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Uni-2—A Dominant Mutation Affecting Leaf Development in *Phaseolus vulgaris*

B. Garrido, R. Nodari, D. G. Debouck, and P. Gepts

Uni-2, a dominant mutation that affects leaf development in *Phaseolus vulgaris,* is described. The mutant type is characterized by the production of unifoliolate true leaves as opposed to the trifoliolate true leaves that characterize the wild-type phenotype of this species. Segregation data from the F_2, F_3, and F_4 generations of crosses between the unifoliolate mutant and trifoliolate genotypes suggest that the trait is coded by a single dominant gene with some deleteriousness in unifoliolate homozygotes. A new polymorphism for the enzyme methylumbelliferyl esterase was controlled by a single locus (*Mue*). No linkages were found between the unifoliolate trait and other molecular and morphological markers.

In its normal development, common bean (*Phaseolus vulgaris,* Fabaceae; $2n = 2x = 22$) produces first two opposite cotyledons and two opposite unifoliolate primary leaves and subsequently alternate trifoliolate true leaves. The former are produced during embryogenesis in the parental pod, and the latter are formed after germination. Common bean shares the presence of trifoliolate true leaves with many other members of the tribe Phaseoleae, including other crop plants such as soybean (*Glycine max*) and hyacinth bean (*Lablab purpureus*) (Maréchal et al. 1978). Several reports describe leaf mutants in common bean with altered leaflet characteristics (Bassett 1981; Lamprecht 1935b; Moh 1969; Moh and Alan 1970; Moh and Nanne 1969; Nagata and Bassett 1984; Singh and Gutiérrez 1982). We describe the genetic control of a common bean mutation in which the trifoliolate true leaves are replaced by simple (unifoliolate) leaves. This mutation is phenotypically very similar to one described by Lamprecht (1935b) but has a different genetic control and does not affect fertility.

Materials and Methods

D. Debouck identified a spontaneous mutant with simple true leaves in a commercial seed lot of the snap bean cultivar Cordonco; Dr. R. Maréchal of the Faculté des Sciences Agronomiques, Gembloux, Belgium, provided seeds of the original trifoliolate genotype (NI1031) and the derived simple-leaved (unifoliolate) mutant (NI1032). We crossed NI1032 to NI1031 as well as to cultivar ICA-Pijao, a standard common bean genotype, to investigate the genetic control of this novel trait in F_2, F_3, and F_4 generations obtained by self-pollinations in greenhouse conditions free of insect pollinators. NI1031 and NI1032, with the exception of true leaf shape, exhibit the same phenotype, which consists of a determinate, bush, nontwining growth habit, with four to six true leaves on the main stem, white flowers, and absence of flower blotch. ICA-Pijao exhibits an indeterminate and twining growth habit and has purple flowers with a dark purple blotch at the basis of the banner petal.

We scored 65 F_2 plants from the cross NI1031 × NI1032, grown in the greenhouse, for the trifoliolate-unifoliolate trait; we also took measurements of petiole and rachis length. We scored 60 F_2 greenhouse-grown plants of the cross ICA-Pijao × NI1032 for the trifoliolate-unifoliolate trait and for other morphological traits (determinacy, flower color, flower blotch, and twining). At the primary leaf (V2) stage (Gepts 1987), we conducted allozyme analysis as described by Koenig and Gepts (1989a). Isozymes revealing polymorphisms between KI1032 and ICA-Pijao consisted of diaphorase (DIAP), leucine aminopeptidase (LAP), and methylumbelliferyl esterase (MUE). The tissue used for enzyme extraction, the buffer systems, and staining procedures for DIAP and LAP were described previously (Koenig and Gepts 1989a). MUE is run from leaf extracts in a histidine buffer (0.065 M, pH 6.5) and stained as in Pasteur et al. (1988), where the acetate buffer is replaced by a potassium phosphate buffer at pH 6.0. The observed F_2 data were tested for goodness-of-fit to the appropriate genetic ratios by chi-square tests and for linkage by maximum likelihood estimation using LINKAGE-1 (Suiter et al. 1983).

Results

The wild-type trifoliolate true leaves of common bean consist of a basal (i.e., close to the stem node) pulvinus, a petiole (which can be distinguished from the pulvini by its nonfleshy, lighter green, and grooved appearance), two lateral leaflets each with a basal pulvinus and a stipel, a rachis, and a terminal leaflet also with its basal pulvinus and two stipels (Figure 1). With the exception described below, true leaves of the unifoliolate mutant consist of a basal pulvinus, a short (sometimes nonexistent) petiole, and a single (terminal) leaflet with its pulvinus and usually two stipels. Compared with the terminal leaflet of the trifoliolate wild type, the single terminal leaflet of the unifoliolate mutant exhibits a larger size (Figure 1). The unifoliolate true leaves are more acuminated than the unifoliolate primary leaves (Figures 1 and 3).

