Indian J. Genet., **62**(1): 5-10 (2002)

Inheritance and linkage relationship between morphological and RAPD markers in lentil (*Lens culinaris* Medik.)

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(Received: August 2001; Accepted: January 2002)

Abstract

Inheritance and linkage relationship between eight morphological and RAPD markers were studied in lentil based on the analysis of F_2 population of the cross L 6163 × L 830. All morphological traits showed monogenic mode of inheritance. Joint segregation analysis between morphological markers revealed that the traits leaf, stem and pod pigmentation were linked to each other. One hundred and fourty RAPD decamer primers were screened for detection of polymorphism in the two parents viz., L 6163 and L 830. Only nine primers showed reproducible polymorphism among the parents. Out of nine polymorphic primers only two primer (OPB 02_{60c} and OPM 06_{900}) gave expected monogenic mode of inheritance. Joint segregation analysis between RAPD marker OPM 06_{900} and other morphological markers revealed that the traits leaf and stem pigmentation are linked with marker OPM 06_{900} at the map distance of 7.23 and 12.50 Kosambi unit, respectively. The other marker OPB 02_{600} did not show any linkage among the morphological traits under study.

Key words : *Lens culinaris*, linkage, morphological markers, RAPD

Introduction

Genetic maps of agricultural crop are valuable tools for plant geneticists and breeders and can be used to improve breeding efficiency. For example, one area where practical significance of genetic map has been realized is the tagging of gene(s) of economic importance (disease resistance or male sterility). An important use of such markers would be in markers based selection in plant breeding.

The current lentil map consists of small number of markers, mainly isozymes and RFLPs, covering a relatively small portion of the lentil genome [1-3]. RAPD analysis has been considered as the most rapid method for constructing genetic map of any crop [4, 5]. It has also been demonstrated to be an efficient marker detection system, particularly for disease resistance gene [6]. Moreover, RAPD analysis is comparatively easy, cost effective and require comparatively minimum time.

The linkage map in lentil is still rudimentary [1]. To make a beginning in genetic mapping of a crop, it is essential to identify and map many more markers. The exact position of several morphological markers has not been precisely determined in lentil. Many genes have not yet been located on the linkage map. Keeping the above consideration in view, the present investigation was initiated to study the linkage relationship between morphological and RAPD markers.

Materials and methods

The experiments were conducted at the Research Farm, Division of Genetics, Indian Agricultural Research Institute, New Delhi during 1998-2001. The parents L6163 and L830 wre choosen for this study as they were differing for as many as eight morphological markers (Table 1).

Table 1. Morphological markers of the parental strains

Sl. No.	Markers	Gene symbol	P_1 (L 6163)	P_2 (L 830)
1.	Brown leaf pigmentation	*Bl*	Non-pigmented	Pigmented
2.	leaf size	*Blf*	Broad	Narrow
3.	Leaf shape	*Ola*	Oval	acute
4.	Stem pigmentation	*Gs*	Non-pigmented	Pigmented
5.	Tendril formation	*Tnl*	Non-tendrilled	Tendrilled
6.	Pod pigmentation	*Rdp*	Non-pigmented	Pigmented
7.	Pod size	*Lpd*	Large	Small
8.	Cotyledon colour	*Y-B-*	Yellow	Orange

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The cross L 6163 × L 830 was made during *rabi* 1998-99 at the Research Farm, Division of Genetics, IARI, New Delhi. The F_1 seeds were sown in National Plytoron Facility, IARI, New Delhi for advancing the generation. The F_2 plants along with parents and F_1 were raised in winter 1999-2000 at the Research Farm, Division of Genetics, IARI, New Delhi, keeping a 50 cm spacing between rows and 25 cm from plant to plant. Observations on morphological traits were recorded at appropriate time of their proper expression.

The total genomic DNA was isolated from 3-5 g of young expanding fresh leaves of 50 individual F_2 plants of the cross and also from the parental strains using the protocol [7] with few modifications.

