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INHERITANCE OF BROWN LEAF PIGMENTATION IN LENTIL (*LENS CULINARIS*)

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ABSTRACT

Inheritance of brown pigmentation of the leaves was studied in 11 crosses involving 9 genotypes with pigmented and 4 with nonpigmented (green) leaves, which were crossed in direct (6 crosses) and reciprocal (4 crosses) combinations. Analysis of 4468 F_2 plants under field conditions revealed perfect monogenic segregation into 3 brown : 1 green ratio. It was a rare case of absence of deviations from expected numbers in F_2, resulting into $\chi^2 = 0$. The development of brown pigmentation is, thus, controlled by a single dominant gene and its recessive condition results in formation of normal green leaves through the entire plant life. The gene symbol Bl (brown leaf) is proposed for this trait. The pattern of stage-dependent synthesis of brown pigment and its disappearance is described.

Key words: Lentil, *Lens culinaris*, inheritance, brown leaf pigmentation.

Lentil, although cultivated over large areas all over the global surface, has been a generally neglected object for genetic studies. Even though the first report on the inheritance of a qualitative trait, i.e. cotyledon colour, appeared in 1928 [1], the next similar publication on the inheritance of flower colour became available 47 years later [2]. Ladizinsky [3] reported the inheritance of epicotyl colour, growth habit, flower colour, pod dehiscence, and seed coat colour. Inheritance of pod pubescence, pod pigmentation, and tendril formation at leaf apex was reported by Slinkard [4]. Brown pigmentation of leaves is another easy-to-observe qualitative trait. However, no published record is available on its inheritance. As the study under report revealed, the stage-dependent formation of brown pigment and its subsequent disappearance, either due to plant age or environmental influence, could be one reason why precise results leading to definite conclusion about the inheritance of this trait could not be obtained if observations were recorded only once in F_2 generation.

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The seedlings of lentil emerge from the ground without brown pigmentation and therefore appear as green as the young plants of any other genotype which is not able to synthesize leaf pigment at all. However, about 30–40 days after sowing, when the seedlings are about 3–4 weeks old, the leaves of pigment-potent plants start turning brown and remain pigmented till about flowering time, after which the brown pigment disintegrates and the plant reverts to nonpigmented state. Since the brown pigment develops gradually on the leaves, all plants of a genotype do not become brown at the same time. Similarly, the reversion of pigmented plants to nonpigmented state is also a gradual process. The brown pigment does not form even in the plants having potential for pigment synthesis if they are kept in partial shade where chlorophyll synthesis can still take place but synthesis of brown pigment is blocked. Thus, for normal expression of the gene responsible for brown coloration exposure to sun is essential. All these peculiarities of brown pigment synthesis in lentil leaves can vitiate the results in the absence of proper care.

MATERIALS AND METHODS

The following brown- and green-leaf strains, used as parents in hybridization, were selected from the germplasm and breeding materials maintained in the collection of the Division of Genetics, I.A.R.I., New Delhi: pigmented—Lens 4149, LS 106, Lens 4602, Pant L 406, PKVL 1, LC-68-17-3-5-1, UK-1 (a strain of unknown origin), and two mutants of the cv. Lens 830–globe and fasciated; and nonpigmented—Eston, Precoz, LC-74-1-5-1, and Lens 6163. Out of the 11 crosses made between these genotypes, the strains with brown leaf were used as female parents in 6 crosses, whereas these genotypes were used as pollinators in 5 crosses. The segregation pattern was remarkably similar in direct as well as reciprocal crosses. The parent strains, and F_2 progenies were raised at the experimental farm of I.A.R.I., New Delhi in rabi 1994–95 and 1995–96, respectively. The F_1 generation was raised during summer 1995 at the Off-Season Nursery in Lahaul Valley (Himachal Pradesh) where meticulous observations on pigment development and its degradation could not be recorded. Since the parent strains also differ in cotyledon colour, the hybridity of F_1 plants was confirmed on the basis of segregation for orange (red), yellow, brownish yellow, or green cotyledons in the seed harvested from each F_1 plant separately (see another article in this issue, pp. 357–371).

With a view to avoid interplant competition and shadowing of leaves from neighbouring plants, and to ensure maximum possible development of each plant and its exposure to sunlight from all sides under field conditions, the F_2 plants were raised at a distance of 30 x 30 cm. Wide spacing also facilitated proper visualization of each plant at all developmental stages.

Each F_2 plant was visited four times during its life beginning 50 days after sowing at 5-day intervals. Since all the pigment-potent plants manifested leaf coloration during this

period and no additional plants formed colour in their leaves, subsequent observations for this purpose were not necessary.

RESULTS AND DISCUSSION

As stated above, all the plants capable of synthesizing brown pigment in their leaves do not manifest coloration at the same time. About 50% of the F_2 plants were recorded as pigmented in the first observation itself. The proportion of plants with brown leaves increased in all crosses to 60–65% and 70–75% in the second and third observations, respectively. A few more plants were added to the pigmented group in the last observation when the situation became stabilized and no additional plants with brown leaves were observed. The plants not capable of producing brown pigment remained green through their life. The pigmentation was gradually diluted during the phase of rapid vegetative growth and almost completely disappeared at flowering or soon after. Beyond this point, it is impossible to distinguish the pigment-potent plants from green ones.

As can be seen from Table 1, the F_2 segregation into brown : green plants gives a very good fit to 3 : 1 ratio, with the χ^2 values ranging from 0.017 to 0.585 (all values nonsignificant).

Table 1. Segregation for brown leaf pigmentation in F_2 generation of lentil crosses

Cross		F_2 segregation		d.f.	χ^2
		pigmented	nonpigmented		
Pigmented (female) x nonpigmented (male)					
Lens 4149	Eston	111	33	1	0.333
LS 106	Precoz	235	74	1	0.182
PKVL 1	Precoz	485	173	1	0.585
Lens 830–fasciated	Lens 6163	479	153	1	0.211
UK-1	LC-74-1-5-1	40	16	1	0.381
Lens 4602	LC-74-1-5-1	198	69	1	0.101
Nonpigmented (female) x pigmented (male)					
Precoz	Pant L 406	372	116	1	0.393
Precoz	Lens 4149	358	121	1	0.017
LC-74-1-5-1	LC-68-17-3-5	429	138	1	0.132
LC-74-1-5-1	Lens 830–globe	237	82	1	0.084
Lens 6163	PKVL 1	407	142	1	0.219
Total		3351	1117	11	2.638
Deviation from 3:1 ratio		0	0	1	0.000
Heterogeneity				10	2.638

The pooled data gave complete fit into 3 brown : 1 green ratio, with 3351 plants pigmented and 1117 nonpigmented ($\chi^2 = 0$). This is a rare example of a biological phenomenon having perfect expression without any deviation from expected.

These results confirm that brown pigmentation of leaves in lentil is controlled by a single dominant gene. In the recessive state of this gene, the plant loses its ability to synthesize the pigment. The gene symbol Bl is proposed for this trait (brown leaf).

With this hypothesis, it can be concluded that the F_1 plants with Blbl genotype should have been brown, although the observations were not recorded in the high mountainous location.

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