhouse at the Research and Extension Center, Kimberly, Idaho, during Summer 1993. Two plants of each parent were represented in each replicate. F_1 seed from crosses made in 1994 were planted individually in 1.8-L pots and arranged in a randomized complete block design with four replications in the greenhouse at Moscow, Idaho, in Summer 1994. Greenhouse temperatures ranged from 23 to 28 °C, and natural light was supplemented with metal halide lamps to maintain an irradiance of at least 1000 μmol·m^{-2}·s^{-1}. Long days inhibited flowering of some parents and crosses, necessitating covering plants to reduce the photoperiod to 12 h.

F_2 seed harvested from the F_1 plants grown at Kimberly in 1993 were sown in the field at the University of Puerto Rico-Mayaguez research station at Juana Diaz near Fortuna, Puerto Rico. One to 25 plants per plot were arranged in a randomized complete block with four replications. One F_3 seed from each F_2 plant was bulked by cross for analysis. Five F_2 seed harvested from F_1 plants grown in the greenhouse, Summer 1994 in Moscow, Idaho were chosen at random for sugar analysis. Remnant F_1 seed from the 1994 crosses were ground and analyzed individually for sugar content.

Sunshine potting mix (Cascade Seed Co., Spokane, Wash.) was used for production of plants in the greenhouse at Kimberly. The plants were fertilized about every 2 weeks with a 15N–7P–14.1K water soluble fertilizer (Peters 15–16–17, J.R. Peters, Inc., Allentown, Pa.). In Puerto Rico, bean plants were grown on a San Anton Clay Loam soil (fine-loamy, mixed, superactive, isohyperthermic Typic Haplustoll) with standard cultural practices used for dry bean production.

Soluble sugar analysis. Total soluble sugars were extracted from 50 mg of bean flour in 1.7-mL Eppendorf microcentrifuge tubes using 750 mL of 80% ethanol. The microcentrifuge tubes were floated in a water bath at 70 °C for ≈10 min. Tubes were vortexed four times during the 10 min incubation, and then were centrifuged at 6,000 g_n for 1 min. The sample was rinsed twice with 500 mL 80% ethanol, centrifuged at 6,000 g_n for 1 min, the supernatants combined, and 160 mg of ß-D-phenylglucopyranoside (Sigma-Aldrich Co., St. Louis, Mo.) added as an internal standard. This solution was dried in a 2-mL glass vial using a heat block (60 °C) and gentle airflow.

Trimethylsilyl derivatization was performed following a procedure modified from Sweeley et al. (1963). A 400 mL aliquot of pyridine with hydroxylamine-HCl (Kodak Co.) at 30 mg·mL^{-1} and 15 mL of dimethylaminoethanol (Sigma-Aldrich Co.) was added to each sample. Samples were incubated in a hot water bath (70 to 80 °C) for 40 to 50 min, then cooled to 21 °C, at which time 400 mL of hexamethyldisilazane was added followed immediately by 20 mL of trifluoroacetic acid. The reaction mixture was poured into 1.7-mL microcentrifuge tubes and centrifuged at 6,000 g_n for 1 min to pellet the ammonium chloride precipitate. Each sample was poured into 2 mL autosampler vials (E and K Scientific Products, Inc., Campbell, Calif.), capped, and stored at 4 °C until analysis.

Gas–liquid chromatography was performed on a gas chromatograph (HP 5830A; Hewlett Packard, Palo Alto, Calif.) equipped with a Hewlett Packard 18850 integrator, an OV-17 packed column, and a flame ionization detector. The run time was 35 min. The F_1 samples were analyzed on the OV-17 column while the F_2 and F_3 samples were analyzed on an OV-11 column with a modified temperature ramp to allow complete detection of the stachyose peak.

Standard samples of fructose, sucrose, raffinose, and stachyose (Sigma-Aldrich Co.) were used to determine the retention times and calculate response factors for use in quantifying the amount of sugar in each sample. The retention times for fructose, sucrose, raffinose, and stachyose were 5.30, 14.16, 25.03, and 30.72 min, respectively on the OV-17 column. The OV-11 column shifted the retention times to 8.37, 17.47, 26.55, and 32.48 min, respectively.

