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Isoenzymatic characterization of some *Lathyrus* L. and *Vicia* L. species (Leguminosae) occurring in Rio Grande do Sul (Southern Brazil)*

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ABSTRACT

MDH, ME and SOD electrophoretic patterns were studied in 18 accessions of five *Lathyrus* and 18 accessions of nine *Vicia* species, using polyacrylamide gels, Scandalios (*Biochem. Genet.* 3: 37-79, 1969) buffer systems and specific staining solutions for each enzyme. For both genera, more than one pattern for each system for each species was found, with few exceptions. Phenograms constructed using Jaccard's similarity coefficients and the simple linkage grouping method showed that, in general, species relationships determined by isoenzymes are in accordance with taxonomic classification.

INTRODUCTION

Native pastures are still the main basis of cattle feeding in the State of Rio Grande do Sul, Brazil. Legume species are an important part of these pastures and many of them could be improved as forages through genetic breeding. Germplasm characterization, that can be performed by several approaches such as morphology, phenology, agronomic characterization, cytogenetics, biochemistry and geographical distribution, is an essential tool for plant breeding.

Nine *Lathyrus* and 12 *Vicia* species are described for Rio Grande do Sul, many of them of good quality and potential as forages. Taxonomic revision of both genera is underway (Bastos and Miotto, 1994; Miotto, unpublished results). Chromosome numbers, meiotic behavior and pollen grain fertility have been studied in eight *Lathyrus* and 11 *Vicia* species (Schifino-Wittmann *et al.*, 1993, 1994) and esterase patterns analyzed for two *Vicia* taxa (Freitas and Schifino-Wittmann, 1993) occurring in the State.

Literature data on isozymes for both genera show that some enzymatic systems allow species or species group characterization (Yamamoto and Plitmann, 1980; Suso and Moreno, 1986), determination of species relationships (Wolff, 1980; Yamamoto, 1986) as well as detection of hybrids and assessment of the degree of genetic recombination (Yamamoto, 1979; Gates and Boulter, 1980).

This work is aimed at characterizing by isozyme patterns several native, naturalized and exotic *Lathyrus* and *Vicia* species occurring in Rio Grande do Sul and nearby regions.

MATERIAL AND METHODS

Eighteen accessions of five *Lathyrus* (native *L. pubescens* (Figure 1), *L. nervosus*, *L. paranensis* and exotic

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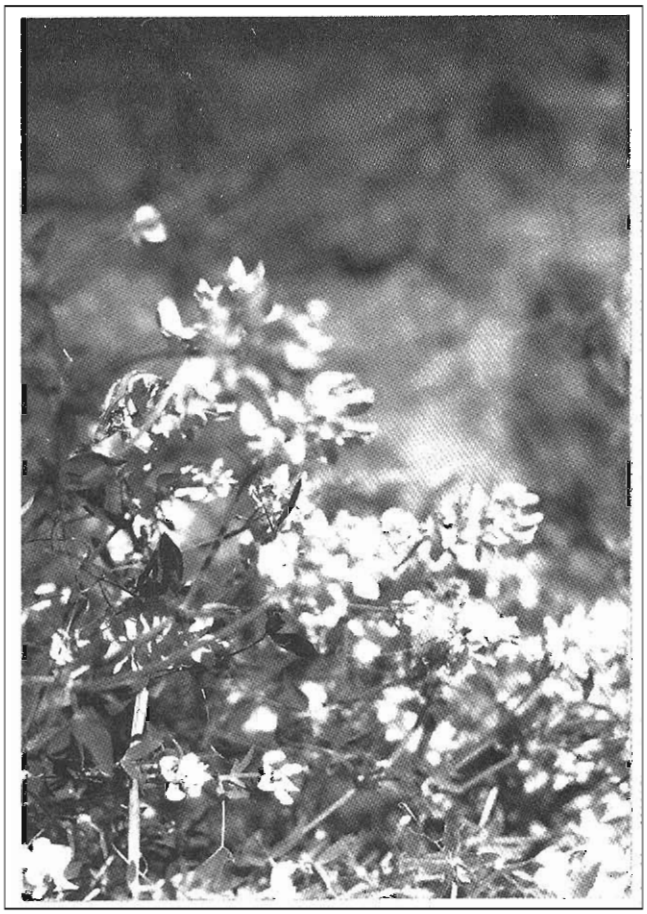

Figure 1 - *Lathyrus pubescens*.