Exceptions to the unifoliolate morphology are observed in the first true leaf of NI1032 plants and progeny plants of crosses between NI1032 and NI1031 or ICA-Pijao. The first true leaf often displays a bifoliolate or trifoliolate morphology (Figure 2). In bifoliolate leaves, one of the lateral leaflets is fused with the terminal leaflet, whereas in the trifoliolate leaves the rachis is often lacking or is substantially shorter. These bifoliolate or trifoliolate leaf types just described have not been observed in wild-type plants. In all cases, the plants exhibiting the simple leaf trait exhibit a significantly shorter petiole than their trifoliolate counterparts, as illustrated by measurements of petiole length in cross NI1031 × NI1032 (Table 1). This reduction in length of the petiole also affects the primary leaves (Figure 3) but is not as marked, however, in those unifoliolate plants whose first true leaves exhibit a bifoliolate or trifoliolate morphology (Table 1).

The F_1 of crosses between the unifoliolate genotype (NI1032) and trifoliolate genotypes (NI1031 or ICA-Pijao) exhibit a unifoliolate phenotype. Analyses of segregation were conducted in the F_2, F_3, and F_4 generations of the crosses NI1031 × NI1032 and ICA-Pijao × NI1032. Results suggest that the simple leaf trait is controlled by a single dominant gene. Out of a progeny of 65 F_2 plants from the cross NI1031 × NI1032, 51 plants exhibited a unifoliolate morphology, whereas 14 were

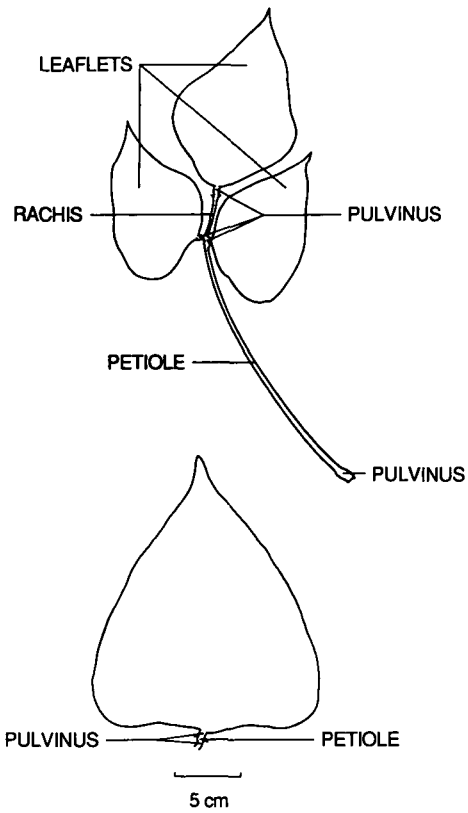

Figure 1. True leaves in the wild-type (**top**) and unifoliolate mutant (**bottom**) common bean plants.

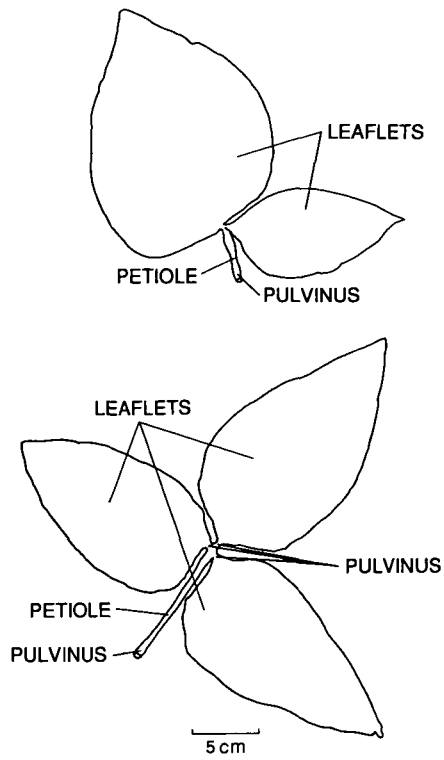

Figure 2. Variant first true leaves of unifoliolate plants exhibiting bifoliolate (**top**) or trifoliolate (**bottom**) morphology.

