RESEARCH NOTE

Genetic studies on morpho-phenological traits in lentil (*Lens culinaris* Medikus) wide crosses

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Introduction

Genetic studies were undertaken in nine intersubspecific and interspecific crosses of lentil to understand the inheritance pattern of morphological characters viz., growth habit, flower colour, cotyledon colour and pod dehiscence. The F_1 and F_2 generations of these wide crosses were assessed and suggested monogenic inheritance of these traits. The segregation pattern of these qualitative traits will also help in the identification of true to type F_1 plants from the interspecific crosses. All nine intersubspecific and interspecific crosses exhibited a wide variability for days to flowering, maturity and duration from flowering to maturity. The results indicated that these characters are governed by independent sets of genes during the growth and development phases. Heritability of both characters has been reported to be high in all wide crosses.

In India, cultivated lentil species have an intrinsically narrow genetic base and that situation limits our plant breeder's progress today (Earskine *et al*. 1998). To attain further breakthrough in increasing yield and improving stability in future crop cultivars, new sources of variation need to be incorporated into the cultivated gene pool. Therefore, some efforts for broadening the genetic base of lentil cultivars using wild *Lens* taxa have been initiated by the National Bureau of Plant Genetic Resources, Pusa, New Delhi, India (Singh *et al.* 2013), and also in some other research organizations (Ahmad *et al.* 1995; Fratini and Ruiz 2006; Gupta and Sharma 2007). Incorporation of different traits of interest in the background of cultivated varieties has helped in the flow of useful genes with increased allelic frequency from wild *Lens* taxa in the common gene pool of cultivated varieties (Ladizinsky *et al.* 1988).

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Material and methods

The genetic materials comprised four cultivated lentil cultivars namely, Precoz, ILL10829, L830 and ILL8006, selected for intersubspecific and interspecific hybridization. These genotypes were intercrossed with an annual wild *Lens* subspecies and species viz, ILWL7, ILWL62 (*Lens culinaris* ssp. *orientalis*), ILWL20, ILWL81 (*L. culinaris* ssp. *odemensis*), ILWL14 (*L. lamottei*) and ILWL30, ILWL55 (*L. ervoides*). All these wild annual *Lens* taxa were obtained from the Biodiversity and Integrated Gene Management Unit at the International Centre for Agricultural Research in Dry Areas (ICARDA), Aleppo, Syria.

Hybridization experiments

Hybridization experiments were conducted at the National Bureau of Plant Genetic Resources (NBPGR), Pusa, New Delhi, India during winter of 2010–2011. A total of nine wide crosses: combinations viz. L830 × ILWL7, ILL10829 × ILWL7, ILL8006 × ILWL62, Precoz × ILWL20, ILL10829 × ILWL81, L830× ILWL14, ILL10829 × ILWL14, ILL10829 × ILWL30 and ILL8006 × ILWL55 were attempted manually by emasculating flower buds between 3.00 and 5.00 pm in the afternoon and pollinating them the next day morning between 8.00 and 9.30 am with fresh pollen of wild *Lens* taxa. Sharp-pointed sterilized stainless steel forceps were used to remove the sepals and the standard. Thereafter, an incision was made on the upper end of the keel, the flower opened and all 10 anthers were gently removed using the forceps. During the emasculation process, special care was taken not to touch the stigma with anthers or forceps to avoid selfing or damaging the stigma. F_1 hybrid seeds were further grown to obtain F_2 seeds of each cross combination.

Keywords. wide hybridization; inheritance; *Lens* species; qualitative traits; genetic studies.

