Electrophoretic analysis of superoxide dismutase and peroxidase isoenzymes in clover species and interspecific hybrids

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We analysed the spectra of peroxidase (PO) and superoxide dismutase (SOD) in clover species and their interspecific hybrids. Comparing *T. pratense* L. and *T. diffusum* Ehrh. species we found specific SOD and PO components, while *T. ambiguum* Bieb. and *T. hybridum* L. spectra were identical. *T. pratense* x *T. diffusum* F_1, B_1F_2 and B_2F_3 hybrids had a complete or partial set of SOD and PO components of parental species, supplemented by original components occurring only in hybrids. In *T. pratense* x *T. diffusum* F_1 hybrids we detected SOD spectra of two types, which give a significant proof of their hybrid origin. In backcross lines derived from *T. pratense* x (*T. pratense* x *T. diffusum*) crossing, hybridity was confirmed in 49.6% of individuals, the other plants had a spectrum identical to parent species. When analysing PO polymorphism in F_1 population, hybridity was confirmed in 96.7% of plants; the rest of the individuals had a spectrum identical to that of *T. pratense*. In backcross B_1F_2 and B_2F_3 populations, 76.0% of plants had a spectrum confirming their hybrid nature, the other 24.0% of clover plants demonstrated only a maternal, *T. pratense* species spectrum. It was determined that in order to identify *T. pratense* x *T. diffusum* hybrids it is necessary to carry out polymorphism analysis of both SOD and PO isoenzymes.

Key words: clover, interspecific hybrids, isoenzymes, peroxidase, superoxide dismutase

INTRODUCTION

Importance of distant hybridisation is increasing in forage grass breeding. Attempts are being made to combine useful characters of both parents in interspecific and intergeneric hybrids. The technique of distant hybridisation is being successfully employed in forage grass breeding [1, 2].

Clover species include self-pollinating, disease- and cold-resistant, re-growing from root, seedy forms, therefore distant hybridisation has quite a promising future here, too. Findings on interspecific clover hybridisation date back to 1953. However, despite abundant attempts, no clover varieties have been released with the help of distant hybridisation yet [3–5].

When interspecific hybrids are developed, it is important to identify them. Cytological analysis in clover hybrids is rather complex, especially in those cases when parental forms have an identical, similar or unstable number of chromosomes. Various biochemical methods, including electrophoretic spectrum analysis of isoenzymes, are being currently used for identification of hybrids [6–10]. It was determined that *T. repens* L. and *T. hybridum* L. clover species had different electrophoretic spectra of the isoenzymes esterase, peroxidase, polyphenol oxidase, superoxide dismutase. Hybrids between *T. repens* L. and *T. hybridum* L. had electophoretic spectra components characteristic of both parental forms, furthermore, new components typical of only hybrids were revealed [11]. Spectral analysis of phosphoglucoisomerase, leucinaminopeptidase and amylase isoenzymes is successfully used for the identification and assessment of hybrids between *T. repens* L. and *T. nigrescens* Viv. [5].

The objective of the present study was to determine the suitability of electrophoretic spectrum analysis of the isoenzymes superoxide dismutase and peroxidase for identification of clover *T. pratense* L.,

T. diffusum Ehrh., *T. hybridum* L. and *T. ambiguum* Bieb. and their hybrids.

MATERIALS

1. *T. pratense* L., 'Liepsna' 2n = 2x = 14 (high-yielding, lacking in seed productivity and disease resistance). **2.** *T. diffusum* Ehrh., 2n = 2x = 16 (wild form, self-pollinating, unproductive, disease-resistant). **3**. Interspecific hybrids (F_1, B_1F_2 and B_2F_3) between *T. pratense* L. and *T. diffusum* Ehrh., 2n = 4x = 30. **4**. *T. hybridum* L., 'Daubiai' 2n = 2x = 16 (unproductive, lacking in seed productivity and disease resistance, can withstand acid soils). **5.** *T. ambiguum* Bieb., 2n = 2x, 4x, 6x = 16, 32, 48 (wild, regrowing from root, persistent, disease-resistant).

