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GENETICS OF COTYLEDON COLOUR IN LENTIL *(Lens culinaris medik.)*

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ABSTRACT
The genetics (inheritance) of cotyledon colour in lentil was studied on the basis of segregation patterns (monogenic, digenic and trigenic) observed in the F_1, F_2 and F_3 generations derived from 36 different crosses involved 31 parents. A monogenic inheritance pattern of cotyledon colour was obtained in the F_2 generation when analysed of orange × yellow, orange × brown orange × dark green and yellow × light green crosses and the F_2 seeds segregated into 3:1 ratio. The digenic inheritance (9:3:3:1) of cotyledon colour was observed in the orange × light green and yellow × brown crosses, and its epistatic (9:3:4) ratio was obtained when analysed F_2 seed derived from yellow × dark green and brown × dark green crosses. The trigenic inheritance pattern, which brought three genes (*Y, B, Dg*) was observed in light green × dark green cross and produced 27 (orange): 9 (yellow): 9 (brown): 3 (light green): 16 (dark green) ratio. The two genes (*Y* and *B*) are responsible for producing yellow and brown, respectively cotyledon colour. The orange colour of cotyledon was received in the dominant homozygous condition (*YYBB*), but remains green in the double recessive homozygous (*yybb*) situation. A third gene '*Dg*' was responsible to block the synthesis of both pigments in recessive state and dark green cotyledons.

INTRODUCTION

Lentil (*Lens culinaris* Medik) is a diploid (2n = 2X = 14) self-pollinating crop. It is rich source of protein (22-25%) and other minerals (Piecyk *et al.,* 2012). India is a largest producer in the world contributes ~ 42% of the total world production. In India, it has been cultivated on 1.27 million hectare area with a production of 0.97 million tonnes grains during 2015-16 (AICRP 2016-17). It is grown different parts of India and farmers' prefer to grow large and small seeded lentil having red cotyledons. However making the genetic improvement in any trait of economic importance, plant breeders are essentially required to know the inheritance of that trait. In lentil, genetics of of seed and plant morphological characters has been reported by a number of researchers (Wilson and Hudson, 1978; Slinkard, 1978; Singh, 1978; Ladizinsky, 1979; Vandenberg and Slinkard, 1987; Vandenberg and Slinkard, 1990; Emami and Sharma, 2000; Haque *et al.,* 2002; Mishra *et al.,* 2007; Saha *et al.,* 2013; Fedoruk, 2013; Cahit Erdoðan, 2015). Inheritance of cotyledon colour in lentil has been studied first time by Tschermak (1928). It has been reported that a inhibitory recessive gene controls the inheritance of green cotyledon (Wilson *et al.,* 1970). The gene symbols *Y* and *B* were assigned to the two distinct yellow phenotypes (Emami and Sharma, 1996). A new gene (Dg) responsible for dark green cotyledon, that acts at an earlier stage in the biosynthesis of cotyledon color in lentil (Sharma and Emami 2002). The five major classes of seed background color and three classes of seed coat pattern were identified in lentil (Erskin and Witcomb, 1984). The monogenic inheritance and linkage among 4 morphological traits was established (Khosravi *et al.,* 2010). Therefore different reported three genes including *Dg* for dark green, *Y* for yellow and *B* for brown for controlling the cotyledon colour in lentil. It has been also confirmed later on by several other works involving few crosses for studying the inheritance of cotyledon colors (Sharma *et al.*, 2004; Mishra, 2004; Kumar *et al.,* 2009). However, present study was undertaken to study the inheritance of cotyledon colours using a large number crosses involving multi-parents having different cotyledon colours.

MATERIALS AND METHODS

The experiment was conducted for the study of inheritance of cotyledon colour in lentil, using F_1, F_2 and F_3 generations derived from 36 crosses different crosses (Table 1). Direct as well as reciprocal crosses were made using 14 parents with orange, 8 with yellow, 2 with brown, 3 with light green and 4 with dark green cotyledons. The inheritance of different cotyledon colour was studied from the crosses between genetically pure parents with different phenotypes: orange ´ yellow (12), orange ´ brown (2), yellow ´ light green (5), orange ´ dark green (2), yellow ´ dark green (3), brown ´ dark green (4), yellow ´ brown (3), orange ´ light green (3) and light green dark green (2). The cotyledons were examined in the F_1 after harvesting the crossed pods and the seed harvested from F_1 plants was used to study segregation pattern of F_2 seeds. Segregation pattern was further confirmed in in F_3 Progenies.

