ISOENZYMATIC CHARACTERIZATION OF NATIVE AND CULTIVATED FORAGE LEGUME SPECIES OF RIO GRANDE DO SUL (SOUTHERN BRAZIL)

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ABSTRACT

Esterase (EST), malic enzyme (ME) and superoxidodismutase (SOD) patterns were studied in 26 accessions of the *Vicia sativa* aggregate, including *V.sativa*, *V.angustifolia* and *V.cordata*. ME was monomorphic but for EST and SOD intra and interspecific and also intrapopulation polymorphism was detected. An EST marker band was detected for *V.cordata*. EST patterns did not allow a clear separation between *V.sativa* and *V.angustifolia* but SOD patterns grouped the taxa in general accordance with taxonomic classification. The results agree with previous reports on polymorphism in European populations of the aggregate and show that isozymes can be useful genetic markers for the study of the *Vicia sativa-V.angustifolia* complex.

KEYWORDS

Forage legumes, isozymes, Vicia sativa aggregate

INTRODUCTION

Many of the native legume species occurring in the grasslands of Rio Grande do Sul as well as the cultivated ones could be improved through genetic breeding. Assessment of existing variability is essential to plant breeding and can be performed by several approaches. In a research line aiming at characterizing by isozyme patterns native and exotic forage legumes our group has been working with Leucaena, Vicia, Lathyrus and Trifolium. The vetches (Vicia) are represented in Rio Grande do Sul by native, naturalized and exotic species. Previous results by Gonzalez and Schifino-Wittmann (in press), showed intra and interespecific variability in nine species analyzed for three isozymes. *V.sativa*, the common vetch, is the most polymorphic species of this genus and its infraspecific taxonomy is not yet fully clarified (Maxted, 1995). European populations of the *Vicia-sativa-V.angustifolia* complex present great morphological, karyotypical and isoenzymatic variability, which led to suggestion that the group could be in an active process of sympatric speciation (Hanelt and Mettin, 1989). *V.sativa* is cultivated in Rio Grande do Sul as forage and *V.angustifolia* is widespread in natural populations. Previous cytogenetical analysis showed variation in chromosome numbers (2n=10, 12 and 14) and karyotipic markers (Schifino-Wittmann *et al*., 1994 and Weber and Schifino-Wittmann, unpublished). The objective of this study was to analyze the isoenzymatic variability of the complex and verify the correspondence of isoenzymatic, cytological and taxonomical data.

METHODS

Twenty-six acessions (individual mother plants, populations and cultivars) of *V.sativa* (9, CTC84E30, PT 89, PT 292 cv.Zypern, IB1078, M1198), *V.angustifolia* (CTC84E01, CTC84E33, CTC84E35, CTC84E47, S1025, S1026, M1078, M1123, M1152, M1153, M1160, M1161, M1322, M1326, M 1329, M1330, M1331, M1354, M1375)) and V. cordata (PT 57) were analyzed. Seeds were germinated in petri dishes, and the plants grown in greenhouse. Electrophoresis was performed in 8% polyacrylamide gels, with Scandalios buffer systems and samples prepared from fresh leaves from grown plants. Details of the technique are described by Gonzalez and Schifino-Wittmann (in press). After previous tests, three enzyme systems were chosen, based on resolution and reproducability of results: EST (esterase), SOD (superoxidudismutase) and ME (malic enzyme). At least five individuals per accession were analyzed. One plant of *V.sativa*) was employed as a standard. In each gel, for each band, the migration rates (rm) were calculated using the distance migrated by the band divided by that of the running front. The rmís presented are the averages of several gels. Isozyme patterns were analyzed considering presence and absence of bands. The accessions were grouped based on similarity indexes (number of common bands divided by the total number of bands) considering the most frequent patterns.

RESULTS

ME. Malic enzyme was monomorphic, all individuals presenting one and the same (rm=0.13) band.