METHODS

Clover was grown in a greenhouse 3 plants per pot (22–25 C°, 16 h photoperiod, 13 thousand lux. light intensity). Fertile interspecific hybrids between *T. pratense* and *T. diffusum* were obtained by the embryo culture method and polyploidy [12, 13]. In our trials we used interspecific hybrids of the first generations (F_1), and backcross hybrids of the first and second generations (B_1 F_2 ir B_2 F_3) were obtained by crossing *T. pratense* with F_1 and B_1 plants.

Enzyme determination method. Enzymes were isolated from young clover leaves. Electrophoretic analysis of isozymes was performed in 7.5% polyacryamid gel. Peroxidase isozymes were stained with dianisidine solution, superoxide dismutase – 0.05 M Tris-HCI in a buffer system containing riboflavine, ethylenediamine tetraacetate and thiazole blue [14].

RESULTS AND DICUSSION

Altogether we found 5 SOD components differing in electrophoretic mobility: one characteristic of *T. pratense* (4); one specific to *T. diffusum* (3); two coinciding in both parental forms *T. pratense* and *T. diffusum* (1 and 2); one found only in hybrids (4a) (Figure A).

In F_1 hybrids we determined I (78.6%) and II (21.4%) electrophoretic SOD spectrum types, which give a significant evidence of the hybridity of the clover studied (Table).

According to SOD spectrum, of 137 backcross hybrids 69 corresponded to *T. pratense* (50.4%) and 1 to *T. diffusum* spectrum (0.7%). The other hybrids had a two-type spectrum: type I – 4 parental components and 1 new component (31.4%); type II – lost *T. diffusum* -characteristic component (17.5%).

While investigating the electrophoretic spectrum of PO isoenzymes, we found 8 components: one characteristic of *T. pratense* (2); one characteristic of *T. diffusum* (1); four present in both parental species (3, 4, 5, 6) and two occurring only in hybrids (1a, 4a) (Figure, B).

The spectra of *T. pratense* and *T. diffusum* PO isoenzymes differed only in the position of the components 1 and 2 with high electrophoretic mobility (Figure, B). In F_1 hybrids we found spectra of 6 types: type spectrum I had components of both parental forms (59.0%); type II spectrum had compo-

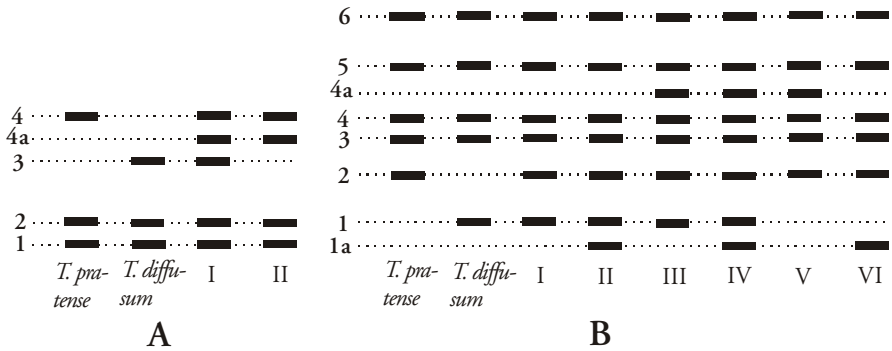

Figure. Electrophoretic spectra of clover *T. pratense, T. diffusum* and their hybrid isoenzymes SOD (A) and PO (B)

Table. **Frequency of isoenzymes SOD and PO electrophoretic spectra types in interspecific clover hybrids *(T. pratense x T. diffusum)***

Spectra types	SOD				PO			
	F_1		B_1 and B_2		F_1		B_1 and B_2	
	number	%	number	%	number	%	number	%
T. pratense L.	0	0	69	50.4	2	3.3	36	24.0
T. diffusum Ehrh.	0	0	1	0.7	0	0	0	0
I	11	78.6	43	31.4	36	59.0	12	8.0
II	3	21.4	24	17.5	3	4.9	26	17.3
III	–	–	–	–	7	11.5	5	3.4
IV	–	–	–	–	5	8.2	26	17.3
V	–	–	–	–	8	13.1	32	21.3
VI	–	–	–	–	0	0	13	8.7
Total	14		137		61		150	
LSD_{05}	4.06		5.50		3.64		3.62	

nents of both parental forms and extra 1a (4.9%); type III spectrum had components of both parental species and extra 4a (11.5%); type IV spectrum had components of both parental species and extra 4a and 1a (8.2%); type V spectrum had *T. pratense* components and extra 4a (13.1%). Of F_1 hybrids, 3.3% had PO spectrum identical to that of *T. pratense* (Table 1).