Crosses were attempted by the emasculating flower buds

between 3.0 to 5.0 and pollinating them in the next day morning with fresh pollen. During emasculation, special care was taken not to touch the stigma with anthers or forceps to avoid or damaging the stigma. F_1 hybrid seeds were further grown to obtain F_2 seeds of each cross. The F_1 plants along with parents and F_2 populations were raised with 20 × 40cm spacing to facilitate individual plant observation. A technique of visualizing cotyledon colour against light in seeds with the testa intact was described earlier (Emami and Sharma *et al.,* 1996 a & b). An instrument for seed screening which improve efficiency of operation was designed by Sharma *et al.* (2005).

To study the inheritance of each trait, the χ^2 was estimated by using the standard formula (Mather, 1951). The segregation of cotyledon color was analyzed by χ^2 test to determine F_2 ratio of phenotypic classes of expected segregation ratios, 3: 1, 9: 3: 3: 1, 9: 3: 4 and 27: 9: 9: 3: 16. The χ^2 value calculated as $\Sigma(O-E)^2/E$, whereas, O = observed, E = expected frequency and Σ = summation over all classes. The null hypothesis assumes that the families are heterogeneous χ^2 value is less than table value for desired d.f. at 5% level of significance then the null hypothesis is accepted, but if the calculated χ^2 value is more than the table value then null hypothesis is rejected.

RESULTS AND DISCUSSION

The present investigation was aimed to study the genetics of cotyledon colour in lentil on the basis of segregation patterns in F_1, F_2 and F_3 generations derived from 36 different crosses. These crosses involved 14 parents with orange, 8 with yellow, 2 with brown, 3 with light green and 4 with dark green cotyledon colour. In these crosses, three different inheritance patterns were observed that have been discussed in the following cross combinations.

Orange *vs* yellow

The relationships between orange and yellow cotyledon color was established in twelve crosses including 7 crosses in one direction (orange × yellow) and 5 crosses in the other direction (yellow × orange). All crosses behaved in the same manner without any maternal effect and F_1 seeds always had orange cotyledons color. The F_2 seed of the individual crosses was segregated into 3 orange: 1 yellow ratio with non-significant χ^2 values (Table 2). The pooled analysis of 31343 F_2 seeds was also segregated into 23481 (orange): 7862 (yellow), which is good fit to the 3:1 ratio with non-significant heterogeneity among crosses (^{2} = 10.30 at 11d.f; P = 0.50). Similar

Table 1: Crosses along with color of parents made for genetics study of cotyledon color in lentil

Cross combination (Female x Male)				Cotyledon colour combination (Female x Male)			
1.	L 3685	x	Precoz	1.	Orange	x	Yellow
2.	L 435	x	Precoz	2.	Orange	x	Yellow
3.	LC 68-17-3-5	x	L 6163	3.	Orange	x	Yellow
4.	P 22115	x	L 6163	4.	Orange	x	Yellow
5.	L 830 globe	x	10-3-Y-26 globe	5.	Orange	x	Yellow
6.	L 830 globe	x	L 6163	6.	Orange	x	Yellow
7.	L 1304	x	25-26	7.	Orange	x	Yellow
8.	Precoz	x	PKVL 1	8.	Yellow	x	Orange
9.	L 6163	x	PKVL 1	9.	Yellow	x	Orange
10.	Precoz	x	L 4076	10.	Yellow	x	Orange
11.	P 22127	x	L 1304	11.	Yellow	x	Orange
12.	L 6163	x	P 33159	12.	Yellow	x	Orange
13.	EC 383087	x	10-2-B-2	13.	Orange	x	Brown
14.	22-B-21	x	P 22211	14.	Brown	x	Orange
15.	PL 406	x	1-B-G-8	15.	Orange	x	Light green
16.	1-B-G-8	x	L 1304	16.	Light green	x	Orange
17.	LC74-1-5-1	x	L830 fasciated	17.	Light green	x	Orange
18.	L 4378	x	L 1304	18.	Dark green	x	Orange
19.	L 4378	x	P 22115	19.	Dark green	x	Orange
20.	EC 383084	x	10-2-B-2	20.	Yellow	x	Brown
21.	Precoz	x	10-2-B-2	21.	Yellow	x	Brown
22.	22-B-21	x	P 22127	22.	Brown	x	Yellow
23.	MC 1	x	LC 74-1-5-1	23.	Yellow	x	Light green
24.	EC 383084	x	1-B-G-8	24.	Yellow	x	Light green
25.	21-Y-13	x	1-B-G-8	25.	Yellow	x	Light green
26.	LC 74-1-5-1	x	Precoz	26.	Light green	x	Yellow
27.	19-B-5 globe	x	10-3-Y-26 globe	27.	Light green	x	Yellow
28.	EC 383084	x	L 4378	28.	Yellow	x	Dark green
29.	10-3-Y-26 globe	x	L 4387	29.	Yellow	x	Dark green
30.	L 4378	x	L 6163	30.	Dark green	x	Yellow
31.	10-2-B-2	x	L 4384	31.	Brown	x	Dark green
32.	22-B-21	x	L 4387	32.	Brown	x	Dark green
33.	8-1	x	10-2-B-2	33.	Dark green	x	Brown
34.	L 4384	x	10-2-B-2	34.	Dark green	x	Brown
35.	L 4378	x	1-B-G-8	35.	Dark green	x	Light green
36.	L 263	x	1-B-G-8	36.	Dark green	x	Light green

