

Inheritance and linkage of isozymes in grasspea.



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Introduction

Although isozyme markers may not be abundant in a species, their codominant inheritance has made them useful in certain situations. In linkage mapping experiments, codominant markers are often preferred over dominant markers as they carry more information on the recombination fraction than dominant markers in the F₂ segregating population⁽³⁾. An inheritance study will provide allelic interpretation of phenotypic variations of isozymes in grasspea and express the phenotypes in terms of genotypes. Knowledge of inheritance will provide more reliable genetic interpretation of the zymogram in this crop. Linkage analysis will allow identification of isozymes that belong to the same linkage group. In the present study we report the inheritance and linkage of 11 isozyme loci in grasspea.

Materials and methods

Four crosses have been made among eight subaccessions (PI 283564c.3 x PI 426885.2, PI 358601.5 x PI 173714.5, PI 426891.1 x PI 172930.4 and PI 283549a.6 x PI 202803a.3). Isozyme data

from 180 F₂ plants in each cross were analysed for the inheritance and linkage study. Starch gel electrophoresis was performed on crude protein extract of freshly germinated cotyledons. Inheritance was determined for each isozyme marker by the Chi-squared goodness-of-fit test⁽³⁾. MAPMAKER⁽²⁾, a linkage software, was used to detect linkage and construct the linkage map. The linkage group was detected on the basis of two point linkage test, using the 'group' command. The criteria for declaring a linkage were LOD (logarithm of odds) = 4 and recombination fraction = 0.30 (≈50 cM map distance).

Results

Polymorphism and Inheritance. Seventeen isozymes have been resolved in eight enzyme systems (Table 1). Of these 17 isozymes, 11 showed polymorphism in at least one F₂ population allowing the determination of inheritance and linkage for these isozymes. The enzyme system EST revealed four zones of enzyme activity representing four different loci (Table 1). Enzyme systems AAT, PGD, TPI, ACO and LAP revealed two zones of enzyme activity representing two different loci. Enzyme systems FDH and SKDH revealed one zone of activity representing one locus. Isozymes AAT-2 and AAT-3 revealed overlapping zones of enzyme activity. Except for EST-3 all isozymes in the present study fitted well to 1:2:1 F₂ ratio indicating a co-dominant inheritance for these isozymes (Table 2). EST-3 showed dominant inheritance due to the presence of null allele and gave a good fit to 3:1 F₂ ratio.

Linkage. Linkage was analyzed for each individual F₂ population. Only two of the eleven isozymes (AAT-2 and SKDH) showed linkage. AAT-2 and SKDH were linked in the crosses PI 283564c.3 x PI 426885.2 and PI 358601.5 x PI 173714.5. Other markers assorted independently in the respective crosses. AAT-2 and SKDH were linked with a map distance of 24 cM and 31 cM in the crosses PI 283564c.3 x PI 426885.2 and PI 358601.5 x PI 173714.5, respectively (Table 3). The χ^2 test of homogeneity was performed before pooling the data over two crosses (Table 3). A nonsignificant χ^2 value indicated homogeneity of the co-segregating classes over different crosses. The data were pooled over two crosses and a map distance of 28 cM between SKDH and AAT-2 was obtained from the pooled data (Table 3).

Table 1: Characteristics of isozymes segregating in F₂ population(s) of grasspea.

Enzyme system	Zone of activity	No. of bands within a zone of activity in heterozygotes	Quaternary structure	Mode of inheritance
ACO	ACO-1	2	monomeric	co-dominant
	ACO-2	2	monomeric	co-dominant
AAT	AAT-1	3	dimeric	co-dominant
	AAT-2	3	dimeric	co-dominant
	AAT-3	<i>a</i>	<i>a</i>	<i>a</i>
EST	EST-1	<i>a</i>	<i>a</i>	<i>a</i>
	EST-2	<i>a</i>	<i>a</i>	<i>a</i>
	EST-3	1	not known	dominant
	EST-6	2	monomeric	co-dominant
FDH	FDH	3	dimeric	co-dominant
LAP	LAP-1	2	monomeric	co-dominant
	LAP-2	<i>a</i>	<i>a</i>	<i>a</i>
PGD	PGD-1	<i>a</i>	<i>a</i>	<i>a</i>
	PGD-2	3	dimeric	co-dominant
SKDH	SKDH	2	monomeric	co-dominant
TPI	TPI-1	3	dimeric	co-dominant
	TPI-2	<i>a</i>	<i>a</i>	<i>a</i>

a - isozymes did not segregate due to the absence of polymorphism between the parents.

Table 2: Chi-squared (χ^2) test of goodness-of-fit to single locus ratio for 11 isozyme loci in four F₂ populations of grasspea.