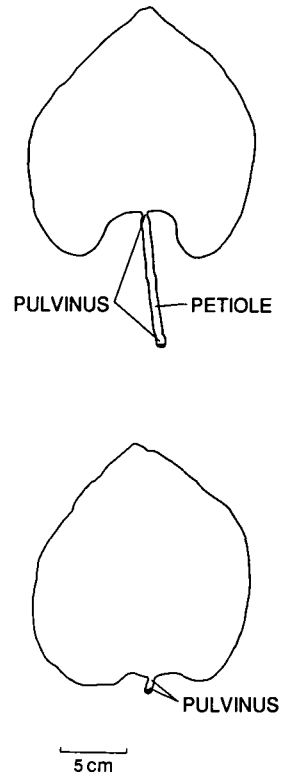

Figure 3. Primary leaves of wild-type (**top**) and unifoliolate (**bottom**) plants.

trifoliolate, corresponding to a χ^2 value of 0.25 ($P > .5$) for a 3:1 ratio. In the F_2 generation of the cross ICA-Pijao × NI1032, the observed segregation also fit a 3:1 ratio (Table 2). Segregations were further analyzed in an F_3 generation consisting of selfed progenies of randomly chosen F_2 plants of the cross NI1031 × NI1032. Each F_3 progeny consisted of 11 individuals, allowing us to identify a homozygous recessive individual at the $P = .95$ level in the case of a 3:1 segregation ratio. All selfed progenies of trifoliolate F_2 plants gave rise exclusively to trifoliolate plants. Among the selfed progenies of 20 unifoliolate F_2 plants, two gave rise exclusively to unifoliolate plants and 18 segregated for the leaf trait. This observed 18:2 ratio is significantly different from the expected 2:1 ratio ($\chi^2 = 3.90$; $.05 > P > .01$). This deficiency in the homozygotes for the unifoliolate trait was also observed in the F_4 generation of the cross ICA-Pijao × NI1032. This particular generation was obtained by single seed descent from the F_2 generation and is expected to consist of 9/16 individuals with a unifoliolate phenotype and 7/16 with a trifoliolate phenotype, assuming the unifoliolate trait is coded by a dominant allele at a single locus. Observations showed 20 unifoliolate individuals and 27 trifoliolate individuals. The χ^2 for a 9:7 ratio was 3.01 ($P < .10$).

The genotypes ICA-Pijao and NI1032 differ for a number of morphological and isozyme traits, in addition to the leaf trait. Flower color and flower blotch segregated according to a 3:1 and 9:7 ratio, respectively (Table 2), consistent with previous reports (Leakey 1988; Vieira and Shands 1969). The segregation observed for determinacy did not fit the ratios 3:1 and 15:1 previously reported for this trait (Arndt and Gepts 1989; Lamprecht 1935a; Norton 1915) but, rather, a 9(determinate) : 7(indeterminate) (Table 2). Likewise, twining exhibited a 7(twining) : 9(nontwining) ratio (Table 2), which is at variance with the previously reported 3:1 ratio (Norton 1915). The three allozymes segregated according to the expected 1:2:1 ratio (Table 2). The segregations and loci for the enzymes DIAP and LAP were described previously (Koenig and Gepts 1989b; Sprecher 1988). This is the first time

Table 1. Petiole and rachis length (mm) in trifoliolate vs. unifoliolate progenies of the cross NI1031 × NI1032

True leaf position	Trifoliolate segregants[a]	Unifoliolate segregants[a]		
		Unifoliolate[b]	Bifoliolate[b]	Trifoliolate[b]
1: Petiole	8.9 (1.2)	4.2 (1.5)	6.1 (1.0)	6.7 (1.1)
Rachis	2.4 (0.4)	–	–	0.8 (0.4)
Number	14	11	8	31
2: Petiole	10.0 (1.4)	1.0 (1.0)	1.9 (1.4)	1.9 (0.9)
Rachis	3.2 (0.5)	–	–	–
Number	14	11	8	31
3: Petiole	12.0 (1.8)	1.0 (0.9)	1.5 (0.9)	2.0 (1.2)
Rachis	3.5 (0.3)	–	–	–
Number	12	11	8	31
4: Petiole	10.3 (1.5)	1.0 (1.1)	1.9 (1.9)	2.0(1.4)
Rachis	3.3 (0.7)	–	–	–
Number	12	11	8	31
5: Petiole	7.3 (2.0)	0.6 (0.3)	1.0 (0.8)	1.9 (1.5)
Rachis	2.2 (0.6)	–	–	–
Number	12	9	7	29

[a] First number = length in mm; number in parentheses = standard deviation.

