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Addition of Genes for Dwarf Seed (*ds*) and Spindly Branch (*sb*) to the Linkage Map of Common Bean

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Abstract. **Linkage detection and estimation procedures based on deviation from expected F_2 segregation ratios in common bean (*Phaseolus vulgaris* L.) were used to localize two genes. The product ratio method of estimation was used with four-class segregations, and the maximum likelihood method was used with three-class segregations and for combining multiple sets of data. A tight linkage of 1.6 ± 1.5 map units (m.u.) was found between dwarf seed (*ds*) and dark green savoy leaf (*dgs*), two genes in linkage group VII. A third gene in linkage group VII, stipelless lanceolate leaf (*sl*), was found to be 18.7 ± 1.6 m.u. from *ds*. The distance between *dgs* and *sl* was found to be 21.2 ± 1.0 m.u., thus establishing that *ds* is located between *dgs* and *sl*. This location of *ds* supports the contention that *ds* and tenuis (*te*), a gene described by Lamprecht, are the same gene. In linkage group IX, an estimate of 4.6 ± 1.5 m.u. was obtained for the linkage between diamond leaf (*dia*) and progressive chlorosis (*prc*). Spindly branch (*sb*) was found to be 15.4 ± 0.7 m.u. from *prc* and 11.4 ± 1.1 m.u. from *dia*. Thus, *dia* is located between *sb* and *prc*. The independence of linkage groups VII and IX is demonstrated by the independence of representatives of the two groups.**

The gene linkage map of common bean is rather rudimentary. Lamprecht published a summary of all gene linkages reported in common bean prior to 1961 (6). There were 26 genes in eight linkage groups. Fewer than a dozen genes have been mapped in common bean since 1961. The lack of an inviting foundation of good-quality marker genes probably discouraged interest in mapping genes in bean. Most of the genetic markers in Lamprecht's map are expressed late, i.e., at flowering or later. Also, many of the genes are involved in complex epistatic relationships, which makes them difficult to classify in segregating populations. Furthermore, many of Lamprecht's lines have been lost. Crop species such as corn (*Zea mays* L.), tomato (*Lycopersicon esculentum* L.), barley (*Hordeum vulgare* L.), and pea (*Pisum sativum* L.) are more attractive to researchers, in part because of the availability of fairly detailed gene maps.

Nagata and Bassett (9) reported a linkage of 38 centimorgans (cM) between yellow wax (*y*), a gene in Lamprecht's linkage group VII, and dark green savoy leaf (*dgs*). Linkage intensities of 21 cM between *dgs* and stipelless lanceolate leaf (*sl*) and 12 cM between *sl* and round leaf (*rnd*) also were reported (9). The three new genes mapped to Lamprecht's linkage group VII—*dgs, sl,* and *rnd*—are induced mutants that had been described previously (8). A gene of uncertain origin (2), named dwarf seed (*ds*), also was found to be 29 cM from *rnd,* but its relationship with other genes in linkage group VII was not determined. One of the two possible orientations of *ds* relative to *rnd* would place it at about the same location as tenuis (*te*), a gene mapped by Lamprecht (6). The expression of *ds* (2) reduces pod length by ≈50% and is similar in phenotype to *te* described by Lamprecht (5). These observations lead to the hypothesis that *ds* and *te* may be the same gene. The unavailability of stocks carrying *te* precludes a direct allelism test. However, if *ds* and *te* are the same gene, then *ds* should be located a few map units (m.u.) from *dgs* and between *dgs* and *sl* (9). Such a location implies a linkage between *ds* and *sl* of a few units less than 21 cM, the linkage distance between *dgs* and *sl*.

Nagata and Bassett (9) also reported a linkage between diamond leaf (*dia*) and progressive chlorosis (*prc*), two induced mutants (8). The progressive chlorosis gene was first designated *pc* by Nagata and Bassett (8), but the symbol was changed to *prc* to avoid confusion with the use of *pc* for the persistent green pod color character (3). The linkage between *dia* and *prc* was estimated from two different sets of repulsion phase linkage data to be 1 cM in one instance and 9 cM in the other. The wide variation in estimates raises questions about the reliability of the combined estimate of 6 cM. Continuing efforts to add other genes to the linkage map of common bean have uncovered the linkage of the spindly branch (*sb*) gene to the *dia–prc* linkage group. A description of this marker and evidence supporting its map location are presented here.

