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INHERITANCE OF MORPHOLOGICAL CHARACTERS ASSOCIATED WITH PLANT AND DRIED SEEDS IN LENTIL (*LENS CULINARIS* MEDIK.)

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Abstract

Inheritance of testa colour, testa pattern, cotyledon color and stem color was studied in crosses of lentil involving parents with brown, grey, green testa color, red and yellow cotyledon color, and with green and colored seedling stem. Analysis of F_1 plant populations of crosses between green and colored (pink) stem revealed that colored stem was dominant over green stem. Similarly red cotyledon was dominant over yellow cotyledon. The F_2 segregation patterns indicated red cotyledon and colored stem to be monogenic traits. The analysis of F_2 and F_3 seeds (having F_1 and F_2 testa, respectively) revealed that spackled testa was dominant to even (without speckles) testa, grey testa was dominant to green testa and brown testa was dominant to both grey and green testa. The F_2 segregation ratio from cross involving parents with brown and grey testa was 9 brown: 3 grey: 4 green. The crosses between parents with grey and green testa color segregated to fit a ratio of 3:1 between dominant and recessive color. These segregating patterns revealed involvement of two genes and a recessive inhibitor in the regulation of testa color.

Introduction

Lentil is an important cool season food legume in Pakistan that is cultivated on 31 thousand hectares annually (Anon., 2009-10); mainly under rain-fed conditions. There has been decline in the area of lentil in Pakistan despite moderate improvement in its productivity. This declining trend in area may be reversed by the release of high yielding cultivars. Studies on lentil in Pakistan were mainly focused on seed diseases (Hussain *et al*., 2007, Rahim *et al*., 2010) and genetic diversity among genotypes prior to variety development (Asghar *et al*. 2010). Most varieties were released without giving consideration to seed traits. The ethnic preferences for specific seed traits such as testa colour, testa pattern and cotyledon colour determine the acceptability and market value of new cultivars. In order to breed varieties with desired combination of morpho-yield traits, an understanding of genetic mechanism controlling these traits is essential. Five major classes of seed background color and three classes of seed coat pattern have been identified in lentil (Erskin & Witcomb, 1984). The genetics of seed and plant morphological characters in lentil has been reported by a number of researchers (Wilson & Hudson, 1978; Slinkard, 1978; Singh, 1978; Ladizinsky, 1979; Vandenberg & Slinkard, 1987; Vandenberg & Slinkard, 1990; Emami & Sharma, 2000, Sharma & Emami, 2002; Haque *et al*., 2002 and Mishra *et al*., 2007 have also reported the genetics of morphological traits in lentil. Initially Tschermak Seysenegg (1928) reported green seed coat with purple spot to be dominant to light bland seed coat which was found to be monogenic (Ladzinsky, 1979).

Monogenic dominance of brown testa color over tan testa color and green testa color has also been reported by Emami & Sharma (2000). They suggested that testa colour was influenced by cotyledon colour; probably due to diffusion of a soluble pigment from the cotyledons to the testa. On the contrary, Wilson & Hudson (1978) reported black seed coat color to be under the control of two independent genes. Digenic inheritance of seed coat color was also reported by Vandenberg & Slinkard (1990); however they reported that testa pattern was inherited by multiple alleles of a single gene. The red cotyledon has been found to be under the control of single gene with green cotyledon recessive to red. A recessive color inhibitor was also found to be involved in the inheritance of green cotyledon (Wilson *et al.,* 1970; Slinkard, 1978 and Singh, 1978). Sharma & Emmami (2002) reported a new gene (Dg) responsible for dark green cotyledon that acts at an earlier stage in the biosynthesis of cotyledon color. More recently, the monogenic inheritance and linkage among 4 morphological characters was established on the basis of F_1 observations and analysis of F_2 plants in five crosses (Khosravi *et al*., 2010).

The aim of the present study was to investigate the genetic mechanism regulating seed and morphological characters in lentil, together with assessing the number of factors involved in phenotypic expression of these traits.

