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CONFIRMATION OF DIGENIC INHERITANCE OF COTYLEDON COLOUR IN LENTIL (*LENS CULINARIS*)

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ABSTRACT

The F_3 seeds harvested from F_2 populations of five crosses between parents with orange and light green cotyledons were analysed. The F_3 segregation confirmed the earlier reported digenic control of cotyledon colour in lentil. Further, four new crosses were made between F_2 plants of different crosses which were raised from seeds with yellow and brown cotyledons. All F_1 seeds had orange cotyledons. The F_2 seeds harvested from F_1 plants raised in phytotron were analysed immediately for cotyledon colour. The F_2 seeds segregated in the ratio of 9 orange : 3 yellow : 3 brown : 1 green. The results from the new crosses also confirm digenic control of cotyledon colour in lentil.

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

Digenic control of cotyledon colour in lentil was reported earlier on the basis of analysis of F_2 seed (harvested from F_1 plants) [1]. Two new phenotypes, viz., yellow and brown, were obtained in F_2 populations of five crosses between genotypes with orange and light green cotyledons. Based on the segregation obtained in F_2 (9 orange : 3 yellow : 3 brown : 1 light green), two genes, Y (for yellow cotyledons) and B (for brown cotyledons), were proposed. The double dominant condition (Y—B—) results in orange phenotype and double recessive state (yybb) gives rise to light green cotyledons. To confirm the inheritance pattern, F_3 seed (harvested from F_2 plants) was analysed. Under such a situation, it was expected that crosses between genotypes with yellow and brown cotyledons would produce F_1 (i.e. crossed) seed with orange cotyledons. Such crosses were made between F_2 plants raised from seeds having yellow and brown cotyledons to confirm the earlier results and the hypothesis of digenic control of cotyledon colour in lentil.

MATERIALS AND METHODS

The F_2 generations of the five crosses described in [1] were raised during rabi (November–April) 1995–96 in the field. The cotyledon colour of seed from which each F_2

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plant was raised and used in the new crosses was known. As expected, such plants could be homozygous (YYbb and yyBB) or heterozygous (Yybb and yyBb) for the two cotyledon colours. Therefore, the seed harvested from F_2 plants was analysed and each F_3 seed progeny was identified as true breeding or segregating for the different phenotypic classes.

The seeds of segregating F_3 progenies were classified into different phenotypic classes and counted to test again and confirm F_2 segregation (Table 1). Besides, random samples of F_3 progenies in four crosses and all F_3 progenies in one cross were placed in the true breeding or segregating categories of various types to determine F_3 segregation (Table 2).

In addition, four crosses were made between F_2 plants which were raised from seeds with yellow and brown cotyledons. The parental plants were selected in the F_2 populations of different crosses raised for linkage studies [2] (see Table 2). The parent plants in cross No. 1 (Table 3) were selected from the F_2 population derived from the cross Lens 830-globe (orange) x LC 74-1-5-1 (light green). Crosses No. 2 and No. 4 were made between F_2 plants derived from the cross LC 74-1-5-1 (light green) x LC 68-17-3-5 (orange). Cross No. 3 was made between F_2 plants of the cross UK-1 (orange) x LC 74-1-5-1 (light green). Detailed information about the parental strains used in the original crosses is given in [2], a part of which was published earlier [1].

The crossed seeds harvested from the female parents were sown to raise F_1 generation during May–September 1996 under controlled environment in the phytotron at IARI, New Delhi. During crop growth, the photoperiod was maintained at 14 h, and the day and night temperatures at 20°C and 10°C, respectively. The relative humidity was maintained at 70% during growth period. The F_2 seeds harvested from F_1 plants were examined and classified into different groups of cotyledon colour by the same method as described by [1].

It was simultaneously confirmed as to which of the F_2 plants used as yellow- or brown-cotyledon parents in the new crosses were homozygous or heterozygous for Y and B genes, as the F_3 seeds harvested from them were either uniform yellow/brown or segregated into 3 yellow : 1 green or 3 brown : 1 green, respectively. As a result, the crossed seeds had either orange, yellow, brown or green cotyledons. The F_2 seeds were analysed for segregation immediately after harvest.

Since the relationship between yellow and light green cotyledon colours as proposed in the published literature has been confirmed by elaborate analysis in the present study, the yellow and green F_1 seeds obtained from these crosses were not sown. Since brown cotyledon colour is a new phenotype discovered, the F_1 seeds with brown cotyledons were also sown along with the orange ones to verify the relationship between brown and light green phenotypes.

RESULTS AND DISCUSSION

As mentioned above, the digenic control of cotyledon colour was proposed to be confirmed by two approaches: analysis of F_3 seed borne on the F_2 plants, and by crossing F_2 plants carrying genes for yellow and brown cotyledons in isolation.