The extract was treated with RNAase to degrade the RNA. Quantification and quality checking were done by 0.8 per cent agarose gel. RAPD analysis was conducted on Perkin Elmer 2400 Thermalcycler. The 25 µl reaction mixture contained of 100 µmoles of each dNTP (promega), 0.2 µmoles of a given 10-mer primers. 0.5 unit of Taq DNA polymerase (Bangalore Genei) and 20 ng of genomic DNA in 1 × Taq polymerase assay buffer (Bangalore Genei). The reaction was carried out in the following temperature cycles : holding at 94°C for 5 min. at start, followed by 45 cycles of 92°C for 1 min., 37°C for 1 min. and 72°C for 2 min. and a final additional extension at 72°C for 7 min. The primer kits namely, OPA 1 to 20, OPB 1 to 20, OPS 1 to 20, OPM 1 to 20, OPU 1 to 20, OPV 1 to 20 and OPW 1 to 20 were used from Operon Technologies Inc., Alameda, CA. USA. The PCR products were electrophoretically separated in 1.5 per cent agarose gel in 1 × TAE buffer and visualized by ethidium bromide staining. The gel was photographed under UV light using T 667 ISO 3000 polaroid film.

To study the inheritance of each trait, χ^2 was estimated by using the standard formula. Linkage was detected based on joint segregation analysis, taking two characters at a time, using the procedure [8]. Recombination fraction was calculated by product-ratio method. Map distance was estimated by Kosambi unit using the formula [9].

Results and discussion

The PCR analysis was carried out to detect polymorphism for the marker binding site among the parents, viz. L 6163 and L 830. The parents were screened for presence or absence of band. Out of 140 primers used for detecting polymorphism, only 9 perimers showed polymorphism among the two parents (Table 2). The polymorphism were reliable and reproducible as because each polymorphic fragment was amplified at least two times on the parental DNA.

Table 2. List of the polymorphic RAPD primers in lentil

Sl. No.	RAPD primer	P_1 (L 6163)	P_2 (L 830)	Number of polymorphic band
1.	OPB 02	+	-	1
2.	OPB 05	-	+	1
3.	OPM 06	-	+	1
4.	OPS 05	-	+	2
5.	OPU 03	+	-	2
6.	OPU 05	+	-	1
7.	OPV 06	-	+	1
8.	OPV 12	-	+	1
9.	OPV 20	-	+	1

+ = Presence of band; - = Absence of band

Only major amplified fragments were used for analysis. High (> 1500 bp) and low (< 500 bp) molecular weight fragments were not considered as because they were usually monomorphic and unclear.

A marker that shows polymorphism between the two parents is expected to be linked with any morphological trait of the parents for which they are contrasting to each other. Therefore, to study the mode of inheritance of polymorphic RAPD primers F_2 population comprising 50 plants was screened on the basis of amplification i.e. presence or absence of band (Table 3).

Table 3. F_2 segregation pattern of RAPD markers in lentil cross L 6163 × L 830

RAPD markers	F_2 segregation			χ^2 (3:1)	d.f.	P value
	Presence (+)	Absence (-)	Total			
OPB 02	37	13	50	0.001	1	>0.95
OPB 05	45	5	50	6.000*	1	> 0.01
OPM 06	36	14	50	0.240	1	>0.80
OPS 05	29	21	50	7.706**	1	>0.001
OPU 03	44	6	50	4.506*	1	>0.02
OPU 05	28	22	50	9.626**	1	>0.001
OPV 06	46	4	50	7.706**	1	>0.001
OPV 12	27	23	50	11.760***	1	>0.0001
OPV 20	30	20	50	6.000*	1	>0.01

* = Significant at 5% level, ** = Significant at 1% level,
*** = Significant at 0.01% level

Out of nine polymorphic primers, only two primers OPB 02 and OPM 06 showed the expected Mendelain pattern of inheritance. The markers OPB 02 gave amplification in 37 plants and it was absent in 13 plants out of total 50 plants in F_2 population. These segregation showed excellent for 3:1 ratio with non-significant χ^2 value (χ^2 = 0.001; P > 0.95). The DNA amplification was found in 36 F_2 plants and absent in 14 F_2 plants in case of primer OPM 06 (Fig. 1). It