Figure 2 - Places of origin of the *Lathyrus* and *Vicia* accessions and species analyzed. 1. San José (ROU). 2. Maldonado (ROU). 3. Pedro Osório. 4. Bagé-Caçapava road. 5. Caçapava do Sul. 6. Eldorado do Sul. 7. Porto Alegre. 8. São Vicente do Sul. 9. Santa Maria. 10. São Francisco de Paula. 11. Torres. 12. Augusto Pestana. 13. Bom Jesus. 14. BR 116 km 284. 15. Lages (SC).

L. latifolius and *L. sativus*) and 18 accessions of nine *Vicia* species (native *V. epetiolaris*, *V. linearifolia*, *V. nana*, naturalized *V. disperma* and exotic *V. hirsuta*, *V. faba*, *V. narbonensis*, *V. pannonica* and *V. villosa*) (Table I), most of them collected in several places of Rio Grande do Sul, some in Santa Catarina and Uruguay (Figure 2) and one in New Zealand, were analyzed.

Seeds were germinated in Petri dishes after mechanical scarification. Seedlings were transplanted to pots with garden soil in a greenhouse, where the plants were grown.

After several tests, three enzymatic systems were studied: malatedehydrogenase (MDH, E.C. 1.1.1.37), malic enzyme (ME, E.C. 1.1.1.40) and superoxidedismutase (SOD, E.C. 1.15.1.1). An average of five individuals per accession were examined. Polyacrylamide (8%) gels and Scandalios (1969) buffer systems were employed.

MDH and ME staining followed Weeden and Wendel's (1990) procedures and SOD was stained according to Harris and Hopkinson (1976) apud Murphy *et al.* (1990). Homogenates were prepared from young leaves. Migration was performed with 200 V (10 V/cm), at 4°C, for four hours. One individual of *L. sativus* was employed as a standard. Species relationships were established using Jaccard's similarity coefficient and phenograms designed by the sample linkage grouping method (Crisci and Armengol, 1983).

RESULTS AND DISCUSSION

MDH, ME and SOD patterns for the *Lathyrus* species are presented in Figures 3 to 5 and those for *Vicia* species in Figures 6 to 8. In general, more than one pattern for each enzymatic system, for each species was found, with common and characteristic bands.

Considering *Lathyrus*, a total of seven different MDH bands were found among the species analyzed, ranging from four to five per species. The rm = 0.27 band was common among all the species and that of rm = 0.44 was a genetic marker for *L. sativus*. For ME, a total of seven bands were also detected, ranging from two to four per species. The slowest band (rm = 0.09) was present in all the taxa but *L. sativus*, that showed an exclusive rm = 0.10 band. Eleven different SOD bands were found ranging from two to four per species, no one being common to all of them.

Regarding *Vicia*, a total of fifteen different MDH bands were found, ranging from three to five per species. The rm = 0.27 band was common to all taxa but

Table I - *Lathyrus* and *Vicia* accessions and species analyzed.

Species	Abbreviation	Origin[a]	Collector number	ICN[b] number	Place of collection	Number[c] of map	Data of collection
L. cf. latifolius	*L. la*	e	S s/no.	–	New Zealand	–	Feb 93
L. nervosus	*L. ne*	n	M 1394	98440	Torres - Guarita	11	Jan 93
			M 1395	98441	Torres - Farol	11	Jan 93
			M 1206	93199	Morro da Polícia	7	Nov 91
			IB s/no.	–	BR 116 - km 284 Vacaria - Lages	14	Nov 92
			M 1388	98452	Bagé - Caçapava road	4	Nov 92
			Marchesi s/no.	MVFA[d]	Maldonado- Uruguay	1	Dec 92
L. paranensis	*L. pa*	n	S 997	93784	S. Fco. de Paula	10	Nov 90
			M 1257	93403[e]	Bom Jesus	13	Dec 91
			Z s/no.	SMDB	Sta. Maria	9	Nov 91
			Machado 1659	SMDB	S. Fco. de Paula	10	Nov 91
L. pubescens	*L. pu*	n	S 1034a	93817	Caçapava do Sul	5	Dec 90
			S 1034b	93817	Caçapava do Sul	5	Dec 90
			S 1034c	93817	Caçapava do Sul	5	Dec 90
			Machado 817	SMDB	–		Dec 90
			Marchesi s/no.	MVSA	S. José - Uruguay	1	Dec 92
L. sativus	*L. sa*	e	CTC 84505 F_2	–	A. Pestana	12	Nov 91
			CTC 84505	–	A. Pestana	12	Dec 89
V. disperma	*V. di*	nt	CTC 90E50 F_2	–	A. Pestana	12	Nov 91
			CTC 90E50	–	A. Pestana	12	Nov 91
V. epetiolaris	*V. ep*	n	Z 245 F_2	SMDB	Caçapava do Sul	5	Nov 91
			Z 245	SMDB	Caçapava do Sul	5	Dec 90
			RD s/no.	SMDB	Eldorado do Sul	6	Dec 92
			Z s/no.	SMDB	Ipiranga - POA	7	Nov 92
			Z s/no.	SMDB	Caçapava do Sul	5	Nov 92
			M 1197	93190	S. Vicente do Sul	8	Oct 91
V. hirsuta	*V. hi*	e	RD s/no.	SMDB	Eldorado do Sul - EEA	6	Dec 92
V. linearifolia	*V. li*	n	Z s/no.	SMDB	P. Osório	3	Dec 91
			Z 635	SMDB	Caçapava do Sul	5	–
			IB 963	–	Bairro Três Figueiras - POA	7	Sep 91
V. nana	*V. na*	n	M 1334	96538	A. Pestana	12	Oct 91
			Z s/no.	SMDB	Caçapava do Sul	5	Nov 91
V. faba	*V. fa*	e	PT 53 F_2	–	EPAGRI - Lages	15	Nov 91
V. narbonensis	*V. nb*	e	PT 62	–	EPAGRI-Lages	15	Dec 89
V. pannonica	*V. pn*	e	PT 54	–	EPAGRI-Lages	15	Dec 89
V. villosa	*V. vi*	e	PT 47	–	EPAGRI-Lages	15	Dec 89