Table 2: F_2 segregation for orange and yellow cotyledon colours

Cross			F_1 phenotype	Total F_2 seeds	F_2 segregation		?² (3 : 1)	P
					Orange	Yellow		
Female	x	Male parent						
Orange	x	Yellow						
L 3685	x	Precoz	Orange	710	540	170	0.42	0.18
L 435	x	Precoz	Orange	790	587	203	0.20	0.66
LC68-17-3-5	x	L 6163	Orange	2343	1733	610	1.34	0.25
P 22115	x	L 6163	Orange	3650	2704	946	1.64	0.20
L 830globe	x	10-3-Y-26globe	Orange	1159	884	275	1.0	0.32
L 830globe	x	L 6163	Orange	5163	3845	1318	0.77	0.40
L 1304	x	25-26	Orange	8099	6057	2042	0.02	0.67
Yellow	x	Orange						
Precoz	x	PKVL 1	Orange	3720	2815	905	0.90	0.36
L 6163	x	PKVL 1	Orange	419	306	113	0.87	0.37
Precoz	x	L 4076	Orange	4205	3191	1014	1.76	0.19
P 22127	x	L 1304	Orange	150	103	47	0.08	0.78
L 6163	x	P 33159	Orange	935	716	219	1.24	0.27
Pooled over 12 crosses				31343	23481	7862	0.12	0.74
Heterogeneity (11d.f.)							10.30	0.50

Table 3: F_2 segregation for orange and brown cotyledon colours

Cross			F_1 phenotype	Total F_2 seeds	F_2 segregation		?² (3 : 1)	P
					Orange	Brown		
Female	x	Male parent						
Orange	x	Brown						
EC 383087	x	10-2-B-2	Orange	2912	2175	737	0.15	0.70
Brown	x	Orange						
22-B-21	x	P 22211	Orange	1853	1424	429	0.03	0.88
Pooled over 2 crosses				4765	3599	1166	0.71	0.42
Heterogeneity (1 d. f.)							0.54	0.47

Table 4: F_2 segregation for orange and light green cotyledon colours

Cross			F_1 phenotype	Total F_2 seed	F_2 segregation				?² (9:3:3:1)	P
					Orange	Yellow	Brown	Light green		
Female	x	Male parent								
Orange	x	Light green								
PL 406	x	1-B-G-8	Orange	1239	693	224	244	78	0.91	0.82
Light green	x	Orange								
1-B-G-8	x	??L 1304	Orange	562	318	92	114	38	2.65	0.46
LC74-1-5-1´Fasciated mut			Orange	761	450	132	135	44	2.61	0.46
Pooled over 3 crosses				2562	1461	448	493	160	2.79	0.44
Heterogeneity (6 d. f.)									3.38	0.76

