Theor Appl Genet (1993) 86:417–426



# Inheritance and linkage relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.)

**K. Kazan [1], F. J. Muehlbauer [1], N. F. Weeden [2], G. Ladizinsky [3]**

[1] Department of Crop and Soil Sciences and USDA Grain Legume Genetics and Physiology Unit, Washington State University Pullman, WA 99164, USA
[2] Department of Horticultural Sciences, Cornell University, Geneva, NY 14456, USA
[3] Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel

Received: 29 June 1992 / Accepted: 20 October 1992

**Abstract.** Inheritance and linkage relationships of several morphological and isozyme loci are described in chickpea (*Cicer arietinum* L.). Segregation data obtained from several $F_2$ families confirmed the previously observed mode of inheritance for most of the morphological loci. Additional morphological markers in chickpea are also described. Most of the isozyme loci studied showed codominant expression and fit expected Mendelian segregation ratios. However, distorted ratios were also observed for some loci. Linkage was found between *Pgd-c*, the locus encoding the cytosolic form of 6-phosphogluconate dehydrogenase, and *Hg*, the locus controlling plant growth habit. These 2 loci were separated by approximately 18 recombinational map units. A similar linkage between comparable loci was previously reported in pea (*Pisum sativum* L.) (Weeden and Wolko 1990). Linkage was also detected among 3 isozyme loci; the cytosolic form of phosphoglucomutase (*Pgm-c*), glucose-1-phosphate transferase (*Gpt1*), and the plastid specific form of 6-phosphogluconate dehydrogenase (*Pgd-p*). The linkage of 2 loci (*Pgm-c* and *Pgd-p*) in this cluster is also conserved in pea and lentil (*Lens* Miller). The linkage between an acid phosphatase locus (*Acp3*) and the locus specifying the cytosolic form of glucosephosphate isomerase (*Gpi-c*) in chickpea suggested another linkage group in common with pea. Additionally, other linkages that were not previously observed in chickpea or related genera included the linkage of the cytosolic form of aconitase (*Aco-c*) with adenylate kinase (*Adk1*) and fructokinase (*Fk3*), and the linkage of a locus encoding the mitochondrial specific aconitase (*Aco-m*) with a seed protein locus (*Spr1*). The loci determining flower color (*P*), epicotyl color (*Gst*), seed coat color ($T^3$), and seed surface (*Rs*) were associated with the locus encoding glucose-1-phosphate transferase (*Gpt2*). These results, along with previous studies, suggest that pea, lentil and chickpea have several common linkage groups consisting of homologous genes. This also indicates that linkages found in one genus can be used to predict similar linkages in related genera in the development of linkage maps.

**Key words:** Genetic linkage – Isozyme markers – Morphological markers – Linkage conservation

Communicated by A. L. Kahler
*Correspondence to:* F. J. Muehlbauer

## Introduction

The potential use of genetic markers to establish linkage maps has increased dramatically over the last decade. Relatively well-developed linkage maps that include loci encoding various isozymes, restriction fragment length polymorphisms (RFLPs), and qualitative and quantitative trait loci are available in crops such as wheat (*Triticum aestivum* L.) (Hart and Gale 1990), tomato (*Lycopersicon esculentum* L.) (Tanksley and Mutschler 1990), pea (*Pisum sativum* L.), (Weeden and Wolko 1990), lentil (*Lens* Miller) (Havey and Muehlbauer 1989; Muehlbauer et al. 1989), soybean (*Glycine max* L.) (Palmer and Kiang 1990), lettuce (*Lactuca sativa* L.) (Kesseli et al. 1990), and maize (*Zea mays* L.) (Coe et al. 1990). An important use of these maps would be to develop marker-based selection in plant breeding programs. To date, several isozyme and RFLP markers closely linked to the genes conferring pest resistance have been mapped in their respective linkage groups. For example, the locus for the plastid-specific form of phosphoglucomutase (*Pgm-p*) was found to be closely linked to the gene for resistance to bean yellow mosaic virus (*Mo*) in pea (Weeden et al. 1984). Similarly, an alcohol dehydro-

genase locus (*Adh1*) was linked to a gene for resistance to pea enation mosaic virus (*En*) in pea (Weeden and Provvidenti 1987). A close association was also identified between an endopeptidase locus and strawbreaker foot rot resistance in wheat (McMillin et al. 1986), and an acid phosphatase locus and nematode resistance in tomato (Rick and Fobes 1974). Recently, RFLP loci linked to disease resistance genes have been reported in maize (McMullen and Lovie 1989) and tomato (Sarfatti et al. 1989).

An established linkage map can also provide useful information for the detection of analogous genes and linkage groups in related genera. For example, the chromosomal locations of isozymes in wheat, including related species, were determined based on the high degree of conservation among the genera (Ainsworth et al. 1984, 1986; Salinas et al. 1984). Similarly, several linkage groups previously identified in pea were found to have counterparts in lentil. For example, the gene specifying the cytosolic form of aspartate aminotransferase (*Aat-c*) is linked to the alcohol dehydrogenase (*Adh1*) locus in both pea and lentil (Weeden et al. 1988). The association between the genes responsible for the production of anthocyanin at the base of the stem (*D* in pea and *Gs* in lentil) and the gene encoding the plastid-specific form of aspartate aminotransferase (*Aat-p*) has been shown in both genera (Muehlbauer et al. 1989; Weeden and Marx 1987). Two other conserved linkages that have been identified in both genera are the linkage between a leaf peroxidase locus (*Prx1*) and a locus encoding for *N*-acetyl-glucoseaminidase (*Nag1*) and the linkage groups of four loci encoding for isozymes of phosphoglucomutase, fructose kinase, peptidase, and 6-phosphogluconate dehydrogenase (Muehlbauer et al. 1989; Weeden and Marx 1987).

Chickpea (*Cicer arietinum* L.), similar to related genera such as pea and lentil, has a wide range of variability for qualitative and quantitative traits (Moreno and Cubero 1978). Several spontaneous and induced mutant genotypes affecting traits such as leaf morphology (Davis et al. 1990; Rao and Pundir 1983), number of flowers per peduncle (Pundir et al. 1988), polycarpy in flowers (Pundir and Van der Maesen 1981), seed protein content (Oram et al. 1987), and nodulation (Davis 1988; Davis et al. 1985, 1986) have been reported in chickpea. Muehlbauer and Singh (1987) reviewed the genetics of several traits in this crop and pointed out the redundancy of the gene symbols used for certain traits. Several linkages have been reported among morphological markers (reviewed by Muehlbauer and Singh 1987), and a rudimentary gene map for *Cicer* which includes 26 isozyme and 3 morphological loci has been reported (Gaur and Slinkard 1990). The objective of the research reported here was to examine the linkage relationships of the genes for several isozyme and morphological traits and to extend the linkage map of chickpea. Although the development of a linkage map for chickpea is still in the initial stages, it is believed that the existence of homologous loci and conserved linkage groups in pea and lentil can be utilized to predict similar linkages and where they might occur in chickpea.

