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Linkage relationships of 19 protein coding genes in watermelon

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Summary. Segregation of seed proteins and isozymes was analysed in two *Citrullus* crosses. In the first cross an F1 hybrid between *C. lanatus* and the wild species *C. colocynthis* was used as a female parent in a backcross to *C. lanatus*. In this interspecific cross the segregation of 17 markers was analysed. Four linkage groups were identified: linkage group 1 includes the genes *Est-2, Skdh-2, Tpi-1, Fdp-1, Sod-1* and *Prx-1*; linkage group 2 – *Got-1, Got-2* and *Sp-4*; linkage group 3 – *Pgm-1* and *Gdh-2*; linkage group 4 with *Pgi-1* and *Pgi-2*. In the second cross an F1 hybrid between two *C. colocynthis* accessions was backcrossed to one of its parents. Seven loci were scored and no new linkages were found.

Key words: *Citrullus* – Isozymes – Seed proteins – Linkage

Introduction

The genus *Citrullus* of the Cucurbitaceae family includes three diploid species (2n=22) originating in southern Africa (Jeffrey 1975). The cultivated watermelon *C. lanatus* is of a world-wide distribution; the wild species *C. colocynthis* is found mainly in northern Africa and Asia (Zamir et al. 1984) while *C. ecirrhosus* is restricted to the Namibian desert (Meeuse 1962). Twenty-five genes have been described in watermelon, most of them affecting the morphology and color of fruit and seed (Robinson et al. 1976). In comparison with the genetic information about other economically important crops the watermelon has been somewhat neglected. We describe here segregation patterns and linkage relationships of two seed protein and seventeen isozymic genes in *Citrullus*.

Materials and methods

Plant material

The following *Citrullus* accessions were used for the crosses: *C. colocynthis*-10 from the coastal plain of Israel, *C. colocynthis*-2 from the Sinai desert, and a local cultivar, *C. lanatus*-60 'Mallali' (Zamir et al. 1984). Two backcrosses were analysed: in the first, the interspecific F1 hybrid between *C. lanatus*-60 and *C. colocynthis*-10 was used as the female parent in a cross with *C. lanatus*-60 as the pollen parent. This cross will be referred to as cross I. In the second cross (cross II) the intraspecific F1 hybrid between *C. colocynthis*-10 and *C. colocynthis*-2 was used as the pollen donor in a cross with *C. colocynthis*-2.

Each seed from the backcross generations was cut in half; the cotyledon section was used for extraction of total seed proteins and the radicle section for sowing. Halved seeds were dipped in an antifungal powder mixture (Captan + PCNB 1:1) and germinated in the dark in vermiculite-covered trays at a temperature of 28–32 °C. After 24 h the germinating seeds were treated with the same antifungal mixture and transferred to seedling trays filled with a 1:1 mixture of peat and vermiculite. Seedlings were grown in the greenhouse and fertilised weekly with half-strength Hoagland's solution.

Electrophoresis

SDS polyacrylamide gel electrophoresis (PAGE) for the analysis of seed proteins was carried out using 18% bis-acrylamide gels. Extraction of proteins, preparation of gels, running conditions, staining and destaining were according to Galili and Feldman (1983).

Isozymes were subjected to starch gel electrophoresis using crude extracts obtained by macerating 1 cm^2 leaf discs on ice in one of the following three buffers: buffer A and buffer B, both prepared according to Zamir and Ladizinsky (1984), and buffer C containing 0.2 M KH_2PO_4 (pH 7.5), 0.1 M 2-mercaptoethanol and 12% PVP-40.

Three different gel and electrode buffer systems were used to resolve the enzyme bands. System 1 was a Tris citrate/lithium borate mixture (Gottlieb 1981) which was used to assay the following enzymes extracted with buffer A: glutamate oxaloacetate transaminase (GOT), superoxide dismutase

(SOD), esterase (EST) and glutamate dehydrogenase (GDH). System 2 was a Tris citrate/boric acid mixture (Tanksley 1979) which was used to assay the following enzymes extracted with buffer B: triose phosphate isomerase (TPI), fructose 1,6 diphosphatase (FDP), acid phosphatase (APS) and peroxidase (PRX). System 3 was a histidine/Tris citrate mixture (Zamir and Ladizinsky 1984). Using this system the following enzymes extracted with buffer C were assayed: phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphoglucanate dehydrogenase (6PGD), malic enzyme (ME), shikimic acid dehydrogenase (SKDH) and leucine aminopeptidase (LAP). Staining for enzyme activity was done according to Vallejos (1983), except for EST and FDP which were stained according to Shaw and Prassad (1970).