In backcross individuals, besides the already mentioned 5 PO spectra types, we determined another type VI, which had *T. pratense* spectrum components and extra 4a. Types of spectra confirming the hybrid nature of the investigated plants were characteristic of 76.0% of plants. Quite a great number of plants (24.0%) were identical with maternal species of *T. pratense* according to the location of PO spectrum components.

Thus, the results of SOD and PO electrophoretic spectra analysis allow to identify interspecific hybrids between *T. pratense* and *T.diffusum.* While identifying F_1 hybrids, assessments of hybridity according to SOD and PO spectra coincided in 92.3%, in backcross hybrids in 66.7%. Spectrum analysis of one enzyme is not sufficient to confirm hybridity. In those cases when analysis of one enzyme does not prove the hybrid nature, the results of the other enzyme spectrum analysis can identify the individual as an interspecific hybrid.

SOD and PO electrophoretic spectra of clover *T. ambiguum* and *T. hybridum* were identical, therefore we did not do any tests in their interspecific hybrids.

CONCLUSIONS

1. Specific components were found in the electrophoretic spectra of isoenzymes PO and SOD in the clover species *T. pratense* L. and *T. diffusum* Ehrh., identical spectra were found in the species *T. ambiguum* Bieb. and *T. hybridum* L.

2. Two SOD and six PO isoenzyme electrophoretic spectra types were determined in the interspecific hybrids between *T. pratense* and *T. diffusum*.

3. Isoenzyme spectra confirming the hybrid nature were found in 100% (according to SOD) and in 96.7% (according to PO) F_1 individuals as well as in 49.6% (according to SOD) and 76.0% (according to PO) B_1, B_2 individuals.

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DOBILŲ RŪŠIŲ IR TARPRŪŠINIŲ HIBRIDŲ IZOFERMENTŲ SUPEROKSIDISMUTAZIŲ IR PEROKSIDAZIŲ ELEKTROFORETINIAI TYRIMAI

S a n t r a u k a

Ištirti peroksidazių ir (PO) superoksidismutazių (SOD) spektrai dobilų rūšyse ir jų tarprūšiniuose hibriduose. Lyginant *T. pratense* L. ir *T. diffusum* Ehrh. rūšis, rasta specifinių SOD ir PO komponentų, tuo tarpu *T. ambiguum* Bieb. ir *T. hybridum* L. spektrai tapatūs. *T. pratense* x *T. diffusum* F_1, B_1F_2 ir B_2F_3 hibridai turėjo visą arba dalinį tėvinių rūšių SOD ir PO komponentų rinkinį, kurį papildė orginalūs, tik hibriduose atsirandantys komponentai. *T. pratense* x *T. diffusum* F_1 hibriduose aptikti dviejų tipų SOD spektrai, kurie patikimai patvirtina hibridinę jų prigimtį. Bekrosinėse linijose, kilusiose iš *T. pratense* x (*T. pratense* x *T. diffusum*) kryžminimo, hibridiškumas buvo patvirtintas 49,6% individų, kiti augalai turėjo spektrą, identišką motininei rūšiai. Tiriant PO polimorfizmą F_1 populiacijoje, hibridiškumas patvirtintas 96,7%, visi kiti individai turėjo *T. pratense* identišką spektrą. Bekrosinėse B_1F_2 ir B_2F_3 populiacijose 76,0% augalų turėjo hibridinę prigimtį patvirtinantį spektrą, kiti 24,0% dobilų rodė vien motininės *T. pratense* rūšies spektrą. Nustatyta, kad *T. pratense* x *T. diffusum* hibridams identifikuoti būtina abiejų izofermentų, SOD ir PO, polimorfizmo analizė.