Inheritance and Linkage Relationships of Days to Flower and Morphological Loci in Lentil (*Lens culinaris* Medikus subsp. *culinaris*)

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Lentils from south Asia differ markedly in days to flower and several other traits from lentils in other major lentil producing areas of the world such as west Asia. Single-gene inheritance and a polygenic system were observed to control days to flower in lentil (*Lens culinaris* Medikus subsp. *culinaris*). Early flowering was determined by a single recessive gene (*sn*). Early flowering transgressive segregants occurred in F_2 populations due to the interaction of *sn* and minor genes for earliness. Pubescent peduncle (*Pep*) was inherited as a single gene dominant to glabrous peduncle (*pep*). Tendrilless leaf (*tnl*) was controlled by a single gene recessive to tendrilled leaf (*Tnl*). The *Sn, Scp* (seed coat pattern), and *Pep* loci were linked together in linkage group 5 and *Tnl* was linked with *Gs* (colored stem) in linkage group 1 of the lentil genome.

Lentil landraces exhibit specific adaptation to their ecological environment and have evolved into distinct ecotypes in different geographical regions (Barulina 1930; Erskine et al. 1989). Adaptation for an appropriate phenology apparently is the main evolutionary force behind this differentiation (Erskine et al. 1989).

Lentils from south Asia (exclusively of the *pilosae* ecotype) are quite distinct from lentils from other countries in their qualitative morphological characters and group together with those from Ethiopia in terms of quantitative characters (Erskine et al. 1998). According to isozyme and RAPD studies, south Asian germplasm clusters tightly with that from Afghanistan (Ferguson et al., in press). Associated with the uniqueness of south Asian germplasm is a very restricted genetic base, as evident from agromorphological and phenological characters (Erskine et al. 1989, 1990), as well as isozyme and RAPD analyses (Ferguson et al., in press). This may be due to a bottleneck when lentil was first introduced into India around 2000 B.C. (Erskine et al. 1998).

A reconstruction of the phenological problems associated with the initial spread of the crop into the Indo-Gangetic plain was inadvertently made with the introduction into the Indo-Gangetic plain of lentil selected in west Asia through international nurseries distributed in the late 1970s by the International Center for Agricultural Research in the Dry Areas (ICARDA). Lentil selected in west Asia, when sown in India and Pakistan, mostly came into flower as the indigenous lentils were maturing (Ceccarelli et al. 1994).

A major effort has been made to broaden the genetic base in south Asian lentil by crossing *pilosae* lentils with genotypes from other origins (Erskine et al. 1998). The introduction of an early flowering line, Precoz (ILL 4605), into south Asia in the early nineties and its use in hybridization with indigenous *pilosae* lentil has resulted in the selection of extra-early genotypes (Sharma et al. 1993). However, before planning a cross-breeding program with the objective of identifying extra-early segregants, it is essential to investigate the genetic control of flowering time to effectively choose parents and construct an appropriate breeding scheme. The two qualitative morphological traits that define the *pilosae* ecotype are pubescence and tendril development. The *pilosae* group has dense soft hairs on plant parts, which give a gray-green color to the canopy, in contrast to the greener vegetative canopy of the west Asian lentils. Lentils of west Asian origin have tendrilled leaves, but *pilosae* lentils are either devoid of tendrils or have rudimentary ones.

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Table 1. Origin and morphological traits of lentil parents

Parent (ILL)	Origin	Peduncle pubescence	Presence of tendril	Stem pigmentation	Cotyledon color	Seed coat pattern
2501	India	−	+	+	R	+
4605	Argentina	−	+	+	Y	−
5773	ICARDA	−	+	−	Y	+
6037	ICARDA	−	+	+	Y	−
7555	India	+	−	+	R	+
7557	India	+	−	+	R	+
7665	India	+	−	−	R	+

+ = present; − = absent; R = red; Y = yellow; ILL = international legume lentil

This investigation aimed to determine the genetic control and linkage relationships of genes that confer days to flower with genes for morphological traits such as peduncle pubescence and tendril development in a diverse set of lentil parental lines, including *pilosae* lines from south Asia and representatives of other ecotypes.