## Materials and methods

Seeds of desi- and kabuli-type chickpeas were obtained from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) located at Hyderabad, India. Seeds of two wild *Cicer* species, *C. reticulatum* (PI 489777) and *C. echinospermum* (PI 489776), were obtained from the United States Department of Agriculture, Regional Plant Introduction Station, Pullman, Wash. Although these two species are in the same crossability group with the cultivated species, they differ from the cultivated species by several morphological traits and alternate alleles for certain isozyme loci. Crosses were made between the two cultivated lines and between the cultivated lines and the wild species in the greenhouse. $F_2$ seeds of two families (C288-119 and C288-120) were provided by D. Foote of the US Department of Agriculture, Regional Plant Introduction Station at Pullman, Wash. $F_1$ hybrids and $F_2$ families were grown in the greenhouse. Relevant morphological markers were scored visually on the $F_2$ plants (Table 1). Isozyme analyses for segregating loci also were conducted on $F_2$ plants.

Enzyme extracts were obtained by grinding two or three leaflets of young seedlings in 100 µl of a potassium phosphate, pH 7, extraction buffer according to Soltis et al. (1983). Extractions from roots soaked 18 hours in water were used for alcohol dehydrogenase (ADH, EC 1.1.1.1), peroxidase (PRX, EC 1.11.1.7) and *N*-acetyl-glucosaminidase (NAG, EC 3.2.1.30). Mercaptoethanol was omitted from the extraction buffer used for the peroxidase assay. Electrophoresis was carried out in 10% starch gels for all gel systems. A pH 6.1 histidine gel and electrode buffer (Cardy et al. 1980) was used to resolve ADH, aldolase (ALD, EC 4.1.2.13), mannose 6-phosphate isomerase (MPI, EC 5.3.8.1), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), and phosphoglucomutase (PGM, EC 2.7.5.1). A TRIS-citrate/lithium borate, pH 8.1, gel system (Selander et al. 1971) was used to resolve alanine aminopeptidase (AAP, EC 3.4.11.12), aspartate aminotransferase (AAT, EC 2.6.1.1), aconitase (ACO, EC 4.2.13), amylase (AMY, EC 3.2.1), glucose-1-phosphate transferase (GPT, EC 3.1.3.10), glucosephosphate isomerase (GPI, EC 5.3.1.9), PRX, and seed protein (SPR). A citrate/*N*-3-aminopropyl-morpholine gel system, pH 6.1, (Clayton and Tretiak 1972) was used to resolve acid phosphatase (ACP, EC 3.1.3.2), fluorescent esterase (EST, EC 3.1.1.2), and β-D-galactosidase (β-GAL, EC 3.2.1, pH 8.5). Histidine gels were run for 4 h at a constant voltage of 310 mA; other gels were run at 45 mA for 4 h. All gels were run in a refrigerated cabinet. AAT, ALD, PGI, and PGM were assayed according to Weeden and Gottlieb (1980). The β-GAL and NAG assays were identical to those described by Muehlbauer et al. (1989). PRX was assayed according to Weeden and Marx (1984). Amylase bands were detected in slices assayed for ACO that were kept overnight in the refrigerator. The ACO assay has been described by Cardy and Beversdorf (1984); MPI was assayed by the technique of Nichols and Ruddle (1973). The AAP assay consisted of 0.1 *M* potassium phosphate (pH 6.0), 0.1 *M* $MgCl_2$, 10 mg fast black K salt, and 10 mg L-alanyl-β-napthylamide dissolved in 1 ml dimethyl formamide. The SPR was assayed with a napthol blue black solution containing 20 mg Napthol blue black and 20 ml methanol wash solution (5:5:1 methanol, water and Acetic acid). The remaining assays were identical to those described by Soltis et al. (1983).

**Table 1.** Morphological markers used in the study

| Marker | Description |
|---|---|
| *Blv/blv* | Normal green/bronze foliage[a] |
| *Br/br* | Basal/umbrella branching[a] |
| *Chl-1/chl-1* | Chlorina chlorophyll deficiency[b] |
| *Str/str* | Sterility/fertility[b] |
| *Glv/glv* | Normal/enlarged leaflets[a] |
| *Gr/gr* | Brown/green testa[a] |
| *Gst/gst* | Purple/green epicotyl[b] |
| *Hg/hg* | Erect/prostrate plant growth habit[a] |
| *Nlv/nlv* | Normal/narrow leaflets[a] |
| *P/p* | Pink/white corolla color[a] |
| *Pin/pin* | Dehiscent/indehiscent pod[b] |
| *Rp/rp* | Elliptical/round pod[a] |
| *Rs/rs* | Rough/smooth testa[a] |
| *Shp/sph* | Angular/round seed[b] |
| $T^3/t^3$ | Brown/yellow testa[a] |
| $T^4/t^4$ | Black/brown testa[a] |
| *Ycot/ycot* | Yellow/green cotyledon[a] |

[a] See Muehlbauer and Singh (1987) for complete description
[b] Kazan and Muehlbauer (unpublished); see text for details

The subcellular location of the AAT, ACO, ADH, ALD, PGD, PGI, and PGM isozymes was determined by comparing the electrophoretic patterns of pollen leachate, leaf extract, and subcellular organelles prepared according to the method of Weeden and Gottlieb (1980) and Muehlbauer et al. (1989).

The system of nomenclature as suggested by Weeden (1988) was used to designate isozymes and loci. This is the same system as that used by Guar and Slinkard (1990). Accordingly, the locus specifying the most anodally migrating isozyme was designated as 1, the next 2 and so on. A letter, p, c, or m, was used after the name of the locus to indicate whether the subcellular location of isozymes was plastid, cytosolic or mitochondrial, respectively.

Linkage analyses were performed on $F_2$ progenies. The LINKAGE-1 computer program of Suiter et al. (1983) was used for the segregation and linkage analyses.