(a) n = Native; nt = naturalized; e = exotic. (b) Herbarium of the Instituto de Biociências (UFRGS). (c) Figure 1. (d) MVFA = Herbarium, Departamento de Botánica, Facultad de Agronomia, Universidad de la Republica Oriental del Uruguay. (e) Herbarium, Departamento de Botânica, Universidade Federal de Santa Maria.

V. faba. Eight ME bands were detected, ranging from one to two per species with the exception of *V. epetiolaris*, that presented three bands. Exclusive specific bands were found for *V. villosa* (rm = 0.07), *V. hirsuta* (rm = 0.09), *V. linearifolia* (rm = 0.12) and *V. faba* (rm = 0.15). For the SOD system 11 bands were detected, ranging from 2 to 5 per species, no one being common to all the taxa or exclusive to any of them.

For both genera, ME and MDH systems were generally more conservative (less variable), considering number of bands and different patterns per species. It could be argued that this conservativeness is probably due to the role of these enzymes in the respiratory metabolism. SOD is the most intra- and interspecific variable system, which could be related to its role in the periferic metabolism. In *L. paranensis*, species of palustris habitats, only one SOD pattern was detected, which suggests a correlation between a specific habitat and an isoenzyme pattern.

Phenograms for *Lathyrus* (Figure 9) and *Vicia* (Figure 10) show species grouping according to presence and absence of bands for the three enzymatic

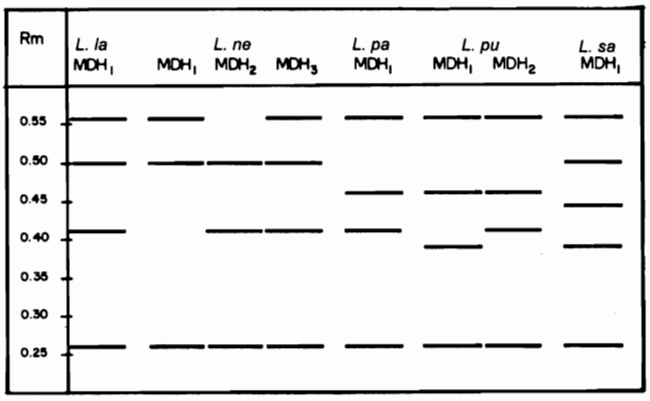

Figure 3 - MDH patterns for *Lathyrus* species.

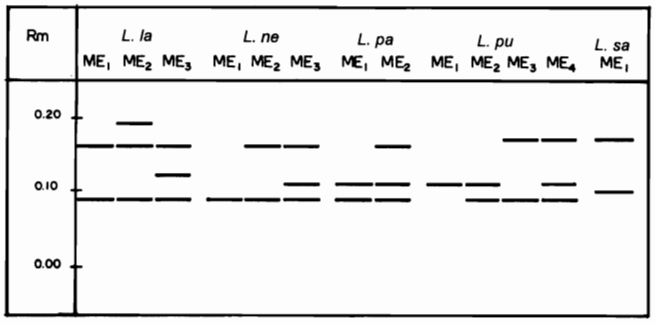

Figure 4 - ME patterns for *Lathyrus* species.