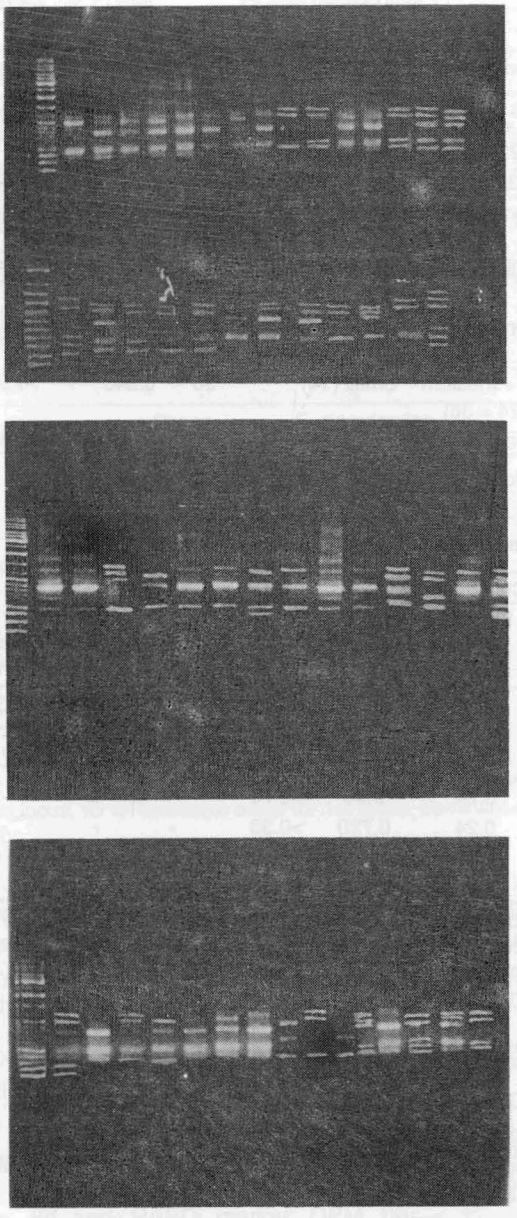


Fig. 1. F_2 segregation pattern of the cross L 6163 × L 830 with RAPD marker OPM 06900
M = DNA ladder mix (10,000 to 100 bp)
P_1 = L 6163 and P_2 = L 830
1-50 = Individual F_2 plants

also gave a good fit of ratio 3:1 with non-significant χ^2 value (χ^2 = 0.240; P > 0.80).

The marker OPB 05 gave amplification in 45 plants and it was absent in 5 plants, it showed significant χ^2 value (χ^2 = 6.00) at 5% level of probability. Highly significant χ^2 value (χ^2 = 7.706) at 1 per cent level was also observed in case of primer OPS 05, where amplification was found in 29 plants and absent in 21 plants. The marker OPU 03 showed significant χ^2 value (χ^2 = 4.506) at 5% level of probability.

Amplification was found in 27 plants and absent in 23 plants in case of primer OPV 12, which showed more highly significant χ^2 value (χ^2 = 11.760 at 0.1 per cent level of probability). The primer OPU 05 and OPV 06 gave highly significant values (9.626 and 7.706, respectively) at 1% level of probability. The primer OPV 20 also gave significant χ^2 value (χ^2 = 6.000) at 5 per cent level of probability. From the above observation it revealed that only two primer OPB 02 and OPM 06 showed the expected monogenic pattern of inheritance. Therefore, only these two markers used for joint segregation analysis with morphological markers of the cross L 6163 × L 830. It was observed that the marker OPB 02 was polymorphic for 600 bp of fragment and the marker OPM 06 was polymorphic for 900 bp of fragment.

The probable cause of segregation distortion in other primers may be that parents were polymorphic for non-coding region of the genome or may be simple mutation which alter the primer binding site resulting polymorphism but such polymorphism may not necessarily important at gene expression level and hence the segregation distortion could occur. Out of 14 polymorphic RAPD primers, 12 primer gave segregation distortion of 3:1 ratio in the cross between wild and cultivated lentil [9].

The parents of the cross L 6163 × L 830 were contrasting for 8 morphological markers. Contrasting traits along with their F_2 segregation are presented in Table 4. All the traits showed monogenic pattern of inheritance with good fit ratio of 3:1, where pigmented leaf was dominant over non-pigmented leaf, broad leaf showed incomplete dominance over narrow leaf, oval leaf shape was dominant over acute leaf shape, pigmented stem was dominant over non-pigmented stem, tendrilled plant was dominant non-tendrilled plant, pigmented pod was dominant over non-pigmented pod, large pod size was dominant over small pod size and orange cotyledon was dominant over yellow cotyledon. In all the cases non- significant χ^2 values were observed which ranged from 0.026 to 2.160 at 5 per cent level of probability (Table 4). The gene symbol *Blf* for leaf size, *Ola* for leaf shape and *Lpd* for large pod are reported for the first time.

