

# Inheritance of Crippled Trifoliolate Leaves Occurring in Interracial Crosses of Common Bean and Its Relationship With Hybrid Dwarfism

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Crippled trifoliolate leaves were observed in two lines from interracial populations of common bean (*Phaseolus vulgaris* L.). The lines, WA 7807-305 and TY 5578-220, were F<sub>2</sub>-derived F<sub>6</sub> recombinant inbreds from populations Rio Tibagi/ABA 58 and Brasil 2/ICA L 23, respectively. Rio Tibagi and Brasil 2 are small-seeded, grow in tropical lowlands, and belong to the race Mesoamerica. In contrast, ABA 58 and ICA L 23 belong to the large-seeded Andean race Nueva Granada. The two lines were crossed with each other, with one of their normal parents, and with tester stocks. The normal parents and tester stocks carried either the *DI1* or *DI2* gene. These genes are complementary dominant, dosage dependent lethal, and known to cause hybrid dwarfism or plant stunting. The recessive gene *lcr* causes trifoliolate leaf crippling in the presence of either *DI1* or *DI2*. When both *DI1* and *DI2* are present, a dwarf-lethal or semilethal phenotype is produced, irrespective of the alleles at the *lcr* locus. Thus, the genotypes of the lines are, for WA 7807-305: *DI1 DI1 dl2 dl2 lcr lcr*, and for TY 5578-220: *dl1 dl1 DI2 DI2 lcr lcr*. Other reported cases of developmental abnormalities are reviewed and their significance for common bean improvement is discussed.

Burkholder and Muller (1926) reported two types of morphological abnormalities in common bean (*Phaseolus vulgaris* L.): leaf abnormalities, which they called "pseudomosaic," and seedling wilt. In crosses with parents of normal growth and development, the inheritance of each abnormality was found to be controlled by duplicate recessive genes. Leaf variegation, resembling mosaic (Zaumeyer 1938, 1942); seedling wilt and leaf rolling (Providenti and Schroeder 1969); crippled morphology, resembling viral symptoms (Coyne 1965); and foliage variegation (Aggour and Coyne 1989; Coyne and Steadman 1977; Finke et al. 1986) were also each reported to be controlled by duplicate recessive genes. Moreover, Aggour and Coyne (1989) and Finke et al. (1986) reported

segregation for crippled growth in the same crosses that segregated for foliage variegation. The crippled growth was inherited by three recessive genes acting additively (Finke et al. 1986). Developmental expression of the character for crippled growth varied considerably, and linkage between foliage variegation and crippled growth was found. Wade (1941) found a variegation that was inherited through the homozygous recessive interacting at any of the three loci with dominant alleles from other loci, or through the recessive alleles interacting among themselves at all three loci. Thus the F<sub>2</sub> segregation ratio was 27 normal:37 variegated. In contrast, a single recessive gene controlled the inheritance of a pale phenotype, resulting from chlorophyll deficiency (Smith 1934), and a mosaic-like, crinkled-leaved, variegated rogue (Coyne 1969).

Since 1979 we have observed crippling, that is, trifoliolate leaves that are either deformed or with virus-like symptoms, in plants from intergene-pool and interracial segregating populations and in some advanced generation lines derived from such populations. The symptoms (Figure 1B-D,G) begin with narrowing or acuteness at the base (i.e., above the pulvinus) of newly formed developing trifoliolate leaves, followed by the appearance of fine yellow streaks (Singh and Molina 1991). Primary leaves, however, are normal. Thus the normal cordate or ovate leaflet shape is modified to a lanceolate shape. Symptom expression may be delayed, and although large variations in severity occur, marked stunting and death of young seedlings is unusual. Nevertheless, in more severe and advanced stages, the interveinal laminate section of the leaf blade is shrunken, corrugated, crinkled, deformed, or crippled, and is yellowish-green (resembling viral symptoms). The leaves also may develop one or more holes or scars, thus adversely affecting leaf photosynthesis. Plants with crippled leaves usually flower and produce pods (Figure 1C), but they have an abnormal appearance and their overall vegetative growth (Figure 1G) and seed yield are greatly reduced.

