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A linkage group of four genes related to morphological traits in lentil (*Lens culinaris* Medik.)

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Abstract

Monogenic inheritance and linkage among four morphological traits of lentil were established on the basis of F_1 observations and analysis of 204 F_2 plants in a cross for development of pubescence on peduncle, 228 F_2 plants in a cross for tendril formation, 574 F_2 plants in 5 crosses for flower numbers per peduncle and 464 F_2 plants in 4 crosses for flower color under field conditions. Development of pubescence on peduncle was found to be dominant over glabrous, two flowers per peduncle was dominant over three flowers, purple flower color was dominant over white flower. Linkage was estimated from joint segregation analysis, taking two characters at a time in all possible combinations as significant χ^2 values were recorded for these genes. Gene symbols *Tnl*, *Pub*, *Fn* and *P* were used for these four traits, respectively. The genes were arranged in the order of *Pub-Tnl-Fn-p* with the map distance of 33, 29 and 41 cM between them in coupling phase and 27, 36 and 47 cM in repulsion phase, respectively.

Keywords: Inheritance; Lentil; Linkage group; Morphological traits.

Introduction

Lentil (*Lens culinaris* Medik.), an important grain legume, has been remained a neglected crop in genetic studies. Knowledge of inheritance pattern and linkage between different morphological traits is of major interest of plant breeders and geneticists because it facilitates the efficient utilization of marker genes in crop improvement programs. Knowledge about linkages of economic attributes with easily scorable breeding-neutral traits can be used to improve breeding efficiency. Only a few genes for specific traits have been reported so far (Emami and Sharma, 1999; Kumar et al., 2005). In the absence of linkage between molecular markers and visible morphological, physiological or biochemical traits, molecular mapping alone cannot be used fruitfully. Information has been

reported on the inheritance of plant cotyledon color (*Y*, *B*, *Dg*) (Emami and Sharma, 1996a; Emami and Sharma, 1996c; Sharma and Emami, 2002); pubescence development on the pod (*Glp*) (Vandenberg and Slinkard, 1989), peduncle (*Pdp*) (Emami, 1996), and whole plant (*Pep*, *Pub*) (Sarker et al., 1999; Hoque et al., 2002); pigmentation of stem (*Gs*) (Ladizinsky, 1979), pod (*Grp*, *Pdp*, *Rdp*) (Vandenberg and Slinkard, 1989; Havey and Muehlbauer, 1989; Emami, 1996b), and leaf (*Bl*) (Emami and Sharma, 1996); pod dehiscence (*pi*) (Ladizinsky, 1979b); tendril formation (*Tnl*) (Vandenberg and Slinkard, 1989); flower color (*V*, *p*) (lal and Srivastava, 1975); flower number per peduncle (*Fn*) (Gill and malhotra, 1980); flowering time (*Sn*) (Sarker et al., 1999); seed coat color (*Ggc*, *Tgc*) (Vandenberg and Slinkard, 1990); seed coat pattern (*Scp*, *Mot*, *Spt*) (Vandenberg and Slinkard, 1990; Emami, 1996); black testa (*Blsc*, *Blt*) (Vaillancourt and Slinkard, 1992; Emami and Sharma, 2000); and biochemical markers (Tahir and Muehlbauer, 1994). Short sequences of few linked genes have also been published (Tahir et al., 1993; Tahir and Muehlbauer, 1994; Emami and Sharma, 1999). Four genes, *Gs-Rdp-Bl-Ert* with total genetic distance of 33.9 cM in coupling and 41.2 cM in repulsion phase. (Kumar et al., 2004) and *Ph-Gl-Pub-H* with genetic distance 17.5, 28.9, 21.1 cM respectively. (Kumar et al., 2005) are also reported to be linked. The present study reports a short sequence of another four linked genes which may be combined with previous maps to derive a larger linkage group.

Material and Methods

The experiments were conducted during the years 2007 and 2008 at Research Farm, Shahrekord University, Iran. A total of 2, 2, 5 and 6 genotypes were involved in crossing program to study the inheritance of pubescence development on peduncle, tendril formation, number of flowers per peduncle and flower color, respectively. Names and origin of parental lines are described in Table 1. All the crosses were made in the field and some F_1 plants were raised in greenhouse to obtain F_2 seeds in 2007. The F_2 populations along with the parents and F_1 hybrids were planted spaced out (40×40 cm) in field to ensure easy single plant observation. Observations on peduncle pubescence, tendril formation, number of flowers per peduncle and flower color were recorded at flowering stage to before maturity of the plants. To study the inheritance of each trait, χ^2 values were estimated by the standard formula using SPSS. 11.5 software. Linkage was detected from joint segregation analysis as described by Mather (1951) and map distances were estimated by Maximum likelihood and Product ratio method (Mather, 1951).