## Results

### *Inheritance*

Segregation results confirmed the previously observed mode of inheritance for plant growth habit, *Hg*, (Argikar and D'Cruz 1963; Rao 1980), corolla color, *P*, (Bhapkar and Patil 1963), seed surface, *Rs*, (More and D'Cruz 1970), pod shape, *Rp*, (Athwal and Brar 1967), leaf morphology, *Glv* and *Nlv*, (Argikar 1952; Singh 1962), foliage color, *Blv*, and branching, *Br*, (Argikar and D'Cruz 1962, 1963). Although several genes influencing seed-coat color in chickpea have been reported (reviewed by Muehlbauer and Singh 1987), it seems likely that there is a redundancy of gene symbols used for this trait. Black seed-coat color ($T^4$) was dominant to both brown and yellow seed-coat color. Similarly, brown ($T^3$) appeared to be dominant to yellow ($t^3$), although distorted segregation was observed in two out of three families (Table 2).

A lethal chlorophyll mutation (chlorina) was detected in one of the $F_2$ families (C289-17) from a intraspecific hybrid between a kabuli (PI 518245), and a desi (PI 518257). Mutant seedlings had yellow foliage and died within a few weeks. Monogenic segregation with normal seedlings being dominant to chlorina was observed (Table 2). A base symbol of *chl* for chlorina is proposed with the gene to be designated as *chl-1*. The same symbol is also used in pea (Weeden and Wolko 1990) and lentil (Ladizinsky 1979) for similar phenotypes. Seed-shape variation was observed in families C288-119 and C289-247 and appeared to be determined by a single gene, with angular (*Shp*) being dominant to round (*shp*). Epicotyl color and pod indehiscence were also controlled by single genes, with purple being dominant to green and dehiscence being dominant to indehiscence. The same gene symbols for epicotyl color (*Gst/gst*) and pod indehiscence (*Pin/pin*) are proposed as have been designated in lentil for the same traits (Ladizinsky 1979; Zamir and Ladizinsky 1984). Some sterility was observed in the $F_2$ plants from two crosses between *C. arietinum* and *C. echinospermum* (families C289-239 and C289-257). The ratio of sterile to fertile plants fit the expected 3:1 ratio. A base symbol of *Str* is proposed for this trait, although the mechanism for sterility is not known.

Our results also confirmed the monogenic inheritance of the genes encoding ADH and PGD (Table 2) isozymes described previously (Tuwafe et al. 1988). However, the ADH1 and PGD1 isozymes of our study correspond to the ADH2 and PGD2 isozymes of Tuwafe et al. (1988) as they designated the slowest migrating isozyme as 1 and progressively faster migrating isozymes as 2, 3 and so on. However, we chose to conform to the system suggested by Weeden (1988) and used by Guar and Slinkard (1990).

A single AAP band was observed when leaf extracts were electrophoresed using a TRIS-citrate/lithium borate gel system. Crosses between the plants possessing alternate allozyme phenotypes gave hybrid bands showing both forms. A good fit to the expected 1:2:1 ratio was obtained in three $F_2$ families, indicating a single gene with two codominant alleles (Table 2).

Two aconitase isozymes, ACO1 and ACO2, were observed. The slower migrating ACO2 showed activity in the mitochondrial extracts, whereas ACO1 was associated with the cytosolic fraction. The $F_1$'s and heterozygous $F_2$ individuals possessed a double-banded isozyme phenotype, indicating the monomeric nature of the enzyme (Table 2). The offspring genotypes from all families fit the expected 1:2:1 ratio for *Aco-c*, but distorted segregation ($P < 0.05$) was observed for *Aco-m* in two $F_2$ families (C289-239 and C289-250) that originated from interspecific crosses. Segregation for *Aco-m* fit the expected 1:2:1 ratio in the $F_2$ family (C289-17) of an intraspecific cross in *C. arietinum* (Table 2).

**Table 2.** Single locus goodness-of-fit to either a 3:1 or a 1:2:1 ratio for genes used in the linkage analysis

| Family | Locus | Number observed with designated phenotype | | | | |
|---|---|---|---|---|---|---|
| | | Dominant or fast | Heterozygous | Recessive or slow | $\chi^2$ | $P$ |
| C288-119 | *Aap1* | 9 | 10 | 7 | 1.69 | 0.43 |
| | *Aco-c* | 23 | 49 | 27 | 0.33 | 0.85 |
| | *Aco-m* | 35 | 46 | 19 | 5.76 | 0.06 |
| | *Acp1* | 5 | 25 | 14 | 4.50 | 0.11 |
| | *Acp3* | 11 | 17 | 8 | 0.61 | 0.74 |
| | *Acp4* | 6 | 29 | 15 | 4.52 | 0.10 |
| | *Adk1* | 4 | 18 | 15 | 6.57* | 0.04 |
| | *Ald-p* | 9 | 17 | 24 | 14.12** | 0.00 |
| | *Gal1* | 30 | 51 | 19 | 2.46 | 0.29 |
| | *Est7* | 21 | 52 | 27 | 0.88 | 0.64 |
| | *Gpi-c* | 19 | 46 | 35 | 5.76 | 0.06 |
| | *Gpt1* | 2 | 20 | 7 | 5.90 | 0.05 |
| | *Pgd-c* | 31 | 54 | 15 | 5.76 | 0.06 |
| | *Pgm-c* | 18 | 57 | 25 | 2.94 | 0.23 |
| | *Spr1* | 3 | 15 | 15 | 9.00* | 0.01 |
| | *P/p* | 72 | – | 27 | 0.27 | 0.60 |
| | *Pin/pin* | 72 | – | 27 | 0.27 | 0.60 |
| | *Rs/rs* | 78 | – | 22 | 0.48 | 0.49 |
| | *Shp/shp* | 62 | – | 37 | 8.08* | 0.00 |
| | $T^3/t^3$ | 50 | – | 49 | 31.68** | 0.00 |
| C288-120 | *Aap1* | 7 | 21 | 3 | 4.94 | 0.08 |
| | *Aco-m* | 23 | 48 | 29 | 0.88 | 0.64 |
| | *Acp1* | 10 | 19 | 15 | 1.95 | 0.38 |
| | *Ald-p* | 11 | 24 | 10 | 0.24 | 0.88 |
| | *Gal1* | 25 | 50 | 23 | 0.12 | 0.94 |
| | *Gal2* | 14 | 55 | 20 | 5.76 | 0.06 |
| | *Est1* | 8 | 49 | 37 | 18.06** | 0.00 |
| | *Gpt1* | 5 | 34 | 4 | 14.58** | 0.00 |
| | *Gpi-c* | 22 | 45 | 33 | 3.42 | 0.18 |
| | *Pgm-c* | 17 | 52 | 31 | 4.08 | 0.13 |
| | *Pgd-c* | 28 | 57 | 12 | 8.26 | 0.02 |
| | *Spr1* | 10 | 23 | 15 | 1.13 | 0.57 |
| | *Gr/gr* | 71 | – | 21 | 0.23 | 0.63 |
| | *Pin/pin* | 72 | – | 21 | 0.29 | 0.59 |
| | *Rs/rs* | 67 | – | 25 | 0.23 | 0.63 |
| | $T^3/t^3$ | 51 | – | 41 | 18.78** | 0.00 |
| C289-15 | *Pgd-p* | 11 | 25 | 9 | 0.73 | 0.69 |
| | *Nlv/nlv* | 37 | – | 8 | 1.25 | 0.26 |
| | *P/p* | 37 | – | 8 | 1.25 | 0.26 |
| C289-17 | *Aco-m* | 10 | 27 | 10 | 1.04 | 0.59 |
| | *Chl1/chl1* | 36 | – | 11 | 0.06 | 0.80 |
| | *P/p* | 33 | – | 14 | 0.57 | 0.45 |
| | *Silv/silv* | 36 | – | 11 | 0.06 | 0.59 |
| C289-239 | *Aat-p* | 18 | 30 | 13 | 0.84 | 0.66 |
| | *Aco-m* | 7 | 33 | 21 | 6.84* | 0.03 |
| | *Amy1* | 19 | 28 | 14 | 1.23 | 0.54 |
| | *Gpt1* | 13 | 35 | 13 | 1.33 | 0.51 |
| | *Pgd-p* | 12 | 37 | 12 | 2.77 | 0.25 |
| | *Pgd-c* | 13 | 26 | 22 | 3.98 | 0.14 |
| | *Str/str* | 49 | – | 11 | 1.42 | 0.23 |
| | *Glv/glv* | 44 | – | 16 | 0.09 | 0.77 |
| | $T^4/t^4$ | 50 | – | 11 | 1.58 | 0.21 |

