Inheritance and Linkage of Isozyme and Morphological Markers in Fenugreek (*Trigonella foenum-graecum*)

H. Nair and A.E. Slinkard, University of Saskatchewan.

ABSTRACT

Fenugreek,a potential new crop for western Canada, has not been studied genetically. Inheritance and linkage studies are required to facilitate subsequent breeding research in fenugreek. Forty-eight fenugreek lines were screened for isozyme polymorphisms in 20 enzyme systems using starch gel electrophoresis. Isozyme polymorphisms were found in seven enzyme systems. Morphological markers (cotyledon colour, pubescence, zero tannin seed coat and single vs. double pods per leaf axil) were also studied. Crosses were made between selected parental lines to obtain F_2 populations segregating for isozyme and morphological markers. Seven such F_2 populations were analysed for genetic linkages. Close genetic linkage was observed between the gene locus controlling cotyledon colour, *Yc*, and the loci coding for two isozymes of esterase, *Est-2* and *Est-3*. The data also suggest weak linkage between the locus coding for triose phosphate isomerase, *Tpi-1*, and both *Yc* and *Est-3*.

Key words: Fenugreek, genetics, inheritance, isozymes, linkage, markers, *Trigonella foenum-graecum*

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is a self pollinating, annual, diploid (2n = 16), leguminous crop widely cultivated in India, the Mediterranean countries, the Middle East, and parts of Africa. It is also cultivated in parts of California on a limited scale. Fenugreek is well suited to regions with cool temperate growing conditions without extremes of temperature and low to moderate rainfall. However, it can tolerate -10 to -15°C (Duke *et al.* 1981) and can survive low moisture conditions well. It is most suited to well-drained, deep loamy soil and is fairly tolerant of salt. Fenugreek pods do not shatter easily and it can be direct combined at maturity. In Saskatchewan fenugreek is reasonably well adapted to the Brown and Dark Brown soil zones. Small plot tests at Saskatoon have resulted in seed yields from 1500 to 2416 kg/ha (Slinkard, personal comm.). At present the area under fenugreek cultivation in western Canada is minimal. Market opportunities must be expanded before fenugreek production is increased in western Canada. Fenugreek is a crop with much potential for food, pharmaceutical and industrial uses and in many parts of the world it is used as a forage. The seeds are used extensively as a condiment and as an ingredient in most spice mixtures. The seeds are also a source of diosgenin

used in the production of steroids in the pharmaceutical industry.The presence of high amounts of galactomannans in the fenugreek endosperm is of interest to the food industry. Galactomannans in the diet reduce blood and plasma glucose and cholesterol levels. The seeds are a very good source of dietary fibre. Mature fenugreek hay is comparable to early-cut alfalfa hay in nutrient content and digestibility (Mir *et al.* 1993)

Isozymes, the varying forms of an enzyme distinguishable through electrophoresis, have been used as molecular markers in the study and breeding of a number of major crops (Soltis and Soltis 1989, Weeden 1989). Isozymes show Mendelian inheritance and are co-dominant, enabling separation of heterozygotes from homozygotes. They are unaffected by the environment and, therefore, the genotype and phenotype can be equated except in the case of null alleles. Isozymes can be used by plant breeders and geneticists for a number of purposes, e.g., as genetic markers for characters which are difficult to screen for, genotype and cultivar identification and phylogenetic studies.

The objectives of this study were to: 1) study the inheritance patterns of isozyme and morphological trait loci in fenugreek and 2) screen for any linkages among or between isozyme and morphological trait loci.

MATERIALS AND METHODS

Forty-eight fenugreek lines from various origins were screened for isozyme polymorphisms and morphological variation. On the basis of the variation observed 11 lines were selected as parental lines and crosses were made among them. Seven F_2 populations were selected on the basis of the number of loci segregating in each and further analysed for segregation of isozymes and morphological characters. The enzyme systems studied for segregation patterns were aconitase (ACO), amylase (AMY), aspartate amino transferase (AAT), esterase (EST), glutamate dehydrogenase (GDH), peroxidase (PRX) and triose phosphate isomerase (TPI). The morphological traits studied were cotyledon colour (*Yc*), pubescence (*Pub*), single vs double pods/leaf axil (*Spd*) and zero-tannin seed coat (*Tan*).

Cotyledons and young leaves were used as source tissue for enzyme extraction. The samples, absorbed on paper wicks, were loaded on a horizontal starch gel and subjected to an electrical potential for 2 to 5 h. See Nair (1994) for details on electrophoretic techniques used. On completion of electrophoresis the gels were sliced and stained to visualize the isozyme pattern. The data obtained were analysed using the PASCAL computer program, LINKAGE-1 (Suiter *et al.* 1983), to determine inheritance ratios and linkages between the loci.