Materials and Methods

The seven parental lines involved in the crosses, their origin, and various different morphological characters are presented in Table 1. All possible cross-combinations among the seven parents were made at ICARDA, Aleppo, Syria, in the 1993/1994 season. The F_1 was grown in an off-season nursery at Terbol, Lebanon, in 1994. The parents, F_1s, and F_2s were grown at ICARDA, Tel Hadya, Syria (36°01′N and 37°20′E) and at New Delhi, India (28°08′N and 77°12′E). In Syria, the experiment was sown on 1 January 1996 and in India it was sown on 20 November 1995. A split-plot design was used at both locations, with crosses as main plots and generations as subplots in two replications. The P_1, P_2, and F_1 were grown in two-row plots, and the F_2s were sown in eight-row plots, 3.0 m long, 0.3 m between rows, and a plant distance of 0.15 m. A total of 21 and 20 (ILL 7555 × ILL 7557 cross missing) cross-combinations were grown in India and Syria, respectively.

Days to flower were measured in days from sowing to the first opened flower on an individual plant basis. Morphological traits including peduncle pubescence, tendrillar leaf and stem color [green (*gs*) versus colored (*Gs*)] were scored visually for individual plants. Seeds from individual plants were examined for seed coat pattern [presence (*Scp*) versus absence (*scp*)] and cotyledon color [red (*Yc*) versus yellow (*yc*)]. For the Mendelian analysis a chi-square test was used to test the F_2 ratios. Joint phenotypic segregation was studied to determine gene relationships. When the presence of linkage was established at $P < .05$, recombination values between loci were estimated by the product ratio method of Fisher and Balmukund (1928).

To confirm the inheritance of flowering time and peduncle pubescence, four crosses segregating for flowering time in the F_2 (ILL 2501 × ILL 4605, ILL 2501 × ILL 6037, ILL 4605 × ILL 5773, and ILL 5773 × ILL 6037) and three crosses segregating for peduncle pubescence (ILL 4605 × ILL 7557, ILL 5773 × ILL 7557, and ILL 6037 × ILL 7557) were sown at the F_3 in separate experiments in two replications in single-row plots, 1 m long with 0.3 m between rows, on 2 January 1997 at Tel Hadya, Syria. Observations were made on five plants for days to flower and all plants were scored for presence or absence of peduncle pubescence in each F_3 family.

Table 2. Segregation for days to flower in lentil at Tel Hadya, Syria

Cross (ILL)	Generation	Observed segregation			Expected ratio	χ^2 value	P
		Late	Segregating	Early			
2501 × 4605	F_1	All					
	F_2	64		22	3:1	0.015	.95–.90
	F_3	20	36	22	1:2:1	0.562	.90–.75
2501 × 6037	F_1	All					
	F_2	74		24	3:1	0.010	.95–.90
	F_3	22	47	21	1:2:1	0.199	.95–.90
5773 × 4605	F_1	All					
	F_2	110		28	3:1	1.632	.25–.10
	F_3	31	58	26	1:2:1	0.443	.90–.75
5773 × 6037	F_1	All					
	F_2	81		25	3:1	0.113	.75–.50
	F_3	19	45	18	1:2:1	0.804	.75–.50

Days to flower of the parents: ILL 2501, 135 ± 2.7; ILL 4605, 100 ± 1.6; ILL 5773, 116 ± 2.1; ILL 6037, 102 ± 1.2

Results

Among environmental factors, temperature and photoperiod modulate time to flower in lentil (Summerfield et al. 1985). The two environments differed greatly with respect to temperature but not for photoperiod. In New Delhi, the mean temperature during vegetative growth was higher (15°C) than in Aleppo (9.9°C); but the mean photoperiod was similar between the environments (10.77 h in New Delhi and 11.59 h in Tel Hadya) because of the different sowing dates.

Inheritance of Days to Flower

In both India and Syria the days to flower in the F_1 in all crosses was much later than the midparental value and almost equal to that of the late parent, indicating that late flowering is dominant over early flowering. Variance in the F_2 was much higher than in the respective parents at both locations, indicating segregation for days to flower (Figures 1 and 2). The F_2 distributions were normal in most of the crosses, but showed bimodality in crosses with ILL 4605 and ILL 6037. In Syria, within the two arrays (arrays of parents 2 and 4), in four crosses between extremes of days to flower (ILL 2501 × ILL 4605, ILL 2501 × ILL 6037, ILL 4605 × ILL 5773, and ILL 5773 × ILL 6037) the bimodality was so extreme as to be discontinuous. The F_2 plants were classified into two distinct classes, early flowering (<108 days) and late flowering (>114 days). No such discrete segregation was observed in the F_2 distributions at New Delhi. The Mendelian analysis of the F_2 population in each of these four crosses from Tel Hadya provided a good fit to 3:1 (late flowering : early flowering) segregation ratio (P = .10–.95). This was further confirmed by the F_3 progeny test (1 homozygous dominant : 2 segregating : 1 homozygous recessive) with high probability levels (P = .50–.95; Table 2).