**Table 2** (continued)

| Family | Locus | Number observed with designated phenotype | | | | |
|---|---|---|---|---|---|---|
| | | Dominant or fast | Heterozygous | Recessive or slow | $\chi^2$ | $P$ |
| C289-247 | *Aap1* | 16 | 54 | 24 | 3.45 | 0.18 |
| | *Aco-c* | 24 | 40 | 24 | 0.73 | 0.70 |
| | *Aco-m* | 23 | 49 | 16 | 2.25 | 0.32 |
| | *Acp1* | 13 | 35 | 13 | 1.35 | 0.51 |
| | *Adk1* | 26 | 37 | 25 | 2.25 | 0.32 |
| | *Ald-p* | 21 | 39 | 24 | 0.64 | 0.75 |
| | *Fk3* | 26 | 37 | 25 | 2.25 | 0.32 |
| | *Gpt2* | 17 | 35 | 26 | 2.90 | 0.23 |
| | *Gpi-c* | 17 | 55 | 22 | 3.26 | 0.20 |
| | *Spr1* | 19 | 46 | 23 | 0.55 | 0.76 |
| | *Prx1* | 12 | 23 | 17 | 1.65 | 0.44 |
| | *Pgd-c* | 26 | 48 | 17 | 2.05 | 0.36 |
| | *Br/br* | 70 | – | 21 | 0.60 | 0.05 |
| | *Gst/gst* | 53 | – | 41 | 17.37** | 0.00 |
| | *Hg/hg* | 68 | – | 23 | 0.00 | 0.95 |
| | *Shp/sph* | 63 | – | 31 | 3.19 | 0.07 |
| | *P/p* | 63 | – | 31 | 3.19 | 0.07 |
| | *Rp/rp* | 61 | – | 32 | 4.39* | 0.04 |
| | *Rs/rs* | 64 | – | 30 | 2.40 | 0.12 |
| | $T^3/t^3$ | 67 | – | 27 | 0.70 | 0.40 |
| C289-250 | *Aco-m* | 17 | 40 | 6 | 8.43* | 0.01 |
| | *Adh1* | 13 | 43 | 7 | 9.54** | 0.01 |
| | *Adh2* | 42 | – | 21 | 2.33 | 0.13 |
| | *Ald-p* | 12 | 19 | 15 | 1.78 | 0.41 |
| | *Fk3* | 15 | 38 | 16 | 1.19 | 0.60 |
| | *Gpt1* | 17 | 34 | 12 | 1.19 | 0.55 |
| | *Gpt2* | 12 | 33 | 18 | 1.29 | 0.53 |
| | *Pgd-c* | 19 | 32 | 12 | 1.57 | 0.46 |
| | *Gpi-c* | 17 | 33 | 13 | 0.65 | 0.72 |
| | *Pgm-c* | 12 | 31 | 14 | 0.59 | 0.75 |
| | *Gr/gr* | 42 | – | 17 | 0.46 | 0.50 |
| | *Gst/gst* | 44 | – | 19 | 0.89 | 0.34 |
| | *Hg/hg* | 43 | – | 20 | 1.53 | 0.22 |
| | *P/p* | 47 | – | 16 | 0.01 | 0.94 |
| | *Ycot/ycot* | 38 | – | 26 | 2.25 | 0.36 |
| C289-257 | *Aat-p* | 14 | 24 | 15 | 0.51 | 0.78 |
| | *Gpt1* | 12 | 30 | 15 | 0.47 | 0.79 |
| | *Pgd-p* | 12 | 24 | 21 | 4.26 | 0.12 |
| | *Pgd-c* | 15 | 21 | 21 | 5.21 | 0.07 |
| | *Pgm-p* | 12 | 30 | 15 | 0.47 | 0.79 |
| | *Str/str* | 45 | – | 12 | 0.47 | 0.49 |

* $P<0.05$, ** $P<0.001$

Abbreviations for families: C288-119 (PI 360177 × PI 489777); C288-120 (PI 360348 × PI 489777); C289-15 (PI 518253 × PI 518260); C289-17 (PI 518249 × PI 518257); C289-239 (PI 374080 × 489776); C289-247 (PI 374080 × PI 489777); C289-250 (PI 360168 × PI 489777); C289-257 (PI 451432 × PI 489776)