Statistical analysis. Initial assumptions for diallel analysis

Table 2. Generation means and overall mean for fructose, sucrose, raffinose, stachyose, total sugar, and total oligosaccharides from a diallel analysis of soluble sugars in common bean seed.

Variable	Generation [mg·g^{-1} dry wt) (mean ± SE)]			Overall
	F_1	F_2	F_3	Mean
Fructose	1.0 ± 0.0	3.2 ± 0.0	3.0 ± 0.0	2.4
Sucrose	31.6 ± 0.2	23.5 ± 0.2	22.6 ± 0.2	25.9
Raffinose	1.4 ± 0.0	2.1 ± 0.0	2.2 ± 0.1	1.9
Stachyose	25.1 ± 0.2	15.4 ± 0.3	14.8 ± 0.2	18.4
Total sugar	59.1 ± 0.5	44.2 ± 0.4	42.6 ± 0.4	48.6
Total oligosaccharides	26.5 ± 0.2	17.4 ± 0.3	17.0 ± 0.2	20.3

were diploid segregation, homozygous parents, no reciprocal differences [Hayman's (1954) analysis], no epistasis, no multiple alleles, and no linkage disequilibrium. Because common bean is self-pollinating and highly inbred, and we selected the parents nonrandomly for the diallel, a descendant reference population and fixed model were assumed (Christie and Shattuck, 1991; Wright, 1985). The diallel data from all three generations were analyzed according to Griffing's Method 1 analysis (Griffing, 1956) using the diallel program created by Burow and Coors (1994). Missing values in the diallel data were estimated using the least squares method (Kirk, 1982), and the error mean squares of the output were adjusted to reflect the appropriate degrees of freedom. Hayman's (1954) analysis was applied to F_2 and F_3 data in addition to Griffing's (1956) analysis. This analysis was performed in a Lotus 1-2-3 spreadsheet (Lotus Development Corp., Cambridge, Mass.) designed by the authors. We examined the potential for reciprocal effects using Griffing's (1956) analysis to test the hypothesis that reciprocal effects might arise due to the oligosaccharide biosynthetic pathway being under joint control of the maternal parent and embryo.

The diallel program was unable to calculate an error term for the F_1 data due to missing values. Therefore, an error term based on a one-way analysis of variance (ANOVA) of the entries was used to test the main effects in the F_1 analysis. Adjusted error mean squares reflecting the reduced number of degrees of freedom due to estimated values was calculated and used to test main effects in the F_2 and F_3 generations. The adjusted error term was used to calculate narrow-sense heritability (h^2) and provided a more conservative test because it was larger than the error calculated from the diallel program.

Results

Four soluble sugars (fructose, sucrose, raffinose, and stachyose) were detected in the accessions used in this study. Some studies have reported the presence of verbascose in common bean (Abdel-Gawad, 1993; Fleming, 1981; Kuo et al., 1988; Sathe et al., 1984) whereas others did not (Barampama and Simard, 1993). Verbascose was not detected in the present study and in many of the aforementioned studies it was present in only trace amounts; therefore, it appears to be a minor constituent of bean seed.

In the F_1 generation, values for fructose, sucrose, raffinose, and stachyose ranged from 0.1 to 2.5, 17.1 to 56.5, 0.1 to 4.1, and

Table 3. ANOVA tables for Griffing's (1956) analysis of F_1, F_2, and F_3 data from a diallel of common bean.

Source	df	Mean squares				
		Sucrose	Raffinose	Stachyose	Total sugar	Total oligosaccharides
F_1						
Crosses	63	92.7*	1.5*	77.1*	163.5*	70.3***
GCA	7	449.2*	8.0*	437.5*	878.9***	370.8***
SCA	28	24.9	0.5	31.4	47.2	30.6
Reciprocal	28	71.3*	0.9*	32.7	100.9	34.8
Error	149	45.9	0.4	31.0	107.7	34.0
F_2						
Block	3	175.9***	1.3*	407.1***	1040.9***	394.1***
Crosses	63	48.5**	3.6**	58.1**	99.9**	47.0***
GCA	7	249.7***	26.6***	343.1***	355.5***	213.5***
SCA	28	30.6*	0.6	13.5	66.3	14.8
Reciprocal	28	16.1	0.7*	31.3*	69.5	37.6
Error	146	11.8	0.4	20.2	56.9	24.2
Adjusted error	31		0.3	12.1		
F_3						
Block	3	1063.6***	14.6***	1055.0***	4658.0***	1217.2***
Crosses	63	18.8**	3.6***	38.5***	47.7	31.7***
GCA	7	50.4***	26.9***	263.2***	134.1**	180.1***
SCA	28	21.6**	0.9	13.3	48.2	16.9
Reciprocal	28	8.1	0.5	7.5	24.9	9.5
Error	171	10.9	0.7	11.5	48.1	15.6
Adjusted error	31		0.2	12.4		