So far crippled leaves have been found only in interracial and intergene-pool crosses. For example, the population Carioca/Guanajuato 31 gave rise to lines A 252 and A 445, which show crippled leaves in certain cool environments (e.g., at 1750 m altitude, Popayán, Colombia). Similarly, line MAM 13, derived from population A 175/Zacatecas 48, shows this trait at Aguascalientes, Mexico (2040 m eleva-

tion). Both Carioca and A 175 are small seeded, grow in tropical lowlands, and belong to the Mesoamerica race of common bean. Guanajuato 31 and Zacatecas 48, in contrast, grow in highlands and belong to the Durango race from Mexico (Singh et al. 1991).

The symptoms of pseudomosaic described and photographed by Burkholder and Muller (1926) are similar to those described for leaf "distortion" by Rabakoarihanta and Baggett (1983), which in turn resemble those reported for crippled leaves in this article (Figure 1B). Any differences in symptoms can be attributed to differences in growing environments and the extent to which the genotypes showing crippled leaves possess characteristics from Andean races (as in Burkholder and Muller 1926) or Middle American races (as in Rabakoarihanta and Baggett 1983; Figure 1B-D,G). However, whereas the first authors reported the trait as controlled by two recessive genes with F<sub>2</sub> segregation ratios of 15 normal:1 pseudomosaic, Rabakoarihanta and Baggett (1983) reported F<sub>2</sub> segregation ratios of 13 normal:3 leaf "distortion" or 1 normal:3 leaf "distortion," depending on the parents used in crosses.

York and Dickson (1975) reported a "semilethal" or "crippled" condition and van Rheenen (1979) a "sublethal" character in common bean as being controlled by two complementary dominant genes. The seedlings germinate normally, but their growth becomes markedly reduced, the primary and trifoliolate leaves become chlorotic, and the young seedlings usually die. These symptoms are similar to those of seedling wilt and leaf rolling, apparently controlled by two recessive genes (Burkholder and Muller 1926; Providenti and Schroeder 1969).

Shii et al. (1980, 1981) also studied in detail these dominant developmental abnormalities and found that, first, the two complementary dominant lethal genes, *DI1* and *DI2*, had differential expression in plant organs: the effect of *DI1* was expressed only in roots and that of *DI2* only in shoots. Second, the severity of plant stunting depended on allelic dosage: double homozygotes (*DI1 DI1 DI2 DI2*) are severely stunted, turn chlorotic, and may die a few days after germinating. In contrast, double heterozygotes (*DI1 dl1 DI2 dl2*) exhibit comparatively less stunting, survive for longer periods, and occasionally produce a few flowers and pods. Third, the genes' adverse effects on plant growth and development could be minimized or over-



**Figure 1.** Trifoliolate leaf crippling and hybrid dwarfism observed in interracial and intergene-pool crosses of common bean. A, E, F, H, and J are normal leaf and/or plants; B, C, D, and G show the crippled leaf trait; I shows F, hybrid dwarfism between two normal parents H and J.

come by changing growing temperatures (moving stunted genotypes from warm to cool environments), by hormonal treatments, or by grafting scions of stunted seedlings a few days old on to appropriate root stocks that do not possess the *D11* allele.

Subsequently, Singh and Gutiérrez (1984) demonstrated that the *D11* and *D12* were found only in small-seeded and large-

seeded races, respectively. Gepts and Bliss (1985) reported that common bean accessions carrying *D11* possessed the S type (for cv. Sanilac) of phaseolin seed protein, whereas *D12* carriers were of the T (for cv. Tendergreen) or C (for cv. Contender) phaseolin types, occurring in Middle American and Andean gene pools, respectively. Since then, Vieira et al. (1989) reported hybrid dwarfism in crosses of

small- and large-seeded Brazilian common bean cultivars, and Koinange and Gepts (1992) reported it in crosses between wild common bean populations from Middle America and the Andes. Most of the crosses used by the earlier researchers were, as far as we could tell, between different races (Table 1) (Singh et al. 1991) or gene pools (Gepts and Bliss 1985).