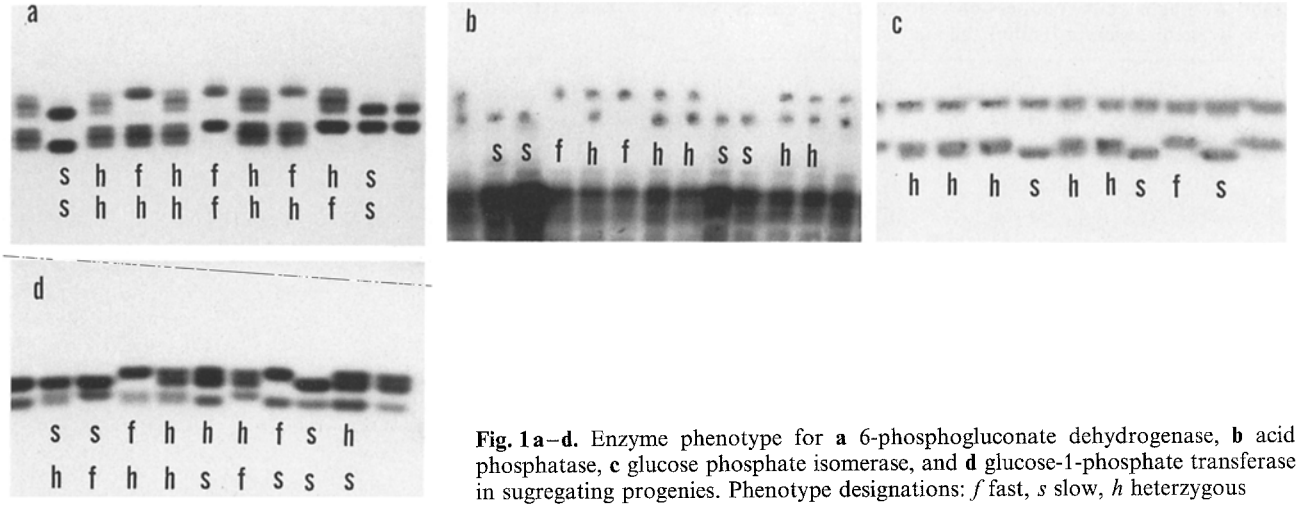


**Fig. 1 a–d.** Enzyme phenotype for **a** 6-phosphogluconate dehydrogenase, **b** acid phosphatase, **c** glucose phosphate isomerase, and **d** glucose-1-phosphate transferase in sugregating progenies. Phenotype designations: *f* fast, *s* slow, *h* heterzygous

Four zones of enzyme activity were observed for AAT in the leaf and root extracts of chickpea. Among these, only the plastid-specific isoenzyme (AAT-3) gave consistently darker staining bands. An overlap was observed between the slower migrating isozyme of AAT-3 and AAT-4, which was monomorphic in all of the families studied. Segregation for *Aat3*, designated as *Aat-p*, was observed in two $F_2$ families. This isozyme was dimeric, as suggested by the three-banded pattern observed in heterozygous individuals. Segregation of *Aat-p* fit the expected 1:2:1 ratio for a single gene.

Although several zones of anodal and cathodal activity for ACP were observed, only the most anodal ACP-1 (Fig. 1 b), the anodal-migrating ACP-3 and the cathodal ACP-4 isozymes gave scorable banding patterns. All 3 of these isozymes were monomeric and showed a good fit to the expected 1:2:1 segregation ratio for a single gene.

Three zones of activity were found for ADH isozymes in root and seed extracts. One of the parents (*C. reticulatum*) had a null allele for ADH-2 and exhibited only one band. Like in many other species, there were 2 loci (*Adh1* and *Adh2*) encoding ADH isozymes in chickpea. The middle zone observed in the homozygous plants was taken to be the intergenic band that resulted from the association of polypeptides produced by *Adh1* and *Adh2* loci. This conclusion was further supported by the fact that no intergenic band was formed in *Adh2* null activity variants. *Adh1* was functionally dimeric and showed distorted segregation. Only two phenotypic classes were observed for *Adh2*, and segregation at this locus fit the expected 3:1 (presence or absence of the activity) ratio.

Three ADK isozymes were observed in leaf extracts. Among these only the most anodal isozyme showed polymorphism. This enzyme was monomeric and showed distorted segregation ($P<0.05$) in one family (C288-119).

The aldolase gene duplication described by Kazan et al. (1991) was observed. The duplication was identified as a fixed heterozygote enzyme phenotype with a five-banded pattern. This five-banded pattern indicated the tetrameric nature of the enzyme. This phenotype did not segregate upon selfing, but segregation was obtained in crosses between two parents differing by 1 of the alleles at the duplicated locus. Other evidence of a gene duplication was that both isozymes were expressed in the same subcellular compartment (plastid), as is expected for a duplicated gene system, and that the asymmetrical banding intensities suggested gene dosage effects observed in heterozygous individuals. Segregation at the duplicated locus showed significant deviation from the expected 1:2:1 ratio ($P<0.01$) in one of the families studied.

One achromatic band for amylase was observed. The enzyme was monomeric, and segregation gave a good fit to the expected 1:2:1 segregation ratio for a single gene, designated *Amy1*.

Two $\beta$-galactosidase (pH 4.5) isozymes were observed. Of these, GAL-1 migrated anodally and GAL-2 migrated cathodally on the pH 6.1 gel. The loci encoding these isozymes (*Gal1* and *Gal2*) exhibited a good fit to the expected 1:2:1 ratio in two families (Table 2).

Several zones of activity for esterase isozymes were observed in leaf and seed extracts of chickpea. Only the most anodal (Zone EST-1) and one cathodal isozyme (Zone EST-7) in two families were studied. Both isozyme systems were monomeric. Segregation at the *Est7* locus gave a good fit to the expected ratio for a single locus, but that of *Est1* showed distorted segregation.

We observed three FK isozyme zones, and the least anodal isozyme segregated in two families. This isozyme zone was designated locus *Fk3*. Segregating progeny gave a good fit to the expected 1:2:1 ratio.