Considerable numbers of early transgressive segregants for days to flower were observed from 15 crosses at New Delhi (Figure 2). However, the 10 crosses involving the parents ILL 4605 and ILL 6037 yielded more early transgressive seg-

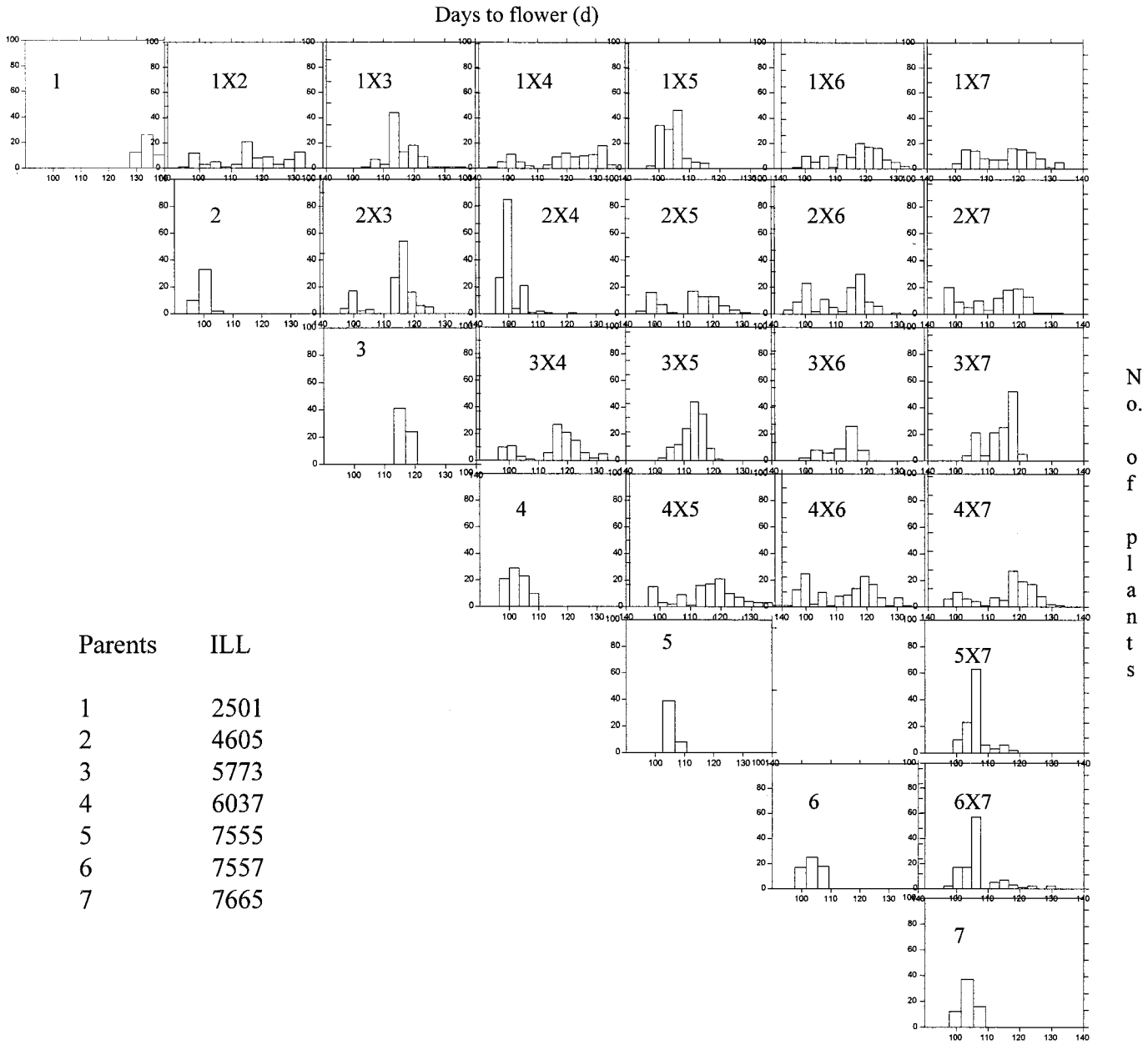

Figure 1. Frequency distribution of days to flower in parents and F_2 lentil plants at Tel Hadya, Syria.

regants (10.96% ± 8.86) than the remaining five crosses (0.87% ± 0.13). By contrast, very few early transgressive segregants occurred in Syria.

