Theor Appl Genet (1990) 80:433–436

Genetic variability for esterase enzyme in *Onobrychis* species*

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Received March 25, 1990; Accepted April 3, 1990
Communicated by H. F. Linskens

Summary. Understanding polymorphism at the enzyme level is basic to its use in population and genetic studies. However, no such information is available on the variability among different sainfoin (*Onobrychis*) species. Therefore, our objective was to study the existence of genetic polymorphism for esterase in 17 *Onobrychis* species and three cultivars of *O. viciifolia* Scop. Three regions of banding were observed in all the materials tested, with the number of bands varying from 0 to 3, 3 to 14, and 1 to 2 bands in each of these zones, which have been designated EST1, EST2, and EST3 respectively. All the materials studied had unique banding patterns, the only common feature being that all of them, except one species, had isozyme 1. Identification was possible only for four species (*O. iberica, O. kachetica, O. transcaucasica,* and *O. bieberstenii*) and one cultivar ('Nova') based on the banding patterns. Large diversity was evident from the wide range of percent similarity values (0%–79%). Subsequent studies should be directed in using these isozyme banding patterns as markers to the desirable agronomic and quality traits of different germplasm lines.

Key words: Esterase – *Onobrychis* spp. – Polymorphism – Sainfoin – Similarity index

Introduction

Isozymes (molecularly distinct patterns of a single enzyme) have long been used to study polymorphism both within and among plant species (Brown and Weir 1983). An advantage of using isozymes in studies of polymorphism is that an array of enzymatic loci can be studied easily on one individual, using small amounts of material with minimal preparation and cost.

The term esterase refers to a group of enzymes that includes a host of ester hydrolases. Esterase activity can be readily assayed in plant extracts. Cubadda and Quattrucci (1974) concluded that esterase isozymes that act upon α- and β-naphthylacetate are carboxyl esterases (E.C. 3.1.1.1). Esterase zymograms characteristically contain a high number of bands (frequently more than ten) controlled by multiple gene loci.

Cultivated sainfoin (*Onobrychis viciifolia* Scop.) is a cross-pollinated perennial forage legume adapted to the dry, calcareous soils of the western United States (Ditterline and Cooper 1975). Sainfoin is nonbloat-inducing (Melton 1973), resistant to alfalfa weevil (Ditterline and Cooper 1975), comparable to alfalfa in both forage quality (Carleton et al. 1968) and average daily gain of cattle (Marten et al. 1987). Sainfoin provides earlier spring grazing or hay production than alfalfa (Smoliak and Hanna 1975), but generally has lower regrowth and total seasonal yield than alfalfa (Bolger and Matches 1990).

Though the gene pool of the wild relatives of sainfoin is large, little information is available about enzyme variability among different sainfoin species. Our objective was to determine the existence of genetic polymorphism for esterase enzyme in 17 *Onobrychis* species and three cultivars of *O. viciifolia* Scop.

Materials and methods

Plant materials

Seventeen sainfoin species, along with three cultivars of *O. viciifolia* used in the study are listed in Table 1. The seed material for

* This work was supported by USDA Specific Cooperative Agreement No. 58-7MN1-8-143 from the Plant Stress and Water Conservation Unit, USDA-ARS, Lubbock, Texas. Joint contribution of the Texas Tech University, Lubbock, Texas and the USDA-ARS. TTU Journal no. T-4-302

the different species and the three cultivars was provided by the Western Regional Plant Introduction Station, Pullman/WA, USA and Dr. M. Hanna, Lethridge, Alberta, Canada, respectively.

Enzyme extraction

Approximately 0.5 g of young, fresh leaf material was crushed in 1 ml of ice-cold extraction buffer with a mortar and pestle in the presence of quartz sand. The extraction buffer consisted of 1 *M* trizma base [TRIS(hydroxymethyl)aminomethane] (pH 6.8), 6% (v/v) glycerol, 5% (w/v) dithiothreiotol, 1% (w/v) soluble polyvinylpyrrolidone, and 0.5% (w/v) bromophenol blue (tracking dye). The extracts were then centrifuged at 11,000 g for 3 min and the supernatant was stored at −70 °C until further analysis.