We studied (1) the inheritance of crip-

Table 1. Races involved in some crosses segregating for crippled trifoliolate leaves in common bean studies by early researchers (1926–1977)

Female		Male		Abnormality	Reference
Identification	Race	Identification	Race		
White Marrow	Chile	Flat Marrow	Durango	Pseudomosaic	Burkholder and Muller (1926)
Well's Red Kidney	Nueva Granada	Robust Pea Bean	Mesoamerica	Seedling wilt <sup>a</sup>	Burkholder and Muller (1926)
Robust	Mesoamerica	Pink	Durango	Pale phenotype	Smith (1934)
Small White	Mesoamerica	Corbett Refugee	Nueva Granada	Leaf variegation	Zaumeyer (1942) <sup>b</sup>
Perry Marrow	Chile	Great Northern	Durango	Leaf variegation	Zaumeyer (1942)
Cranberry	Chile	Pinto	Durango	Leaf variegation	Zaumeyer (1942)
G.N. Nebraska #1	Durango	Yellow Eye PI 209806	Nueva Granada	Crippled	Coyne (1965)
G.N. Nebraska #1	Durango	Dark Red Kidney	Nueva Granada	Crippled	Coyne (1965, 1966)
Red Kidney	Nueva Granada	Scotia	Mesoamerica	Seedling wilt <sup>a</sup>	Provvidenti and Schroeder (1969)
Red Kidney	Nueva Granada	Black Turtle	Mesoamerica	Leaf rolling	Provvidenti and Schroeder (1969)
PI 165426	Mesoamerica	P-73-121-7	Durango	Leaf variegation	Coyne and Steadman (1977)

<sup>a</sup> Likely to give hybrid dwarfism.

<sup>b</sup> Had other crosses that segregated for leaf variegation.

pled leaves found in lines WA 7807-305 and TY 5578-220, and (2) the relationship between crippled leaves and hybrid dwarfism controlled by the *D11* and *D12* genes. We also discuss their significance for common bean improvement.

## Materials and Methods

Three out of 79 random  $F_2$ -derived  $F_6$  lines ( $F_{2.6}$ ) in population WA 7807 and 6 out of 79  $F_{2.6}$  lines in population TY 5578 showed crippled leaves at the CIAT farm at Popayán (1750 m elevation, mean growing temperature 18°C) in 1990. Of these lines, WA 7807-305 and TY 5578-220 from the two respective populations had high and uniform expression of the trait. Population WA 7807 is a cross between Rio Tibagi and ABA 58; and TY 5578 is a cross between Brasil 2 and ICA L 23. Rio Tibagi possesses an indeterminate, upright growth habit and small black seeds. ABA 58 is a determinate line with large white seeds. Brasil 2 has a determinate growth habit with small cream seeds. ICA L 23 is a determinate, erect line with large, reddish-brown, mottled seeds. Rio Tibagi and Brasil 2 belong to the tropical lowland race Mesoamerica (Singh et al. 1991), and ABA 58 and ICA L 23 possess principal characteristics

of the Andean race Nueva Granada. All four parents possess the dominant *I* gene conferring hypersensitive type of resistance to bean common mosaic virus (BCMV).

WA 7807-305 was crossed with TY 5578-220 by using manual emasculation and pollination. The  $F_1$  seed, along with that of the two parents, was sown in the field at Popayán. The  $F_1$  germinated normally but, after a few days, the seedlings were stunted, with pale yellowish-green primary and trifoliolate leaves (i.e., showing typical hybrid dwarfism). Because of this and from previous work (Singh and Gutiérrez 1984), we knew that Rio Tibagi and ICA L 23 were carriers of the *D11* and *D12* genes, respectively. These genes, therefore, seemed to be involved in the expression of crippled leaves in WA 7807-305 and TY 5578-220. Therefore, both lines were crossed with one of their normal parents and with independent tester stocks known either to possess *D11* (Carioca) or *D12* (A 195) (Tables 2, 3). To produce  $F_2$  seed, attempts were made to graft some  $F_1$  seedlings of WA 7807-305/TY 5578-220 onto root stocks of noncarrier genotypes such as Brasil 2, ABA 58, and ICA Pijao. Similarly, to produce seeds for  $F_2$  and/or backcrosses to

both parents of A 195/WA 7807-305, ICA L 23/WA 7807-305, Carioca/TY 5578-220, and TY 5578-220/WA 7807-305, grafting on root stocks of appropriate genotypes was necessary. Also, in each case, some  $F_1$  plants were allowed to grow undisturbed for further observations.