Inheritance of Peduncle Pubescence

Peduncle pubescence as seen on *pilosae* lentils was studied in three crosses (ILL 4605 × ILL 7557, ILL 5773 × ILL 7557, and ILL 6067 × ILL 7557) in Syria. The F_1 plants in all three crosses were pubescent, indicating that pubescence is dominant over glabrous. The F_2 segregation in the three crosses provided a good fit to a 3:1 (pubescence : glabrous) segregation ratio (P = .01–.95; Table 3). The hypothesis of single gene control was further confirmed in these three crosses in F_3 families by the 1:2:1 ratio (homozygous dominant : segregating : homozygous recessive) (P = .25–.90). The total chi-square test for 79 homozygous dominant : 161 segregating : 76 homozygous recessive in F_3 also provided a good fit to a 1:2:1 ratio (χ^2 = 2.224; P = .50–.75).

Inheritance of Tendrilled Leaf

Five crosses were analyzed for inheritance of tendrilled leaf at Tel Hadya. The F_1s between tendrilled and tendrilless parents formed tendrils, indicating that presence of the tendril (tendrilled) is dominant over the terminal leaflet (tendrilless). The F_2 analysis of individual crosses showed that the trait segregated into a 3:1 (tendrilled : tendrilless) ratio with high probability among crosses (P = .10–.90; Table 4). The total chi-square test for the 3:1 (tendrilled : tendrilless) F_2 phenotypic ratio for five individual populations revealed a good fit (801 tendrilled : 275 tendrilless; χ^2 = 3.841; P = .50–.75).

Linkage Observed

The joint segregation of seven pairs of loci was investigated in a varying number of crosses. Of these, four pairs of loci—*Sn*