The subcellular location of different isozymes was determined using the pollen soaking method (Weeden and Gottlieb 1980). This procedure proved effective for isolating the cytosolic forms of the following enzymes: PGI, PGM, GDH and 6PGD.

Statistical analysis

Recombination frequencies between loci were estimated using maximum likelihood equations given by Allard (1956). Chi-square values for 1:1 ratios were calculated using Yates' correction.

Results and discussion

Single locus segregation in cross I

Seed proteins. Six major seed protein bands were stain in SDS PAGE. The segregation patterns of two seed protein bands, tentatively designated *Sp-4* and *Sp-5*, were scored in cross I. Their molecular masses were in the range of 26,000 dalton and 23,000 dalton respectively (Fig. 1). Monogenic segregations for these two loci did not deviate from the expected 1:1 ratio (Table 1).

Isozymes

Monogenic segregations of fifteen isozymes were monitored in this cross (Table 1).

Glutamate oxaloacetate transaminase. Four loci were identified by their activity. *Got-1*, *Got-2* and *Got-4* segregated in this cross, but GOT-4 stained poorly most of the time and was therefore not scored. The more

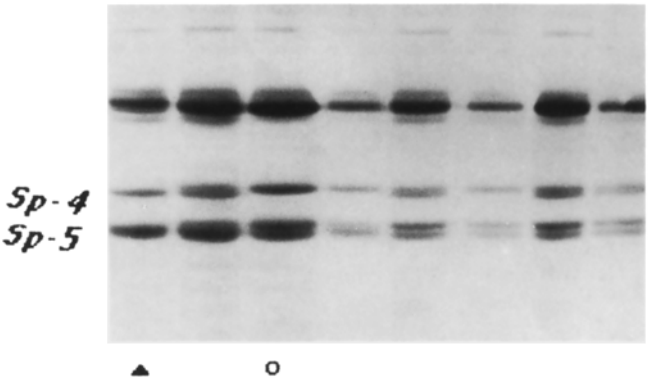

Fig. 1. Polyacrylamide gel electrophoresis pattern of seed proteins of *C. colocynthis* (▲), the F1 hybrid (between the parents), *C. lanatus* (○) and four BC1 progeny showing allelic variation in *Sp-4* and *Sp-5*

Table 1. Chi-square tests of the 1:1 segregation ratio for the seed proteins and isozyme loci in two *Citrullus* backcrosses. None of the chi values is significant at the 0.05 level

Locus	Cross I[a]			Locus	Cross II[b]		
	Homozygous	Heterozygous	χ^2 (1:1)		Homozygous	Heterozygous	χ^2 (1:1)
Got-1	94	82	0.7	*Est-1*	50	47	0.0
Got-2	138	138	0.0	*Est-2*	35	36	0.0
Sod-1	143	140	0.0	*Pgm-1*	62	55	0.3
Est-1	144	139	0.1	*Skdh-2*	52	48	0.1
Est-2	132	153	1.4	*Gdh-2*	43	45	0.0
Gdh-2	136	140	0.0	*Me-1*	55	62	0.3
Tpi-1	136	151	0.7	*6Pgd-1*	63	54	0.5
Fdp-1	135	150	0.7				
Aps-2	153	134	1.1				
Prx-1	152	134	1.0				
Pgi-1	138	142	0.0				
Pgi-2	148	137	0.4				
Pgm-1	127	152	2.1				
Skdh-2	128	128	0.0				
Lap-1	160	127	3.6				
Sp-4	144	120	2.0				
Sp-5	129	135	0.1				

[a] Cross I – (*C. lanatus*-60 × *C. colocynthis*-10) × *C. lanatus*-60
[b] Cross II – *C. colocynthis*-2 × (*C. colocynthis*-10 × *C. colocynthis*-2)

