Hybrid Weakness in Wild Phaseolus vulgaris L.

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Hybrid weakness (HW) is a usually lethal condition appearing in the F₁ of crosses between certain Andean and Middle American common bean (Phaseolus vulgaris) cultivars; it is controlled by two complementary semidominant genes, DI₁ and DI₂, of Middle American and Andean origin, respectively. The objective of this study was to determine whether the DI₁ and DI₂ genes are present in the wild P. vulgaris gene pool and whether they exhibit the same geographic distribution as in their cultivated counterparts. Twenty wild accessions chosen for their geographic range distribution and phaseolin and allozyme diversity were crossed to two testers (G00122 and G04830) known to have genes conditioning HW and to each other in half-diallel fashion. To promote the expression of HW, the F₁ crosses were screened at high temperatures (25°C night, 35°C day). HW appeared in Middle American × Andean crosses but not in Middle American × Middle American or Andean × Andean crosses. Our results suggest that the DI genes arose before domestication as a consequence of geographic isolation, rather than as a consequence of selection pressure to eliminate unadapted hybrids among cultivars. They were introduced among cultivars presumably through domestication. Possible consequences for common bean breeding are presented.
show a sublethal reaction. Individuals that are homozygous recessive at one or both loci will not show hybrid lethality. In addition, HW is expressed strongly at temperatures above 25°C, whereas it is not expressed at temperatures below 20°C (Shii et al. 1980, 1981). The restricted root growth of F₁ hybrids showing HW was alleviated by the addition of cytokinins, suggesting that a hormonal imbalance is involved in the lethality (Shii et al. 1981).

The **Dl** gene is found among Middle American genotypes, whereas the **Dl2** gene is found among Andean genotypes (Gepts and Bliss 1985).

Singh and Gutiérrez (1984) proposed that hybrid weakness appeared as an isolation mechanism among cultivars with differential adaptation to environmental factors, particularly temperature. Domestication in common bean has led to viable and fertile **F₁** and **F₂** descendants. **HW** is expressed strongly at temperatures above 25°C, whereas it is not expressed at temperatures below 20°C (Shii et al. 1981).

An alternative hypothesis suggested by Gepts and Bliss (1985) states that hybrid weakness is a by-product of geographic isolation between Middle American and Andean genotypes. Because divergence between these two groups pre-dates domestication, one would, under this hypothesis, expect to find **Dl** genes in the wild ancestor as well as in the cultivated descendants.

Before this study, **Dl** genes were described exclusively in common bean cultivars (Gepts and Bliss 1985; Shii et al. 1980; Singh and Gutiérrez 1984). A limited number of crosses between wild and cultivated accessions failed to reveal any HW but led to viable and fertile **F₁** and **F₂** individuals (Burkart and Brücher 1953; Harmsen et al. 1987; Koenig and Gepts 1989b; Kornegay and Cardona 1991; Motto et al. 1978; Weiseth 1954). The latter observation confirmed that wild and cultivated common beans from Mesoamerica and the Andes are indeed members of the same biological species. However, the geographic distribution of the wild accessions was too limited and the number of accessions was too small to conclude with a reasonable degree of confidence that **Dl** genes were absent from the wild gene pool of *P. vulgaris*.

The objective of this study was to determine whether the **Dl1** and **Dl2** genes are present among wild common bean and whether they exhibit the same geographic distribution as in their cultivated counterparts. Their existence in a significant proportion of the wild germplasm could affect the increased use of this germplasm for common bean improvement.

### Materials and Methods

We obtained 20 wild *P. vulgaris* accessions from the *Phaseolus* germplasm collection at the Centro Internacional de Agricultura Tropical (Table 1). These accessions were selected to be as representative as possible of the geographic distribution range (Table 1) and of the phaseolin and allozyme diversity observed in the wild germplasm (Gepts and Bliss 1986; Gepts et al. 1986; Koenig and Gepts 1989a). Two cultivars known to possess the **Dl** genes were included as testers. These were **G00122** (‘Jatu Rong’; India), a cultivar derived from Andean domesticates and with a **Dl1Dl2Dl2** genotype, and **G04830** (‘Rio Tibagi’; Brazil), cultivar of Middle American origin and with a **Dl1Dl1Dl2Dl2** genotype.

We grew single plants of each accession and two of the cultivars in the greenhouse in fall 1989. A half-diallel cross was made among the 20 wild accessions. Where possible, we included reciprocal crosses in each pair. Crosses between each wild accession and the two tester cultivars and reciprocal crosses between the two cultivars were also carried out.

