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Dwarfing genes in the genus *Lens* Mill.

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Abstract Dwarfing genes were detected following intra- and interspecific hybridization in *Lens*. Dwarf phenotypes are controlled by two complementary dominant genes, Df_1 and Df_2. These two genes are suppressed by the dominant allele of the dwarf inhibitor genes, *Dfi*. The dominant allele of the *Df* gene was detected in *L. ervoides* from Ethiopia and Uganda and in a cultivated line of *L. culinaris* from Ethiopia, that of the Df_2 gene in a *L. ervoides* accession from Israel. The dominant allele of the *Dfi* gene was detected in segregating populations of hybrids between *L. ervoides* accessions from Israel and Uganda. Using the homozygous dwarf, *dfi dfi*, Df_1Df_1, Df_2Df_2 as the parent in interspecific crosses, we detected the dominant allele of the *Dfi* gene in one accession of *L. nigricans* and another of *L. lamottei*. The appearance of dwarf plants in segregating populations of hybrids between the cultivated line from Ethiopia and *L. ervoides* from Israel indicate that the cultivated line possesses the dominant allele of the *Dfi* gene. Dwarf plants were characterized by short internodes, a short leaf axis and smaller, convex leaflets. Spraying the dwarf plants with gibberellic acid induced internode and lead-axis elongation but had no effect on leaflet shape and size. When the dwarfs and their parental lines were grown in the dark they had the same internode length.

Key words Lentil · Dwarf · Gibberellic acid · Interspecific hybridization

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Introduction

Hybridization of two accessions of the wild lentil, *Lens ervoides* (Bring.) Grande, one from Israel and the other from Ethiopia, yielded F_1 hybrids which were all fertile but dwarf. Segregation in the F_2 indicated that the dwarf genotype is controlled by two complementary dominant genes, Df_1 and Df_2 (Ladizinsky et al. 1992). Since dwarf plants were also detected in progeny originating from a normally growing plant which was obtained via embryo culture of hybrids between the same *L. ervoides* accession from Israel and a cultivated lentil line from Ethiopia, it has been postulated that the cultivated line possesses a gene(s), *Dfi*, which is(are) epistatic to the *Df* genes. As this information was only briefly mentioned by Ladizinsky et al. (1992), more details are given here.

This paper also provides further information regarding the distribution of these dwarfing genes and others related to dwarfism in *L. ervoides* and other *Lens* species.

Materials and methods

The following plant material was used either directly or indirectly in this study: *Lens culinaris* no. 7, a cultivated line from Ethiopia; *L. ervoides*, no.32 from Israel, no.46 from Algeria; no.228 from Ethiopia; no.245 from Uganda; *L. nigricans* (Bieb.) Godr., no.59 from Italy; *L. lamottei* Czefr. no.73 from France and no.244 from Morocco.

Results

Dwarfing genes in *L. ervoides* from Ethiopia and Israel

Although *L. ervoides* is a Mediterranean species it has been reported from two localities in East Africa, near Addis Ababa, Ethiopia and in the Kisoro district of

Uganda. The Addis Ababa population apparently no longer exists because that particular locality is now a dwelling area. However, another population was detected 6 km NE of Alleltu, on the Addis Ababa-Debre-Birhan road.

All of the F_1 hybrids between *L. ervoides* from Israel, no.32, and from Ethiopia were dwarf, regardless of the direction of the cross. The epicotyl and the first leaf were nearly the same size in the parental lines and their hybrids, but upper internodes became shorter in the dwarfs. The third internodes of the dwarf were about one-third shorter than those of the parental plants, and the 6th internodes were only one-sixth as long (Table 1). The hybrid leaf axis was also much shorter than that of the parents. The number of leaflets per leaf was about the same, but they were smaller and very close to one another in the dwarfs. Moreover, the leaflets of the dwarfs were much more convex than the parental leaflets and the distal leaflets of a particular leaf much smaller than the proximal ones. The dwarf plants had a bushy appearance with more branches that the parental lines. At maturity, dwarf plants were no taller than 10 cm, while the normal plants were up to 50 cm high. The hybrids were fertile and produced many seeds, the size of which was similar to that of the parents.