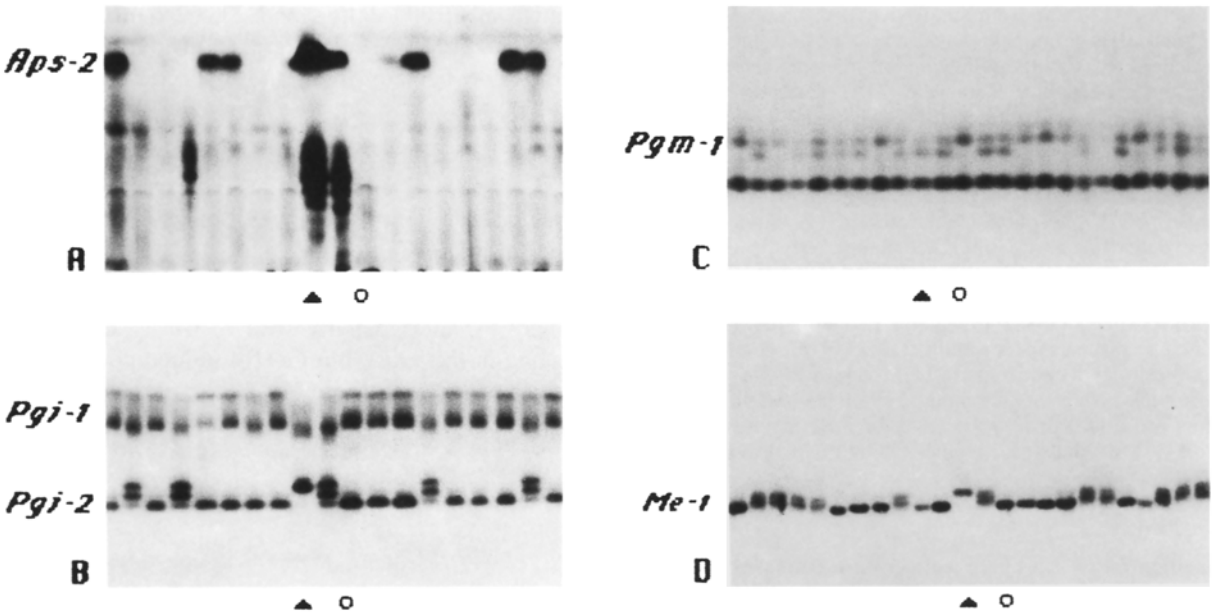

Fig. 2 A–D. Starch gels showing the segregation patterns in parental, F1 and BC1 progeny in the loci *Aps-2* (**A**), *Pgi-1* and *Pgi-2* (**B**), *Pgm-1* (**C**) and *Me-1* (**D**). Banding patterns of *C. colocynthis*-10 (▲), the F1 hybrid (between the parents), and the recurent parent (○ *C. lanatus*-60 in gels A–C and *C. colocynthis*-2 in gel D) is indicated at the bottom of each zymogram. The anode is towards the top of each zymogram

rapidly migrating alleles of both GOT-1 and GOT-2 belong to *C. colocynthis*. Our system of nomenclature designates isozymes according to their relative migration rates, the most anodal isozyme being GOT-1 and its coding gene *Got-1* (Zamir et al. 1984).

Superoxide dismutase. Three zones of activity were detected. *Sod-1* was the only segregating locus. SOD could be resolved in system 2 as well as in system 1.

Esterase. Two major loci were detected, their products staining as two cosegregating bands (*Est-1*) in the anodal gel, and as one band (*Est-2*) in the cathodal gel.

Glutamate dehydrogenase. The cytosolic GDH-1 is monomorphic while the organelle-bound GDH-2 has two alleles, the more anodal allele belonging to *C. lanatus*.

Triose phosphate isomerase. Two bands stained by TPI activity. *Tpi-1* segregated and was scored in this cross. The more anodal allozyme belongs to *C. colocynthis*.

Fructose 1,6 Diphosphatase. One segregating band, designated *Fdp-1*, was scored here. A second more cathodic band stained inconsistently and was not scored.

Acid phosphatase. Several zones of APS activity were detected; the only variable locus was *Aps-2* which was expressed by *C. colocynthis* while *C. lanatus* had a null allele (Fig. 2).

Peroxidase. Three major anodic bands were detected in the parents and their segregation scored in this cross. They cosegregated and were therefore scored as a single locus, with the more anodal pattern being coded by the wild parent.

Phosphoglucoisomerase. Two segregating loci code for this dimeric enzyme: *Pgi-1* codes for the chloroplastic form and *Pgi-2* for the cytosolic form (Fig. 2). It was easier to differentiate between a homozygous and a heterozygous condition at the *Pgi-1* locus when gels were prepared with 13 percent starch instead of the usual 12.5%.

Phosphoglucomutase. Two anodal bands stained: *Pgm-1*, which is the plastid isozyme, segregated and was scored. *Pgm-2*, a more active locus whose product is present in the cytosol, was monomorphic (Fig. 2).

Shikimic acid dehydrogenase. Two loci code for this monomeric enzyme: *Skdh-1* was monomorphic, *Skdh-2* segregated in this cross.

Leucine aminopeptidase. One locus codes for this monomeric enzyme.

Single locus segregations for the enzymatic loci in cross I did not deviate from the Mendelian 1 : 1 ratio.