*,**,***Significant at $P = 0.05$, 0.01, or 0.001, respectively.

7.6 to 43.7 mg·g^{-1} DW, respectively (means in Table 2). Because fructose was a small percentage of total soluble sugars and was not involved directly in the RFO biosynthetic pathway, it was not subjected to diallel analysis. While the levels of sugars are within the range of those observed in our germplasm screen (McPhee and Myers, 1996) they exceed the ranges from previous studies (Barampama and Simard, 1993; Borejszo and Khan, 1992; Fleming, 1981; Kuo et al., 1988; and Sathe et al., 1984). The lowest levels detected for sucrose were similar to previous reports, but highest levels exceeded reported levels by a factor of two. Conversely, we observed levels that were two to three times lower than those reported in the literature for raffinose and stachyose; but our high levels were similar. Differences in experimental technique might lead to the differences that we observed for those reported in the literature, although from our broader germplasm screen (McPhee and Myers, 1996) we believe that similar quantities of oligosaccharides were detected. In the present study, the F_2 and F_3 values are similar, while the F_1 values differed from the latter generations (Table 2). This was most likely caused by the samples being analyzed at different times and on different types of gas chromatography columns.

Griffing's (1956) analysis. From ANOVA (Table 3), general combining ability (GCA) was significant for all sugars in all three generations. Specific combining ability (SCA) was significant only for sucrose for the F_2 and F_3 data. GCA was, on average, 16.5 times greater than SCA suggesting that additive gene action is most important for accumulation of soluble sugars in common bean seed.

Significant reciprocal effects were detected for sucrose and raffinose in the F_1 and for raffinose and stachyose in the F_2 (Table 3). Reciprocal effects were not significant for any of the sugars in the F_3 generation.

Parental lines with high and low GCA effects were identified for each sugar (Table 4). G13817 produced the largest positive GCA effect for sucrose in all three generations. G18354 and G13817 produced negative GCA effects for raffinose in all three generations. PUE 40 produced the largest positive GCA effect for raffinose accumulation and the largest negative GCA effect for stachyose accumulation.

Hayman's analysis. Hayman's (1954) analysis provides additional information on dominance, epistasis, and heritability from the regression of variance among family means within an array (V_r), on the covariance between families within an array, and their nonrecurrent parent (W_r) (Fig. 1). If assumptions of the additive-dominance model are met, then the slope of the V_r–W_r regression line should not differ significantly from unity. Slopes of the V_r–W_r plots for sucrose (F_2), raffinose (F_2 and F_3), stachyose (F_2 and F_3), and total oligosaccharides (F_2) were not significantly different from unity. However, sucrose in the F_3 and total sugar (F_2 and F_3) had slopes significantly different from unity indicating that one or more of the assumptions of Hayman's (1954) analysis were not met. Inspection of the graphs with regressions not having a slope equal to unity revealed that data points were widely dispersed with small and nonsignificant R^2.

The intersection of the regression line with the ordinate indicates the presence of partial dominance (y-intercept > 0),

Table 4. General combining ability effects for sucrose, raffinose, stachyose, total soluble sugar, and total oligosaccharides from Griffing's (1956) analysis of F_1, F_2, and F_3 data of an eight parent diallel of common bean.