Segregation to normal and dwarf plants in the F_2 indicated that two complementary dominant genes control dwarfism in *L. ervoides* (Table 2). Plants homozygous for the two dwarfing genes were selected by progeny testing in the F_3. No morphological differences were found between heterozygous and homozygous dwarfs, although the former was slightly taller (Fig. 1). The two genes were arbitrarily designated Df_1Df_1 in the Ethiopian population and Df_2Df_2 in no.32.

Table 2 Genetics of dwarfism as indicated by the segregation pattern in F_2 populations

Hybrid	Dwarf	Normal	Total	χ^2		P
				9:7	6:1[a] (ca.)	
No.228 × no.32	92	55	147	2.39		0.2–0.1
No.245 × no.32	9	69	78		0.388	0.9–0.5

[a] See text

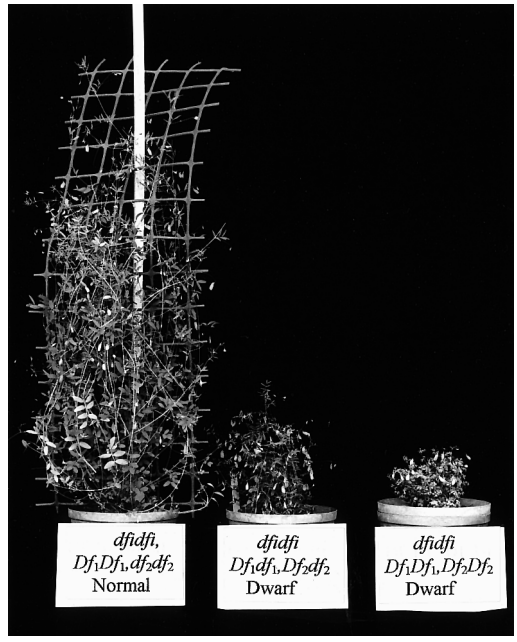

Fig. 1 Plant height at maturity of normal and dwarf phenotypes and their genotype

Response of the dwarf plants to gibberelic acid (GA_3)

Six-week-old homozygous dwarfs were sprayed with 20 ppm commercial gibberellic acid with 0.3% Triton X-100 as the surfactant. Consequently, internodes and leaf axes became considerably longer, although they were still shorter than in normal plants (Table 1). While no tendrils were formed on the dwarf plants, they did develop following the GA_3 treatment. A similar treatment with GA_4 did not affect the growth pattern of the dwarfs.

Table 1 Measurements (cm) of dwarf plants and their parental lines

	No.32	No.228	Dwarf	Dwarf + GA
3rd internode	1.3–1.5	1.2–1.5	0.4–0.5	
6th internode	1.8–2.2	2.0–2.2	0.2–0.3	1.2–1.5
Leaf axis, 6th internode	1.2–1.4	1.8–2.2	0.2–0.5	0.6–0.8
Plant height at flowering	20–25	23–27	5–7	12–15

The dwarfs were further characterized by growing them, together with their parental lines, in complete darkness. After 3 weeks they reached about 8 cm and no difference was noted between the dwarfs and their parents.

Dwarfing genes in *L. ervoides* from Uganda

In Uganda, *L. ervoides* grows in a restricted area in the Kisoro district, on the volcanic slopes of Mt. Muhavura at altitudes of 2500–2800 m. It is the most distantly located population, separated from the main distribution range of this species in the Mediterranean region by about 3500 km and from the Ethiopian population by about 1500 km. The wild lentil plants grow there in small patches, usually along animal paths among perennial grasses, mainly *Pennisetum*

clandestinum (kikuyu grass). Mature plants exhibit a marked climbing habit and may reach 60 cm or even more. The origin of this *L. ervoides* population is obscure, but it must be fairly recent in geological terms because Mt. Muhavura is approximately 200,000 years old.