Single locus segregations in cross II

Segregations of the following seven loci were scored in this cross: *Pgm-1, Skdh-2, Est-1, Est-2, Gdh-2, 6Pgd-2*

Table 2. Two-locus segregations indicating linkage in cross I

Tested loci		A/A[a]	A/B	B/A	B/B	χ^2	Distance cM
1st locus	2nd locus						
Linkage group 1							
Est-2	*Skdh-2*	117	9	9	119	187.1**	7.1±1.6
Skdh-2	*Tpi-1*	78	50	45	83	17.4**	37.1±3.0
Est-2	*Tpi-1*	82	50	53	100	24.2**	36.1±2.9
Tpi-1	*Fdp-1*	88	48	47	102	33.1**	33.3±2.8
Fdp-1	*Sod-1*	98	36	43	104	54.4**	28.1±2.6
Sod-1	*Prx-1*	119	23	31	109	108.5**	19.2±2.0
Fdp-1	*Prx-1*	84	51	67	83	10.2*	41.4±2.9
Linkage group 2							
Got-1	*Got-2*	64	27	23	57	30.2**	29.2±3.5
Got-2	*Sp-4*	106	11	14	122	165.0**	9.9±1.9
Got-1	*Sp-4*	59	28	32	43	14.3**	37.0±3.8
Linkage group 3							
Pgm-1	*Gdh-2*	74	49	55	90	15.6**	38.8±3.0
Linkage group 4							
Pgi-1	*Pgi-2*	108	30	38	104	74.6**	24.3±2.6

[a] A/A – a homozygous condition in the first and second loci; A/B – a homozygous condition in the first locus and a heterozygous condition in the second locus, etc
*,** Significant chi value at 0.05 and 0.01 levels, respectively

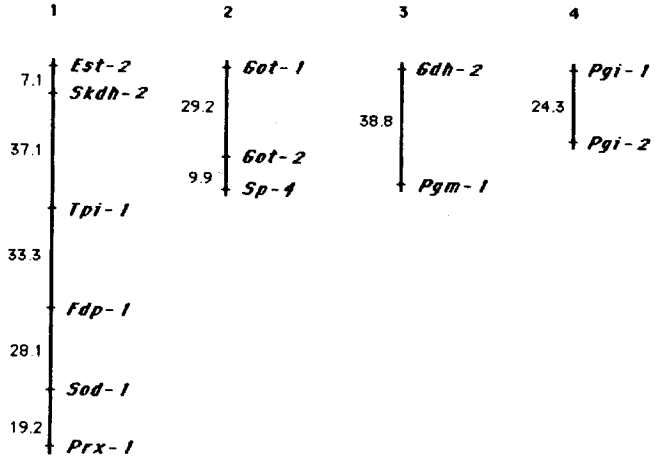

Fig. 3. A schematic linkage map of *Citrullus*. The following loci were independent of the linkage groups: *Aps-2, Lap-1, Est-1, Sp-5, Me-1, 6Pgd-1*

and *Me-1*. The last two were not assayed in cross I (Table 1).

6-Phosphoglucanate dehydrogenase. Two zones of activity stained for 6PGD. The plastid isozyme 6PGD-1 was monomorphic while the cytosolic isozyme 6PGD-2 segregated in the cross.

Malic enzyme. ME activity is coded for by a single locus (Fig. 2).

No deviation from the 1 : 1 ratio was found for any of the loci in cross II.

Linkage analysis

Based on the analysis of the two-locus segregations, four major linkage groups were identified (Table 2); linkage group 1 (six loci) – *Est-2, Skdh-2, Tpi-1, Fdp-1, Sod-1* and *Prx-1*; linkage group 2 (three loci) – *Got-1, Got-2* and *Sp-4;* linkage group 3 (two loci) – *Pgm-1* and *Gdh-2*; linkage group 4 (two loci) – *Pgi-1* and *Pgi-2.*

All the relevant data for determining the linkages between these 13 loci were derived from cross I. In cross II the only linkage found was between *Est-2* and *Skdh-2,* with a distance of 7.0 cM± 3.0 between them, confirming the distance found in cross I. The following loci did not map to any of the linkage groups: *Aps-2, Lap-1, Est-1, Sp-5, Me-1* and *6Pgd-1.* It is possible that *Me-1* and *6Pgd-1* are linked with one of the loci which were assayed in cross I, but not in cross II. A schematic linkage map showing the linked loci placed in their relevant positions is presented in Fig. 3.

Genetic mapping of electrophoretically detectable marker genes has been conducted in a variety of plant species (Tanksley and Orton 1983). The resulting information has proved useful for characterising phylogenetic relationships between species and for tagging genes of economical importance. The probability of discovering such linkages is increased as more of the genome is covered by segregating markers (Soller and Brody 1976). In this study we have described, nineteen segregating electrophoretic markers and their linkage relationships in watermelon.

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