Parents,  $F_1$ ,  $F_2$ , and some backcrosses were sown in the field at Popayán. The distance between rows was 50 cm and between seeds within rows about 20 cm. All seeds were treated with fungicides and insecticides to prevent root rots and damage from insects. The plots were fertilized adequately and kept free of weeds, diseases, and insects throughout the growing season. Data for plant dwarfing, crippled leaves, and normal morphology were recorded, beginning 2 weeks after germination, and verified periodically until flowering began. Although some variation was found within each class of dwarfing and leaf crippling, for data analyses only three classes were recognized: dwarf, crippled leaf, and normal plant. Data were subjected to the chi-square test for goodness-of-fit of the expected segregation ratios.

## Results

When the line WA 7807-305, with crippled leaf trait, was crossed with one of its normal parents, Rio Tibagi, the  $F_1$  hybrid was vigorous, with normal plant height and dark green primary and trifoliolate leaves (Table 2 and Figure 1A,E,F). A similar phenotype was observed when the line was crossed with an unrelated cultivar, Carioca, that possessed the *D11* gene. But when WA 7807-305 was crossed with other normal genotypes carrying the *D12* gene, such as lines ICA L 23 and A 195, all  $F_1$  plants had stunted growth and exhibited hybrid dwarfism (e.g., Figure 1I). The  $F_2$  populations of WA 7807-305 with ICA L 23 and A 195, despite some excess (over expected

Table 2. Phenotypes of the  $F_1$  from crosses between lines with crippled leaf trait, WA 7807-305 and TY 5578-220, and normal parents and tester stocks of known genotypes for *D11* and *D12* genes, evaluated at CIAT, Popayán, Colombia, during 1991 and 1993

Normal leaf	<i>D11</i> and <i>D12</i> genotypes	Crippled leaf	$F_1$ phenotype
Rio Tibagi	<i>D11 D11 d12 d12</i>	WA 7807-305	Normal
Carioca	<i>D11 D11 d12 d12</i>	WA 7807-305	Normal
A 195	<i>d11 d11 D12 D12</i>	WA 7807-305	Dwarf
ICA L 23	<i>d11 d11 D12 D12</i>	WA 7807-305	Dwarf
ICA L 23	<i>d11 d11 D12 D12</i>	TY 5578-220	Normal
A 195	<i>d11 d11 D12 D12</i>	TY 5578-220	Normal
Rio Tibagi	<i>D11 D11 d12 d12</i>	TY 5578-220	Dwarf
Carioca	<i>D11 D11 d12 d12</i>	TY 5578-220	Dwarf
WA 7807-305 <sup>a</sup>		TY 5578-220	Dwarf

<sup>a</sup> Crippled leaf.

**Table 3. Segregation for dwarf, normal, and crippled-leaved phenotypes in F<sub>2</sub> and backcross populations of common bean evaluated at CIAT, Popayán, Colombia, during 1991 and 1993**

Cross	Phenotype segregation			Expected ratio	χ <sup>2</sup>
	Dwarf (D)	Normal (N)	Crippled (C)		
ICA L 23/TY 5578-220	0	1,571	523	3N:1C	0.00
ICA L 23//ICA L 23/TY 5578-220	0	152	0	1N:0C	0.00
TY 5578-220//ICA L 23/TY 5578-220	0	52	57	1N:1C	0.23
ICA L 23/WA 7807-305	210	195	46	36D:22N:6C	18.16 <sup>ab</sup>
ICA L 23//ICA L 23/WA 7807-305	70	58	0	1D:1N	1.12
WA 7807-305//ICA L 23/WA 7807-305	20	12	9	2D:1N:1C	0.46
A 195/TY 5578-220	0	2,349	808	3N:1C	0.59
A 195//A 195/TY 5578-220	0	27	0	1N:0C	0.00
TY 5578-220//A 195/TY 5578-220	0	30	37	1N:1C	0.73
A 195/WA 7807-305	148	119	29	36D:22N:6C	5.04 <sup>a</sup>
A 915//A 195/WA 7807-305	32	45	0	1D:1N	2.19
WA 7807-305//A 195/WA 7807-305	12	4	5	2D:1N:1N	0.52
Carioca/TY 5578-220	33	39	13	36D:22N:6C	11.04 <sup>ab</sup>
Carioca//Carioca/TY 5578-220	35	32	0	1D:1N	0.13
TY 5578-220/WA 7807-305	17	0	15	9D:1N:6C	2.81
Carioca/WA 7807-305	0	147	39	3N:1C	1.61
Carioca//Carioca//WA 7807-305	0	22	0	1N:0C	0.00
WA 7807-305//Carioca/WA 7807-305	0	16	8	1N:1C	2.67