Materials and Methods

Four pure breeding germplasm lines selected on the basis of variation for seed and plant morphological characters were the source material for this experiment. The selected genotypes were Pant L-406, Precoz, Syrian Local Large (SLL) and Flip 86-38L.

Pant-L-406 is a small seeded genotype having Red cotyledon, brown seed coat with spots and anthocyanin pigmentation on stem of young seedlings. Whereas Precoz has Yellow cotyledon, green testa without spots and young seedlings with anthocyanin on their base. Similarly the Syrian Local Large (SLL) has yellow cotyledons, large seeds with green testa and young seedlings without anthocyanin pigmentation. The fourth genotype Flip86-38L produces seed with red cotyledon, grey testa without mottling. Its seedlings are also without anthocyanin pigments.

The above mentioned genotypes were crossed in diallel fashion under green house conditions. The F_1 seeds harvested from maternal plants were scored for cotyledon colour and testa characters. Ten seeds from each hybrid were germinated in pots and placed in green house under the conditions used for growing with parental genotypes. F_2 populations of plants were also raised by germinating 50 F_2 seeds with yellow cotyledon and 50 with red cotyledon from each of the crosses involving parents with yellow and red cotyledon. However, from crosses where both parents had yellow cotyledons, 100 F_2 seeds were randomly selected and germinated. Data were recorded on F_1 and F_2 populations for testa colour, testa pattern, cotyledon colour and anthocyanin pigmentation. Since testa is a maternal tissue, F_2 and F_3 seeds harvested from F_1 and F_2 plants were used to study F_1 and F_2 testa characteristics. The probability of the data fitting known genetic patterns was tested using Chi square test.

Results

For seed traits F_1, F_2 and F_3 seed populations and for morphological traits F_1 and F_2 plant populations were studied through genetic analysis. Neither of the traits in present study showed differences between reciprocal crosses. The study of testa background color revealed F_1 seed coat color to be similar to that of its maternal parent. F_1 plants originating from crosses between brown and grey (Pant-L-406 × F86-38L) or brown and green (Pant-L-406 × SLL or Precoz) produced seed with brown testa (Figs. 1&2). The seed coat color produced by F_1 hybrid plants from crosses between grey and green was grey (Table 1). Green seed coat was produced only by F_1 plants from cross involving both parents with green testa (SLL × Precoz). The segregating pattern for seed coat colour of seeds harvested from F_2 plants with either yellow or red cotyledon was similar. Three phenotypes, brown, grey and green were observed in F_2 seed coat populations harvested from F_2 plant populations obtained from crosses involving brown and green parents that fitted 9:3:4 ratios (Table 1). The cross between grey and green segregated between grey and green to fit 3:1 ratio. The pattern for F_1 testa obtained from crosses between spotted and absent (Pant-L-406 × SLL, Precoz or Flip86-38L) was spotted Plate-1 & Table 2). The F_1 testa of all these crosses segregated between 3 spotted: 1 absent. There was no reciprocal effect on the testa pattern and cotyledon colour. The cotyledon colour of all F_1 hybrid seeds from crosses involving parents with red × yellow cotyledon (Pant-L-406 × Precoz or SLL and Flip86-38L × procoz or SLL) was red (Fig. 3). The F_2 phenotypic frequencies of red and yellow in all the crosses provided a good fit to 3:1 ratio (Table 3). All the F_1 hybrid seedlings germinated from seeds obtained by crossing genotypes with coloured stem (anthocyanin pigments) and green stem (without anthocyanin) had pigmentation on their stem. The F_2 populations of these crosses segregated into 3 coloured and 1 green stem (Table 4).