Table 1. Name, origin and source of lentil parental lines.

Genotype	Origin	Source
L830	India	IARI
Flip98-10l	ICARDA	ICARDA
Kermanshah	Local	Iran (Tehran Univ.)
Lc74-1-5-1	India	IARI
Ghazvin	Local	Iran (Tehran Univ.)
Local 91	Local	Iran (Tehran Univ.)

Results and Discussion

Peduncle pubescence

The presence or absence of pubescence on pod was first investigated by Vandenberg and Slinkard (1989) and gene symbol *Glp* (for glabrous pod) was proposed. Emami (1996) proposed gene symbol *Pdp* for peduncle pubescence. Sarker et al. (1999) used gene symbol *Pep* for pubescence development on the whole plant. Kumar et al. (2005) reported pubescence development is not confined to the inflorescence or pod alone and the whole plant is either pubescent or glabrous. They also reported the same gene symbol *Pub* (Hoque et al., 2002) for pubescent formation on lentil plant. We have also used symbol *Pub* for this trait. The inheritance of pubescence development on the plant was studied in 1 cross (Table 2). The F_1 plants were pubescent. The F_2 segregated with a good fit to the ratio of 3 pubescent: 1 glabrous (χ^2=2.11; p=0.15; df=1), indicating non-significant χ^2. Thus, the presence of pubescence on the lentil plant is dominant over its absence (glabrous) and the trait is monogenically inherited.

Tendril formation

Tendril formation in lentil was first investigated by Vandenberg and Slinkard (1989) and gene symbol *Tnl* (for tendrilled plant) was proposed. In this study we have also used the gene symbol *Tnl* for tendrilled plant. The inheritance of tendril formation on the plant was studied in 1 cross only (Table 3). The F_1 plants were tendrilled. The F_2 segregated with a good fit to the ratio of 3 tendrilled: 1 tendril-less (χ^2=2.33; p=0.13; df=1). Thus, the presence of tendril in lentil plant is dominant over its absence (tendril-less) and the trait is monogenically inherited.

Table 2. F_2 segregation for pubescent peduncle.

Cross	F_1 phenotype	F_2 segregation		χ^2 (3:1)	p
		Pubescent	glabrous		
L830 (pubescent)×Ghazvin (glabrous)	pubescent	144	60	2.11	0.15

Table 3. F_2 segregation for tendril formation.

Cross	F_1 phenotype	F_2 segregation		χ^2 (3:1)	p
		tendrilled	tendril-less		
L830 (tendrilled)×Ghazvin (tendril-less)	tendriled	161	67	2.33	0.13

Flower color

The inheritance of flower color was studied in four crosses with 2 purple and 3 white flowered parents (Table 4). The F_1 plants of all crosses produced purple flowers and the F_2 populations segregated in to plants with purple and white flowers with a good fit to 3:1 ratio with non-significant χ^2 values (χ^2=0.008-2.17; p=0.15-0.93).The ratio was confirmed by the analysis of the pooled population of 464 F_2 plants (χ^2=3; p=0.08) With non-significant heterogeneity among the crosses (χ^2_{Het}=1.97 at df=3; p=0.74) Thus purple flower phenotype is monogenic dominant over white flower. Since the gene symbol *P* was proposed earlier by Lal and Srivastava (1975), we also use the same symbol for this trait.

Table 4. F_2 segregation for flower color.

Cross	F_1 phenotype	F_2 segregation		χ^2 (3:1)	p
		Purple	white		
Purple flower×white flower					
Kermanshah×Ghazvin	Purple	83	37	2.17	0.15
Kermanshah×local 91	Purple	38	18	1.52	0.22
Kermanshah×Flip 98-10-L	Purple	89	37	1.28	0.27
L830×Ghazvin	Purple	121	41	0.008	0.93
Pooled over 4 crosses		331	133	3.0	0.08
Heterogeneity (3 df)				1.97	0.74

Number of flowers per peduncle

The inheritance of flower number per peduncle was studied in five crosses (Table 5). The F_1 plants of all crosses produced two flowered peduncles, and the F_2 populations segregated into plants with two and three flowers with a good fit to 3:1 ratio with non-significant χ^2 values (χ^2=0.02-0.99; p=0.32-0.87).The ratio was confirmed by the analysis of the pooled population of 574 F_2 (χ^2=2.52; p=0.11) With non-significant heterogeneity among the crosses (χ^2_{Het}=2.35 at df=4; p=0.41). Thus two flowers per peduncle phenotype is monogenically dominant over 3-flowered phenotype. The gene symbol *Fn* was proposed earlier by Gill and Malhotra (1980) for this trait. So we also used the same symbol here.