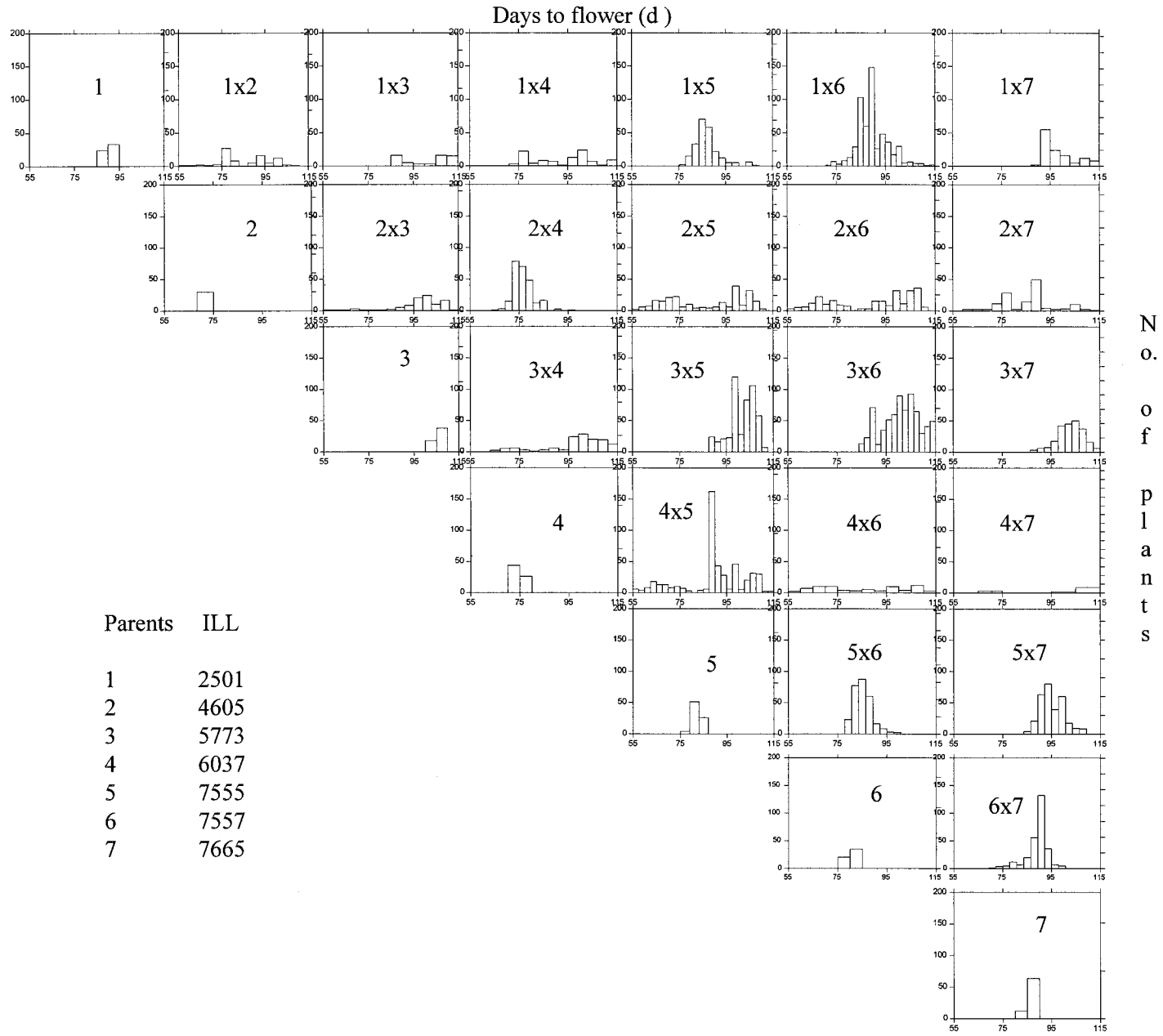

Figure 2. Frequency distribution of days to flower in parents and F_2 lentil plants at New Delhi, India.

Table 3. Frequency and segregation for pubescent versus glabrous peduncle in F_2 and F_3 in three lentil crosses

Cross (ILL)	Parents			F_2 frequency				F_3 frequency	
	Female	Male	F_1	+	−	χ^2 3:1	*P*	+	Segregating
4605 × 7557	+	−	+	132	29	4.190	.05–.01	29	48
5773 × 7557	+	−	+	91	31	0.011	.95–.90	24	53
6037 × 7557	+	−	+	130	42	0.230	.75–.50	26	60
Total	+	−	+	353	102	4.431	.25–.10	79	161

Presence (+) versus absence (−) of pubescent peduncle.

(flowering time) and *Scp* (seed coat pattern), *Pep* (peduncle pubescence) with *Scp, Sn* with *Pep,* and tendrilled leaf (*Tnl*) with red stem color (*Gs*)—significantly deviated from independent assortment (Table 5). The analysis showed that *Sn* was linked with *Scp* with a crossover value of 22%, *Scp* and *Pep* with a crossover value of 18%, and *Sn* was linked to *Pep* with a 38% crossover value (Figure 3). Another loose linkage was detected between *Tnl* and *Gs* with a 36% crossover value. Other pairs of loci—*Yc* (cotyledon color) with *Scp, Sn* with *Yc,* and *Yc* with *Pep*—assorted independently (data not shown).

Table 4. Segregation for tendrils in F_2 generation in lentil

Cross (ILL)	Parents			F_2 frequency			
	Female	Male	F_1	+	−	χ^2 (3:1)	*P*
4605 × 7665	+	−	+	101	27	1.041	.50–.25
5773 × 7665	+	−	+	163	68	2.425	.25–.10
5773 × 7555	+	−	+	339	118	0.146	.75–.50
6037 × 7665	+	−	+	11	3	0.094	.90–.75
6037 × 7555	+	−	+	187	59	0.135	.75–.25
Total	+	−	+	801	275	3.859	.75–.50

Presence (+) versus absence (−) of tendrils.