EST. A variable number of bands, ranging from 2 -7 per plant were detected, but only those with good repeatability were used for comparisons. All these bands exhibited simultaneously ð/ß activity. *V.cordata* (2n=10) has a specific marker band (rm=0.60) absent from any other individual. For *V.sativa* (2n=12) and *V.angustifolia* (2n=12 in the accessions counts were made), the most frequent bands were those of rm=0.90, 0.70 and 0.54 bands but in *V.sativa* the 0.90 band was present in 20 % of the accessions and in *V.angustifolia* in 55%. Intrapopulation polymorphism was detected in one acession of *V.sativa* (IB 1078) and four of *V.angustifolia* (M 1152, M1123, M1160, Loreto). Three groups were formed, mixing *V.sativa* and *V.angustifolia* accessions (Figure 1). It can be concluded that esterase patterns do not allow clear cut distinctions between the taxa.

SOD. For SOD, 2 to 4 bands were detected (rm= 0.60, 0.45, 0.40 and 0.20). The 0.60 band appeared in 100% of the *V.angustifolia* and *V.cordata* individuals but only in 40% of the *V.sativa* plants. Only one population of *V.sativa* (IB 1078) was found to be polymorphic. Four distinct groups were found (Figure 2): one formed by the "typical" V.sativa (cultivated material, with the submetacentric marker chromosome described by Weber and Schifino-Wittmann (unpublished)), and the other mainly with *V.angustifolia*. *V.cordata* grouped with the first *V.angustifolia* group.The accession M1198, classified as *V. sativa* but with a typical *V. angustifolia* karyotype (predominance of acrocentic chromosomes and absence of the submetacentric marker) was distinctly separated. SOD patterns are much more consistent with taxonomic classification and cytological data than the EST ones.

Our results are in agreement with previous reports on the variability of European populations of the complex and show that some isozymes can be useful genetic markers in the *Vicia sativa-V.angustifolia* aggregate.

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Figure 1
Phenograms of accessions grouping based on EST patterns for the Vicia taxa analyzed

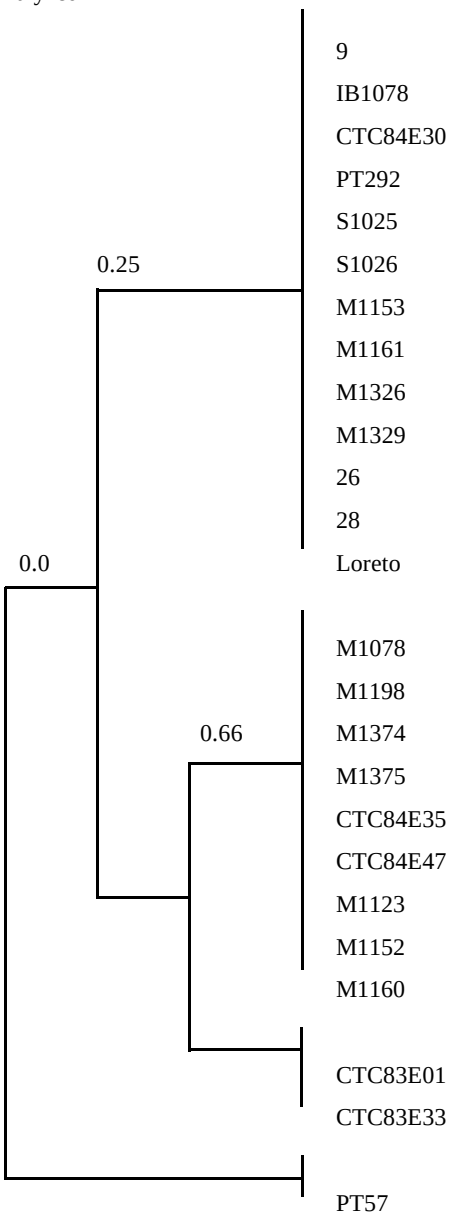

Figure 2 Phenograms of accessions grouping based on SOD patterns for the *Vicia* taxa analyzed