We screened **F₁** plants in a temperature-controlled greenhouse, during summer 1990. The temperature was set at 25°C at night and 35°C during the day. We grew at least four seeds from each cross in each 2-liter plastic pot. We evaluated 240 wild by wild and wild by cultivated crosses for hybrid weakness, together with four reciprocal crosses between the cultivars as con-

### Table 1. Outcome of crosses between wild *Phaseolus vulgaris* accessions and two tester lines with known **Dl** genotype

<table>
<thead>
<tr>
<th>Accession</th>
<th>Origin</th>
<th>Province or State</th>
<th>Evolutionary origin</th>
<th>Tester (Dl genotype; evolutionary origin)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>G23463</td>
<td>Mexico</td>
<td>Chihuahua</td>
<td>M</td>
<td>d1d1d1d1d1</td>
</tr>
<tr>
<td>G10022</td>
<td>Mexico</td>
<td>Durango</td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G12863</td>
<td>Mexico</td>
<td>Jalisco</td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G12866</td>
<td>Mexico</td>
<td>Jalisco</td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G12922</td>
<td>Mexico</td>
<td>Jalisco</td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G12910</td>
<td>Mexico</td>
<td>Guanajuato</td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G12861</td>
<td>Mexico</td>
<td>Michoacán</td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G19907</td>
<td>Guatemala</td>
<td>Sacatepequez</td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G20559</td>
<td>Costa Rica</td>
<td></td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G21117</td>
<td>Colombia</td>
<td>Cundayamarca</td>
<td>M</td>
<td>d1d1d1d1 or d1d1d1d1</td>
</tr>
<tr>
<td>X663</td>
<td>Colombia</td>
<td>Cundayamarca</td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G21245</td>
<td>Peru</td>
<td>Cajamarca</td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G15865B</td>
<td>Peru</td>
<td>Huancou</td>
<td>A</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G10021</td>
<td>Argentina</td>
<td></td>
<td>A</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G19887</td>
<td>Argentina</td>
<td>Lujuy</td>
<td>A</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G19890</td>
<td>Argentina</td>
<td>Salta</td>
<td>A</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G19892</td>
<td>Argentina</td>
<td>Salta</td>
<td>A</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G19894</td>
<td>Argentina</td>
<td>Tucumán</td>
<td>A</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G19899</td>
<td>Argentina</td>
<td>Tucumán</td>
<td>A</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G19902</td>
<td>Argentina</td>
<td>Tucumán</td>
<td>A</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G00122</td>
<td>India</td>
<td></td>
<td>A</td>
<td>d1d1d1d1</td>
</tr>
<tr>
<td>G04830</td>
<td>Brazil</td>
<td></td>
<td>M</td>
<td>d1d1d1d1</td>
</tr>
</tbody>
</table>

* According to Toro et al. 1990.

**HW** = presence of hybrid weakness; 0 = absence of hybrid weakness; — = no data.

As determined by phaseolin and allozyme analysis (Gepts and Bliss 1986; Gepts et al. 1986; Koenig and Gepts 1989a; Koenig et al. 1990): M = Middle American; A = Andean.

As determined by crossing results shown in Tables 1 and 2.

Accessions from the *Phaseolus* collection, Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
Hybridity of the F1 was confirmed by the presence of dominant traits such as pod and seed color or color pattern, stem color, and indeterminacy. The reciprocal crosses between cultivated testers were distributed at random throughout the greenhouse to verify that the environmental conditions were conducive to the expression of HW in the entire greenhouse. At flowering, the presence of adventitious roots, the most characteristic symptom of HW, was scored for presence or absence.

Results

It was possible to obtain F1 progeny from most crosses attempted to identify the presence of the Dl genes. Exceptions can be attributed to the lack of adaptation of certain materials and, hence, their poor quality as parents. In particular, G21245 from northern Peru and G21117 from Colombia produced seed neither as female nor as male parents with the two tester cultivars. For other accessions, one of the reciprocal crosses was absent. However, previous observations by Shii et al. (1980) indicate that HW is expressed irrespective of the direction of the cross, which was confirmed by our own results that are presented here.

The F1 generation was grown under environmental conditions that were adequate for consistent expression of HW. All control F1 plants from the cross G00122 x G04830, distributed throughout the greenhouse, showed the HW symptoms. However, contrary to previous observations (e.g., Shii et al. 1980), HW was observed not at the seedling stage but only at or after flowering. This may have been due to a hereto unreported interaction with photoperiod. Our experiment was conducted during the summer with daylengths greater than 15 h, whereas experiments of Shii et al. (1980) were carried out in growth chambers under a 12-h day-length.

Results of the testcrosses showed that the Dl genes were present in wild beans and that they followed the same geographic distribution pattern as in the cultivars (Table 1). When crossed to G00122 and G04830 as testers with known Dl genotypes, several wild accessions gave rise to F1 plants with HW (Figure 1). G00122, of Andean origin as determined by its phaseolin and allozyme phenotype (Singh et al. 1991a), gave rise to HW when crossed with F1 plants with HW (Figure 1). G00122, of Andean origin as determined by its phaseolin and allozyme phenotype (Singh et al. 1991a), gave rise to HW when crossed with F1 plants with HW (Figure 1). G00122, of Andean origin as determined by its phaseolin and allozyme phenotype (Singh et al. 1991a), gave rise to HW when crossed with G00122 and seven other Middle American wild beans or all eight Andean wild beans tested. Conversely, G04830 yielded HW when crossed with six Andean (Peruvian and Argentinean) wild materials. It did not lead to HW when crossed with an additional Andean wild line (G19902 from Argentina) nor with all nine Middle American wild forms tested.