The five objectives of this report are to a) test the hypothesis that *ds* and *te* are the same gene, b) to establish the map position of *ds* relative to *dgs* and *sl*, c) improve the accuracy of the estimate for linkage between *dia* and *prc*, d) determine the map location of *sb* relative to *dia* and *prc*, and e) report linkage tests

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between representatives of linkage groups VII and IX to establish the independence of these linkage groups.

Materials and Methods

All the mutant genes used in the present study exhibited single-gene inheritance with no cytoplasmic factors involved (ref. 8; M.J.B., unpublished data). The *ds* gene, previously described by Bassett (2), results in a marked reduction in pod and seed size with deep constrictions in the pod surrounding the seeds (Fig. 1). The *sb* mutant is distinctive by the third-true-leaf stage and produces a characteristic reduction in the size and strength of branches and leaf petioles (Fig. 2), resulting in spindly branches that reflex as plants mature. A reduction in internode length results in a compact plant habit and dense foliage at flowering. The phenotypes of the other mutants used in this research have been described (8).

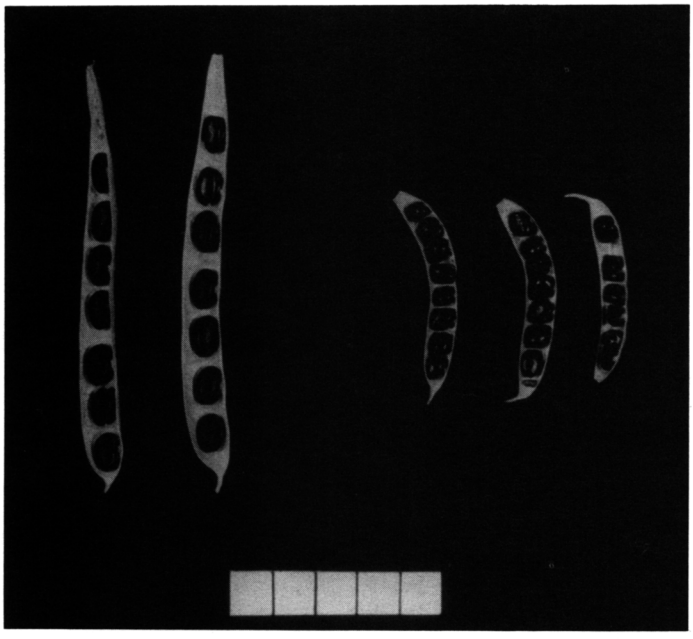

Fig. 1. Pods and seeds from a dwarf seed (*ds/ds*) plant (**right**); pods and seeds from a nearly isogenic normal (*Ds/Ds*) plant (**left**).

Fig. 2. A *spindly branch* plant (**left**) with characteristically thin, drooping, branches and a normal plant (**right**).

Repulsion phase crosses of single mutant stocks (Table 1) were made in the greenhouse in the fall. Whenever double mutants of linked genes were recoverable, coupling phase tests were also conducted (Table 2). The F_1 seed were planted in the greenhouse, and F_2 seed were planted in the field about 1 Apr. in pairs of rows on raised beds. The between- and within-row spacing of plants was 30 and 10 cm, respectively. Within-row spacing of 20 cm was used when the mutant had poor competitive ability with normal plant segregants. The F_2 segregating populations were classified as soon as the different phenotypes in a particular test could be distinguished.

Based on previously established inheritance ratios of 3:1 (normal : mutant) for all the mutant phenotypes involved in the tests, a 9:3:3:1 segregation ratio was expected in crosses involving all unlinked pairs of genes. The F_2 phenotypic ratios were thus tested for deviations from the expected, using the χ^2 test for goodness of fit (7) with a significance level established at $P \leq 0.05$. Precise values of P (the probability that a given deviation from expected could have occurred due to chance) were obtained. When significant P values were found, the product ratio method was used to calculate linkage, provided that all four phenotypic classes were represented. Tables developed by Immer (4) were used to obtain linkage estimates from the product ratio values calculated. In F_2 linkage tests, where the double recessive class was missing, which occurs with tight linkage, the maximum likelihood method was used for estimating linkage intensity. The tables of Allard (1) were employed in applying this method of estimation. The maximum likelihood method, using the tables of Allard, was also used for combining different sets of data (different years, or coupling tests with repulsion tests). The procedure also provides a χ^2 test of homogeneity among the data sets combined. A threshold value of $P \geq 0.25$ was accepted as indicating sufficient homogeneity among data sets.