Table 5: Confirmation of four categories of cotyledon colours in the F3 seeds

F_2 plants No.	Total F_3 seeds Phenotype	F_3 segregation (no. of seeds)					Ratio tested	?²	P
			Orange	Yellow	Brown	Light green			
10	Orange	7567	4330	1388	1398	451	9 : 3 : 3 : 1	3.26	0.36
5	Orange	3650	2770	880	0	0	3 : 1	1.54	0.21
5	Orange	3339	2523	0	816	0	3 : 1	0.56	0.47
5	Yellow	2278	0	1734	0	544	3 : 1	1.52	0.22
5	Brown	2032	0	0	1545	487	3 : 1	1.16	0.29

inheritance pattern also observed when analyzed 5623 F_3 seeds derived from two crosses. In earlier studies, monogenic dominance of orange over yellow cotyledon colour has also been reported in several studies (Wilson *et al.,* 1970; Singh, 1978; Silinkard, 1978; Sinha *et al.,* 1987, Emami, 1996; Saha *et al.,* 2013; Fedoruk, 2013; Cahit Erdoðan, 2015).

Orange *vs* brown

The orange cotyledon colour was obtained in F_1 seeds, while F_2 seeds in each cross as well as pooled over all the crosses

were segregated into 3 orange: 1 brown ratio (χ^2 at 1d.f. = 0.03 to 0.71; *P* = 0.42 to 0.88) with non-significant heterogeneity (Table 3). The monogenic dominance of orange cotyledon colour over brown was also confirmed in the F_3 seeds. The gene symbol *B* for brown cotyledon is used (Emami and Sharma, 1996 a & b).

Orange *vs* light green

A total of three crosses were attempted to study genetics of orange *vs* light green cotyledon color. In F_1 seeds orange cotyledons colour was observed, while F_2 seeds were segregated into 9 orange: 3 yellow: 3 brown: 1 light green ratio with non-significant χ^2 values ranging from 0.91 to 2.65 at (P = 0.46 to 0.82). The pooled analysis of 2562 F_2 seeds over three crosses segregated into 1461 orange: 448 yellow: 493 brown: 160 light green, which fits to 9:3:3:1 ratio and undisputedly establishes digenic inheritance for orange *vs* light green cotyledon colour (Table 4). The F_3 seeds obtained from 10 F_2 plants which turned to be heterozygous for both the colour genes segregated into 4330 orange: 1388 yellow: 1398 brown: 451 light green cotyledon color which fits to 9:3:3:1 ratio and confined digenic control for this trait (**T**able 5).

The first indication of involvement of more than one gene for the expression of cotyledon colour in lentil when available with the crosses between orange / yellow and yellow / green parent gave 3:1 segregation into orange: yellow and yellow: green, respectively (Slinkard, 1978). The yellow colour being recessive to orange and dominant over green was interpreted and a possibility of multiple allelism as orange–yellow-green. A significant model of digenic control of cotyledon colour was proposed by Emami and Sharma (1996a & b) when made crosses between orange and light green cotyledons parents and obtained orange cotyledon colour in F_1 seeds, while F_2 seeds segregated into four groups orange, yellow, brown and light green with good fit to the digenic ratio (9:3:3:1). The new gene symbols *Y-B-* (orange), *Y-bb* (yellow), *yy-B* (brown) and *yybb* (light green) were proposed for these inheritance patterns (Emami and Sharma, 1996a; Kumar *et al*., 2012).

Orange *vs* dark green

Two crosses were attempted between orange and dark green cotyledon colours parents and F_1 seed had orange cotyledon while F_2 seeds of individual crosses segregated into 3:1 ratio with non-significant χ^2value (0.23 to 1.39; P = 0.25 to 0.65). The pooled analysis of 12339 F_2 seeds was also segregated into 9272 orange: 3067 dark green (χ^2 = 0.14; P = 0.71) with non-significant heterogeneity (Table 6). An attempt was made to confirmed 3:1 ratio in F_3 seeds obtained from 20 F_2 plants segregated into 8423 (orange): 2832 (dark green). These observations confirmed monogenic recessive nature of dark green cotyledon colour in relation to orange. Previously, it has been reported that light green colour is under the control of two homozygous recessive genes, while dark green produced by third gene (Sharma and Emami, 2002).