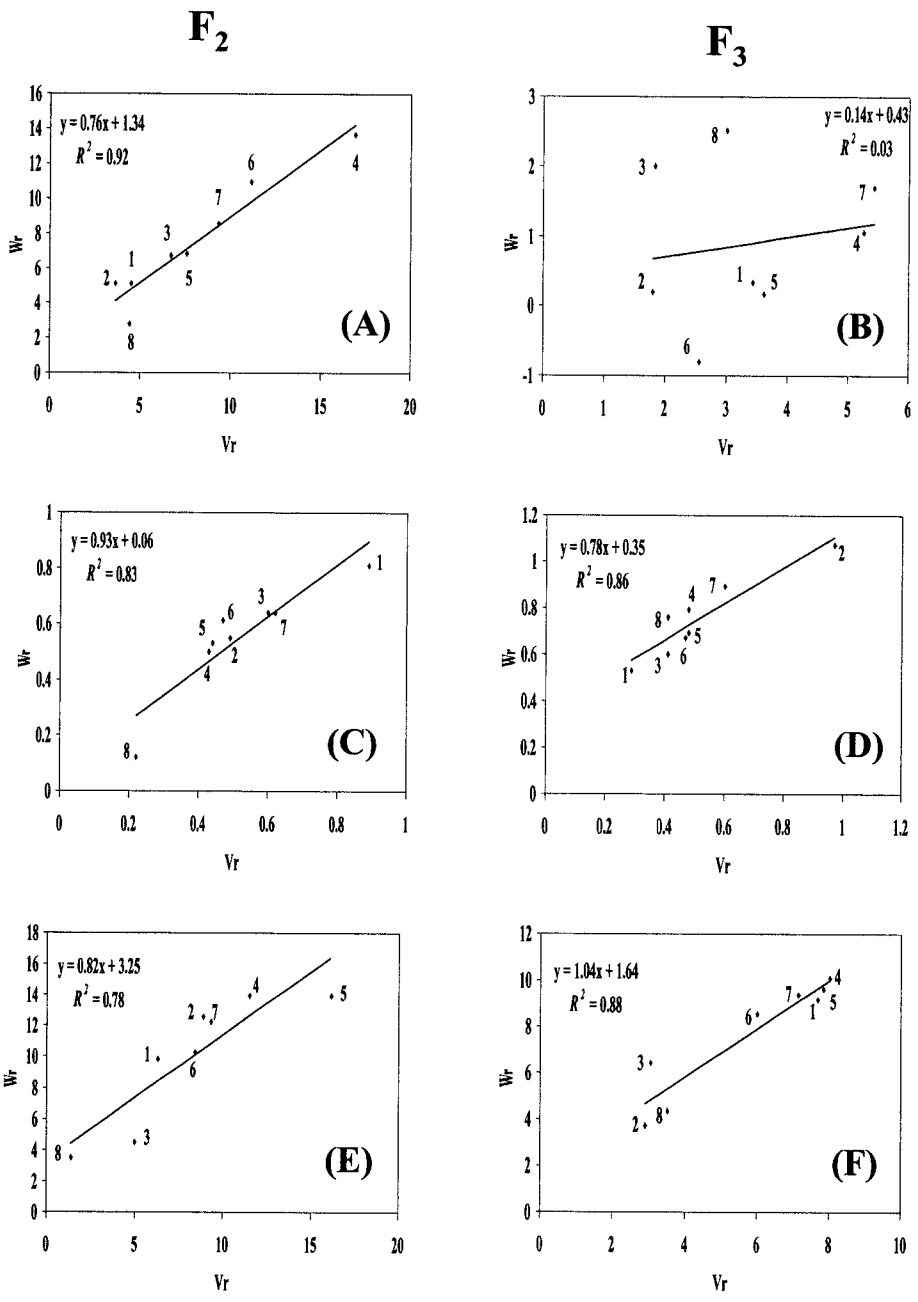
	Sugar (mg·g^{-1} dry wt)				
Parent	Sucrose	Raffinose	Stachyose	Total sugar	Total oligosaccharides
F_1					
Coscorron	–6.6	0.6	1.4	–4.2	2.0
G01106	1.4	0.1	–0.7	0.5	–0.6
G13817	9.9	–0.7	–0.3	9.0	–1.0
G15306A	4.5	–0.3	4.7	8.8	4.4
G18270	–1.2	–0.5	4.4	3.0	3.9
G18354	–0.3	–0.9	0.3	–1.1	–0.6
PR 40	–4.5	0.4	2.2	–2.0	2.6
PUE 40	–3.3	1.2	–11.9	–13.9	–10.7
F_2					
Coscorron	–3.6	0.3	0.1	–3.2	0.4
G01106	–0.8	0.2	1.0	0.5	1.3
G13817	2.4	–0.5	0.4	2.4	–0.1
G15306A	1.6	–0.5	0.6	1.6	0.1
G18270	–0.9	–0.3	2.2	1.0	1.9
G18354	2.0	–0.5	0.2	1.5	–0.3
PR 40	–0.7	0.0	1.0	0.3	0.9
PUE 40	–0.1	1.4	–5.5	–4.1	–4.1
F_3					
Coscorron	–0.4	0.7	1.1	1.7	1.8
G01106	–0.2	0.1	0.2	0.1	0.3
G13817	1.1	–0.5	0.9	1.3	0.4
G15306A	0.9	–0.5	0.5	0.7	0.0
G18270	–1.3	–0.8	1.8	0.1	1.4
G18354	0.1	–0.6	–0.4	–0.9	1.0
PR 40	–1.0	0.0	0.7	–0.2	0.7
PUE 40	0.8	1.2	–4.8	–2.9	–3.6

