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DIGENIC CONTROL OF COTYLEDON COLOUR IN LENTIL (*LENS CULINARIS*)

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ABSTRACT

In spite of reports suggesting digenic nature of inheritance of cotyledon colour in lentil, the precise nature of gene interactions is unknown. A technique has been developed which allows, with some experience, near-perfect identification of cotyledon colour in intact seeds without removing testa. Such screening led to classification of the conventionally known "yellow" cotyledons into two easily distinguished categories: yellow and brown (dirty yellow). These two cotyledon colours are caused by dominant state of two unlinked genes, designated as Y (for yellow) and B (for brownish or dirty yellow). Double dominant condition YB gives orange (or red) cotyledons, while double recessive state yy bb produces light green cotyledons.

Key words: Lentil, *Lens culinaris*, cotyledon colour, digenic inheritance.

Three cotyledon colours are known in lentil: orange (also called red), yellow, and green. Even though the first report on the inheritance of cotyledon colour in lentil was published in 1928 by Tschermak [1], the precise nature of relationship between these traits is not understood. Careful examination of seeds in the present study has revealed two different types of yellow as well as green cotyledons. The typical bright yellow cotyledon colour is easily distinguishable from another colour type, which can be best described as dirty or musty yellow due to brownish tinge (henceforth called brown for brevity). The green cotyledons are also of two types: light green and deep (or dark) green. The light green colour has relatively poor penetrance, may be a leaky situation which allows a slight development of yellow pigment, especially in the core of cotyledons, which becomes visible only on splitting the seed and observing the cotyledons on their inner surface. Possibly, development of yellow pigment results in the lighter intensity of green colour in these genotypes. The light green cotyledon character is controlled by the same system of gene interactions which produce orange (red) and yellow (as well as brown) cotyledons.

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MATERIALS AND METHODS

The study reported is based on five crosses involving parents with orange (Lens 830—globe and fasciated mutants, Lens 4076, UK-1, and LC-68-17-3-5) and light green (LC-74-1-5-1) cotyledons. The orange-cotyledon parents were used as female parents and light-green genotypes as pollinators in four crosses. The fifth cross was reciprocal (female with green and male with orange cotyledons).

The F_1 seeds (obtained after crossing) as well as F_2 seeds (harvested from individual F_1 plants) were examined in a crude but simple device against concentrated light (Fig. 1). The device was made of a bent plastic pipe at the bottom of which an electric bulb of 100 Watt was fixed. A convex lense of 10 cm diameter was fixed at the top of the vertical arm of the plastic pipe, which was covered with an ordinary glass sheet or Petri dish for placement of seeds.

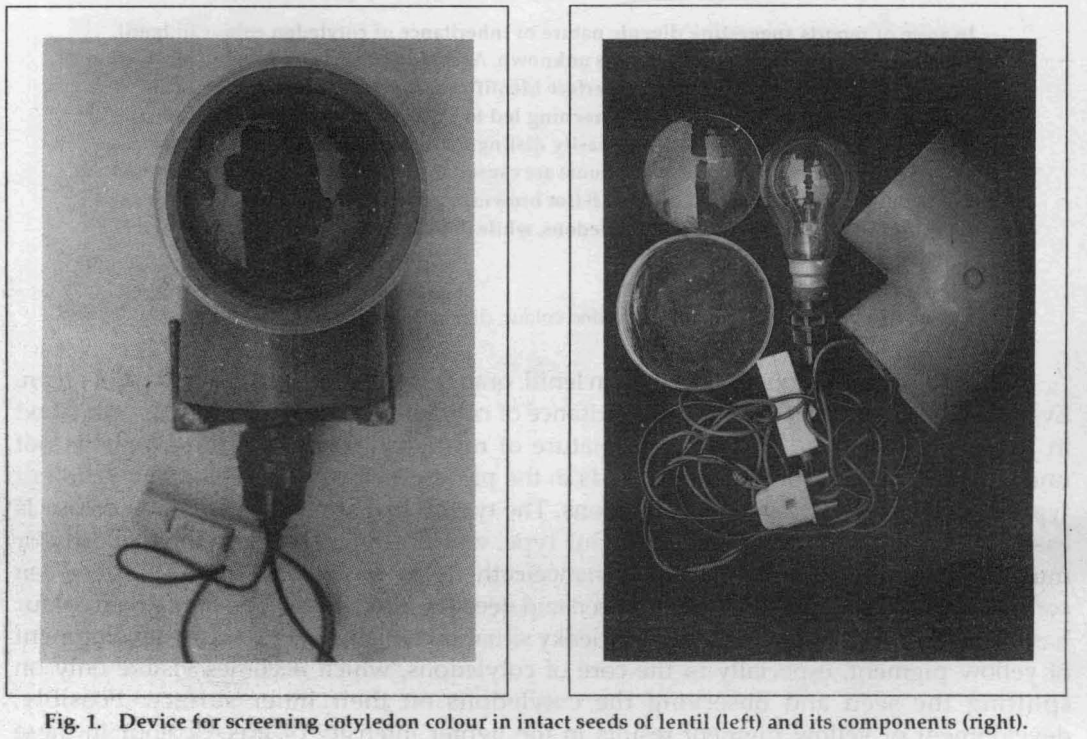

Fig. 1. Device for screening cotyledon colour in intact seeds of lentil (left) and its components (right).