CONFIRMATION OF F_2 SEGREGATIONS IN F_3 GENERATION

The inheritance of cotyledon colour was confirmed by analysing F_3 seed in different groups (Table 1). The F_3 seed harvested from individual F_2 plants raised from seeds with green cotyledons always had green cotyledons. The seed produced on F_2 plants which were raised from yellow- or brown-cotyledon seeds had either uniformly yellow or brown cotyledons, or segregated for yellow and green or brown and green cotyledons, respectively. The analysis based on the pooled data over F_3 seeds harvested from seven heterozygous yellow-cotyledon F_2 plants (total 1095 F_3 seeds) showed segregation into yellow (829 seeds) and light green type (266 seeds) with a good fit to 3:1 ratio ($\chi^2 = 0.29$; $P = 0.61$). Likewise, the 1351 F_3 seeds obtained from six brown-cotyledon F_2 plants segregated into 1036 brown : 315 green. Thus, the seeds harvested from these supposedly heterozygous F_2 plants gave 3:1 segregation with high degree of confidence ($\chi^2 = 2.04$; $P = 0.16$).

Table 1. Confirmation of F_2 segregation for cotyledon colour in F_3 generation of lentil

F_2 phenotype	No. of F_2 plants	F_3 segregation (No. of seeds)				Ratio tested	d.f.	χ^2	P
		orange	yellow	brown	green				
Yellow	7	0	829	0	266	3:1	1	0.29	0.61
Brown	6	0	0	1036	315	3:1	1	2.04	0.16
Orange	3	553	180	0	0	3:1	1	0.08	0.78
Orange	5	1557	0	497	0	3:1	1	0.71	0.42
Orange	17	5327	1697	1686	546	9:3:3:1	3	6.68	0.09

The F_2 plants, which were raised from the seeds with orange cotyledons, produced either uniformly orange seed or the seed harvested from them showed three different types of segregation. In one category of 733 seeds, the segregation of 553 orange and 180 yellow was in the ratio of 3 orange : 1 yellow. The other category of 2054 seeds segregated into 1557 orange and 497 brown, which was also in the ratio of 3 orange : 1 brown. In the third category, 9256 seeds segregated into 9 orange : 3 yellow : 3 brown : 1 green, producing 5327, 1697, 1686 and 546 seeds in the four classes, respectively (Table 1). The seeds analysed in these three categories were harvested from 3, 5 and 17 F_2 plants, respectively. The goodness of fit to the expected segregation ratios was evident from the low χ^2 values in all cases (0.08 and 0.71 at 1 d.f., 6.68 at 3 d.f.; $P = 0.78$ and 0.42 at 1 d.f., 0.096 at 3 d.f.).

CONFIRMATION OF DIGENIC INHERITANCE BY F_3 SEGREGATION

Besides the above confirmation of digenic F_2 segregation ratios in the F_3 progenies of heterozygous F_2 plants, the segregation of F_3 progenies themselves was also verified. Out of the five crosses showing digenic segregation in F_2 generation, random samples of 27, 63,

11 and 19 F_2 plants in the four crosses (total 120) were taken to analyse their F_3 seeds (Table 2). In one cross (Lens 830-globe x LC 74-1-5-1), all the 216 F_2 plants were harvested individually and their F_3 seed analysed to check the error due to random sampling in the remaining four crosses.

The data are presented in Table 2 for each individual cross as well as pooled over the four sampled crosses. The F_3 segregation in digenic inheritance of cotyledon colour with independent assortment is expected to be in the following ratio of F_3 progenies:

True breeding	4 :	1 orange : 1 yellow : 1 brown : 1 green
Monogenic segregation	8 :	2 orange : yellow (3:1)
		2 orange : brown (3:1)
		2 yellow : green (3:1)
		2 brown : green (3:1)
Digenic segregation	4 :	orange : yellow : brown : green (9:3:3:1)

The results obtained (Table 2) clearly show that the segregation of F_3 progenies fits exceedingly well to the above expected ratios. In four crosses, 120 F_3 plants were harvested as random samples. The χ^2 value (11.06; P = 0.10) even in this case was nonsignificant. The segregation of 216 progenies in the cross Lens 836-globe x LC 74-1-5-1 was even closer to the expected ratio in the 9 classes (χ^2 = 7.41; P = 0.31). The difference in the two cases is understandable as the greater deviation in the four sampled crosses could be partly due to smaller sample size (120 F_3 progenies) and partly due to sampling error. The error seems to have magnified in the cross LC 74-1-5-1 x LC 68-17-3- 5, in which the χ^2 value of 15.98 was marginally significant only at 5% level (threshold χ^2 = 15.51), but not at 1% level.