Two PGD isozymes were observed in leaf, seed, and root extracts of chickpea. Both isozymes were dimeric (Fig. 1 a). The product of locus *Pgd2* was active in the cytosolic compartment and was designated *Pgd-c*, while

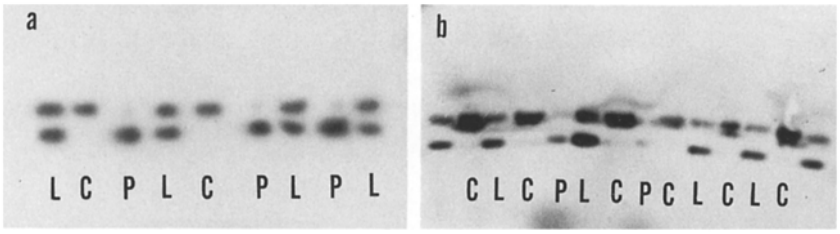


**Fig. 2 a, b.** Subcellular compartmentalization of 6-phosphogluconate dehydrogenase (**a**) and glucose phosphate isomerase (**b**) in chickpea. *P* Extracts from pollen, *C* extracts from chloroplasts, *L* extracts from leaf

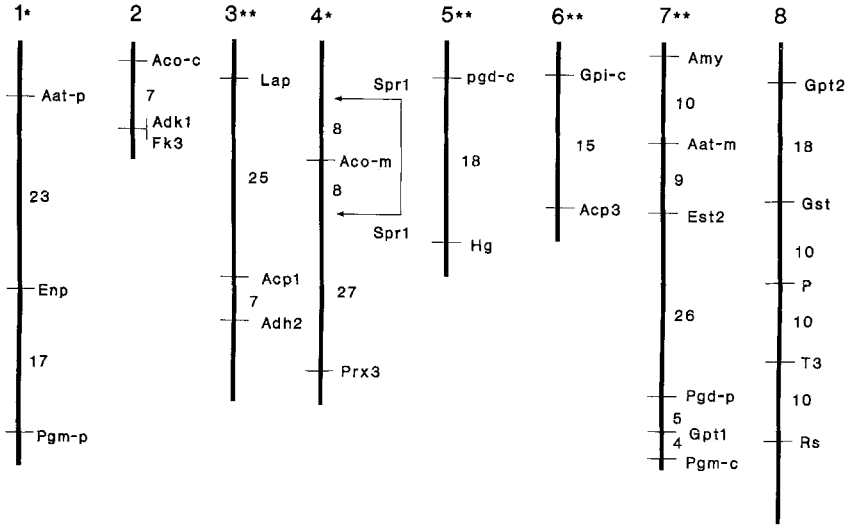


**Fig. 3.** A compiled genetic linkage map of chickpea (after Gaur and Slinkard 1988). ** Linkages conserved in pea, * linkages conserved in lentil

that of locus *Pgd1* was found in the plastids and designated *Pgd-p* (Fig. 2a). We observed allozyme polymorphism for both loci in the progeny of two families (C289-239 and C289-257) (Table 2), and both of these loci fit the expected 1:2:1 ratio.

Two loci were found for PGM: the one which encoded for the plastid-specific form was designated *Pgm-p* and the one which encoded for the cytosolic specific form was designated *Pgm-c*. Polymorphism for *Pgm-c* was found in three families, while polymorphism for *Pgm-p* was present in only one family (C289-257).

Two zones of activity were observed for GPI (Fig. 1c). The more anodal isozyme was monomorphic and was expressed in the plastid compartment (GPI-p); the other isozyme of GPI was dimeric and was expressed in the cytosolic compartment (Fig. 2b). The locus encoding this isozyme (*Gpi-c*) showed normal Mendelian segregation (Table 2).

Two monomeric isozyme zones were resolved for GPT (Fig. 1d), and polymorphisms were observed for both zones. The locus, *Gpt1*, which encoded the most anodally migrating isozymes, showed a distorted segregation pattern that did not fit the expected 1:2:1 ratio in one of the families studied. However, $F_2$ segregation ratios at the *Gpt2* locus showed no significant deviation from the expected 1:2:1 ratio for a single locus.

General protein was assayed in the seed extracts of chickpea. Although the type of proteins scored is not known, it can be assumed that these are the protein fractions that exist in large amounts in the seed extracts. We found variation only for the most anodal migrating zones. Segregation in this zone fit the expected monogenic segregation ratios for a single locus in two families, but showed distorted segregation in another family (Table 2).

Several zones of activity were observed for peroxidase isozymes in the root extracts of chickpea. We studied only the most anodal monomeric isozyme zone, designated PRX1. Segregation for isozymes in this zone fit the expected segregation ratios for a single locus, which was designated *Prx1* (Table 2).

No polymorphism was found for NAG or MPI in any of the families used in this study.

### *Linkage*

We observed several linkages both between isozyme loci and between morphological and isozyme loci. Segregation data from at least two $F_2$ families originating from the same crosses were pooled since it was difficult to obtain a large enough $F_2$ family from a single $F_1$ plant. Joint segregation for pairs of loci that deviated signifi-

