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Linkage mapping of two mutant loci controlling leaf necrosis and glabrousness in chickpea (*Cicer arietinum* L.)

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Abstract

Linkage analysis of two linked mutant loci controlling leaf necrosis (*nec*) and glabrous shoot (*gl*) was done with eleven isozyme loci in two F_2 populations of chickpea (*Cicer arietinum* L.). The *nec* and *gl* loci were located closely linked to the isozyme locus *Amy*, 6.2 ± 1.8 cM and 15.4 ± 2.8 cM apart respectively, in a gene order of *Amy-nec-gl*. As the locus *Amy* is known to be located on linkage group VII, *nec* and *gl* loci were also assigned to this linkage group. The isozyme AMY can be used as marker for necrosis gene in segregating generations.

Key words: Chickpea, leaf necrosis, glabrous shoot, isozymes, linkage mapping

Introduction

Development of a genetic linkage map in chickpea (*Cicer arietinum* L.) and tagging of genes controlling traits of economic importance could facilitate formulation of effective marker assisted selection programmes in this crop. Several studies during the past decade have concentrated on enrichment of genetic map of chickpea. Use of isozyme markers in linkage studies provided an initial momentum to detect genetic linkages in this important food legume crop. The first linkage map of chickpea, published in 1990, was developed based on isozyme markers and contained 26 isozyme and three morphological trait loci [1, 2]. Several additional loci have since been added to this linkage map [3, 4].

A glabrous mutant has been identified in chickpea [5]. The absence of trichomes makes the mutant highly susceptible to black aphids. The mutant may be useful in entomological studies as it provides good medium for rearing large populations of black aphids [5]. A gene for leaf necrosis was later identified in the glabrous mutant [6]. Necrosis reduces the photosynthesis area of the plant by partial or complete drying of leaves and leads to poor vegetative growth, Thus, the utility of glabrous mutant is adversely affected by the presence of necrosis gene [6]. This article reports on mapping of these two mutant loci on the linkage map of chickpea using isozyme markers. The linked isozyme marker can be used to select against the necrosis gene in segregating materials.

Materials and methods

Two F_2 populations derived from the crosses of a *C. arietinum* mutant ICC 15566 (glabrous shoot, leaf necrosis) with two accessions of *C. reticulatum*, ICCW 8 and ICCW 9, were used for linkage analysis. Plants were grown during *Rabi* 1997-98 in normal field conditions following recommended cultural practices.

Isozymes were analysed by starch gel electrophoresis [1,2]. The enzymes assayed included acid phosphatase (ACP; E.C. 3.1.3.2), alcohol dehydrogenase (ADH; E.C. 1.1.1.1), amylase (AMY; E.C.3.2.1._), aspartate aminotransferase (AAT; E.C. 2.6.1.1), esterase (EST; 3.1.1._), glucose-6-phosphate isomerase (GPI; E.C. 5.3.1.9), malate dehydrogenase (MDH; E.C. 1.1.1.37), malic enzyme (ME; E.C. 1.1.1.40), peroxidase (PRX; E.C. 1.11.1.47) and phosphoglucomutase (PGM; E. C. 5.4.2.2). Inheritance and Linkage analyses were performed using the computer programme LINKAGE-1 [7].

Results and discussion

The glabrous mutant (ICC 15566) of chickpea cv 'Chafa' is characterized by almost hairless and lustrous shoot and high susceptibility to black aphid. A single recessive gene, designated *gl*, has been reported to govern the glabrous trait [5].

A gene for leaf necrosis, designated *nec*, has recently been identified from the glabrous mutant ICC 15566 [6]. Plants carrying recessive *nec* gene in homozygous condition start showing leaf necrosis prior to flower initiation. The older leaves show drying of leaflet margins to whole leaflets and the intensity of necrosis decreases toward the apical meristem. The

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necrotic plants grow weak and produce only few pods. The glabrous plants (*gl gl*) and the pubescent plants (*Gl* -) show equal degree of leaf necrosis when they possess *nec* gene in homozygous condition (Fig. 1), The *gl* and *nec* loci have been reported to be linked with a map distance of 16 ± 3 cM [6].