<sup>a</sup>  $P < .05$ , in all others,  $P > .05$ .

<sup>b</sup> The data for these three F<sub>2</sub> populations fit better the expected segregation ratio of 27 dwarf:22 normal:6 crippled (see text for explanation).

values) of normal plants and deficiency of dwarfs, tended to segregate into a ratio of 36 dwarf:22 normal:6 crippled leaf (Table 3). Their respective backcrosses with normal parents gave a good fit to the expected ratio of 1 normal:1 dwarf. The backcrosses with W 7807-305 gave a good fit to the segregation ratios of 2 dwarf:1 crippled leaf:1 normal.

When line TY 5578-220, with the crippled trifoliolate leaf trait, was crossed with normal genotypes of ICA L 23 and A 195, carrying the *Dl2* gene, all F<sub>1</sub> plants were normal. But, when TY 5578-220 was crossed with Carioca and Rio Tibagi, both possessing the *Dl1* gene, the F<sub>1</sub> plants showed stunted growth and other typical symptoms of hybrid dwarfism. Furthermore, the F<sub>2</sub> population between Carioca and TY 5578-220 segregated into a ratio of 36 dwarf:22 normal:6 crippled leaf. The backcross with Carioca gave a good fit to 1 normal:1 dwarf.

The F<sub>1</sub> from the two lines with crippled leaves, TY 5578-220 and WA 7807-305, was stunted with pale yellow primary and trifoliolate leaves. Although the population size in F<sub>2</sub> was small and no normal segre-

gants were observed, a good fit to 9 dwarf:6 crippled leaf:1 normal was found.

### Discussion

In three F<sub>2</sub> populations, namely, A 195/WA 7807-305, ICA L 23/WA 7807-305, and Carioca/TY 5578-220, normal segregants exceeded expected values, whereas dwarf segregants were fewer (Table 3). Some dwarf plants, being slow growing and weak competitors, were probably lost in a mixture of populations also segregating for normal and crippled leaves before they could be scored. But because relatively cooler temperatures help reduce the interaction effects of the *Dl1* and *Dl2* (Shii et al. 1981), some segregants, heterozygous for both genes, also may have resembled normals and hence were possibly misclassified. But an expected segregation ratio of 27 dwarf:22 normal:6 crippled gives a better fit in all three F<sub>2</sub> populations (A 195/WA 7807-305, ICA L 23/WA 7807-305, and Carioca/TY 5578-220), segregating simultaneously at the three loci (*Dl1*, *Dl2*, *lcr*) than the tested ratio of 36 dwarf:22 normal:6 crippled (Table 3). An alterna-

tive explanation could be that the genotype *Dl1-Dl2-lcr lcr* is lethal during postfertilization and embryo formation, so that no mature seed would be formed with this genotype. Thus, one-fourth (or 9 seeds) of 36 dwarf genotypes would be absent from the F<sub>2</sub> populations. Hence, among the seedlings that are planted, the expected ratio would be 27 dwarf:22 normal:6 crippled. Nonetheless, distorted segregations in common bean were also reported by Koenig and Gepts (1989), Nodari et al. (1993), Paredes and Gepts (1995), and Welsh et al. (1995). To resolve these factors in future genetic studies, all seeds should be planted in sterilized soils and in individual pots under controlled humidity and temperatures. Moreover, a complete record should be kept of the number of seeds sown and germinated and the plants scored at different growth stages.