Detection of linkage (linkage study)

Four crosses were analyzed to detect linkage between the genes *Fn* and *P* (Table 5). Joint segregation analysis in the F_2 of each cross as well as in pooled data of 636 F_2 plants revealed significant linkage χ^2 values for the *Fn-P*, gene pair, ranging from 12.6 to 14.27 (P<0.01). The map distance varied from 34 to 36 cM in coupling phase and 33 to 37 cM in repulsion phase. The map distance in pooled data was 41 cM in coupling phase and 42 cM in repulsion phase (Table 6).

The joint segregation of the genes *Fn* and Tnl was studied in one cross (Table 5). The linkage χ^2 value was significant (χ^2_I=8.19; p=0.04). The map distance was 29 cM in coupling phase and 36 cM in repulsion phase.

Linkage between *Pub* and *Tnl* was studied in one cross (Table 6). The linkage χ^2 value was significant (χ^2=9.49; p=0.06). The map distance was 33 cM in coupling phase and 27 cM in repulsion phase (Table 7).

Table 5. F_2 segregation for flower per peduncle.

Cross	F_1 phenotype	F_2 segregation		χ^2 (3:1)	p
		2-flowered	3-flowered		
2-flowered×3-flowered					
Kermanshah×Ghazvin	2-flowered	89	31	0.04	0.83
Kermanshah×local 91	2-flowered	41	15	0.04	0.83
Flip 98-10-L×Ghazvin	2-flowered	78	25	0.02	0.87
Flip 98-10-L×Lc74-1-5-1	2-flowered	90	34	0.38	0.53
L830×Ghazvin	2-flowered	116	46	0.99	0.32
Pooled over 5 crosses		414	160	2.52	0.11
Heterogeneity (4 df)				2.35	0.41

Table 6. Joint segregation and linkage intensity of the gene Fn (flower numbers per peduncle) with P (flower color) and Tnl (tendrilled plant).

Gene pair(x)-(y)	Cross	F_2 segregation				χ^2 Joint Segregation	P (linkage)	Distance (cM)	
		XY	Xy	xY	xy			Coup.	Rep.
Fn-p (coupling)	Kermanshah×Ghazvin	52	31	28	8	2.62	0.45	-	-
	L830×Ghazvin	79	17	27	21	14.27	<0.01	36.16	37.06
	Kermanshah×local 91	22	14	13	6	12.6	<0.01	34.01	33.79
	Pooled analysis	153	62	68	35	9.16	0.02	41.0	42.03
	Heterogeneity (2 df)					20.33	<0.01	-	-
Fn-Tnl (coupling)	L830×Ghazvin	84	34	20	3	8.19	0.04	29.07	36.05

X and Y are standing for the related characters in the crosses in order to indicate recessive (x,y), dominant (X,Y) relations.

Table 7. Joint segregation and linkage intensity of the gene Pub (pubescent plant) and Tnl (tendriled plant).

Gene pair(x)-(y)	Cross	F_2 segregation				χ^2 Joint Segregation	P (linkage)	Distance (cM)	
		XY	Xy	xY	xy			Coup.	Rep.
Pub-Tnl (coupling)	L830×Ghazvin	103	41	53	7	9.49	0.06	33.04	27.12

X and Y are standing for the related characters in the crosses in order to indicate recessive (x,y), dominant (X,Y) relations.

Considering the information presented in Tables 5 and 6, we determined a linkage group of four genes *Pub*, *Tnl*, *Fn* and *P*. The arrangement of genes and their map distance in this linkage group is presented in Figure 1. The presence of linkage among the characters indicates the possibility for indirect selections during breeding programs.

Considering this map and earlier map reported by Kumar (2005), a larger linkage group with gene order *P-Fn-Tnl-Hl-Pub-Gl-Ph* may be determined.

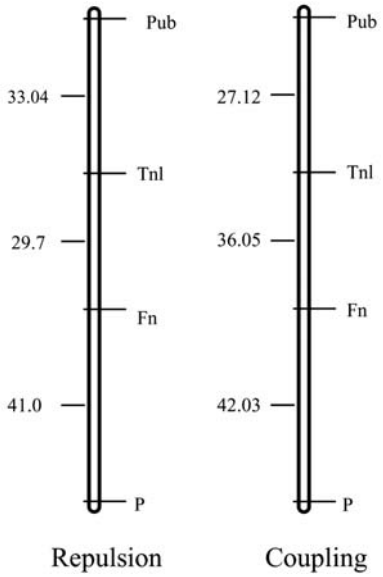

Figure 1. A linkage group of four genes in lentil. Map distances are in cM Tnl, Fn, P and Pub are standing for tendril formation, flower number, flower color and pubsent, respectively.

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