F ₂ population	Locus	Fm ^a	N ^b	Observed ratio F:H:S or A-aa	Expected ratio	χ^2
PI 283564c.3 x PI 426885.2	<i>Aat-2</i>	F	180	48:82:50	1:2:1	1.5
	<i>Est-3</i>	AA	180	45:135	1:3	0.3
	<i>Lap-1</i>	S	180	40:90:50	1:2:1	1.1
	<i>Skdh</i>	S	180	52:92:36	1:2:1	2.9
PI 358601.5 x PI 173714.5	<i>Aat-1</i>	F	180	40:98:42	1:2:1	1.5
	<i>Aat-2</i>	F	180	49:100:31	1:2:1	5.7
	<i>Aco-1</i>	F	180	44:96:40	1:2:1	1.0
	<i>Aco-2</i>	S	180	42:95:43	1:2:1	0.6
PI 426891.1 x PI 172930.4	<i>Skdh</i>	S	180	33:102:45	1:2:1	4.8
	<i>Aat-1</i>	S	180	52:88:40	1:2:1	1.7
	<i>Aat-2</i>	S	180	46:92:42	1:2:1	0.3
	<i>Aco-2</i>	S	180	44:39:180	1:2:1	1.4
	<i>Est-3</i>	AA	180	34:146	1:3	3.6
	<i>Fdh</i>	F	180	40:102:38	1:2:1	3.2
PI 283549a.6 x PI 202803a.3	<i>Pgd-2</i>	S	180	41:106:33	1:2:1	6.4*
	<i>Est-6</i>	F	180	52:82:46	1:2:1	1.8
	<i>Lap-1</i>	AA	180	42:92:46	1:2:1	0.3
	<i>Pgd-2</i>	S	180	38:92:50	1:2:1	1.7
	<i>Tpi-1</i>	S	180	38:86:56	1:2:1	4.0

^aFm = Phenotypes of the female parent, F = homozygotes for fast allele, H = heterozygotes, S = homozygotes for slow allele, AA = dominant homozygotes, A- = either dominant homozygotes or heterozygotes showing bands as opposed to a null phenotype, aa = recessive homozygotes showing null phenotype.

^bN = population size. *Significant at P = 0.05 level

Table 3: Joint segregation of *Aat-2* and *Skdh* loci in two F₂ populations of grasspea.

F ₂ population	Genotype ^a									Map distance (cM)
	FF	FH	FS	HF	HH	HS	SF	SH	SS	
PI 283564c.3 x PI 426885.2	3	20	25	12	60	10	37	12	1	24.0
PI 358601.5 x PI 173714.5	4	19	26	12	72	16	17	11	3	31.0
Pooled data	7	39	51	24	132	26	54	23	4	28.0

^aF = fast homozygotes, H=heterozygotes, S=slow homozygotes.

Discussion

The quaternary structure of the polymorphic isozymes observed in the present study fully agreed with the quaternary structure of the respective isozymes in other plant species^(6,7). As for example, in the present study ACO-1 and ACO-2 both were functional in monomeric quaternary structure and AAT-1 and AAT-2 were functional in dimeric quaternary structure. The numbers of bands observed in heterozygotes suggest the quaternary structure of the isozymes. Due to the absence of multiple bands in heterozygotes the quaternary structure of EST-3 could not be determined. Two bands occur in the heterozygotes for monomeric isozymes, three bands for dimeric isozymes, four bands for trimeric isozymes and so on. For simpler isozyme pattern the zones of isozyme activity are well separated representing different loci. Overlapping of zones of isozyme activity may complicate the interpretation of zymogram. In the present study, AAT-2 and AAT-3 are typical examples where overlapping of the zones of enzyme activity occurred. The activity zone of AAT-1 was well separated from the activity zones of AAT-2 and AAT-3, allowing putative allelic interpretation of AAT-1. The complicated banding pattern of AAT-2 and AAT-3 requires an inheritance study on these two isozymes for their allelic interpretations.

Linkage was detected between two loci (*Aat-2* and *Skdh*) in the present study. These two loci are also linked in other genera of the tribe Viceae. In lentil, linkage between SKDH and AAT-mb was reported⁽⁴⁾. Linkage between AAT-m and SKDH has been reported in lentil⁽⁴⁾ and also in pea. Information about the subcellular localization of AAT in grasspea (data not available) may provide a further clue to its possible homology. The failure to establish more linkages may be due to the limited number of isozymes segregating in each cross. No more than six isozymes were segregating in a single cross in the present study. To establish more linkages, genetically more diverse parents should be selected in a crossing program to allow segregation for a larger number of polymorphic isozyme loci in a single cross, or alternatively, many crosses have to be made, using different parental combinations. Molecular markers could also be included along with isozymes to establish more

linkages among the isozymes. Flower colour has been used to determine outcrossing rate in natural population of grasspea^(1,5). However, dominant inheritance and pollinators' preferences pose limitations to the use of flower colour for an unbiased estimation of outcrossing rate in grasspea. In such cases isozyme markers will provide a better estimate, as isozyme markers are selectively neutral, and are mostly codominant. Moreover, isozymes can be assayed from the cotyledons, allowing early detection of the outcrossed individuals. As the genetic basis of isozymes are now known in grasspea, they can be used for assessing outcrossing rate, surveying genetic polymorphism, measuring genetic diversity or for further linkage studies in grasspea.

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