Results and Discussion

A linkage estimate for *dgs* and *sl* of 20.5 ± 1.34 m.u. obtained by Nagata and Bassett (9) was based on one repulsion phase test and one coupling phase test (included in Tables 1 and 2, respectively). A much larger F_2 population from a repulsion phase test (Table 1) gave a distribution that agreed closely with the two previous tests (Table 3). An improved estimate of 21.2 ± 1.0 m.u. obtained by combining all three sets of data is presented in Table 3.

The initial estimate of linkage between *ds* and *sl* was obtained from a small F_2 population of 481 plants, but a second estimate based on a larger F_2 population of 3205 plants agreed fairly closely with it (Table 1). The χ^2 test of homogeneity (Table 3) indicated similar distributions in the two sets of data and thus increased confidence in the combined estimate of 18.7 ± 1.6 m.u.

Three progressively larger F_2 populations, up to 2688 plants, from repulsion phase crosses of *ds* and *dgs* did not contain any double recessives (Table 1). These results are interpreted to indicate a tight linkage between the two genes. The combined estimate of linkage between *dgs* and *ds* is 1.6 ± 1.5 m.u. The

Table 1. Linkage relationships determined from F_2 segregation data obtained from repulsion phase linkage tests to localize *ds* and *sb*.

Cross	Phenotypic classification: Normal	Mutant 1	Mutant 2	Double mutant	χ^2 (9:3:3:1)	*P*	Product ratio	Map units[z]
dgs x *sl*[y]	271	148	130	9	68.4	<0.001	0.127	23.5 ± 2.7
dgs x *sl*	2033	930	927	47	274.1	<0.001	0.111	22.1 ± 1.0
ds x *sl*	292	91	96	2	28.3	<0.001	0.067	17.6 ± 3.0
ds x *sl*	1603	775	798	29	283.7	<0.001	0.075	18.6 ± 1.1
ds x *dgs*	246	99	111	0	38.7	<0.001	---[x]	0.6 ± 3.2
ds x *dgs*	978	332	279	0	112.3	<0.001	---[x]	2.5 ± 2.5
ds x *dgs*	1645	525	518	0	181.0	<0.001	---[x]	2.5 ± 1.9
dgs x *y*[y]	346	141	136	23	13.4	0.004	0.415	37.7 ± 2.2
prc x *dia*[y]	298	154	110	1	56.7	<0.001	0.018	9.3 ± 2.8
prc x *dia*[y]	499	234	220	0	88.8	<0.001	---[x]	1.1 ± 3.2
prc x *dia*	1477	683	718	1	287.3	<0.001	0.003	3.9 ± 1.3
sb x *prc*	259	122	133	3	50.0	<0.001	0.048	15.0 ± 2.9
sb x *dia*	1763	916	786	11	349.5	<0.001	0.027	11.4 ± 1.1

[z]Map units expressed as percentage of recombination ± SE.
[y]Data obtained from Nagata and Bassett (9).
[x]Indicates crosses in which no product ratio could be calculated because one phenotypic class was missing; map units were calculated using the maximum likelihood equation instead.

Table 2. Linkage relationships determined from F_2 segregation data obtained from coupling phase linkage tests to localize *ds* and *sb*.

Mutants[z] tested	Phenotypic classification: Normal	Mutant 1	Mutant 2	Double mutant	χ^2 (9:3:3:1)	*P*	Product ratio	Map units[y]
dgs + *sl*[x]	639	93	84	160	273.2	<0.001	0.076	20.0 ± 1.0
dgs + *y*	446	84	94	52	36.5	<0.001	0.340	35.5 ± 1.6
dgs + *y*	356	92	82	39	12.1	0.007	0.543	41.6 ± 1.9
sb + *prc*	2108	136	246	402	879.4	<0.001	0.039	15.1 ± 0.5

[z]Double-mutant plants pollinated with normal parent (182-1).
[y]Map units expressed as percentage of recombination ± SE.
[x]Data obtained from Nagata and Bassett (9).

Table 3. Recombination values obtained by combining different linkage tests with the maximum likelihood equation.

Mutant pairs tested	Recombination[z] value (%)	χ^2 test[y]	df	*P*
dgs and *sl*	21.2 ± 1.0	1.6	2	0.443
ds and *sl*	18.7 ± 1.6	0.1	1	0.773
ds and *dgs*	1.6 ± 1.5	0.1	2	0.966
dgs and *y*	37.9 ± 1.6	2.2	2	0.334
prc and *dia*	4.6 ± 1.5	2.1	2	0.340
sb and *prc*	15.4 ± 0.7	0.3	1	0.566

[z]Recombination percentage ± SE.
[y]Test of homogeneity among the data sets used to determine the recombination values.