RESULTS AND DISCUSSION

Polymorphism was observed for 9 isozymes (ACO-2, AMY-2, AAT, EST-1, EST-2, EST-3, GDH, PRX-3, and TPI-1) belonging to 7 enzyme systems in the fenugreek lines studied. In all 7 F_2 populations, segregation of isozyme and morphological phenotypes gave a good fit to the expected 1:2:1 and 3:1 ratios

Table 1. Single locus goodness-of-fit for 1:2:1 or 3:1 F_2 segregation ratios for isozyme and morphological loci in fenugreek

Family	Locus	F_2 phenotype			χ^2
		Dominant / fast	Heterozygous	Recessive / slow	
PI 138685 X	*Aco-2*	15	23	14	0.73
PI 273973	*Aat*	13	28	11	0.46
	Pub	32	--	20	5.03*
9095 X	*Aco-2*	12	28	13	0.21
PI 194019	*Pub*	42	--	11	0.51
PI 286436 X	*Aat*	11	23	16	1.32
PI 194020	*Est-1*	11	23	16	1.32
	Est-2	16	25	9	1.96
	Gdh	11	25	14	0.36
	Tpi-1	38	--	12	0.03
PI 220555 X	*Aat*	11	24	15	0.72
PI 138685	*Est-1*	11	24	15	0.72
	Est-2	18	21	11	3.24
	Est-3	15	26	8	2.18
	Prx-3	14	25	11	0.36
PI 268434 X	*Est-2*	12	25	13	0.04
PI 286436	*Est-3*	10	29	11	1.32
	Tpi-1	40	--	10	0.67
	Yc	37	--	13	0.87
Australian X	*Aat*	9	29	12	1.64
PI 194020	*Gdh*	16	24	10	1.52
BVS X	*Amy-2*	15	21	12	1.12
PI 383791	*Est-1*	17	24	7	4.16
	Est-2	13	25	10	0.46
	Prx-3	15	21	12	1.12
	Tan	36	--	15	0.53
	Spd	33	--	18	2.88

* significant at the 0.05 level

indicating monogenic control and Mendelian inheritance (Table 1).

All isozymes were diallelic and functionally monomeric in their quarternary structure except AAT which was dimeric and had three alleles. A null allele was obtained for TPI in fenugreek.

All morphological loci were monogenically controlled and segregated in a Mendelian fashion. Red cotyledon was dominant over yellow, glabrous was dominant over pubescent, double pods were dominant over single pods and tannin-containing seed coat was dominant over zero-tannin seed coat.

Close linkages were observed between two isozymes of EST and cotyledon colour (Table 2). These loci were also loosely linked with TPI-1. Thus, four loci could be included in one linkage group: *Tpi-1* -- *Est-2* -- *Yc* -- *Est-3* (Figure 1). Close linkage was also observed between *Prx-3* and *Amy-2* in one F_2 population with both isozymes showing identical segregation patterns. Further studies with a larger number of samples is needed to determine possible recombinations between these two loci.

Table 2. Significant contingency χ^2 tests for pairs of loci in fenugreek

Linked loci	Family	N[Z]	df	χ^2	p[Y]	r ± SE
Est-2/Est-3	PI 220555 X PI 138685	49	4	72.31	0.00	0.052 ± 0.023
	PI 268434 X PI 286436	50	4	68.01	0.00	0.062 ± 0.025
Est-2/Yc	PI 268434 X PI 286436	50	2	45.01	0.00	0.020 ± 0.020
Est-3/Yc	PI 268434 X PI 286436	50	2	40.32	0.00	0.039 ± 0.028
Est-3/Tpi-1	PI 268434 X PI 286436	101	2	7.12	0.03	0.340 ± 0.056
Tpi-1/Yc	PI 268434 X PI 286436	50	1	3.74	0.05	0.323 ± 0.124
Prx-3/Amy-2	BVS X PI 383791	48	4	96.00	0.00	---

[Z] Number of individuals studied
[Y] Probability

It can be concluded that significant variability exists for isozymes in fenugreek. When more loci are mapped and a more comprehensive genetic map is prepared, the potential exists for the use of isozymes as genetic markers in this crop.

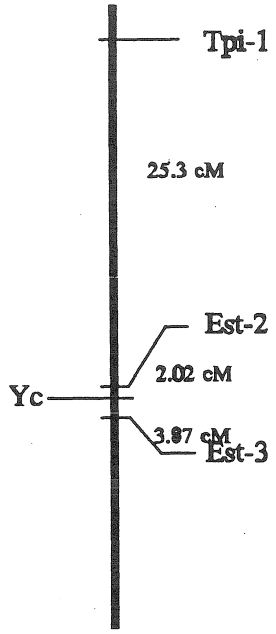

Figure 1. Linkage group identified in fenugreek

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