Yellow *vs* brown

Three crosses were attempted between yellow and brown cotyledon color parents and F_1 seeds always produced orange cotyledon colour (Table 7). A digenic ratio (9:3:3:1) was obtained when 3430 F_2 seeds were segregated into 1929 (orange): 645 (yellow): 627 (brown): 229 (light green) with non-significant χ^2value (0.37 to 1.28; P = 0.74 to 0.95). Appearance of light green colour in double recessive homozygotes category confirms, the absence of both yellow and brown pigments in the non-functioning state of two genes (Emami and Sharma, 1996a).

Yellow *vs* light green

The relationship between yellow and light green cotyledon colours was established in five crosses. The F_1 seeds had yellow cotyledons and F_2 seeds segregated into yellow: light green cotyledons with good fit to the 3:1 ratio (χ^2 = 0.00 to 0.75; *P* = 0.41 to 1.00). The pooled data were also showed the same trend with non-significant heterogeneity (Table 8). The similar results were visually observed in the F_3 seed. Monogenic dominance of yellow over light green cotyledon colour was established in this voluminous study as well.

Yellow *vs* dark green

The genetics of dark green cotyledon over yellow colour was studied in three crosses. F_1 seeds were showed orange cotyledons, while F_2 seeds segregated into 4775 orange: 1575 yellow: 2121 dark green cotyledon which is good fit to 9:3:4 ratio having the χ^2 values of 0.36 to 2.29 at (*P* = 0.32 to 0.84). The pooled data (8471 seeds) was also showed the same trend with non-significant heterogeneity (Table 9)**.** These results indicated two genes are controlled the cotyledon colour in lentil. This inheritance pattern was also observed in the F_3 seeds. In the first category, 3195 seeds obtained from 7 heterozygous F_2 plants which were segregated into 1910 (orange): 417 (yellow): 868 (dark green) ratios (9:3:4)**.** The second category comprising 1598 seeds obtained from 5 heterozygous F_2 plants, segregated into 1215 orange: 383 yellow cotyledons (3:1 ratio)**.** The third category had 3115 seeds from 8 heterozygous F_2 plants again segregated into 2326 orange: 789 dark green-cotyledons showed good fit the 3:1 ratio**.** Similarly, the F_3 seeds were raised with yellow cotyledons produced either uniformly yellow-cotyledon or segregated for yellow and dark green cotyledons colour. Total 479 seed from 3 heterozygous F_2 plants segregated into 365 (yellow): 114 (dark green) cotyledons with a good fit to 3:1 ratio (Table 10). Digenic control of cotyledon colour was earlier reported by Slinkard (1978) in the crosses having green-cotyledon parent. The gene symbol '*O*' for orange and its recessive allele '*o*' for yellow cotyledons was proposed (Singh, 1978). Sharma and Emami (2002) proposed a third gene (*Dg*) which mutated blocks the synthesis of all pigments and creates a phenotype that is distinctly different from the dark green cotyledon colour observed in recessive condition of both *Y* and *B*. The *dg* gene also manifested recessive epistasis when with yellow and dark green cotyledons parents were crossed. The orange phenotype appeared in F_1 and succeeding generations because the dominant genes *Y* and *B* were contributed by the dark green and yellow cotyledons parents

Brown *vs* dark green

Identical results were obtained when brown seeded parents were crossed with dark green and the F_1 plants had orange cotyledons, while F_2 populations were segregated into 9 (orange): 3 (brown): 4 (dark green) ratios with non-significant χ^2 values which ranges from 0.06 to 2.18 at 2 d.f. (*P* = 0.34 to

Table 6: F_2 segregation for orange and dark green cotyledon colours

Cross	F_1 phenotype	Total F_2 seeds	F_2 segregation		X^2 (3 : 1)	P
			Orange	Dark green		
Female x Male parent						
Dark green x Orange						
L 4378 x L 1304	Orange	7420	5547	1873	0.23	0.65
L 4378 x P 22115	Orange	4919	3725	1194	1.39	0.25
Pooled over 2 crosses		12339	9272	3067	0.14	0.71
Heterogeneity (1 d. f.)					1.49	0.23