complete dominance (y-intercept = 0), and overdominance (y-intercept < 0) (Hayman, 1954). While all y-intercepts were positive in the F_2 and F_3 generations, several were not significantly different from zero (Fig. 1).

The position of each parent on the regression line is indicative of the relative number of dominant and recessive alleles possessed by that parent. Those arrays with small V_r and W_r values (clustered near the origin) possess predominantly dominant alleles while those arrays with large V_r and W_r values (clustered distant to the origin) possess predominantly recessive alleles. PUE 40 apparently has dominant alleles for high raffinose and stachyose based on F_2 data. This effect was not as pronounced in the regression plot of F_3 data. Based on F_2 data, Coscorron had recessive alleles for raffinose, but based on F_3 data a shift from alleles behaving as recessive alleles to dominant alleles was observed. The four parents chosen for low raffinose content were grouped near the midpoint of the regression line, suggesting a combination of dominant and recessive alleles.

The parents in this study chosen because of low stachyose content (PUE 40, G13817, 'Coscorron', and G18354) were all grouped near the origin, indicating that low stachyose content is controlled mainly by dominant alleles. The four parents in the study with high stachyose, with the exception of G01106 (which fell near the origin in the F_2 generation), were grouped near the upper right-hand corner of the regression, indicating that high stachyose may be controlled by recessive alleles.

Estimates of h^2 were calculated based on estimates of additive and dominance variation derived from Hayman's (1954) analysis combined with an adjusted error calculated from a two-way analysis of the parents included in the design. Overall, the estimates were low to moderate and differ slightly in magnitude between the F_2 and F_3. Heritability estimates ranged from 0.20 for total sugar accumulation to 0.51 for the raffinose in the F_2, and from 0.11 for sucrose to 0.56 for raffinose accumulation in the F_3 (Table 5). Raffinose had the highest heritability (0.54) and total sugar had the lowest (0.17) when averaged over the two generations.



Discussion

The RFO biosynthesis pathway elucidated by Kandler and Hopf (1980) would suggest a model of a linear chain of substrates acted upon by enzymes specific to each step in the pathway. As such, qualitative differences in gene expression might affect the RFO pathway, but quantitative differences (differences in enzyme efficiency for example), could produce the observed genetic variation. A quantitative approach to the genetic analysis of RFOs was warranted because RFOs are measured as continuous variables, and we had little knowledge of the genetic control and interaction of the maternal and progeny genotypes. The diallel analysis allowed us to examine reciprocal effects from different maternal–paternal combinations. Three generations were investigated to examine the effect of different generations on genetic control of RFOs (including reciprocal effects). Because of time and seed production constraints, all three generations could not be grown in the same environment, which may have led to confounding of environmental and genotypic effects. In general, the three generations showed similar genetic trends for inheritance of RFOs, indicating that while overall levels may vary, the underlying genetic control is similar.

Griffing's (1956) analysis indicated that GCA effects predominate in the RFO pathway. Because of the predominance of additive gene action, it should be possible to select for high or low raffinose oligosaccharides using an appropriate breeding scheme.