Hybrids between accession no.32 from Israel and no.245 from Muhavura were easily obtained. They developed normally and were fertile. Growth habit segregated in the F_2 populations; of a total 78 plants, 69 had normal growth and 9 were dwarfs, indicating that the Muhavura population also possesses the dominant allele of the complementary gene Df_1. More interesting, the segregation pattern confirms the previous assumption (Ladizinsky et al. 1992) of the existence of an epistatic gene, *Dfi*, which suppresses the dwarfing gene's dominant alleles. Genotypically then, a dwarf genotype is *dfidfi*, Df_1df_1 Df_2df_2, or, homozygous dominant in both *Df* genes. Accordingly, accession no.32 can be designated as *dfidfi*, df_1, df_1, Df_2Df_2 and no.245 as *DfiDfi*, Df_1Df_1, df_2df_2. The expected number of dwarfs among the 78 F_2 plants is thus $(1/4 \times 9/16) \times 78 = 10.9$, and of normal plants, $3/4 \times 78 + (1/4 \times 7/16) \times 78 = 67.03$. The observed and expected figures were close, giving $X^2 = 0.388$ and $P = 0.9$–0.5.

Accession no.245 was also crossed with no.46 of *L. ervoides* from Algeria. The hybrids were normal, and among the 26 F_2 plants not even a single dwarf was observed. Hybrids of the no.32 × no.46 combination were normal, but their F_2 populations were not examined. No.46 was also crossed with 228×32 homozygous dwarf, and all the hybrids were normal. Although no.46 appears to possess the *Dfi* gene and lack the Df_2 gene, an examination of a larger F_2 population of no.46 × no.245 may prove otherwise.

Other *Lens* species containing the *Dfi* allele

To determine the occurrence of the *Dfi* allele in other species, we crossed, the homozygous dwarf 228×32 with representatives of two wild species, *L. nigricans* (Bieb.) Godr and *L. lamottei* Czefr. The latter was delimited from herbarium material (Czefranova 1971), and its validity has been confirmed by breeding experiments (Ladizinsky et al. 1984) and more recently by restriction fragment length polymorphism (RFLP) analysis of cpDNA (Van Oss et al. 1997).

Hybrids were produced between a homozygous dwarf and two accessions of *L. lamottei*, no.73 and no.244. Hybrids with the former were normal but sterile, as one would expect of *L. ervoides* × *L. lamottei* hybrids. Their normal growth indicated that no.73 of *L. lamottei* possesses the *Dfi* allele. Only 2 hybrids were obtained with no.224, and both were extreme dwarf. They were much smaller than the parental dwarf, produced three to four leaves and have not progressed beyond that. This growth habit indicates that no.224 possesses the *dfi* allele. The extreme dwarfism of the 2 hybrids suggests not only the existence of several *dfi* alleles which differentially affect the *Df* genes but also of more than two alleles at each of the *Df* genes.

Only a single accession of *L. nigricans*, no.59, was crossed with the homozygous dwarf. Although all the F_1 plants exhibited normal growth, their hybrid origin was confirmed by their low fertility, as is the case in all *L. ervoides* × *L. nigricans* hybrids (Ladizinsky et al. 1984). No.59 therefore appears to possess the *Dfi* allele.

The cultivated lentil, *L. culinaris* Medik., and *L. ervoides* are cross-incompatible and belong to different crossability groups on the basis of the abortion of their hybrid embryos (Ladizinsky et al. 1984, 1985). These embryos can be rescued by transplanting them in a suitable medium (Cohen et al. 1984). Dwarf individuals were detected among the F_5 plants of a normal-growing but partially sterile hybrid between a lentil line, no.7, from Ethiopia and *L. ervoides* no.32 from Israel. The progeny of a dwarf plants segregated dwarf to normal in a 1 : 1 ratio and later bred true. The 32×7 dwarfs responded to a treatment with GA_3 in the same way as the 228×32 dwarf. It can therefore be concluded that the cultivated line from Ethiopia possesses the Df_1 and *Dfi* genes. An attempt to backcross the 32×7 homozygous dwarf to the cultivated line failed because of hybrid-embryo abortion. This indicates that the 32×7 dwarf cannot be assigned to the cultivated lentil crossability group.