Table 7: F_2 segregation for yellow and brown cotyledon colours

Cross	F_1 phenotype	Total F_2 seed	F_2 segregation				X^2 (9:3:3:1)	P
			Orange	Yellow	Brown	Light green		
Female x Male parent								
Yellow x Brown								
EC383084 x 10-2-B-2	Orange	1292	730	240	237	85	0.37	0.95
Precoz x 10-2-B-2	Orange	1059	583	200	202	74	1.16	0.76
Brown x Yellow								
22-B-21 x P 22127	Orange	1079	616	205	188	70	1.28	0.74
Pooled over 3 crosses		3430	1929	645	627	229	1.41	0.70
Heterogeneity (6 d. f.)							1.41	0.96

Table 8: F_2 segregation for yellow and light green cotyledon colours

Cross	F_1 phenotype	Total F_2 seed	F_2 segregation		X^2 (3 : 1)	P
			Yellow	Light green		
Female x Male parent						
Yellow x Light green						
MC 1 x LC 74-1-5-1	Yellow	2782	2086	696	0.00	1.00
EC383084 x 1-B-G-8	Yellow	2109	1574	535	0.15	0.70
21-Y-13 x 1-B-G-8	Yellow	2035	1543	492	0.74	0.41
Light green x Yellow						
LC74-1-5-1x Precoz	Yellow	1568	1171	397	0.09	0.78
19-B-5g x 10-3-Y-26globe	Yellow	3252	2425	827	0.32	0.59
Pooled over 5 crosses		11746	8799	2947	0.05	0.83
Heterogeneity (4 d. f.)					1.24	0.87

Table 9: F_2 segregation in the cross between parents with yellow and dark green cotyledons

Cross	F_1 phenotype	Total F_2 seeds	F_2 segregation			X^2 (9:3:4)	P
			Orange	Yellow	Dark green		
Female x Male parent							
Yellow x Dark green							
EC 383084 x L 4378	Orange	4150	2289	796	1065	2.02	0.38
10-3-Y-26globe x L 4387	Orange	1504	853	273	378	0.36	0.84
Dark green x Yellow							
L 4378 x L 6163	Orange	2837	1633	526	678	2.29	0.32
Pooled over 3 crosses		8471	4775	1575	2121	0.01	1.00
Heterogeneity (4 d. f.)						4.66	0.33

0.97). The pooled data over all the crosses also followed the same trend (χ^2 = 0.37 at 2d.f.; P = 0.83) with non-significant heterogeneity (Table 11). These observations revealed digenic (epistatic) control of cotyledon. The inheritance of orange, brown and dark green cotyledons was also confirmed in F_3 seeds.

Light green *vs* dark green

Another interesting interaction between genes coding for cotyledon colours was observed when two crosses made between dark green and light green of parents. The F_1 seeds produced orange cotyledons and F_2 seeds segregated into orange: yellow: brown: light green: dark green with a good fit to the trigenic ratio of 27:9:9:3:16 (χ^2 = 2.52 and 2.10 at 4d.f.; P = 0.64 and 0.72). The pooled data over two crosses (2250 F_2 seeds) was segregated into 956 (orange): 301 (yellow): 300 (brown): 100 (light green): 593 (dark green) ratio with non-significant heterogeneity (Table 12). This convincingly that three genes are involved to control of cotyledon colour in

Table 10: Confirmation of F_2 segregation for orange, yellow and dark green colours in F_3 seeds

F_2 plants No.	Total F_3 seeds Phenotype	F_3 segregation (no. of seeds) Orange	Yellow	Dark green	Ratio tested	X^2	P
7	Orange	3195	1910	417	868	9 : 3 : 4	1.04	0.60
5	Orange	1598	1215	383	0	3 : 1	0.91	0.35
8	Orange	3115	2326	0	789	3 : 1	0.18	0.68
3	Yellow	479	0	365	114	3 : 1	0.37	0.56

Table 11: F_2 segregation for brown and dark green cotyledon colours

Cross	F_1 phenotype	Total F_2 seeds	F_2 segregation Orange	Brown	Dark green	X^2 (9:3:4)	P
Female x Male parent							
Brown x Dark green							
10-2-B-2 x L 4384	Orange	796	446	144	206	0.43	0.81
22-B-21 x L 4387	Orange	1015	548	203	264	2.18	0.34
Dark green x Brown							
8-1 x 10-2-B-2	Orange	2531	1429	475	627	0.07	0.96
L 4384 x 10-2-B-2	Orange	1257	704	239	314	0.06	0.97
Pooled over 4 crosses		5599	3127	1061	1411	0.37	0.83
Heterogeneity (6 d. f.)						2.37	0.88