Table 2 shows that the occurrence of crippled trifoliolate leaves in common bean is a recessive trait. This conforms with the relatively small frequency of lines with this character found in the original populations, that is, 3 out of 79 F<sub>2,6</sub> random lines in population WA 7807 and 6 out of 79 F<sub>2,6</sub> lines in population TY 5578. Moreover, while the weak cripples are more likely to be lost during advancement of filial generations because of their poor competitive ability, the expression of crippled leaves is dependent on the presence of the *Dl1* or *Dl2* allele such that when both these alleles are absent leaf crippling is not observed. But when both *Dl1* and *Dl2* are present, the phenotype is stunted, confirming the earlier observations of Shii et al. (1980), Singh and Gutiérrez (1984), van Rheenen (1979), and York and Dickson (1975). The symbol *lcr* (for trifoliolate leaf crippling) is assigned to the recessive gene. Thus the genotype of WA 7807-305 is *d11 dl1 dl2 dl2 lcr lcr* and of TY 5578-220 is *d11 dl1 Dl2 Dl2 lcr lcr*.

Genotypes possessing both *Dl1* and *Dl2* would not survive on their own in warm tropical and subtropical environments. Hence, from the genotypes of WA 7807-305 and TY 5578-220, we can conjecture that, at a time, only a maximum of two genes would segregate in the cross between two normal parents to produce progeny with crippled trifoliolate leaves. Moreover, the F<sub>2</sub> segregation ratios would be 13 normal:3 crippled leaves. The F<sub>2</sub> between a crippled leaf genotype (e.g., *d11 dl1 Dl2 Dl2 lcr lcr*) and any of the four possible true-breeding normal genotypes would segregate in ratios as in Table 4.

Similarly, in a cross between two lines

**Table 4. Expected phenotypic segregation ratios in F<sub>2</sub> between a crippled leaf genotype (e.g., *d11 dl1 Dl2 Dl2 lcr lcr*) and the four possible true-breeding normal leaf genotypes**

Normal leaf genotype	F <sub>2</sub> segregation ratios
<i>d11 dl1 dl2 dl2 lcr lcr</i>	3 crippled:1 normal
<i>d11 dl1 dl2 dl2 Lcr Lcr</i>	13 normal:3 crippled
<i>d11 dl1 Dl2 Dl2 Lcr Lcr</i>	3 normal:1 crippled
<i>Dl1 Dl1 dl2 dl2 Lcr Lcr</i>	36 dwarf:22 normal:6 crippled

with crippled leaf of different origins (e.g., WA 7807-305 and TY 5578-220), the  $F_1$  would show hybrid dwarfism and only two genes (*D11* and *D12*) would segregate in the  $F_2$ , as observed in this study. Thus, a cross of crippled leaf (*dl1 dl1 D12 D12 lcr lcr*) with normal leaf (*dl1 dl1 D12 D12 Lcr Lcr*) would explain how Smith (1934) found a single recessive gene controlled chlorophyll deficiency as did Coyne (1969) for the mosaic-like, crinkled leaf, variegated rogue. But how Wade (1941) got an  $F_2$  segregation ratio of 27 normal : 37 variegated in a cross of a variegated  $\times$  normal is unclear to us. He may have been unable to distinguish between the dwarf (probably his Figure 1A,B) and crippled or variegated leaf (Figure 1C–F), thus misclassifying some variegated genotypes as normals. A ratio of 36 dwarf : 22 normal : 6 crippled or variegated leaf would have been expected from his  $F_2$  populations. Moreover, we cannot rule out the possibilities that (1) the evaluation environment affected or modified the gene interactions and (2) Wade was dealing with completely different morphological abnormalities.

Because hybrid dwarfism and trifoliolate leaf crippling are interrelated, we emphasize that two types of developmental abnormalities affect plant morphology in crosses between gene pools and between races of common bean. The first type is a dominant trait and is characterized first by marked stunting of young seedlings (Figure 1I), which otherwise germinate normally. Relatively warmer temperatures (e.g., 24°C) favor expression of this abnormality, which is controlled by two complementary dominant genes, *D11* and *D12*.