Possible variation in the *Df* and *Dfi* genes

Variation in the *Df* and *Dfi* genes may be inferred from the growth habit of the different dwarfs. As already mentioned a dwarf resulted from 228×32 dwarf × *L. lamottei*. no.224 was much smaller than its parental dwarf. When the homozygous 228×32 dwarf was back-crossed to no.32 the hybrids were dwarf as well, but with larger and normal-looking leaflets. The leaf axis was longer than in the dwarf but still shorter than in no.32. It is therefore likely that more dwarfing genes exist, that the known loci possess more than two alleles, or both.

Discussion

As in maize (Phinney 1984), pea (Reid and Ross 1993) and rice (Murakami 1972) dwarf phenotypes in lentil also appear to result from mutations in the GA_1 biosynthetic pathway. Applying GA_3 to homo- and heterozygous dwarfs resulted in stem elongation, although full recovery of the internode length was not achieved. However, unlike dwarfism in maize, pea and rice, in which all the mutants were controlled by a single recessive gene, in lentil dwarfism is controlled

by two complementary dominant genes. In this regard, lentil's dwarfs are probably closer to the grass-clump dwarf of wheat, which results from an interaction between dominant alleles of three loci (Gale and Youssefian 1985). The lentil dwarfs are even more unique in that another dominant gene is super-imposed on and epistatic to the two complementary dwarfing genes, thereby suppressing their activity. It is also worth mentioning that although recessive alleles causing dwarfism have been detected in crop plants, in lentil these alleles are naturally occurring and have been detected in four lentil species. Some wild accessions possess dominant alleles in two of the three genes necessary for the development of dwarf symptoms. A more extensive search for these alleles and probably of more can be made by crossing different accessions with the following three tester lines: (1) *Lens ervoides* no.32 (*dfidfi*, df_1df_1, Df_2Df_2) to detect the Df_1Df_1 allele; and (2) no.228 (*dfidfi*, Df_1Df_1, df_2df_2) to detect the Df_2 alleles, when each of them occurs on the *dfidfi* gene background. (3) The *dfi* alleles may be detected with the homozygous dwarf (*dfidfi*, Df_1Df_1, Df_2Df_2). These tester lines can be used successfully to search for dwarfing alleles in *L. ervoides*, *L. nigricans* and *L. lamottei*. It would be much more difficult to use them in crosses with species of the other crossability group of *Lens*, i.e. *L. culinaris* and *L. odemensis*, because of hybrid embryo abortion. The availability of these tester lines makes the genus *Lens* an appropriate model for further studies on GA_1 biosynthesis.

The occurrence and possible role of individual dwarfing genes in natural populations of at least four *Lens* species in puzzling and intriguing. Obviously, more information regarding the distribution of the dwarfing genes and their role in the GA_1 biosynthesis is required before their evolutionary role can adequately be assessed. One may wonder, however, if it is just a coincidence that the two *L. ervoides* accessions from East Africa possess the Df_1 allele. The population from which no.228 and no.245 were collected grow in completely different habitats than those of *L. ervoides* and other *Lens* species in the Mediterranean region. Furthermore, the Df_1 allele also occurs in the cultivated line from Ethiopia.

The dwarfing genes described here are completely different from the one reported by Sinha and Chowdhury (1991). These authors described dwarfism following irradiation treatment, and the control of the dwarf phenotype by a single co-dominant gene. The different genetic control of different dwarfs in lentil may suggest their mode of action: via interference in GA biosynthesis by the co-dominant gene, and threshold effect by the complementary *Df* genes and the *Dfi* gene.

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