Hybridity testing

Besides morphological markers, true hybridity of F_1 plants was also confirmed using inter simple sequence repeats (ISSR) markers. For this, total genomic DNA was extracted from the F_1s and their respective parents by CTAB method as described by Saghai-Maroof *et al.* (1984) with minor modifications. Subsequently, DNA was purified eliminating various contaminants and made ready for PCR. PCR amplification was carried out with 50 ng of genomic DNA, 1.5 mM $MgCl_2$ (Sigma, Aldrich, MO, USA), 1.2 U *Taq* DNA polymerase (Bangalore Genei, Bangalore, India), 1× PCR buffer (Sigma,), 0.5 μM primer and 0.2 mM of dNTP mix (Sigma,). The volume was diluted to 40 μL with sterile distilled water. PCR was carried out in PTC 200 (MJ Research, MA, USA) thermo cycler. Thermo-cycling conditions consisted of initial denaturation at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and primer extension at 72°C for 1 min. Additionally, final extension was carried out at 72°C for 5 min, followed by storage at 4°C until removing from the machine. Electrophoresis, 1.5% agarose was carried out at 100 V for 2–3 h. The gels were stained with ethidium bromide and visualized under UV light in a Gel Documentation System Uvitac Cambridge, Cambridge, UK.

Field evaluation of morpho-phenological traits

Parental lines and their F_1 and F_2 generations of all nine crosses were studied to infer the inheritance pattern of some important morphological traits. Each F_2 plant was assessed for contrasting characters (table 1) and the chi-square test for goodness of fit was used to determine the genetics of these characters. Yate's correction factor was also used where the population size was too small with one degree of freedom (Yates 1934). Likewise, 10 random plants from the parents and all plants from each cross combination in F_2 were observed for days to flowering, days to maturity and days from flowering to maturity. Phenotypic variability was assessed from the range, mean and coefficient of variation for the above three traits. However, heritability in broad sense (H_b^2) was estimated using the mean of parental and the F_2 variances. Genotypic variance (σ_G^2) was calculated by subtracting the average variance of parents (σ_E^2) from the variance of F_2 (σ_p^2) as follows:

$$H_b^2 = \frac{\sigma_G^2}{\sigma_P^2} = \frac{\sigma_P^2 - \sigma_E^2}{\sigma_P^2}.$$

Results

True nature of hybrids under study was confirmed by the morphological traits in F_1 generation from the donors. ISSR markers were used to test the hybridity and purity of each wide cross combination for developing reliable F_2 populations. A total of 120 ISSR primers was screened and only three primers were found useful for confirming the true hybridity.

Inheritance of morphological traits

Growth habit: Totally four intersubspecific and interspecific cross combinations between erect and spreading type parents were attempted for Precoz × ILWL20, Precoz × ILWL62, Precoz × ILWL81 and Precoz × ILWL30. The F_1 plants had erect growth habit and the segregation data

Table 1. Morphological characters of cultivated and wild *Lens* taxa.

Accession	Growth habit	Flower colour	Pod dehiscence	Cotyledon colour
L830 (*L. culinaris* ssp. *culinaris*)	Semispreading	Purple	Nondehiscence	Orange
ILL10829 (*L. culinaris* ssp. *culinaris*)	Semispreading	White	Nondehiscence	Orange
ILL8006 (*L. culinaris* ssp. *culinaris*)	Erect	Purple	Nondehiscence	Orange
Precoz (*L. culinaris* ssp. *culinaris*)	Erect	White	Nondehiscence	Yellow
ILWL7 (*L. culinaris* ssp. *orientalis*)	Spreading	Purple	High	Olive green
ILWL62 (*L. culinaris* ssp. *orientalis*)	Spreading	Purple	High	–
ILWL20 (*L. culinaris* ssp. *odemensis*)	Spreading	White	High	Yellow
ILWL81 (*L. culinaris* ssp. *odemensis*)	Spreading	Purple	High	Yellow
ILWL14 (*L. lamottei*)	Spreading	Purple	High	Yellow
ILWL30 (*L. ervoides*)	Spreading	Purple	High	Orange
ILWL55 (*L. ervoides*)	Spreading	Purple	High	Yellow

in F_2 generation fitted well in 3:1 (erect : spreading) growth habit phenotypic ratio (table 2). However, the spreading type of growth habit has also been observed to be incompletely dominant over erect growth habit in lentil wide crosses.