Discussion

The genetic diversity in legemes (Sultana *et al.*, 2005; Akhtar *et al.*, 2011; Asim, 2012) provides an opportunity for improvement of economic traits. However, the understanding of genetic mechanism of seed morphological traits in lentil is essential for development of varieties with favorable combination of seed morphological traits that improve the acceptability of this legume by various ethnic groups. The present study on the inheritance of seed coat color, seed coat pattern and cotyledon color in lentil showed that brown seed coat was dominant over both grey and green seed coat and grey was dominant over green. The segregation pattern (9brown: 3grey: 4green) for seed coat color from cross between brown and green suggested that said character was controlled by two genes with involvement of recessive inhibitor. Such a recessive inhibitor for the control of cotyledon color has been reported previously (Slinkard, 1978). The presence of recessive inhibitor may have added to number of green testa. Wilson & Hudson (1980) suggested from crosses between beige and black color that testa color was controlled by two independent genes. Another study showed that testa color was determined by two independent genes, which also showed that brown testa color was dominant over grey, tan and green. The grey testa was dominant over tan and green; and tan was dominant over green (Vandenberg & Slinkard, 1990). An F_2 segregation ratio with 9 brown:3 grey:3 tan:1 green was reported by Vandenberg & Slinkard (1990) from a cross between brown and green. This segregation pattern was different from that obtained in the present study from similar cross but involving different genotypes. They obtained seeds with tan color in F_3 seeds (with F_2 testa) which was not recorded in our study. This may have occurred due to presence of homozygous recessive gene for 0 tennin in parents involved in our cross which converted tan color into green resulting in 9 brown: 3 grey: 4 green segregation pattern. Vandenberg & Slinkard (1990) found tan testa color to be hypostatic to zero tannin gene. The genotypes used for cross between brown and green in the present study could be homozygous recessive for zero tenin. However, Emami & Sharma (2000) reported that black testa was dominant over brown, tan and green; with incomplete penetrance. They reported monogenic dominance of brown testa over green and tan. They also observed in the same study that expression of testa color was influenced by cotyledon color probably due to diffusion of a soluble pigment from cotyledon to the testa. Their study showed that F_2 seeds from crosses of genotypes having brown or green testa with genotypes having orange or green cotyledons showed effect of cotyledon colour on testa. F_2 seeds from their crosses with green testa always had green cotyledons and never orange cotyledons whereas F_2 seeds with brown testa always had orange cotyledons and never green cotyledons. Such relationship between testa and cotyledon color was not revealed in our study. The present study showed cotyledon color, testa pattern and anthcyanin pigmentation on seedling to be monogenic in their inheritance. Red cotyledon was dominant over yellow, anthocyanin pigmentation of stem was dominant over green stem and spotted testa was dominant over even testa (spots absent). These findings are in line with those reported by Slinkard (1978); Singh (1978) and Ladizinksky (1979). Metz *et al.*, (1992) studied inheritance of epicotyls color in lentil and faba bean and found it monogenic and dominant over green epicotyle. The review of studies on inheritance of morphological traits reported by Mishra *et al*., (2007) revealed almost similar findings.

Fig. 1. Inheritance of testa color in lentil.

Table 1. F_1 phenotypes, F_2 phenotypes, F_2 frequencies and expected phenotypic ratios in lentil crosses involving Brown, Green and Grey seed coat color Set all tables as Table 1.

Cross	F_1 phenotype	F_2 phenotypic frequencies			Expected ratio	X^2	p-value
		Brown	Grey	Green			
Pant-l-406 x SLL	All brown	58	16	19	9:3:4	1.52	0.25-0.50
Pant-l-406 x precoz	All brown	60	17	23	9:3:4	0.57	0.25-0.50
Pant-l-406 x Flip86-38L	All brown	66	19	-	3:1	0.23	0.50-0.75
SLL x Precoz	All green	-	-	97	-		
SLL x Flip86-38L	All grey	-	70	21	3:1	0.17	0.50-0.75
Precoz x Flip86-38L	All grey	-	79	21	3:1	0.85	0.25-0.75
Precoz (Precoz x Flip86-38L)	-	-	17	15	1:1	0.12	0.50-0.75

Pant-L-406 = Brown seed coat color, SLL = Green seed coat color, Precoz = Green seed coat color, Flip86-38L= Grey seed coat color

Fig. 2. Inheritance of testa pattern in lentil.

Table 2. F_1 phenotypes, F_2 phenotypes and frequencies and expected phenotypic ratios in lentil crosses involving spotted and absent testa pattern.