The second type of morphological abnormality—crippled leaves—is recessive, is expressed only in trifoliolate leaves (Figure 1B–D,G), and is not usually accompanied by marked plant stunting (especially in early growth stages) or death. Moreover, symptom expression may be delayed and varies according to the plant's developmental stage, growing environment, whether the genotype is more Andean or Middle American, and probably the allelic dosage at the *D11* or *D12* locus and type of interaction (i.e., whether the *lcr* gene is interacting with the *D11* or *D12*). Relatively cooler temperatures favor expression of this trait. This abnormality has so far been observed in crosses between races, that is, Mesoamerica with Durango, Mesoamerica with Nueva Granada, Durango and Nueva Granada, and Nueva Granada with Chile (Table 1).

Some early investigators, for example,

Aggour and Coyne (1989), Burkholder and Muller (1926), Finke et al. (1986), and Zau-meyer (1942) recorded the two types of developmental abnormalities but were unable to establish the interrelationship between them. They failed because they lacked knowledge on the evolutionary origins of the *D11* and *D12* genes and the effect of interaction between them. They also lacked appropriate tester (genetic) stocks in crosses. The growing environment, agronomic management of field-grown nurseries, and data registration were also probably inappropriate. Some crippled leaf recombinants were lost because of their poor competitive ability and/or misclassification, resulting from delayed or mild symptom development, leading to some deficiency of that class and hence an  $F_2$  segregation ratio of 15 normal : 1 crippled leaf. The  $F_2$  data from Rabakoarihanta and Baggett (1983) on the cross Oregon 4594  $\times$  Gallatin 50 also gave a good fit to the ratio 15 normal : 1 distorted leaf. But by evaluating the population in different environments and by using additional crosses, they obtained  $F_2$  ratios of 13 normal : 3 distorted, or 3 distorted : 1 normal, depending on the genotypes of the parents used in the crosses. Our results support their findings, provided their results are interpreted in terms of present evidence.

The action and role of the *D11* and *D12* genes are well documented by Shii et al. (1980, 1981) and demonstrated again by our study. The dominant gene described by Rabakoarihanta and Baggett (1983) as *Ld* and as controlling leaf distortion was either *D11* or *D12*, and was present in severely distorted Oregon lines 4594 and 4622. Oregon 1604 possessed its recessive alleles as claimed by the authors. But the dominant gene *Ds* in Gallatin 50, which suppressed the expression of leaf distortion is equivalent to the dominant gene *Lcr* in our study. The likely genotype of Gallatin 50 would then be *dl1 dl1 dl2 dl2 Lcr Lcr*, that of Oregon 1604 would be *dl1 dl1 dl2 dl2 lcr lcr*, and that of the severely distorted lines 4594 and 4622 either *D11 D11 dl2 dl2 lcr lcr* or *dl1 dl1 D12 D12 lcr lcr*. Hence, the  $F_2$  of crosses of Oregon 1604 and Gallatin 50 with lines 4594 and 4622 would segregate according to ratios of 3 distorted leaf : 1 normal and 13 normal : 3 distorted, respectively, as reported by the authors. Thus, we can conclude that leaf distortion, deformation, or crippling results from the interaction of a recessive gene, *lcr*, independently with *D11* and *D12*.

Individuals with different degrees of leaf

Table 5. Possible homozygotes\* for all three genes involved in hybrid dwarfism and trifoliolate leaf crippling in common bean and their phenotypes

Genotype	Phenotype
<i>D11 D11 D12 D12 Lcr Lcr</i>	Dwarf
<i>D11 D11 D12 D12 lcr lcr</i>	Dwarf
<i>D11 D11 dl2 dl2 lcr lcr</i>	Crippled trifoliolate leaves
<i>dl1 dl1 D12 D12 lcr lcr</i>	Crippled trifoliolate leaves
<i>D11 D11 dl2 dl2 Lcr Lcr</i>	Normal
<i>dl1 dl1 D12 D12 Lcr Lcr</i>	Normal
<i>dl1 dl1 dl2 dl2 Lcr Lcr</i>	Normal
<i>dl1 dl1 dl2 dl2 lcr lcr</i>	Normal

\* The first four would have selective disadvantage in natural environments.