Reciprocal differences were observed in the F_1 and F_2, but were absent in the F_3 generation. Reciprocal effects may have

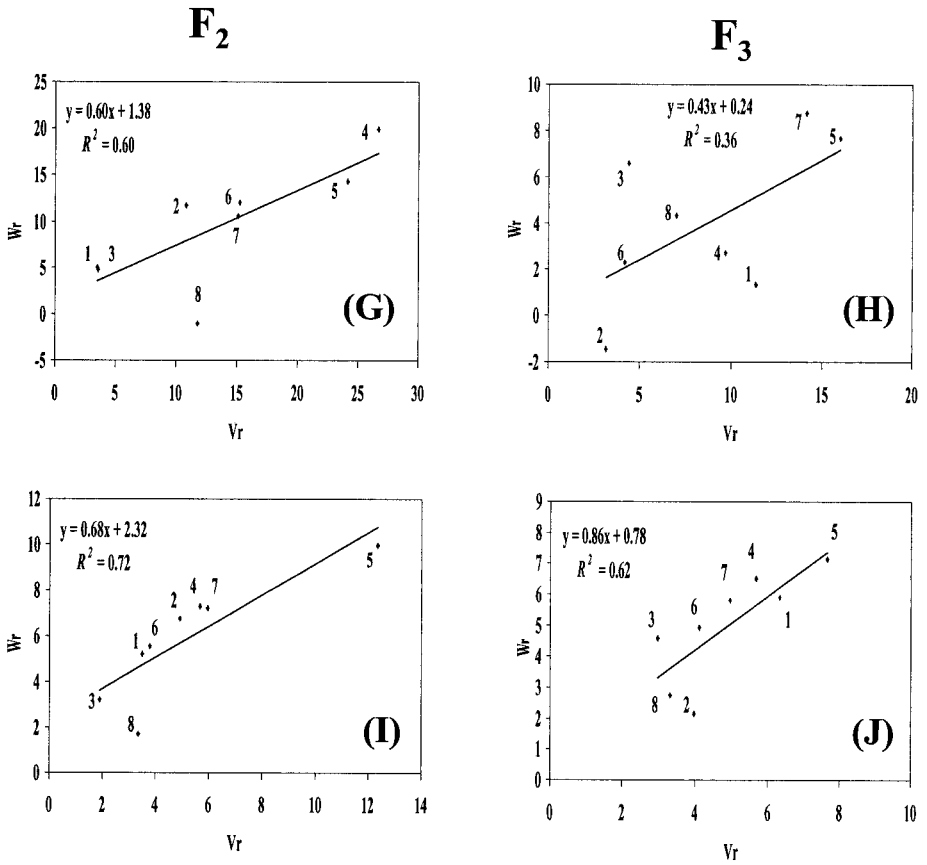


Fig. 1. Vr-Wr regressions for (**A** and **B**) sucrose, (**C** and **D**) raffinose, (**E** and **F**) stachyose, (**G** and **H**) total sugars, and (**I** and **J**) total oligosaccharides for an eight parent dry bean diallel. Graphs for F_2 generation are shown in the first column and graphs for the F_3 generation are in the second column. Points representing individual genotypes are numbered as 1 = 'Coscorron'; 2 = G01106; 3 = G13817; 4 = G15306A; 5 = G18270; 6 = G18354; 7 = PR 40; and 8 = PUE 40.

been due to sampling error, although it would be difficult to attribute reciprocal effects entirely to sampling error when they occurred in two consecutive generations. Reciprocal effects may have been masked in the F_3 by use of a larger sample size (a bulk of 12 seeds vs. five in the F_2). An alternate explanation for reciprocal effects may lie in the nature of the relationship between the parent and embryo genotypes regarding sucrose and RFO accumulation. RFO precursors (galactose, inositol, and sucrose) are synthesized in maternal tissues, then transported to the embryo where galactinol, raffinose and stachyose are synthesized sequentially (Castillo et al. 1990; Saravitz et al. 1987). Galactinol is produced by galactinol synthase (Kandler and Hopf 1980); raffinose and stachyose are then synthesized by the reaction of galactinol with sucrose, and raffinose with galactinol in a stepwise fashion. The genotype of the embryo must control RFO accumulation, but this would not exclude the possibility for the maternal tissues to regulate the pathway. In the present study, reciprocal effects on RFO accumulation could be expected because of genotypic differences between the maternal tissue and the embryo. These effects would disappear with advancing generations, as the maternal and embryonic generations became more similar genetically.