Table 12: F_2 segregation for light green and dark green cotyledon colours

Cross	F_1 phenotype	Total F_2 seeds	F_2 segregation Orange	Yellow	Brown	Light green	Dark green	X^2 (27:9:9:3:16)	P
Female x Male parent									
Dark x Light green									
L 4378 x 1-B-G-8	Orange	1123	467	153	150	50	303	2.52	0.64
L 263 x 1-B-G-8	Orange	1127	489	148	150	50	290	2.10	0.72
Pooled over 2 crosses		2250	956	301	300	100	593	3.59	0.47
Heterogeneity (4 d. f.)								1.03	0.31

Table 13: Confirmation of F_2 segregation into 5 classes of cotyledon colours in F_3 seeds

F_2 plants No.	F_3 seeds Phenotype	Orange	Yellow	Brown	Light green	Dark green	Ratio tested	X^2	P
2	Orange	234	64	0	0	0	3 : 1	1.97	0.17
3	Orange	376	0	115	0	0	3 : 1	0.65	0.44
7	Orange	1770	0	0	0	600	3 : 1	0.36	0.57
6	Orange	1176	391	0	0	563	9 : 3 : 4	2.38	0.31
2	Orange	449	0	138	0	219	9 : 3 : 4	2.70	0.24
5	Orange	1219	2297	396	126	0	9 : 3 : 3 : 1	0.75	0.86
5	Orange	955	287	328	100	576	27:9:9:3:16	3.91	0.43
2	Yellow	0	304	0	0	112	3 : 1	0.82	0.36
3	Yellow	0	456	0	152	0	3 : 1	0.00	1.00
5	Yellow	0	553	0	115	273	9 : 3 : 4	3.21	0.20
3	Brown	0	0	363	137	0	3 : 1	1.54	0.22
2	Brown	0	0	323	0	104	3 : 1	0.09	0.76
2	Brown	0	0	411	93	251	9 : 3 : 4	3.58	0.17
2	Light green	0	0	0	372	120	3 : 1	0.10	0.76

lentil. Confirmatory results of F_2 segregation for 5 classes of cotyledon colours in F_3 seeds were represented of all possible types. Segregations were obtained in monogenic ratio for the pairs of phenotypes orange–yellow, orange–brown, orange–dark green, yellow–dark green, yellow–light green, brown–light green, brown–dark green and light green–dark green. Digenic segregation with recessive epistasis (9:3:4) was obtained when *Y* or *B* genes were combined with *dg*, while independent assortment (9:3:3:1) resulted when gene *dg* excluded (Table 13).

The F_1 hybrid from the crosses between parents with light green and dark green cotyledons produced seeds with orange colour because dark green-cotyledon parents were dominant for *Y* and *B* genes, which did not produce any pigment due to the epistatic effect of the recessive *dg* gene. Involvement of three genes for cotyledon colour was amply demonstrated by trigenic F_2 segregation into 27 orange: 9 yellow: 9 brown: 3

light green: 16 dark green. The genes (*Y, B* & *Dg*) are functionally associated in the metabolic pathway of pigment synthesis. Emami (1996) provided a model to explanation the role of three genes leading to synthesis of three kinds of pigments in the developing seeds of lentil. According to their hypothesis, the yellow and brown pigments are synthesized from a common precursor (P) through the evolvement of genes *Y* and *B,* respectively. A mutation in the gene *Dg* results in the loss of both pigments. This would happen only if it is assumed that gene *Dg* acts a stage prior to the point of action of the genes responsible for synthesis of the final product (yellow and brown pigments) at the last stage. In such a scheme, the substrate P for the action of the genes *Y* and *B* will not be available and two pigment products will be knocked down by a single mutation in gene *Dg*. The two pigments will also not be produced when both *Y* and *B* genes are mutated to their recessive alleles. However, the phenotypes of double recessive *yybb* and monogenic *dgdg* homozygotes are not identical, and for that reason they have been called light green and dark green, respectively. It appears that a certain amount of leaky pigment synthesis occurs in the double recessive *yybb* situation, leading to light green colour homozygous *dgdg* situation when the last precursor is eliminated, hence the deep green phenotype.

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