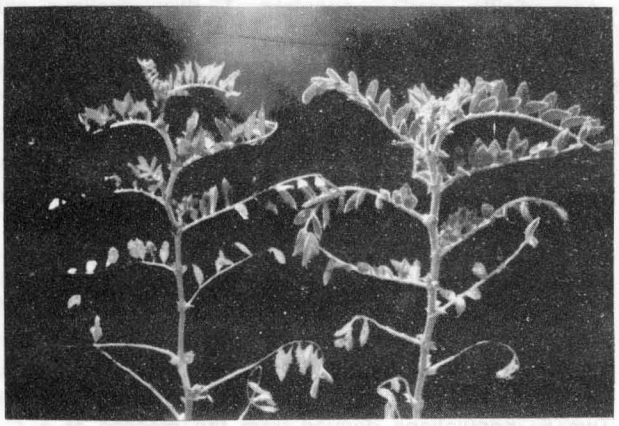

Fig. 1. Glabrous necrotic, *gl gl nec nec* (left), and pubescent necrotic *Gl_nec nec* (right) chickpea plants

This study confirms the previously reported mode of inheritance for glabrous and leaf necrosis traits (Table 1). Each of these traits was recessive and controlled monogenically. Inheritance was studied for 11 isozymes which included ACP-1, ADH-2, AMY, AAT-3, GPI-2, MDH-2, ME-1, PRX-2, PRX-3, PGM-1 and PGM-2. The allozymes of each isozyme showed codominance in the heterozygotes and segregated in a ratio of 1 FF : 2 FS : 1 SS in the F_2 (Table 1). These results confirm the earlier reports [1, 2] that each of these loci is controlled by a single locus with codominant alleles.

The Loci *gl* and *nec* were found linked and a map distance of 12.9 ± 6.8 cM was estimated between these loci from the cross ICC 15566 × ICCW 8 and 10.0 ± 7.0 cM from the cross ICC 15566 × ICCW 9 (Table 2). This linkage was first detected by Gaur and Gour [6] who studied five intervarietal crosses of *C. arietinum* and estimated a map distance ranging from 12.7 ±7.1 to 18.3 ±5.8 between these loci.

The linkage relationships of *gl* and *nec* loci were determined with 11 isozyme loci in this study. Ten of these isozyme loci were with known location on linkage map of chickpea. The loci *Aat-p* and *Pgm-p* are located on linkage group I, *Acp-1* and *Adh-2* on linkage group III, *Prx-2* and *Prx-3* on linkage group IV, *Gpi-c* on linkage group VI, *Amy* and *Pgm-c* on linkage group VII and *Me-1* on linkage group VIII [2, 3]. The locus *Mdh-2* has not been assigned to any linkage group. The loci *gl* and *nec* showed a linkage with *Amy* and segregated independently of other isozyme loci. The banding patterns of AMY are shown in Fig. 2. A map distance of 6.2 ± 1.8 cM was estimated between *Amy*

Table 1. Goodness-of fit test for F_2 segregation of isozyme and morphological traits in chickpea

Trait	Number of crosses	F_2 phenotype			Expected genetic ratio	Goodness-of-fit χ^2	P	Hetero-geneity χ^2	P
Isozyme		FF*	FS	SS					
ACP-1	9	220	495	227	1:2:1	2.55	0.28	9.57	0.89
ADH-2	5	111	225	104	1:2:1	0.45	0.80	4.56	0.80
AMY	5	150	309	141	1:2:1	0.81	0.67	2.75	0.95
AAT-3	4	75	173	94	1:2:1	2.16	0.34	0.73	0.99
GPI-2	4	77	181	84	1:2:1	1.46	0.48	3.80	0.70
MDH-2	3	104	224	90	1:2:l	3.09	0.21	3.00	0.56
ME-I	3	100	220	98	1:2:1	1.18	0.55	4.16	0.38
PRX-2	4	134	243	139	1:2:1	1.84	0.40	3.05	0.80
PRX-3	5	108	213	105	1:2:1	0.04	0.98	5.81	0.67
PGM-1	5	97	205	96	1:2:1	0.37	0.83	2.27	0.97
PGM-2	4	126	236	140	1:2:1	2.57	0.28	4.02	0.67
Morphological trait		dominant	-	recessive					
Glabrous shoot	2	318 (pubescent)	-	89 (glabrous)	3:1	2.13	0.14	0.29	0.60
Leaf necrosis	2	316 (healthy)	-	91 (necrotic)	3:1	1.51	0.22	0.39	0.5

*FF = both fast allozymes, FS = fast and slow allozymes, SS = both slow allozymes