Results from the half-diallel crosses confirmed the results from the testcrosses (Table 2). Based on the evolutionary origin of the wild accessions (as determined by phaseolin and allozyme analyses by Gepts and Bliss 1986, Gepts et al. 1986, Koenig and Gepts 1989a, and Koenig et al. 1990), and taking into account the results of the testcrosses, one would expect HW to appear only in the upper right quadrant of Table 2. This prediction was borne out by our results with one exception, which concerns accession G19902. According to the testcross, this accession was not a carrier of a Dl gene; in the half-diallel cross, however, it led to HW when it was crossed with G19902, an accession carrying a Dl gene, according to the testcross to G00122 (Table 1). The half-diallel crosses also allowed us to infer the Dl genotype of those accessions for which no testcross could
be obtained. For example, accession G21117 may be a carrier of the D1l gene as it led to HW when crossed with G12856B, a carrier of D12. On the other hand, none of the crosses between G21117 and the D12-carrying accessions (G10021, G19877, G19890, G19892, and G19894) showed HW.

Based on a joint analysis of the test and half-diallel crosses, a D1 genotype can be proposed for all the wild accessions included in this study (Table 1).

Discussion

Our results clearly establish for the first time the presence of the D1 genes in wild P. vulgaris, whereas previously they had only been reported in cultivated P. vulgaris. Two possible origins can be suggested for these D1 genes in wild common beans: either they originated in wild common beans and were introduced into the cultivated gene pool, presumable through domestication, or they originated among cultivars and they were introduced into the wild gene pool through occasional outcrosses. Arguing against the latter alternative is the observation that the accessions carrying the D1 genes (D11 or D12) do not show any signs of past hybridizations to cultivars, such as large seed size. In addition, most of them were found in natural, undisturbed vegetation (Debouck 1985; Vanderborght 1987). D1 genes therefore, may have been introduced into the cultivated gene pool from the wild ancestor of P. vulgaris, presumably through domestication, although outcrosses subsequent to domestication cannot be excluded.

If D1 genes originated among wild beans, they more likely arose as a consequence of geographic isolation rather than through selection pressure to eliminate unadapted hybrids. For selection to operate, the latter hypothesis requires the possibility of hybridization, an event highly unlikely to take place in geographically separated populations. In addition, there are more efficient reproductive isolation mechanisms than F1 lethality, such as prezygotic or early postzygotic barriers.

There are two possible explanations for the inconsistencies in the results of the test- and half-diallel crosses involving G21117 and G19902. It is possible that these accessions are both heterozygous or heterogeneous, the former for the D11 gene and the latter for the D12 gene. Alternatively, there may be other lethality systems that are present among wild beans but not among cultivated beans.

In our sample, the frequency of the D12 gene among Andean wild beans was much higher than that of the D11 gene among Middle American wild beans. However, caution should be exercised before extrapolating these frequencies to the entire Andean or Middle American wild bean populations. Because of the number of crosses involved, our sample included only 20 wild bean accessions. In addition, many of the Andean accessions originated in three contiguous provinces of Argentina, because of the unavailability at the onset of this study of materials from other areas, particularly Bolivia and Peru. It is possible that if the Andean sample had been as uniformly distributed as the Middle American sample, the frequencies of the D11 and D12 genes would have been more similar.

What are the consequences for common bean breeding of the identification of D1 genes in wild common bean? Several observations indicate that domestication has induced a marked bottleneck in genetic diversity in common bean. For example, there is considerably more diversity for phaseolin among wild common beans than among cultivated common bean (Gepts and Bliss 1986; Gepts et al. 1986; Koenig et al. 1990). Arcelin, a seed protein that confers resistance to the weevil Zabrotes subfasciatus, is found only in certain Middle American wild common bean accessions, but is absent in cultivars (Osborn et al. 1986). Wild beans are, therefore, a potential source of additional genetic diversity in common bean improvement. The pres-
ence of the DI genes may obviously interfere with gene transfer between wild and cultivated beans when they belong to different gene pools as they have done among cultivars (e.g., Kelly 1988). However, there are ways to circumvent HW such as selecting a cultivated recipient parent without DI genes, such as cultivar ICA-Pijao (Kelly 1989; Singh 1990). Alternatively, the early generations can be grown at lower temperatures to avoid expression of HW and to allow for recombination to occur. In later generations, higher temperatures can be used to eliminate individuals carrying a dominant allele at both DI loci. Whether this strategy will lead to satisfactory recombinants will depend partly on the linkage intensity between DI loci and the loci that control the agronomic traits of interest.

References
Vanderborght T, 1987. The study of common bean (Phaseolus vulgaris L.) variability by the use of multivariate statistical methods applied to a data base. Diss Abstr 47:3190B–3191B.