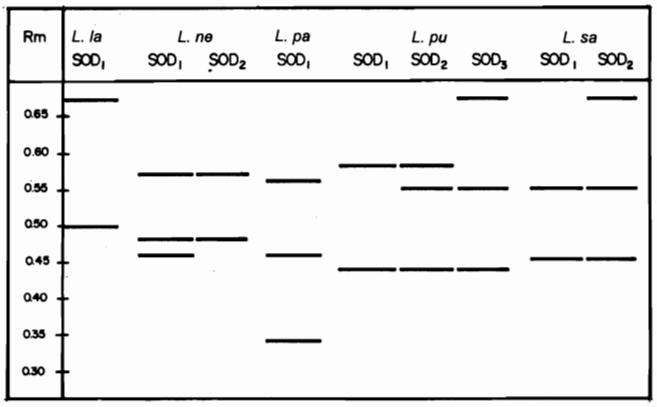

Figure 5 - SOD patterns for *Lathyrus* species.

systems together. Values in the Figures are those of Jaccard's similarity coefficient calculated for species or groups of species. There is a general agreement between these phenograms and taxonomic classification, with some exceptions.

As expected, the native *Lathyrus nervosus* and *L. paranensis* grouped together, with the highest similarity coefficient (0.54), uniting them to exotic *L. latifolius* (0.43). The grouping of native *L. pubescens* and exotic *L. sativus* (0.38) is totally unexpected considering the taxonomical situation of these taxa.

As for *Vicia*, the agreement between isoenzyme similarities and taxonomical classification is more accurate. The introduced *V. disperma* and *V. hirsuta*

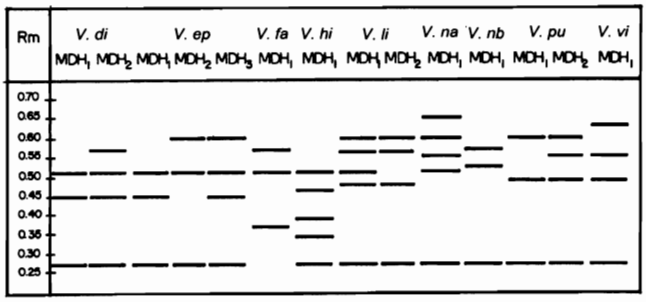

Figure 6 - MDH patterns for *Vicia* species.

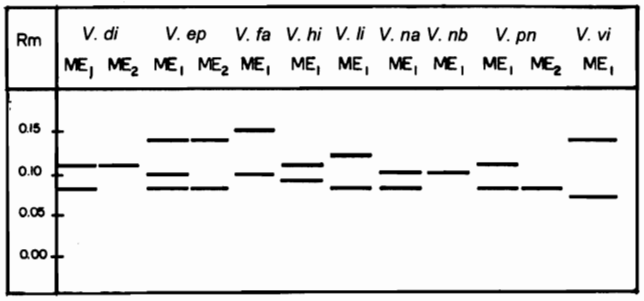

Figure 7 - ME patterns for *Vicia* species.

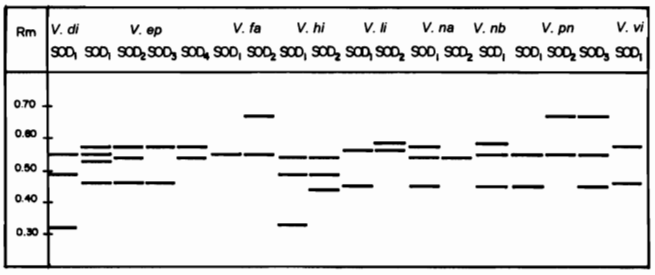

Figure 8 - SOD patterns for *Vicia* species.