Griffing's (1956) analysis for calculation of GCA and SCA effects requires no additional assumptions other than those that are necessary for ANOVA (Christie and Shattuck, 1991). On the other hand, Hayman's (1954) analysis assumes that an additive-dominant model is appropriate to explain variation in the data. The significant deviation of slopes from unity of the V_r–W_r regression for certain variables indicated that one or more of the assumptions (nondiploid segregation, heterozygous parents, reciprocal differences, epistasis, multiple alleles and linkage disequilibirum) were not met. Of these assumptions, one can ignore the first two because common bean is a self-pollinated diploid. Based on Griffing's (1956) analysis one knows that reciprocal effects were present, although correspondence between significant reciprocal effects and lack of fit to the V_r–W_r regression model was not perfect (Tables 3, Fig. 1). In terms of epistasis, SCA effects for sucrose were significant in the F_2 and F_3. Based on the dispersion of data points on the V_r–W_r regression plots, linkage disequilibrium may have contributed to deviations in the regression equation as well (Christie and Shattuck, 1991). Our conclusion is that one or both of epistasis and linkage disequilibrium were the most likely factors causing lack of fit to the V_r–W_r graph.

High raffinose and low stachyose content both appear to be controlled by dominant alleles. This combined with the fact that raffinose is a precursor for stachyose suggests that accumulation of these two sugars may be closely related and possibly controlled by a common mechanism. We hypothesize that the controlling mechanism is based on a single gene for galactinol : raffinose-6-galactosyltransferase, which is responsible for stachyose synthesis (Kandler and Hopf 1980; Tanner and Kandler 1966). If low stachyose content was controlled by a dominant allele that was inefficient in biosynthesis of stachyose, then raffinose would accumulate and appear to be controlled by a dominant allele—the same dominant allele. If a single gene controlled this trait, then the apparent quantitative behavior may be explained by a combination of difference in efficiency of enzymes contributed by each parent and microenvironmental variation in RFO accumulation. There may also be minor genes that contribute to the quantitative inheritance of this trait.

Overall heritability estimates were higher for the individual RFOs than for sucrose or total soluble sugar accumulation. These results are compatible with the idea of more complex genetic control of sucrose and total sugars than for the individual RFOs,

Table 5. Narrow-sense heritability estimates for sucrose, raffinose, stachyose, total sugar, and total oligosaccharides based on estimates of additive and dominance variation obtained from Hayman's (1954) graphical analysis of data from a common bean eight-parent diallel.

Variable	F_2	F_3	Mean
Sucrose	0.32	0.11	0.22
Raffinose	0.51	0.56	0.54
Stachyose	0.42	0.46	0.44
Total sugar	0.20	0.14	0.17
Total oligosaccharides	0.26	0.27	0.27

as suggested by the analyses of Griffing (1956) and Hayman (1954).

Manipulation of sucrose and RFOs in common bean is feasible because for most variables, the major form of genetic variance is additive and is moderately heritable. In the case of reducing levels of RFOs, it would have been desirable to identify null mutations in galactinol synthase or galactinol : raffinose-6-galactosyltransferase that would completely prevent accumulation of RFOs. However, such mutations were not identified in the present study and are not known in common bean. PUE 40 had the largest negative GCA effect on stachyose, and because stachyose is the prevalent oligosaccharide in bean seed, it would contribute to the greatest overall decrease in RFOs. Parents that appear to increase sucrose but block raffinose accumulation would be G13817, G15306A, and G18354. These parents could be used in recurrent selection based on S_1 progeny means (Hallauer and Miranda, 1981) to decrease levels of RFOs.

Increasing levels of RFOs appears quite feasible, and may have fairly simple genetic control. Ignoring PUE 40 (which had the largest positive GCA effect for raffinose but very large negative GCA effects for stachyose), the parents with the greatest genetic potential to increase RFOs would be 'Coscorron', G01106, and PR 40. In transferring high RFO accumulation to snap beans from dry beans, the inbred-backcross method (Bliss, 1981; Wehrhahn and Allard, 1965) could allow recovery of genetic variation for RFO accumulation in a snap bean background.

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