Discussion

In Syria, crosses between early (ILL 4605 and ILL 6037) and late parents (ILL 5773 and ILL 2501) indicated monogenic segregation for days to flower (Table 2). The flowering behavior of the parents from these four crosses revealed that ILL 4605 and ILL 6037 possessed a single gene for earliness. Evidence of a single recessive gene controlling earliness in flowering has also been reported in chickpea (Kumar and van Rheenen 1997), sweet pea (Little and Kantor 1941), pea (Murfet 1971; Rowlands 1964; Tedin and Tedin 1923). In Tel Hadya, the discrete classes (late and early) observed in progeny from crosses between early (ILL 4605 and ILL 6037) and late parents (ILL 5773 and ILL 2501) could not be detected in other crosses because of small differences between parents for days to flower. The F_2 distributions in crosses with these parents showed bimodality at Tel Hadya, and to a lesser extent at New Delhi (Figures 1 and 2). However, two distinct classes (late and early) were not observed at New Delhi, owing to the masking effect of environmental factors, particularly temperature. We noticed negligible segregation in the ILL 4605 × ILL 6037 cross at both locations, indicating that the parents carry the same gene for early flowering. This is not surprising because ILL 6037 is a selection from an ILL 4605 × Laird cross.

The continuous distribution among F_2 segregants in other crosses suggested that a polygenic system is also operating to control days to flower. The occurrence of early transgressive segregants is due to the interaction between a major early flowering gene and minor genes contributing to earliness. The concept that flowering is controlled by both major and minor genes has been suggested by earlier workers (Barber 1959; Murfet 1975; Rasmusson 1935; and Rowlands 1964). The gene symbol *Sn* for flowering was first assigned by Tedin and Tedin (1923) and was later used by Barber (1959). Murfet (1971) designated *Sn* for late and *sn* for early flowering in pea. We also propose the *Sn* gene symbol for flowering time in lentil. Therefore homozygous late plants have the genotype *Sn*/*Sn*; homozygous early plants are *sn*/*sn.* We also conclude that ILL 4605 and ILL 6037 could be used extensively by the south Asian lentil-breeding programs to generate early genotypes, since earliness is a desirable trait in the intensive cropping pattern in that region.

Peduncle pubescence is a new morphological character, controlled by a single gene with pubescent peduncle dominant over glabrous as revealed by F_2 and F_3 segregation (Table 3). We propose the gene symbol *Pep* for pubescent peduncle in lentil. Thus the homozygous genotypes are *Pep*/*Pep* (pubescent peduncle) and *pep*/*pep* (glabrous peduncle). In the genetic control of tendrilled leaf we found a monogenic system in which tendrillar growth was dominant over nontendrillar leaf in five crosses (Table 4). Our findings are in agreement with the work of Vandenberg and Slinkard (1989) and they assigned the gene symbol *Tnl* for tendrilled leaf. Therefore the genotype of a homozygous dominant plant is *Tnl*/*Tnl* and a homozygous recessive plant is *tnl*/*tnl.*

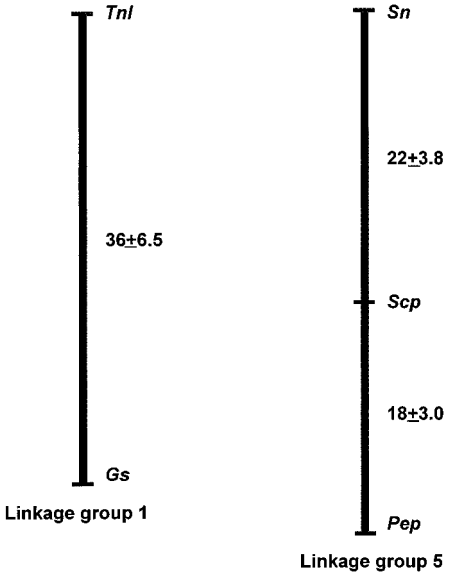

Figure 3. Linkage map of five loci in lentil.

The linkage analysis showed that the *Sn, Scp,* and *Pep* loci are located in the same linkage group with the *Scp* locus situated between the *Sn* and *Pep* loci (Figure 3). This linkage association could be assigned to linkage group 5 of the lentil genome, where the gene *Scp* has previously been located (Muehlbauer et al. 1995). This is the first report of linkage between a flowering gene and morphological loci in lentil. Linkages between major flowering genes and morphological loci have been reported in pea (Hoshino 1915; Tedin and Tedin 1923). The *Tnl* locus could be assigned to linkage group 1 where the *Gs* locus is located. Our present finding expands the existing linkage map of the lentil genome.