[b] Morphology of the first true leaf (see text for additional explanations).

Table 2. Goodness-of-fit test for segregations in the F_2 generation of cross ICA-Pijao × NI1032

Trait	Offspring phenotypes	Expected ratios	χ^2	df	*P*
Leaf type	Unifoliolate: 46; trifoliolate: 13	3:1	0.28	1	.60
Determinacy	Indeterminate: 22; determinate: 37	7:9	0.75	1	.41
Twining	Twining: 25; nontwining: 34	3:1	33.50	1	.00
Flower color	Purple: 43; white: 16	3:1	0.14	1	.71
Flower blotch	Present: 32; absent: 27	9:7	0.03	1	.75
Leucine aminopeptidase	*Lap-3^{103}Lap-3^{103}*: 4; *Lap-3^{103}Lap-3^{100}*: 9; *Lap-3^{100}Lap-3^{100}*: 4	1:2:1	0.06	2	.97
Methylumbelliferyl esterase	*Mue^{103}Mue^{103}*: 8; *Mue^{103}Mue^{100}*: 19; *Mue^{100}Mue^{100}*: 2	1:2:1	5.28	2	.07
Diaphorase	*Diap-1^{100}Diap-1^{100}*: 13; *Diap-1^{100}Diap-1^{95}*: 27; *Diap-1^{95}Diap-1^{95}*: 20	1:2:1	2.23	2	.33

that a segregation for MUE is reported in common bean. The staining procedure reveals a single band of activity in homozygotes and two in heterozygotes suggesting that MUE is a monomeric enzyme. We propose the symbol *Mue* to designate the gene for this enzyme. None of the genes segregating in the ICA-Pijao × NI1032 cross exhibited linkage.

Discussion

The F_2 data of the two crosses—NI1031 × NI1032 and ICA-Pijao × NI1032—suggest that the unifoliolate trait is controlled by a dominant allele at a single locus. Further generations of both crosses, however, show a deficiency of the homozygous dominant individuals. This deficiency might be due to the deleterious nature of the unifoliolate trait or of a closely linked gene. From the 59 F_2 plants of the ICA-Pijao × NI1032 cross, 47 F_4 lines were recovered by single seed descent from individual F_2 plants and 12 remained sterile either during the F_2 or F_3 generations. Nine F_2 plants, all of which were unifoliolate, did not produce seed. Three additional lines, which were also unifoliolate, did not set seed in the F_3 generation. The fact that this deficiency was also observed in the F_3 generation of the cross between the two lines NI1031 and NI1032, which differ only for the leaf trait, suggests that the deleteriousness resides at the locus controlling the unifoliolate trait rather than at a closely linked locus.

The larger size of the lamina of the simple unifoliolate leaves suggests that the wild-type allele corresponding to this mutation causes the subdivision of the entire leaf lamina into the two lateral leaflets and the terminal leaflet, in addition to an elongation of the petiole and rachis. The wild-type allele appears to be expressed during or after imbibition of the seed because the mutation affects only those leaves formed after germination. Exceptions to the unifoliolate morphology that affect the first true leaf could therefore be interpreted as a premature incipient trifoliolate morphogenesis that occurs before seed desiccation.

Lamprecht (1935b) described a unifoliolate mutant with a phenotype very similar to the one described here. His mutant, however, was controlled by a single recessive gene (*uni* for *unifoliata*, or unifoliate leaf) and was highly sterile. The stock carrying this mutation has since been lost and, therefore, no test of allelism with the mutation can be performed. To avoid any confusion with the original symbol proposed by Lamprecht (1935b), we propose to name the locus coding for this mutant phenotype *Uni-2*.

The unexpected ratios observed for determinacy and twining could be attributed to a number of causes, including chance due to the relatively small population sizes used in this study. Alternatively, they could be due to the existence of genetic controls that are distinct from the ones reported earlier (Arndt and Gepts 1989; Lamprecht 1935a; Norton 1915). A third possibility is that they represent segregation distortion favoring, in this particular cross, the determinate, nontwining parent. Distorted segregation ratios were observed earlier by Koenig and Gepts (1989b). It is possible that the divergent evolutionary origins of the two parents as determined by allozyme analysis (ICA-Pijao: Mesoamerica; NI1032: Andean; Koenig and Gepts 1989a) may have contributed to this distortion.

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