To investigate the linkage relationship between the gene *Bl* (brown leaf pigmentation) and other morphological markers *viz.*, stem pigmentation (*Gs*),

Table 4. F_2 segregation for different morphological markers in lentil cross L6163 x L830

Morphological marker with gene symbol	P_1 L 6163	P_2 L 830	F_1 phenotype	F_2 segregation 1	2	3	χ^2 (3:1)	d.f.	p value
Leaf pigmentation (*Bl*)	Non-pigmented	Pigmented	Pigmented	Pigmented (38)	Non-pigment (12)	50	0.026	1	>0.80
Leaf size (*Blf*)	Broad	Narrow	Medium	Broad + medium (12 + 23 = 35)	Narrow (15)	50	0.680	1	>0.30
Leaf shape (*Ola*)	Oval	Acute	Oval	Oval (39)	Acute (11)	50	0.240	1	>0.50
Stem pigmentation (*Gs*)	Non-pigmented	Pigmented	Pigmented	Pigmented (36)	Non-pigment (14)	50	0.24	1	>0.50
Tendril (*Tnl*)	Non-tendrilled	Tendrilled	Tendrilled	Tendrilled (39)	Non-tendrilled (11)	50	0.240	1	>0.50
Pod pigmentation (*Rdp*	Non-pigmented	Pigmented	Pigmented	Pigmented (42)	Non-pigment (8)	50	2.160	1	>0.10
Pod size (*Lpd*)	Large	Small	Medium	Large + medium (12 + 24 = 36)	Small (14)	50	0.240	1	>0.50
Orange cotyledon (Y-B-)	Yellow	Orange	Orange	Orange (38)	Yellow (12)	50	0.026	1	>0.80

Note : Values within parenthesis indicate the number of segregating population

Table 5. Joint F_2 segregation of the gene *Bl* with other morphological markers in lentil cross L6163 x L830

Gene pairs	Phase	F_2 segregation DD	Dr	rD	rr	Total	Chi-square Locus 1 (3:1)	Lucas 2 (3:1)	Joint Seg.	P (Linkage)	RF %	S.E. %	Map dis. in Kosambi unit
Bl-Gs	C	33	5	3	9	50	0.026	0.24	18.008	<0.0001	23.409	7.00	25.386
Bl-Tnl	C	28	10	11	1	50	0.026	0.24	1.502	>0.20	-	-	-
Bl-Blf	R	25	13	10	2	50	0.026	0.68	3.208	>0.05	-	-	-
Bl-Ola	R	30	8	9	3	50	0.026	0.24	0.80	>0.70	-	-	-
Bl-Lpd	R	27	11	10	2	50	0.026	0.24	0.720	>0.30	-	-	-
Bl-Rdp	C	32	6	4	8	50	0.026	2.16	12.168	<0.0001	36.302	8.80	46.01
Bl-Y-B	C	29	9	9	3	50	0.026	0.026	0.001	>0.95	-	-	-

Note: C = Coupling phase, R = Repulsion phase, DD = Dominant alleles for both the loci, Dr = Dominant allele for 1st locus, recessive allele for 2nd locus, rD = recessive allele for 1st locus, dominant allele for 2nd locus, rr = Recessive alleles for both loci; RF = Recombination Fraction

tendril formation (*Tnl*), leaf size (*Blf*), leaf shape (*Ola*) and orange cotyledon (Y-B-), the F_2 population comprising fifty plants was used for joint segregation analysis (Table 5). A highly significant χ^2_L value between the gene pairs *Bl-Gs* (χ^2_L = 18.00; P < 0.0001) was observed indicating presence of linkage between them. The recombination fraction was 23.409 ± 7.00 per cent and the estimated map distance of 25.386 Kosambi unit could be established. Non-significant χ^2_L value was observed between the gene pairs *Bl-Tnl, Bl-Blf, Bl-Ola, Bl-Lpd* and *Bl-Y-B* (χ^2_L = 1.502; P > 0.20, χ^2_L = 3.208; P > 0.05, χ^2_L = 0.80; P > 0.70, χ^2_L = 0.720; P > 0.30 and χ^2_L = 0.001; P > 0.95, respectively) indicating absence of linkage between these gene pairs. Linkage was also established between the gene pairs *Bl-Rdp* because of significant χ^2_L value (χ^2_L = 12.168; P < 0.0001) was observed in joint segregation analysis. The recombination fraction was estimated as 36.302 ± 8.80 in coupling phase. The map distance was as calculated as 46.01 Kosambi unit.