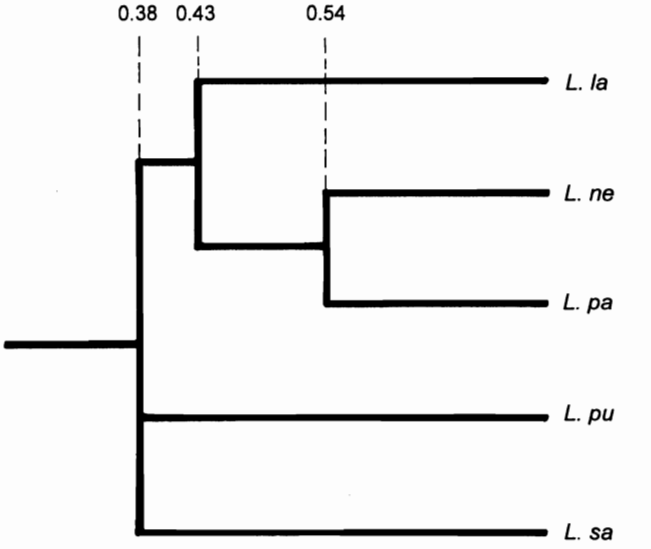

Figure 9 - Phenogram for 18 *Lathyrus* accessions, using the simple linkage method, based on MDH, ME and SOD data.

(Cracca Section) grouped together (similarity coefficient 0.33), as the native *V. nana* and *V. epetiolaris* (0.47) (Australes Section, Kupicha, 1976). Exotic *V. faba* and *V.*

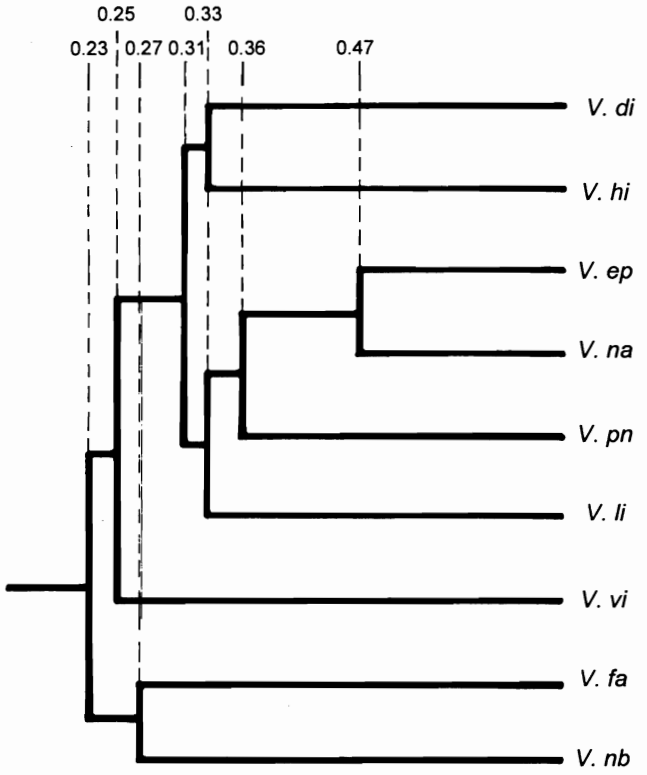

Figure 10 - Phenogram for 18 *Vicia* accessions, using the simple linkage method, based on MDH, ME and SOD data.

narbonensis (Faba Section) grouped together (0.27), which is expected especially considering the suggestion that *V. narbonensis* is one of the putative ancestors of *V. faba*. The great distance of *V. villosa* to the Cracca group as well the greater similarity coefficient of *V. pannonica* than that of *V. linearifolia* to the *V. nana-V. epetiolaris* group disagree with taxonomical classification.

According to Yamamoto and Plitmann (1980), isoenzymatic similarities in *Vicia* were generally not related to taxonomic affinities. Yet, Van der Ven *et al.* (1993) found that molecular analyses (RFLPs and PCR) are good indications of taxonomic affinities, even considering the existing exceptions. As stated by Hunziker (1971), different approaches must always be used for classification, in order to obtain more accurate results.

In conclusion, our results, the first study on isozymes of native *Lathyrus* and *Vicia* species of Southern Brazil, support the utilization of isoenzymatic data for germplasm characterization and establishment of species affinities.

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RESUMO

Padrões eletroforéticos para MDH, ME e SOD foram estudados em 18 acessos de cinco espécies de *Lathyrus* e 18 acessos de nove espécies de *Vicia*. Foram utilizados géis de poliacrilamida, sistemas de tampões de Scandalios (*Biochem. Genet. 3*: 37-79, 1969) e soluções corantes específicas para cada enzima.

Para ambos os gêneros, foram detectados mais de um padrão por espécie, para cada sistema, com poucas exceções. Fenogramas foram construídos usando o coeficiente de similaridade de Jaccard e o método de agrupamento simples. De modo geral, as relações interespecíficas determinadas pelos dados isoenzimáticos estão de acordo com a classificação taxonômica.

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