Table 5. Joint F_2 segregation of pairs of loci that significantly deviated from independent assortment in lentil

Loci	No. of crosses	Total no. of plants	F_2 phenotypic frequency				χ^2 overall	χ^2 heterogeneous	Recombination fraction	SE (±)
			P_1	P_2	R_1	R_2				
Sn/*Scp*	4	367	230	57	44	36	95.78	10.14**	22	3.8
Pep/*Scp*	2	215	140	37	24	14	70.68	2.56*	18	3.0
Sn/*Pep*	5	564	248	94	129	83	130.52	7.72**	38	8.7
Tnl/*Gs*	4	444	202	82	96	64	131.5	9.98**	36	6.5

*, ** P = .05 and .01, respectively.

Sn = early versus late flower; pep = presence versus absence of peduncle pubescence; Scp = presence versus absence of seed coat pattern.

References

Barber HN, 1959. Physiological genetics of *Pisum* II. The genetics of photoperiod and vernalization. Heredity 13: 3–60.

Barulina H, 1930. Lentils of the USSR and other countries. Bull Appl Bot Genet Plant Breed Leningrad Suppl 40:265–304.

Ceccarelli S, Erskine W, Hamblin J, and Grando S, 1994. Genotype by environment interaction and international breeding programs. Exp Agric 30:177–188.

Erskine W, Adham Y, and Holly L, 1989. Geographical distribution of variation in quantitative traits in a world lentil collection. Euphytica 40:97–103.

Erskine W, Ellis RH, Summerfield RJ, Roberts EH, and Hussain A, 1990. Characterization of responses to tem-

perature and photoperiod for time to flowering in a world lentil collection. Theor Appl Genet 80:193–199.

Erskine W, Chandra S, Chaudhry M, Malik IA, Sarker A, Sharma B, Tufail M, and Tyagi MC, 1998. A bottleneck in lentil: widening the genetic variation in south Asia. Euphytica 101:207–211.

Ferguson ME, Robertson LD, Ford-Lloyd BV, Newbury J, and Maxted N, in press. Genetic relationship of lentil landraces from sixteen countries. Euphytica.

Fisher RA and Balmukund B, 1928. The estimation of linkage from offspring of selfed heterozygotes. J Genet 20:79–92.

Hoshino Y, 1915. On the inheritance of flowering time in peas and rice. J Coll Agric Hokkaido Imp Univ 6:229–288.

Kumar J and van Rheenen HA, 1997. A major locus for time of flowering in chickpea. In: Proceedings of the International Food Legume Research Conference III, Adelaide, Australia, 22–26 September 1997; 95.

Little TM and Kantor JH, 1941. Inheritance of earliness in flowering in the sweet pea. J Hered 32:379–383.

Muehlbauer FJ, Kaiser WJ, Clement SL, and Summerfield RJ, 1995. Production and breeding of lentil. Adv Agron 54:283–332.

Murfet IC, 1971. Flowering in *Pisum*: three distinct phenotypic classes determined by the interaction of a dominant early and a dominant late gene. Heredity 26: 243–257.

Murfet IC, 1975. Flowering in *Pisum*: multiple alleles at the lf locus. Heredity 35:85–98.

Rasmusson J, 1935. Studies on the inheritance of quantitative characters in *Pisum* I. Preliminary note on the genetics of time of flowering. Hereditas 20:161–180.

Rowlands DG, 1964. Genetic control of flowering in *Pisum sativum* L. Genetica 35:75–94.

Sharma B, Tyagi MC, and Asthana AN, 1993. Progress in breeding bold-seeded lentil in India. In: Lentil in south Asia. Proceedings of the Seminar on Lentil in South Asia, New Delhi, India, 11–15 March 1991 (Erskine W and Saxena MC, eds). Aleppo, Syria: ICARDA; 22–38.

Summerfield RJ, Roberts EH, Erskine W, and Ellis RH, 1985. Effects of temperature and photoperiod on flowering in lentils (*Lens culinaris* Medic.). Ann Bot 56:659–671.

Tedin H and Tedin O, 1923. Contributions to the genetics of *Pisum* III. Internode length, stem thickness and place of the first flower. Hereditas 4:351–362.

Vandenberg A and Slinkard AE, 1989. Inheritance of four qualitative genes in lentil. J Hered 80:320–322.

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