Flower colour: Flower colour in lentil has been observed to be a variable character viz; violet, white, pink, blue and purple. The cultivated genotypes viz; L830 and ILL8006 have purple flower colour, while ILL10829 and Precoz have white flower colour. Likewise, wild *Lens* taxa parental lines, namely ILWL7, ILWL62, ILWL81, ILWL14, ILWL30 and ILWL55 exhibited purple flower colour, while ILWL20 had white flower colour. For this trait, data could be recorded only in two crosses for purple × white flower colour between cross combination of L830 × ILWL20 and ILL8006 × ILWL20 (table 2). The segregation pattern indicated that purple flower colour is controlled by single dominant gene.

Table 2. Segregation pattern of morphological traits in cultivated × wild crosses.

Cross	Generation	Observed segregation		Expected ratio	Chi-square	*P* value
		Growth habit				
		Erect	Spreading			
Precoz × ILWL 20	F_1	20	–	–	–	–
	F_2	345	131	3:1	1.43	0.2–0.3
Precoz × ILWL 62	F_1	22	–	–	–	–
	F_2	230	68	3:1	0.84	0.3–0.5
Precoz × ILWL 81	F_1	20	–	–	–	–
	F_2	60	22	3:1	2.65	0.1–0.2
Precoz × ILWL 30	F_1	15	–	–	–	–
	F_2	32	10	3:1	0.40	0.5–0.7
		Flower colour				
		Purple	White			
L830 × ILWL 20	F_1	18	–	–	–	–
	F_2	237	69	3:1	1.36	0.3–0.5
ILL8006 × ILWL20	F_1	16	–	–	–	–
	F_2	155	51	3:1	1.43	0.2–0.5
		Cotyledon colour				
		Orange	Yellow			
L830 × ILWL 14	F_1	22	–	–	–	–
	F_2	498	179	3:1	1.06	0.3–0.5
L830 × ILWL 55	F_1	21	–	–	–	–
	F_2	1116	351	3:1	1.43	0.2–0.3
		Pod dehiscence				
		Dehiscent pods	Nondehiscent pods			
L830 × ILWL 7	F_1	21	–	–	–	–
	F_2	332	102	3:1	0.90	0.3–0.5
ILL10829 × ILWL 7	F_1	23	–	–	–	–
	F_2	217	73	3:1	0.55	0.9–0.95
ILL8006 × ILWL 62	F_1	12	–	–	–	–
	F_2	294	99	3:1	0.45	0.7–0.9
Precoz × ILWL 20	F_1	16	–	–	–	–
	F_2	131	41	3:1	0.15	0.7–0.9
ILL10829 × ILWL 81	F_1	16	–	–	–	–
	F_2	226	68	3:1	0.65	0.3–0.5
L830 × ILWL 14	F_1	12	–	–	–	–
	F_2	149	39	3:1	2.22	0.1–0.2
ILL10829 × ILWL 14	F_1	15	–	–	–	–
	F_2	68	21	3:1	0.10	0.2–0.3
ILL 10829 × ILWL 30	F_1	10	–	–	–	–
	F_2	122	44	3:1	0.00	0.9–0.95
ILL8006 × ILWL 55	F_1	12	–	–	–	–
	F_2	120	44	3:1	0.15	0.7–0.9

Table 3. Range, mean, coefficient of variation (CV%) and heritability (H^2 b) for three characters in lentil wide crosses.