Cross	F_1 phenotype	F_2 phenotypic frequencies		Expected ratio	X^2	p-value
		Spotted	Absent			
Pant-l-406 x SLL	Spotted	68	26	3 : 1	0.36	0.25-0.50
Pant-l-406 x precoz	Spotted	73	27	3 : 1	0.21	0.50-0.75
Pant-l-406xFlip86-38L	Spotted	66	22	3:1	0	1.00
SLL x Precoz	Absent	-	97	-		
SLL x Flip86-38L	Absent	-	91	-		
Precoz x Flip86-38L	Absent	-	100	-		

Pant-L-406 = spotted testa, SLL = Testa without pattern, Precoz = Testa without pattern, Flip86-38L = Testa without pattern

Table 3. F_1 phenotypes, F_2 phenotypes and frequencies and expected phenotypic ratios in lentil crosses involving spotted and absent testa pattern.

Cross	F_1 phenotype	F_2 phenotypic frequencies		Expected ratio	X^2	p-value
		Red	Yellow			
Pant-l-406 x SLL	Red	204	62	3 : 1	0.40	0.25-0.50
Pant-l-406 x precoz	Red	183	60	3 : 1	0.12	0.50-0.75
Pant-l-406xFlip86-38L	Red	257	-	All red		1.00
SLL x Precoz	Yellow	-	186	All yellow		
SLL x Flip86-38L	Red	196	78	3 : 1	1.75	0.10-0.25
Precoz x Flip86-38L	Red	157	53	3 : 1	0.006	0.90– 0.99

Pant-L-406 = Red cotyledon, SLL = Yellow cotyledon, Precoz = Yellow cotyledon, Flip86-38L= Yellow cotyledon

Table 4. F_1 phenotypes, F_2 phenotypes and frequencies and expected phenotypic ratios in lentil crosses involving spotted and absent testa pattern.

Cross	F_1 phenotype	F_2 phenotypic frequencies		Expected ratio	X^2	p-value
		Purple	Green			
Pant-l-406 x SLL	Purple	72	28	3 : 1	0.48	0.50 – 0.75
Pant-l-406 x precoz	Purple	100	-	All purple	-	0.50-0.75
Pant-l-406xFlip86-38L	Purple	63	24	3:1	0.31	0.50-0.75
SLL x Precoz	Purple	80	20	3 : 1	1.33	0.10-0.25
SLL x Flip86-38L	Green	-	97	All green	-	
Precoz x Flip86-38L	Purple	79	21	3 : 1	0.85	0.10-0.25

Pant-L-406 = Purple seedling stem, SLL = Green seedling stem, Precoz = Purple seedling stem, Flip86-38L = Green seedling stem

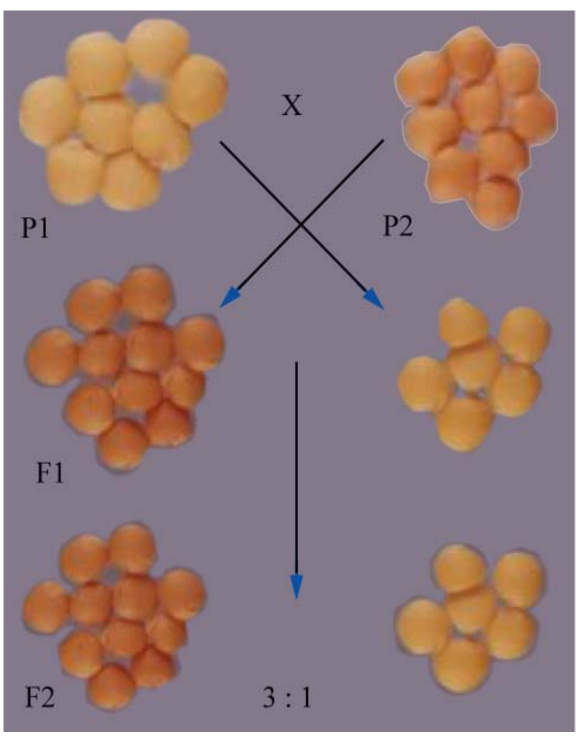

Fig. 3. Inheritance of cotyledon colour in lentil.

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