Table 1. List of the 13 species and three cultivars of sainfoin studied for esterase isozymes, along with their country of origin

Code	Species	Origin	PI no.
1.	*Onobrychis altissima*	Russia	312907
2.	*O. antasiatica*	Russia	314541
3.	*O. arenaria*	Czechoslovakia	372804
4.	*O. argentea*	Spain	280259
5.	*O. bieberstenii*	Russia	312932
6.	*O. grandis*	Russia	440568
7.	*O. kachetica*	Russia	314469
8.	*O. iberica*	Russia	312934
9.	*O. inermis*	Russia	312944
10.	*O. oxyodonata*	Russia	312945
11.	*O. petraea*	Russia	312946
12.	*O. tanaitica*	Russia	312970
13.	*O. transcaucasica*	Russia	273758
14.	*O. vassilczenkoi*	Russia	300580
15.	*O. hajastana*	Russia	312933
16.	*O. michauxii*	Turkey	383712
17.	*O. viciifolia*	Washington	212241
18.	*O. viciifolia*	(Nova)	
19.	*O. viciifolia*	(Eski)	
20.	*O. viciifolia*	(Remont)	

Electrophoresis procedure

Enzyme extracts (100 µl) were loaded onto 10% polyacrylamide gels with a 5% polyacrylamide stacking gel prepared according to Laemmli's (1970) procedure. The dimensions of the gel were 8 × 16 × 0.15 cm. Electrophoresis was carried out at 4 °C for approximately 3.5 h at 4 mA per well. The gels were run again after a week, using fresh leaf material.

Enzyme staining

Esterase activity was localized on the gels according to Shaw and Prasad (1970) with minor modifications. Eighty milligrams of α-naphthylacetate was dissolved in 4 ml of acetone and the volume was made up to 100 ml with 100 m*M* phosphate buffer (pH 6.0). Then 200 mg of Fast Blue RR salt was dissolved by vigorous stirring. Gels were incubated at 35 °C for 20 min, then washed with distilled water.

Similarity index

Relative mobility (Rf) values were calculated for individual bands based on the average migration distances of the bands in duplicate gels. The similarity between various profiles was expressed in the similarity index. The percent similarity was computed for the different species following the procedure of Whitney et al. (1968).

Percent similarity:

$$\frac{\text{number of pairs of similar bands}}{\text{total number of bands in both species}} \times 100.$$

Enzyme nomenclature

Each isozyme activity was named with the abbreviation of the enzymatic system in capitals (EST) followed by a number, 1, 2, or 3, depending on the relative mobilities. The numerical values 1, 2, and 3 respresent the slower, intermediate, and faster migration, respectively. Isozymes were also numbered from slower to faster migration in gels.

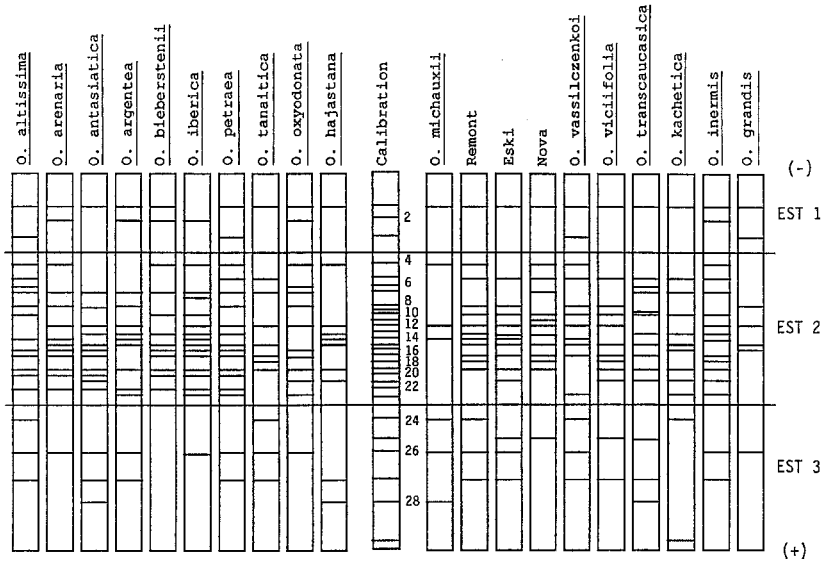

Fig. 1. Zymogram showing the banding patterns of 17 *Onobrychis* species and three cultivars of *O. viciifolia* for esterase enzyme. Isozymes 1–3, 4–23, and 24–29 are in EST1, EST2, and EST3, respectively