Parent/population	Days to flowering				Days to maturity				Days from flowering to maturity			
	Range	Mean	CV (%)	H^2_b	Range	Mean	CV (%)	H^2_b	Range	Mean	CV (%)	H^2_b
L830	36.0–38.0	37.0	2.7		84.0–87.0	85.3	1.0		48.0–49.0	48.3	2.3	
ILWL7	95.7–100.0	98.6	2.0		132.0–137.0	135.2	1.4		36.3–37.0	36.6	1.3	
L830 × ILWL7 (F_2)	95.0–106.0	99.2	2.8	0.84	144.0–159.0	150.0	2.9	0.81	49.0–53.0	50.8	10.4	0.78
ILL10829	42.0–46.0	44.3	4.2		79.0–83.0	80.9	2.5		36.0–37.0	36.6	1.5	
ILWL7	95.7–100.0	98.6	2.0		132.0–137.0	135.2	1.4		36.3–37.0	36.6	1.3	
ILL10829 × ILWL7 (F_2)	111.0–113.0	112.0	0.5	0.81	154.5–161.0	158.1	1.2	0.79	43.5–48.0	46.1	4.6	0.77
ILL8006	38.0–42.0	40.0	4.5		70.0–74.0	71.6	3.2		31.0–32.0	31.6	1.8	
ILWL62	91.0–99.0	94.9	3.1		120.0–123.0	121.2	1.0		24.0–29.0	26.3	13.3	
ILL8006 × ILWL62 (F_2)	107.5–113.0	111.2	1.1	0.79	147.0–162.5	155.7	2.1	0.81	39.5–49.5	44.5	5.8	0.80
Precoz	39.0–42.0	40.5	4.1		77.0–80.0	78.8	1.9		38.0–39.0	38.3	1.4	
ILWL20	91.8–96.0	93.3	1.9		131.0–135.0	132.4	1.4		39.0–39.2	39.1	0.3	
Precoz × ILWL20 (F_2)	90.5–98.0	93.9	2.4	0.69	143.0–158.0	150.9	4.0	0.73	52.5–60.0	57.0	11.4	0.77
ILL10829	42.0–46.0	44.3	4.2		79.0–83.0	80.9	2.5		36.0–37.0	36.6	1.5	
ILWL 81	62.9–67.0	64.8	2.5		124.0–126.0	124.8	0.6		59.0–61.1	60.0	2.4	
ILL10829 × ILWL81 (F_2)	126.5–132.5	128.7	2.0	0.83	156.5–162.5	159.4	1.6	0.88	30.0–32.0	30.7	3.0	0.81
L830	36.0–38.0	37.0	2.7		84.0–87.0	85.3	1.0		48.0–49.0	48.3	2.3	
ILWL14	92.0–98.0	95.0	3.0		130.0–135.0	132.7	1.6		37.0–38.0	37.7	1.8	
L830 × ILWL14 (F_2)	97.0–105.5	98.4	2.5	0.66	142.0–150.0	145.6	2.1	0.72	44.5–52.0	47.2	6.3	0.75
ILL10829	42.0–46.0	44.3	4.2		79.0–83.0	80.9	2.5		36.0–37.0	36.6	1.5	
ILWL14	92.0–98.0	95.0	3.0		130.0–135.0	132.7	1.6		37.0–38.0	37.7	1.8	
ILL10829 × ILWL14 (F_2)	114.0–116.0	114.7	0.5	0.69	156.0–160.5	157.5	0.9	0.74	42.0–44.5	42.8	4.0	0.79
ILL10829	42.0–46.0	44.3	4.2		79.0–83.0	80.9	2.5		36.0–37.0	36.6	1.5	
ILWL30	89.0–95.0	92.1	2.8		121.0–124.0	122.6	1.2		29.0–32.0	30.5	6.9	
ILL10829 × ILWL30 (F_2)	92.0–108.0	101.9	5.0	0.70	140.0–156.5	147.3	2.6	0.74	48.0–48.5	45.4	13.8	0.78
ILL8006	38.0–42.0	40.0	4.5		70.0–74.0	71.6	3.2		31.0–32.0	31.6	1.8	
ILWL55	95.0–100.0	97.5	2.2		133.0–135.0	133.7	0.7		35.0–38.0	36.2	5.8	
ILL8006 × ILWL55 (F_2)	95.0–107.5	103.0	4.4	0.78	148.0–157.0	152.7	1.8	0.71	49.5–53.0	49.7	9.0	0.79

Cotyledon colour: Cotyledons are sporophytic tissues and their colours can be visualized in the freshly harvested and threshed seeds. Two cross combinations were attempted between orange and yellow cotyledon colours for L830 × ILWL14 and L830 × ILWL55 revealed that all the F_1 plants had orange cotyledon colour and the F_2 analysis of each cross combination exhibited that the character segregated into 3:1 (orange:yellow cotyledon colour) Mendelian segregating ratio (table 2).