This device makes it possible to determine cotyledon colour in intact seeds as the concentrated rays of light pass through the papery testa. With experience, the technique gives very reliable results. The resolution of cotyledon colour is perfect if the seed coat is

colourless. However, difficulty is faced in the genotypes producing testa with dark background colour or dense mottling. Extreme care is required in classifying the seeds for cotyledon colour in such cases.

The crosses were made during rabi 1993–94, and the F_1 and F_2 generations were raised in 1994–95 and 1995–96, respectively. All experimental materials were raised in the fields of I.A.R.I., New Delhi. The results reported here are confined to the F_1 seeds (i.e. seeds obtained after hybridization) and F_2 seed (i.e. seeds harvested from F_1 plants). It must be borne in mind that the cotyledon and testa tissues in these seeds are of F_1 and maternal genotypes, respectively.

RESULTS AND DISCUSSION

The results are based on the analysis of 1604 F_2 seeds (Table 1). The F_1 seeds from all the crosses between orange- and green-cotyledon genotypes (direct as well as reciprocal crosses) were always orange. This is in agreement with all other reports [2–5]. However, appearance of typical yellow and brownish yellow (= brown) cotyledons in the F_2 seeds of these crosses forced us to re-examine the situation. Distinguishing the two types of yellow cotyledons appears to explain the gene interactions involved.

Table 1. Segregation for cotyledon colour in lentil crosses

Cross	F_1		F_2 segregation				d.f.	χ^2 value (9:3:3:1)
	pheno-type	No. of seeds	orange	yellow	brown	green		
Lens 830-globe x LC-74-1-5-1 (orange) (light green)	Orange	8	269	78	67	29	3	5.051
UK-1 x LC-74-1-5-1 (orange) (light green)	Orange	10	73	27	25	13	3	2.632
Lens 830-fasciated x LC-74-1-5-1 (orange) (light green)	Orange	15	241	76	73	27	3	0.582
Lens 4076 x LC-74-1-5-1 (orange) (light green)	Orange	6	277	82	84	25	3	1.872
LC-74-1-5-1 x LC-68-17-3-5 (light green) (orange)	Orange	6	73	20	27	8	3	1.055
Total	Orange	45	933	283	276	102	15	11.192
Expected (in 9:3:3:1 ratio)			902.25	300.75	300.75	100.25		
Deviation			40.75	17.75	24.75	1.75	3	4.125
Heterogeneity							9	7.067

The segregation of F_2 seeds into groups of orange, yellow, brown and green cotyledons fits the digenic ratio of 9:3:3:1 with very high degree of probability as the χ^2 values calculated for each individual cross as well as pooled over the five crosses are very low. This confirms that the yellow and brown cotyledon colours are controlled by two independent unlinked genes in dominant condition. Double dominant situation produces orange (red) and double recessive gives light green cotyledons.

Assigning the gene symbols Y for yellow and B for brown cotyledon colours, the genotypes for the four cotyledon types can be written as follows:

Y – B –	:	orange (red) cotyledon
Y – b b	:	yellow cotyledon
y y B –	:	brown cotyledon
y y b b	:	light green cotyledon

This formulation suggests that a cross between a double dominant homozygous genotype with orange cotyledons (YY BB) will always produce F_1 seed with orange cotyledons when crossed with genotypes homozygous for yellow (YY bb), brown (yy BB), or green (yy bb) cotyledons. The F_2 segregation will be monogenic (3:1) in the crosses with homozygous yellow- or brown-cotyledon parents, as was observed by Wilson [2]. Distinction of brown cotyledon colour was never made in the published literature.

Slinkard [5] also reported dominance of orange (red) cotyledon character over yellow as well as green cotyledon colours. Interestingly, however, in the crosses of orange-cotyledon parents with some green-cotyledon parents, he observed F_2 segregation in the ratio of 9 red : 3 yellow : 4 green, and concluded dihybrid control of cotyledon colour with an inhibitor gene. This appears to be a complicated scheme. Nevertheless, appearance of 4 green seeds out of 16 F_2 seeds cannot be explained by the relatively simpler model of digenic interitance proposed here based on much larger volume of experimental material.

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