**Table 3.** Joint segregation of pairs of loci that deviated significantly from random assortment

| Family | Loci | Number of $F_2$ plants with designated phenotype[a] | | | | | | | | | $\chi^2$ | $r$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | −/− | −/H | −/+ | H/− | H/H | H/+ | +/− | +/H | +/+ | | |
| C288-119 | *Adk1* : *Aco-c* | 13 | 1 | 1 | 1 | 14 | 3 | 0 | 0 | 4 | 41.1 | 0.10 |
| | *Spr1* : *Aco-m* | 0 | 2 | 13 | 1 | 13 | 1 | 3 | 0 | 0 | 44.4 | 0.06 |
| | *Acp3* : *Gpi-c* | 0 | 3 | 5 | 2 | 12 | 3 | 9 | 2 | 0 | 26.4 | 0.15 |
| | *Shp* : *P* | 20 | – | 17 | – | – | – | 7 | – | 55 | 21.4 | 0.24 |
| C288-120 | *Spr1* : *Aco-m* | 0 | 5 | 10 | 3 | 19 | 1 | 8 | 2 | 0 | 43.7 | 0.12 |
| C289-239 | *Gpt1* : *Pgd-p* | 11 | 2 | 0 | 1 | 33 | 1 | 0 | 2 | 11 | 86.2 | 0.05 |
| C289-247 | *Adk1* : *Aco-c* | 23 | 1 | 1 | 0 | 37 | 0 | 1 | 2 | 23 | 146.3 | 0.04 |
| | *Fk3* : *Aco-c* | 23 | 1 | 1 | 0 | 37 | 0 | 1 | 2 | 23 | 146.3 | 0.04 |
| | *Fk3* : *Adk1* | 25 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 23 | 151.9 | 0.00 |
| | *Spr1* : *Aco-m* | 1 | 2 | 20 | 1 | 44 | 1 | 14 | 3 | 2 | 113.3 | 0.08 |
| | *Pgd-c* : *Hg* | 4 | 2 | 17 | – | – | – | 13 | 46 | 9 | 33.5 | 0.22 |
| | *Gpt2* : *P* | 1 | 14 | 13 | – | – | – | 25 | 21 | 4 | 24.6 | 0.24 |
| | *P* : *Gst* | 31 | – | 10 | – | – | – | 0 | – | 53 | 59.8 | 0.09 |
| | $T^3$ : *P* | 24 | – | 3 | – | – | – | 7 | – | 60 | 53.6 | 0.10 |
| | $T^3$ : *Gst* | 24 | – | 3 | – | – | – | 17 | – | 50 | 31.6 | 0.20 |
| | $T^3$ : *Rs* | 20 | – | 2 | – | – | – | 13 | | 59 | 51.0 | 0.10 |
| | *Gpt2* : *P* | 0 | 8 | 8 | – | – | – | 18 | 25 | 4 | 16.9 | 0.21 |
| | *Gpt2* : *Gst* | 0 | 9 | 10 | – | – | – | 18 | 24 | 2 | 24.0 | 0.18 |
| | *P* : *Gst* | 14 | – | 5 | – | – | – | 2 | – | 42 | 33.48 | 0.11 |
| C289-257 | *Pgd-p* : *Gpt1* | 15 | 6 | 0 | 0 | 24 | 0 | 0 | 0 | 12 | 89.57 | 0.05 |
| | *Pgm-c* : *Gpt1* | 14 | 0 | 1 | 1 | 29 | 0 | 0 | 1 | 11 | 94.41 | 0.04 |
| | *Pgm-c* : *Pgd-p* | 14 | 0 | 1 | 6 | 24 | 0 | 1 | 0 | 11 | 75.76 | 0.09 |

[a] –, Recessive phenotypes or homozygous slow; H, heterozygotes; +, dominant or homozygous fast

cantly from random assortment and maximum likelihood estimates of recombination frequencies are presented in Table 3. A compiled genetic linkage map of chickpea is also given in Fig. 3.

A tight linkage was found between *Aco-c* and 2 loci, *Adk1* and *Fk3*, with approximately 7% recombination. No recombination was detected between *Adk1* and *Fk3*. The location of *Adk1* and *Fk3* in pea and lentil is not known. However, only 1 FK isozyme has been resolved in lentil (Muehlbauer et al. 1989), and the locus encoding this isozyme is polymorphic and located between the genes encoding the cytosolic form of phosphoglucomutase (*Pgm-c*) and the plastid form of 6-phosphogluconate dehydrogenase (*Pgd-p*). The *Fk3* locus we studied did not show linkage with either of these 2 loci, indicating that it is probably not homologous to the *Fk* locus previously mapped in lentil (Muehlbauer et al. 1989).

A distance of 8 map units was detected between *Aco-m* and *Spr1*. Previously, *Aco-m* was reported to be linked to the *Prx3* locus and was assigned to the linkage group 4 of chickpea (Gaur and Slinkard 1990). In this study, we were not able to test the three-point linkage among *Aco-m*, *Spr1*, and *Prx3*; therefore, the order of these 3 loci is not known.

Linkage was found between *Pgd-c* and *Hg*. The 2 loci were separated by a map distance of approximately 18 recombination units.

Linkage was detected among *Pgd-p*, *Gpt1*, and *Pgm-c*. The genes in this cluster were closely linked with *Pgd-p* and *Pgm-c*, estimated to be 9 map units apart, whereas *Gpt1* was in the middle and separated by 3–4 recombination units from each of the 2 other genes.

Also identified was a linkage group including a glucose-1-phosphate transferase locus (*Gpt2*) and 4 morphological markers, flower color (*P*), epicotyl color (*Gst*), seed coat color ($T^3$), and seed surface (*Rs*). The linkage among these morphological loci has also been reported by Pawar and Patil (1983).

Another linkage was detected between an acid phosphatase locus (*Acp3*) and the cytosolic form of the glucose phosphate isomerase (*Gpi-c*). These 2 loci are separated by a map distance of 15 recombination units.

## Discussion

There were distorted segregation ratios for some of the isozyme and morphological markers in chickpea. A misclassification of heterozygotes and small family size might account for some of these distortions. The distorted segregation of isozyme loci in interspecific crosses has also been reported in lentil (Muehlbauer et al. 1989; Zamir and Tadmor 1986), pea (Weeden and Marx 1984), and common bean (*Phaseolus*) (Koenig and Gepts 1989).

Zamir and Tadmor (1986) indicated that distorted segregation may be the result of linkages between markers and the genes operating in the pre- and postzygotic phases of reproduction. Although we did not examine the causes of distorted segregation, other possible explanations might include preferential chromosome elimination (Tanksley 1984), preferential fertilization, and the selective elimination of particular zygotes (Koenig and Gepts 1989).

Previous studies have shown that isozyme loci can be used to compare linkage maps of species in closely related genera. In order to do so, it is important that homologous loci be compared. Due to the high degree of conservation of metabolic pathways, two taxa derived from a common ancestor may possibly have homologous isozymes with the same substrate specificity, the same subcellular localization, and the same quaternary structure. The position of homologous loci relative to other known markers on linkage groups may also be conserved. Accordingly, one can expect a higher degree of conservation in the closely related taxonomic groups since their divergence from a common ancestor is relatively recent. For example, the conservation of similar linkage groups was observed in pea and lentil belonging to the same tribe (Vicieae) and in chickpea belonging to the closely related tribe (Cicereae). It is apparent from the map given in Fig. 3 that at least six linkage groups containing 17 loci are conserved in pea and lentil. The linkage groups of chickpea were numbered on the basis of similarities with the pea linkage map. In this map, linkage group 2 of Gaur and Slinkard (1990) was reassigned to linkage group 8, and 3 loci, *Aco-c – Adk1 – Fk3*, were tentatively assigned to linkage group 2.