Pod dehiscence: Inheritance of pod dehiscence was studied in nine inter subspecific and interspecific crosses (L830 × ILWL7, ILL10829 × ILWL7, ILL8006 × ILWL62, Precoz × ILWL20, ILL10829 × ILWL81, L830 × ILWL14, ILL10829 × ILWL14, ILL10829 × ILWL30 and ILL8006 × ILWL55) of lentil. F_1 generations of each cross combination revealed that dehiscence trait was dominant over non-dehiscence (table 2). In the F_2 generation of all crosses, the character segregated into 3:1 phenotypic ratio (dehiscence: nondehiscence pods).

Variability studies of phenological traits

The range, coefficient of variability and heritability of F_2 populations of all nine wide crosses were studied for days to flowering, days to maturity and days from flowering to maturity (table 3). Mean number of days to flowering and maturity in F_2 generations were greater and wider than the mid-parent values in majority of crosses. However, large portion of F_2 population of most of the wide crosses matured later than the parental lines. Likewise, the pattern for length of flowering–maturity period in most of the wide crosses was towards higher side than the parents. Heritability was also high in magnitude for almost all F_2 cross combinations.

Discussion

In the present investigation, wide hybridization was attempted between cultivated and wild *Lens* taxa with the objective of transferring useful traits from the wild annual *Lens* species into cultivated varieties for broadening the genetic base (Singh *et al.* 2013). Establishing the true hybrid nature of wide crosses in the beginning of an experiment is very important to develop reliable segregating populations for mapping of genes controlling desirable trait of interest. We made use of ISSR markers for this purpose. ISSRs are dominant markers and in order to rule out the possibility of selfing, only the male parent specific markers, which express in the hybrid, are useful.

Segregation pattern of growth habit revealed that the trait is controlled by single dominant gene. Ladizinsky (1979) had also reported the inheritance of growth habit and suggested that it is incompletely dominant over erect growth habit and proposed gene symbol *Gh* for erect growth and symbol *ghgh* for semispreading type. In contrast, spreading growth habit was suggested to be completely dominant over erect type and proposed gene symbols *Ert* (dominant) for spreading type and symbols *ert ert* (homozygous recessive) for erect type in intervarietal crosses (Emami and Sharma 1999; Kumar 2002; Mishra 2004) of lentil. Likewise, flower colour between purple and white parents indicated that the trait is also controlled by single dominant gene. Crosses between the parents of orange and yellow cotyledon colour showed that the F_1 generation had orange cotyledon colour and in F_2 generation, the trait appeared in the segregation of 3:1 phenotypic ratio, suggesting that orange cotyledon colour is dominant over yellow. Similar observations were reported by other researchers also in cultivated × cultivated lentil crosses (Vandenberg and Slikard 1989; Malaviya and Shukla 1990 Emami 1996; Hoque *et al.* 2002; Kumar 2002). Vandenberg and Slikard (1989) proposed *Yc* gene symbol for orange and *yc* for yellow cotyledon colour. A digenic control of cotyledon colour in lentil was concluded by Emami and Sharma (1996). Pod dehiscence in wild lentil is a common problem and the inheritance pattern of this important trait was estimated in all the nine crosses and the data exhibited that pod dehiscence character is under the control of a single dominant gene. Further, the genetics of the above four qualitative characters suggested their monogenic inheritance of plant growth habit (erect growth habit being dominant over spreading habit), flower colour (purple is dominant over white), cotyledon colour (orange colour being dominant over yellow colour) and pod dehiscence (dehiscent pods being dominant over nondehiscent pods). This study also helped in understanding the genetics of these morphological traits in intersubspecific and interspecific crosses. The inheritance pattern of these results also helped us to identify true F_1 hybridity of wide crosses through morphological markers.

Relationship among days to flowering, maturity and flowering–maturity period

All nine cross combinations were also assessed for days to flowering, maturity and flowering-maturity period using range, mean and coefficient of variation of parents and F_2 population of each cross. The results revealed sufficient variability and differences in range of F_2 generation for majority of crosses in days to flowering and maturity due to early and late segregants and the appearance of late flowering and maturity segregants in almost all F_2 crosses, suggesting fixing of late maturing wild alleles in the segregant populations. The study further suggested that the numbers of days taken to flowering and from flowering to maturity are governed by independent sets of genes. However, high heritability values indicated less environmental influence on these important phenological traits.

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