Even though the pattern of segregation did not change when the data for all the five crosses were pooled, the value of χ^2 was 12.56, indicating a greater deviation (although nonsignificant) from the expected as compared to several crosses taken individually. This was because the phenotypic classes homozygous or segregating for light green and brown cotyledons were recorded at frequencies lower than expected. This situation was repeated in the four sampled crosses as well the cross analysed in totality.

CONFIRMATION BY YELLOW x BROWN TYPE CROSSES

The results from the new crosses are based on the analysis of 738 F_2 seeds harvested from 7 F_1 plants (Table 3). The F_1 seeds in all the crosses between brown- and yellow-cotyledon genotypes (direct as well as reciprocal crosses) were always orange. This confirms that the presence of both the dominant genes Y and B leads to orange phenotype, as reported earlier [1].

The F_2 seeds segregated into four classes in which 403 seeds had orange, 139 yellow, 152 brown, and 44 light green cotyledons. The segregation fits well into the digenic 9:3:3:1

Table 2. Segregation of F_3 progenies for four types of cotyledon colour in lentil (ratio for digenic inheritance indicated in parentheses under each phenotypic class)

Cross	True breeding				Segregating for					Total F_3 progenies	χ^2 (8 d.f.)	P
	orange (1)	yellow (1)	brown (1)	light green (1)	orange and yellow (2)	orange and brown (2)	yellow and green (2)	brown and green (2)	all four phenotypes (4)			
Random samples of F_3 progenies analysed												
Lens 4076 x LC 74-1-5-1	1	3	2	0	4	3	4	2	8	27	4.00	0.85
LC 74-1-5-1 x LC 68-17-3-5	9	3	1	0	9	12	9	6	14	63	15.98*	0.02
Lens 830-fasciated x LC 74-1-5-1	0	3	1	1	1	2	0	1	2	11	10.82	0.29
UK-1 x LC 74-1-5-1	1	2	0	3	6	1	3	0	3	19	13.81	0.06
Pooled over four crosses												
Observed	11	11	4	4	20	18	16	9	27	120	11.06	0.10
Expected	7.5	7.5	7.5	7.5	15	15	15	15	30	120		
All F_3 progenies analysed												
Lens 830-globe x LC 74-1-5-1												
Observed	16	18	11	13	30	29	20	19	58	216	7.41	0.31
Expected	13.5	13.5	13.5	13.5	27	27	27	27	54	216		
Pooled over all the five crosses												
Observed	27	29	15	17	50	47	36	28	85	336	12.56	0.16
Expected	21	21	21	21	42	42	42	42	84	336		

*Significant at 5% level but not significant at 1% level.

ratio with high degree of probability as the P values for each individual cross as well as pooled over the four crosses were very high. This again confirms that the yellow and brown cotyledon colours are controlled by two unliked dominant genes and interaction of these genes gives orange phenotype. Double recessive state for the two genes results in elimination of both (yellow and brown) pigments and the seeds acquire natural green colour.

Table 3. Digenic segregation in F_2 populations of crosses between yellow- and brown-cotyledon genotypes

Cross	S. No. of cross	F_1 pheno-type	No. of F_1 plants	F_2 phenotypes and frequencies				Total F_2 seeds	d.f.	χ^2	P
				orange	yellow	brown	green				
Brown female x yellow male	1	Orange	4	272	97	107	25	501	3	3.53	0.32
	2	Orange	1	63	20	22	8	113	3	0.27	0.96
Yellow female x brown male	3	Orange	1	54	14	16	7	91	3	1.07	0.78
	4	Orange	1	14	8	7	4	33	3	3.58	0.31
Pooled over crosses			7	403	139	152	44	738	3	1.80	0.62
Heterogeneity								9	6.65	0.67	

The dominant–recessive relationship between the brown and light green cotyledon phenotypes was also confirmed by the results obtained from the new crosses as some of the F_1 seeds had brown cotyledons. Evidently, such seeds did not have the dominant Y gene for yellow cotyledon colour. These F_1 seeds would have yyBb genotype and segregate in F_2. Segregation was observed in four plants which produced a total of 330 seeds. Their segregation into 246 brown and 84 light green classes fits well the 3 : 1 ratio ($\chi^2 = 0.04$, P = 0.85). Dominance of brown cotyledon colour over light green is thus once again confirmed.

In conclusion, the detailed analysis for inheritance of cotyledon colour based on F_2 and F_3 segregations as well as crosses between yellow- and brown-cotyledon genotypes proves beyond doubt that this trait is governed by two unlinked genes, designated by gene symbols Y and B [1].

REFERENCES

1. M. K. Emami and B. Sharma. 1996. Digenic control of cotyledon colour in lentil (*Lens culinaris*). Indian J. Genet., **56**(3): 357–361.

2. M. K. Emami. 1996. Genetic Mapping in Lentil (*Lens culinaris* Medic.). Ph. D. Thesis. I.A.R.I., New Delhi.