Table 2. Similarity indices for esterases in 17 *Onobrychis* species and three cultivars of *O. viciifolia* (in %)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean
O. altissima (1)	–	50	71	47	62	42	34	55	42	40	51	36	39	52	22	25	41	40	39	50	44.11
O. antasiatica (2)		–	69	57	59	36	52	55	45	43	73	46	62	28	56	27	44	50	55	47	50.21
O. arenaria (3)			–	74	77	48	31	73	47	59	63	40	36	43	42	29	38	44	43	48	51.32
O. argentea (4)				–	56	40	32	62	41	54	52	33	37	30	43	20	40	38	44	42	44.32
O. bieberstenii (5)					–	32	50	79	50	48	60	35	46	31	36	7	50	56	38	44	48.21
O. grandis (6)						–	21	26	9	40	40	33	19	48	24	0	32	20	29	36	30.26
O. kachetica (7)							–	43	57	32	47	47	61	31	45	0	50	56	54	44	41.42
O. iberica (8)								–	56	48	65	37	33	40	46	26	36	55	47	52	49.16
O. inermis (9)									–	55	47	52	53	47	54	39	50	69	67	65	49.74
O. oxyodonata (10)										–	45	33	37	22	35	20	40	31	37	36	39.74
O. petraea (11)											–	62	63	50	57	32	40	58	50	55	53.16
O. tanaitica (12)												–	56	48	48	33	43	67	48	62	45.21
O. transcaucasica (13)													–	43	58	19	69	67	36	55	46.79
O. vassilczenkoi (14)														–	42	48	46	52	64	76	44.26
O. hajastana (15)															–	59	36	52	58	56	45.74
O. michauxii (16)																–	0	30	29	55	26.21
O. viciifolia (17)																	–	64	69	59	44.58
'Nova' (18)																		–	67	71	51.95
'Eski' (19)																			–	76	50.00
'Remont' (20)																				–	54.16

Results and discussion

The results of esterase isozyme pattern in different species tested in the present study are reported in Fig. 1. In all, a maximum of 29 isozymes was noted. These were distributed in three zones, viz., EST1, EST2, and EST3, with the first 3 isozymes in EST1, isozymes 4–23 in EST2, and isozymes 24 to last in EST3. The number of isozyme bands in the different species ranged from 7 (*O. michauxii* and *O. grandis*) to 17 (*O. altissima* and *O. petraea*). Isozyme 1 was common to all the species, with the exception of *O. iberica*. Macdonald and Brewbaker (1975) reported that maize esterase isozymes were coded by ten loci, while Stuber and Goodman (1982) found two to nine alleles at each locus. Nakai (1976) found a total of 33 esterase isozymes in wheat. At least ten loci have been described in barley (Hvid and Nielsen 1977), and some of them are highly polyallelic. Quiros (1980) reported eight different zymograms in alfalfa for esterase involving bands in seven different positions.

In outcrossing species, any one population is often highly representative of the species as a whole; that is, the most frequent allele for a given gene in one population is usually also most common in other populations of the species, as indicated by Gottlieb (1975) on subspecies of *Staphanomeria exigua*.

EST1

The number of isozyme bands in this zone varied from 1 to 2 (Fig. 1). Isozyme 1 was the only isozyme form present in 50% of the species tested, while isozyme 2 was the only form in *O. iberica*. Both isozymes 1 and 2 are present in 25% (5), while both 1 and 3 were present in 20% (4) of the species.

EST2

The EST2 region was the most active zone with high intensity bands and the number of isozyme bands varied between 3 and 14. Isozymes 4, 5, 7, 11, 12, 14, 16, 18, and 20, which were present in 55%–85% of the species tested, are more common than the other isozymes. *O. transcaucasica* and Nova can be identified on the basis of the presence of isozymes 9 and 11, respectively.

EST3

The EST3 region is less complex than EST2, with the number of bands varying from zero to three. Isozymes 26 and 27 are present in 70% and 60% of the species tested, respectively, while isozyme 29 is unique to *O. kachetica*. Nova can be easily recognized by the presence of isozyme 25, while *O. bieberstenii* can be identified by the absence of any isozyme bands in this region.

Similarity index

To estimate the variation in esterase banding pattern among the different sainfoin species, the percent similarity was determined (Table 2). The homology, or the similarity between species, varied from 0% to 79%. *O. arenaria* resembled *O. altissima* (71%), *O. argentea* (74%), *O. bieberstenii* (77%), and *O. iberica* (73%). *O. iberica* and *O. bieberstenii* also had similar banding patterns (79%). *O. michauxii* had the most unique banding pattern, as evidenced by the lowest mean similarity (26.21%)

value. The banding patterns of *O. michauxii* did not match those of *O. bieberstenii, O. grandis, O. kachetica,* and *O. viciifolia.* As expected, *O. viciifolia* and the three cultivars of this species had high similarity values within their group. Although the three cultivars had mean similarity values exceeding 50%, only Nova can be identified by the presence of isozyme 11 in EST2 and isozyme 25 in EST3 regions.

In conclusion, all of the species and the three cultivars studied had unique banding patterns, however, all of them, except one species (*O. iberica*), had isozyme 1. Identification was possible only for one species in EST1, one species and a cultivar in EST2, and two species and a cultivar in EST3 regions. Large genetic diversity among the species tested was evident from the wide range of percent similarity values (0%–79%). Subsequent studies are needed to investigate the presence of desirable agronomic and quality traits of different germplasm lines. Relating these traits to the isozyme banding patterns might reduce the time required for interspecific gene transfer by the use of isozyme selection.

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