crippling were observed in segregating populations, as well as among  $F_{2,6}$  lines from populations WA 7807 and TY 5578. Such variation most probably results from interaction between the allelic dosage at the *D11* and *D12* loci, growing environment, and the *lcr* allele. Moreover, because the *D11*, *D12*, and *lcr* genes alone do not cause plant stunting or leaf crippling, they should be free of any selection pressure. But the plant dwarfism and leaf crippling that occur in crosses between races and between gene pools due to interactions between these genes also affect seed yield (reproductive fitness) and adaptation, becoming expressed at high temperatures (plant dwarfism) and low temperatures (trifoliolate leaf crippling). Thus, the genes involved might have played an important role in the evolution of the domesticated common bean by increasing reproductive isolation between races and gene pools. Four of eight possible homozygotes at all three loci are either dwarf or crippled (Table 5) and hence have a selective disadvantage. These would be gradually eliminated from natural populations. The possibility that each of *D11*, *D12*, and *lcr* are members of an independent favorable multilocus linkage block, associated with reproductive fitness and adaptation cannot be ruled out either. The interactions between the three genes would form a powerful force to eliminate undesirable recombinants in crosses between different races and gene pools. Similarly, we should investigate any specific selective advantage in given environments for each of the four homozygotes at all three loci that give rise to normal plant morphology. In rice (*Oryza sativa* L.), for example, a gene (*Hwc-2*) involved in hybrid weakness is believed to be associated with gene(s) controlling adaptability to high latitude, elevation, or upland environment (Sato and Morishima 1987).

Unlike  $F_1$  hybrid dwarfism, which so far

has been recorded only between the small- and medium-seeded common beans of Middle America (*D11* carriers) and large-seeded counterparts from the Andes (*D12* carriers) (Gepts and Bliss 1985; Singh and Gutiérrez 1984), crippled leaves have also been found in crosses between races Mesoamerica (e.g., cv. Carioca) and Durango (e.g., cv. Guanajuato 31), both of Middle American origin, and between races Chile and Nueva Granada, both of Andean origin. The race Mesoamerica occurs in relatively warm lowland tropics of Middle America, whereas the race Durango is indigenous to the cool, semiarid highlands of Mexico (Singh et al. 1991). Similarly, Nueva Granada germplasm is better adapted to the midaltitudes (650–2000 m) of the Andes, whereas race Chile is indigenous to the higher latitudes of the southern Andes (Singh 1989; Singh et al. 1991).

Occurrence of hybrid dwarfism and/or trifoliolate leaf crippling in segregating interracial and intergene-pool populations reduces available useful genetic variation for breeders and geneticists. Also, if environments used for evaluation, selection, and development of lines do not encourage expression of leaf crippling and hybrid dwarfism in early segregating generations, an unnecessary genetic load will be carried to advanced generations, thus wasting scarce resources, and possibly adversely affecting subsequent release and adoption of new cultivars in environments conducive to the expression of either trait. For example, line MAM 13 was released as a new cultivar, Azufrado Tapatio, in the Mexican State of Jalisco, but cannot be recommended for cultivation in the adjoining state of Aguascalientes where leaf crippling was expressed. Similarly, lines A 252 and A 445, although very high yielding in some environments and highly resistant to anthracnose [caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib.] could not be commercially used in Brazil because of the appearance of leaf crippling.

The positive aspects of the occurrence of hybrid dwarfism and/or leaf crippling in segregating populations is that they indicate the populations' broad genetic base. Every attempt should be made to identify and select rare recombinants possessing favorable alleles in these populations. Growing early generation segregating populations and families alternately in relatively warm and cool environments will help identify and discard both types of morphological abnormalities. Moreover, common bean lines, with genotype *d11 d11*

*d12 d12 Lcr Lcr* and possessing complementary desirable agronomic traits, should be identified in different races and gene pools of common bean for hybridization. These lines would produce comparatively higher proportions of nondwarfing and noncrippling recombinants.

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## Electrophoretic Evidence for Tetrasomic Inheritance in the Dioecious Tree *Maclura pomifera* (Raf.) Schneid.

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Although polyploidy is recognized as an important feature of chromosomal evolution, natural autopolyploids rarely occur compared to allopolyploids. Enzymes coded by four electrophoretic loci (*Lap*, *Pgm-1*, *Pgi-2*, and *Tpi-2*) in adults and full-sib progeny indicate tetrasomic inheritance in the dioecious tree *Maclura pomifera*, strongly suggesting that this species is an autopolyploid. This study is the first to document tetrasomic inheritance in a di-