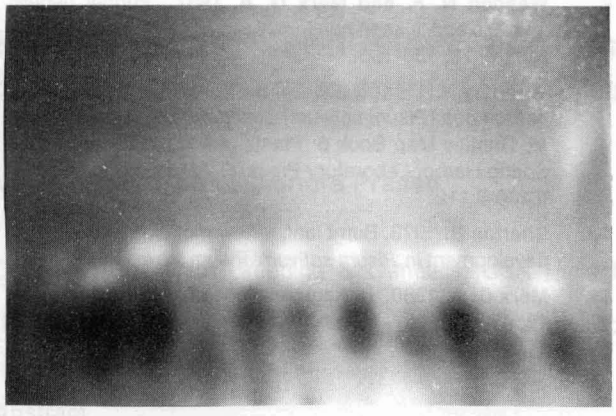

Fig. 2. The white bands represent the segregating isozyme *AMY* in chickpea. The two single-banded (lower single band and the upper single band) phenotypes represent the two parental type plants, whereas the double-banded phenotypes represent heterozygous plants

The exact side of the locus *Amy* to which the linkage *nec* - *gl* is located could not be determined. In relation to *Amy*, this linkage may be located toward either *Gal-2* or *Aat-m*. Analysis of joint segregation of *gl* or *nec* with *Gal-2* or *Aat-m* could resolve precise location.

Chickpea and pea are closely related and have several common linkage groups consisting of homologous genes [1,2,3,4]. A gene for leaf necrosis, designated *bulf* (burnt leaf), has been reported in pea [10] and mapped on chromosome 3 [11]. This gene does not appear homologous to the *nec* gene of chickpea as the *bulf* gene affects the growing tips more severely than the lower part of the plant, whereas opposite was the case with *nec* gene of chickpea. The locations of *bulf* and *nec* loci in different linkage groups further support that these loci are not homologous.

Table 2. Contingency X^2 test for F_2 segregation for pair of loci in the linkage group *gl-nec-Amy* in chickpea

Segregating loci and cross	F_2 phenotype						Total	χ^2	r ± SE
gl / nec	PU/HL	GL/HL			PU/NC	GL/NC			
ICC 15566 × ICCW 8	151	14	-	-	10	34	209	92.70*	12.89 ± 6.80
ICC 15566 × ICCW 9	145	6	-	-	12	5	198	108.48*	9.99 ± 7.02
Total	296	20	-	-	22	69	407	189.95*	11.46 ± 4.88
Heterogeneity								11.43	
gl / Amy	PU/1	GL/1	PU/H	GL/H	PU/2	GL/2			
ICC 15566 × ICCW 9	45	2	96	8	16	31	198	77.09*	15.39 ± 2.75
nec / Amy	HL/1	NC/1	HL/H	NC/2	HL/2	NC/2			
ICC 15566 × ICCW 9	47	0	98	6	6	41	198	l37.85*	6.20 ± 1.76

*Significant at P = 0.001; Note: GL = glabrous, HL = healthy, NC = necrotic, PU = pubescent, H = heterozygous, 1 allozymes of parent 1, 2 allozymes of parent 2.

and *nec* and 15.4 ± 2.7 cM between *Amy* and *gl* (Table 2). These results suggest a gene order of *Amy-nec-gl*.

The loci *nec* and *gl* were assigned to linkage group VII of chickpea as the locus *Amy* belongs to this linkage group. A linkage group consisting of nine isozyme loci, i.e., *Ald-p1 - Glu-3 - Gal-2 - Amy - Aat-m - Est-2/Est-3 - Pgd-p - pgm-c*, was first reported by Gaur and Slinkard [2]. This linkage group was designated as linkage group II of chickpea [2] because of its high similarity with linkage group II of pea reported earlier [8]. As the linkage group II of pea was later redesignated as linkage group VII [9], the linkage group II of chickpea was also redesignated as linkage group VII [3]. An additional isozyme locus *Gpt-1* (Glucose-phosphate transferase-1) has been assigned on this linkage group between the loci *Pgd-p* and *Pgm-c* [3].

Necrosis adversely affects plant growth and ultimately yield. As it is controlled by recessive gene, progeny testing is required to identify heterozygous healthy plants. The closely linked isozyme locus *Amy* can be used as marker for necrosis gene. It will help in distinguishing between the dominant and the heterozygous healthy plants and facilitate elimination of necrosis gene without need for progeny testing.

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