Joint segregation analysis between the gene pairs *Gs-Tnl, Gs-Blf, Gs-Ola, Gs-Lpd* and *Gs-Y-B* indicated absence of linkage between these gene pairs because of non-significant χ^2_L values were observed. The χ^2_L value varied from 0.001 to 3.208 and P > 0.95 to P > 0.05 (Table 6). A highly significant χ^2_L value (χ^2_L = 14.942; P < 0.0001) was observed between the gene pairs *Gs* and *Rdp*, indicating linkage relationship between them. The recombination fraction was found to be 21.47 ± 1.14 and the estimated map distance of 22.95 Kosambi unit (Table 6) could be established.

Table 6. Joint F_2 segregation of the gene *gs* with other morphological markers in lentil cross L6163 x L830

Genepairs	Phase	F_2 segregation					Chi-square			P (Linkage)	RF %	S.E. %	Map dis. in Kosambi unit
		DD	Dr	rD	rr	Total	Locus 1 (3:1)	Locus 2 (3:1)	Joint seg.				
Gs-Tnl	C	26	10	13	1	50	0.24	0.24	2.568	>0.10	-	-	-
Gs-Blf	R	24	12	11	3	50	0.24	0.68	0.720	>0.30	-	-	-
Gs-Ola	R	29	7	10	4	50	0.24	0.24	0.435	>0.50	-	-	-
Gs-Lpd	R	25	10	11	4	50	0.24	0.24	0.001	>0.95	-	-	-
Gs-Rdp	C	34	2	6	8	50	0.24	2.16	14.942	>0.0001	21.47	1.14	22.95
Gs-Y-B-	C	25	11	13	1	50	0.24	0.026	3.208	>0.05	-	-	-

Note: C = Coupling phase, R = Repulsion phase, DD = Dominant alleles for both the loci; Dr = Dominant allele for 1st locus, recessive allele for 2ns locus, rD = Recessive allele for 1st locus, dominant allele for 2nd locus; rr = Recesive alleles for both loci; RF = Recombination Fraction

Table 7. Joint F_2 segregation of RAPD marker (OPM 06_{900}) with other morphological markers in lentil cross L6163 x L830

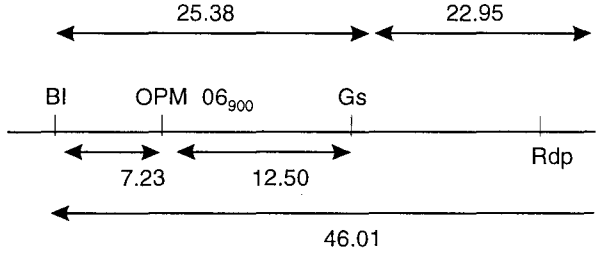
Gene pairs	Phase	F_2 segregation					Chi-square			P (linkage)	RF %	SE %	Map dis. in Kosambi unit
		DD	Dr	rD	rr	Total	Locus 1 (3:1)	Locus 2 (3:1)	Joint seg.				
Bl-OPM 06_{900}	C	35	3	1	11	50	0.026	0.240	33.075	0.001	7.187	3.816	7.237
Blf-OPM 06_{900}	R	28	7	8	7	50	0.680	0.240	2.702	>0.02	-	-	-
Ola-OPM 06_{900}	R	29	10	7	4	50	0.240	0.240	0.435	>0.50	-	-	-
Gs-OPM 06_{900}	C	33	3	3	11	50	0.240	0.240	28.880	0.0001	12.248	4.110	12.502
Tnl-OPM 06_{900}	C	30	9	6	5	50	0.240	0.240	2.000	>0.10	-	-	-
Rdp-OPM 06_{900}	C	31	11	5	3	50	2.160	0.240	0.222	>0.50	-	-	-
Lpd-OPM 06_{900}	R	28	8	8	6	50	0.240	0.240	2.568	>0.10	-	-	-
Y-B--OPM 06_{900}	C	29	9	7	5	50	0.026	0.240	1.502	>0.20	-	-	-