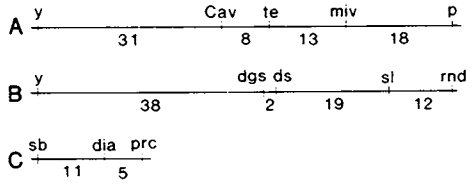

Fig. 3. (**A**) A portion of Lamprecht's linkage group VII showing the location of *y*, tying it to the linkage group shown in B. (**B**) The new markers in linkage group VII, showing the addition of *ds* (probably a synonym for *te*). (**C**) Linkage group IX, showing the addition of *sb* and an improved estimate for *dia*–*prc*.

similarity of the three data sets is indicated by a high *P* value for the χ^2 test of homogeneity (Table 3).

Rounding the linkage estimates discussed above to the nearest centimorgan gives the following values: 19 cM for *ds* to *sl*, 21 cM for *dgs* to *sl*, and 2 cM for *ds* to *dgs*. These estimates preclude any arrangement of the three genes other than that indicated in Fig. 3B, where *ds* is located between *dgs* and *sl*. This location of *ds* corresponds closely to that of *te* (Fig. 3A) and thus lends credence to the hypothesis that tenuis and dwarf seed are synonyms for the same gene.

The report of linkage between *dgs* and *y*, indicating that the linked genes *dgs*, *ds*, *sl*, and *rnd* are in Lamprecht's linkage group VII (Fig. 3A), was based on a single set of repulsion phase data (9). These data are reproduced in Table 1 and confirmed by two sets of coupling phase data in Table 2. Since the combined estimate of 37.9 ± 1.6 m.u. (Table 3) is not different from the original estimate of 37.7 ± 2.2 m.u. (9), the approximation of 38 cM is confirmed.

Nagata and Bassett (9) obtained a combined estimate of 6.35 ± 2.55 m.u. for the linkage between progressive chlorosis (*prc*) and diamond leaf (*dia*) from two sets of repulsion phase data (Table 1). The one double-mutant plant observed did not flower. In a third repulsion phase test involving 2879 F_2 plants, we

Table 4. Data from F_2 populations showing the independence of linkage groups VII and IX.

No.	Cross[z]	Phenotypic classification: Normal	Mutant 1	Mutant 2	Double mutant	χ^2 (9:3:3:1)	*P*
1	*sb* x *y*	267	91	86	31	0.21	0.976
2	*dgs* x *sb*	287	84	86	21	4.36	0.225
3	*dgs* x *dia*	303	115	106	38	1.66	0.645
4	*rnd* x *dia*	334	107	95	32	2.25	0.523
5	*dgs* x *prc*	324	130	87	36	2.69	0.443
6	*rnd* x *prc*	207	76	78	19	2.32	0.508
7	*prc* x *sl*	358	123	113	37	0.60	0.896

[z]Crosses 3 through 7 obtained from unpublished work by Nagata and Bassett.

expected to produce several double mutant segregants that could be used for a coupling phase test, but the F_2 contained only one such phenotype and it did not flower. The three repulsion phase tests (Table 1) were combined to produce an estimate of 4.6 ± 1.5 m.u. (Table 3).

A repulsion phase cross of spindly branch (*sb*) and *prc* indicated a linkage of 15.0 ± 2.9 m.u. (Table 1). A large F_2 population from a coupling phase cross confirmed this linkage with the almost identical estimate of 15.1 ± 0.5 m.u. (Table 2). The combined estimate was 15.4 ± 0.7 m.u., which had an acceptable homogeneity χ^2 value (Table 3).

A single repulsion phase test gave a linkage estimate of 11.4 ± 1.1 m.u. between *sb* and *dia* (Table 1). Rounding of the estimates of linkage in this group produced the following values: 5 cM for *prc* to *dia*, 15 cM for *sb* to *prc*, and 11 cM for *sb* to *dia*. The only gene order consistent with these data is *sb* – *dia* – *prc* (Fig. 3C).

The independence of linkage group IX from Lamprecht's linkage group VII is supported by the independent segregation of genes from the two groups (Table 4). The possibility of linkage group IX being a part of linkage group VII cannot be ruled out completely until markers covering one or both chromosomes are available, or appropriate primary trisomic stocks are available. Until then, it is proposed that the *sb* – *dia* – *prc* group be designated linkage group IX, to maintain continuity with previously published work (9).

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