The linkage between *Pgd-c* and *Hg* suggested the possibility of a conserved linkage group among the members of *Vicia*. We assigned these 2 loci tentatively onto linkage group 5 of chickpea. The gene "*creep*" for prostrate growth habit in pea was also linked to a *Pgd-c* locus (Weeden and Wolko 1990). These 2 genes are located at the distal end of chromosome 5 in pea and separated by approximately 10 map units. The *Nag* locus also appears to be located between these 2 loci in pea, whereas *Prx1* is more distantly associated with the *creep – Nag – Pgd-c – Mpi* linkage group (Weeden and Wolko 1990). In this study, the lack of polymorphism for *Nag* and *Mpi* loci prevented us from testing the linkage among the 4 loci, *Hg*, *Nag*, *Pgd-c*, and *Mpi*. However, we were able to test the linkage between *Prx1* and *Hg – Pgd-c*, but no linkage was found. Although linkage has been detected between *Nag* and *Prx1* in pea and lentil, the map distance between these 2 loci was not very tight ($r=0.20$) (Muehlbauer et al. 1989). Therefore, the existence of such a linkage in chickpea should not be excluded. A parallel linkage group for a growth habit gene and *Pgd-c* may also be present in lentil. A gene with incomplete dominance is responsible for plant growth habit in lentil (Ladizinsky 1979), and a variant for *Pgd-c* has also been recently identified and can be used for genetic analyses (Erskine and F. J. Muehlbauer unpublished).

There is evidence suggesting that the gene for early flowering (*Sn*) in pea may have a counterpart in chickpea. This gene, which is located at the distal end of chromosome 7, is closely linked to *Amy1* and *Gal2* in pea (Weeden and Wolko 1990). Although initial observations suggested a similar association between *Gal1* and a possible gene for early flowering in chickpea, the action of these genes has yet to be determined.

The linkage between *Pgd-p*, and *Pgm-c* in chickpea was also conserved in pea and lentil. Although this linkage was reported by Gaur and Slinkard (1990), the location of *Gpt1* between these 2 loci was first found in this study. Gaur and Slinkard (1990) detected linkage between the cluster involving *Amy*, *Aat-m*, and *Est2* and the cluster carrying *Pgd-p* and *Pgm-c* (Fig. 3). No linkage has been reported between *Gpt1* and *Pgd-p – Pgm-c* in other related genera. This might be due to a lack of polymorphism for *Gpt1*. However, a conserved linkage cluster containing *Pgm-c – Fk – Pep – Pgd-p* has been found in pea and lentil. In this study a similar linkage cluster including all 3 loci was not confirmed since polymorphism was not found for the peptidase (*Pep*) locus. In addition, the polymorphism found for fructose kinase (*Fk3*) showed an association with *Aco-c*.

Linkage between *Pgd-c* and *Acp1* was not detected in chickpea, whereas a linkage has been found in pea between these 2 loci (Weeden and Marx 1984). The *Acp* locus we studied may not be the homologous locus of *Acp1* in pea since several ACP isozymes exist in both genera. However, we observed a linkage between *Gpi-c* and *Acp3* that is possibly conserved in pea. Although no recombination was detected between *Gpi-c* and *Acp4* in pea, these 2 loci were separated by a map distance of 15 recombination units in chickpea. We tentatively assigned these 2 loci to linkage group 6 of chickpea as they are found on the same linkage group of pea.

Although several loci for morphological traits were assigned separately to linkage group 8 of chickpea, it seems likely that there is 1 locus for anthocyanin production, which could be either *P*, *Gst*, or $T^3$. This locus, as with the *A* gene in pea (Weeden and Wolko 1990), is likely to be associated with anthocyanin production in the corolla, epicotyl, and seed coat. The remaining 2 genes in this linkage group may modify the expression of this major gene for pigmentation.

The linkages of loci that are not conserved in all three genera might be due to the fact that chromosomal repatterning may have changed the location of the genes after their divergence from a common ancestor. Chromosomal translocations observed in pea (Lamm 1951), lentil (Tadmor et al. 1987), and chickpea (Ladizinsky and Adler 1976) and differences in chromosome number ($n=7$ for

pea and lentil and n = 8 for chickpea) indicate the role of chromosomal arrangements in the evolution of these genera.

Additional morphological loci such as mutant genotypes affecting leaf morphology, the number of flowers per peduncle, seed protein content, nodulation, and loci conferring disease resistance are available in chickpea and should be used in linkage studies. Additional isozyme polymorphisms can also be found by screening a large number of cultivated lines and wild *Cicer* species. The location of disease resistance genes in pea should be more carefully examined to detect common linkages in chickpea. Kusmenoglu et al. (1989) reported two major genes for resistance to ascochyta blight (*Ascochyta rabiei*) in chickpea, although linkage relationships between the resistance genes and other available genetic markers are not known. A gene for resistance to *Ascochyta pisi* has been located on the linkage group 1 of pea (Darby et al. 1985). Linkage group 1 of chickpea also shares homologous markers (*Aat-p*, *Est1*) with the first linkage group of pea. This indicates the possibility that a locus for ascochyta blight resistance might be located on this linkage group in chickpea. A tight linkage between ascochyta blight resistance and a genetic marker would be very useful in selecting for disease resistance in plant breeding programs. Linkage group 1 of pea also carries a cluster of genes (*Sym-2*, *Sym-5*) for nodulation (Weeden and Wolko 1990). Genes affecting ineffective nodulation (Davis 1988) and nodule formation (Davis et al. 1986) might also be located on the same linkage group in chickpea.

The linkage map of chickpea proposed in this paper contains 23 molecular and 5 morphological loci. The arrangement proposed was based on linkages observed between the loci studied and on the possibilities of conserved linkages between *Cicer* and closely related genera, *Lens* and *Pisum*. Extension of the linkage map through the addition of RFLP and RAPD loci is currently underway and should greatly extend the amount of the genome covered by marker loci. The usefulness of the mapped marker loci should be realized when loci affecting resistance to ascochyta blight (incited by *Ascochyta rabiei* Pass. Lab.) and certain viruses and other economically important genes are added to the map. The use of closely linked markers should facilitate breeding by giving important genes a unique identity and by providing a means of selection in the absence of nurseries and screening procedures that can be costly and time consuming.

*Acknowledgements*. The authors wish to acknowledge Ms. D. Foote for providing $F_2$ seeds of two populations from crosses of *C. arietinum* with *C. reticulatum*, and Mrs. D. Tinnemore for laboratory assistance. This work is supported in part by US-992-85-C from BARD, The United States- Israel Binational Agricultural Research and Development Fund.

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