Note : C = Coupling phase, R = Repulsion phase, DD = Dominant alleles for both the loci, Dr = Dominant allele for 1st locis, recessive allele for 2nd locus, rD = Recessive allele for 1st locus, dominant allele for 2nd locus, rr = Recessive alleles for both loci, RF = Recombination Fraction

The linkage relationship were also analysed between the RAPD locus OPM 06_{900} and eight morphological markers (Table 7). Joint segregation analysis in F_2 population revealed that there was a highly significant linkage between the gene Bl and OPM 06_{900} (χ^2_L = 33.075; P < 0.0001). The recombination fraction was estimated as 7.187 ± 3.816 per cent. The map distance was estimated to be 7.237 Kosambi unit between these two genes. The same F_2 population was also analysed for joint segregation regarding the gene *Blf* and RAPD marker OPM 06_{900} to detect linkage between them. The χ^2_L value was found to be non-significant, indicating absence of linkage between these two loci (χ^2_L = 2.702; P > 0.02). Absence of linkage was also demonstrated between the gene Ola and OPM 06_{900} locus, based on joint segregation analysis in F_2 population of the same cross as the χ^2_L value was non-significant (χ^2_L = 0.435; P > 0.50). The joint segregation analysis on F_2 population of the cross L 6163 × L 830 gave a highly significant linkage for the gene *Gs* and OPM 06_{900} (χ^2_L = 28.880; P < 0.0001). The recombination fraction between the was estimated as 12.248 ± 4.110 per cent. The map distance was found to be 12.502 Kosambi unit. The joint segregation of the characters tendril formation and CPM 06_{900} locus was analysed in F_2 population of the same cross (L 6163 × L 830). The non-significant χ^2_L value (χ^2_L = 2.000; P > 0.10) indicated absence of linkage between *Tnl* and OPM 06_{900} locus. The non-significant χ^2_L value (χ^2_L = 0.222; P > 0.50) was obtained between the gene *Rdp* and OPM 06_{900} locus, indicating absence of linkage between them. The joint segregation of *Lpd* gene and OPM 06_{900} locus showed non-significant χ^2_L value indicating absence of linkage (χ^2_L = 2.568; P > 0.10) between them. Joint segregation analysis of orange cotyledon (Y-B) and OPM 06_{900} locus indicated absence of linkage between them. (χ^2_L = 1.502; P > 202).

The above observations revealed that the RAPD marker OPM 06_{900} was linked with two morphological markers *viz.*, brown leaf pigmentation (*Bl*) and stem pigmentation (*Gs*) having the map distance of 7.237 and 12.502 Kosambi unit, respectively.

The two genes *Bl* and *Gs* showed linkage based on morphological analysis having the map distance 25.38 Kosambunit. The summation of map distance between *Bl*-OPM 06_{900} and *Gs*-OPM 06_{900} is smaller than the map distance between the genes *Bl*-Gs. It indicated that the RAPD marker OPM 06_{900} is located between the genes *Bl* and *Gs*. Therefore, the combined map of the three morphological (leaf, stem and pod pigmentation) markers and one RAPD marker could be developed as follows:



This linkage relationship has been reported for the first time in lentil.

Earlier studies also indicate that the three gene *Bl* (leaf pigmentation), *Gs* (stem pigementation) and *Rdp* (pod pigmentation) are located in same linkage group based on morphological analysis [10] supporting the present findings. On the basis of morphological and molecular (RAPD + RFLP) analysis, epicotyl colour (stem pigmentation) gene (Gs) could be located in linkage group 1 [11]. Based on morphological analysis it revealed that the leaf pigmentation gene (*Bl*) was linked with stem pigmentation gene (*Gs*) [12]. Therefore, from the above discussion it can be concluded that the three gene (*Bl*, *GS* and *Rdp*) and one RAPD marker (OPM 06_{900}) are located in the same linkage group of lentil genome.

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