BIOLOGIJA. 2005. Nr. 3. P. 54–58

Evaluation of wild red clover (*Trifolium pratense* L.) ecotypes and hybrid populations (*Trifolium pratense* L. × *Trifolium diffusum* Ehrh.) for clover rot resistance (*Sclerotinia trifoliorum* Erikss.)

G. Dabkevièienë

Z. Dabkevièius

Lithuanian Institute of Agriculture,
LT-58344 Akademija,
Këdainiai distr., Lithuania
E-mail: genetik@lzi.lt,
dabkevicius@lzi.lt

Forage grass breeders are intensively searching for qualitatively novel initial breeding material, more attention is being paid to wild ecotypes' phenomenon of polymorphism within the species, and the application of interspecific hybridisation is becoming increasingly widespread. The present study was designed to investigate the variability of clover rot resistance (*Sclerotinia trifoliorum* Erikss.) in wild red clover (*Trifolium pratense* L.) ecotypes and hybrid populations (*Trifolium pratense* L. × *Trifolium diffusum* Ehrh.). Red clover ecotypes were found to possess a different resistance to the clover rot causal agent: 38.5% of the ecotypes were attributed to the moderately resistant clover group, whereas the plants of the standard variety 'Liepsna' were found to be non-resistant. Compared with non-resistant ecotypes, moderately resistant wild ecotypes exhibited a better overwinter survival and better digestibility characteristics, however, they accumulated lower contents of crude protein and green material. Interspecific hybrids, compared with the plants of the standard varieties 'Liepsna' and 'Vyliai', were characterised by increased (on average 1.4 times higher) resistance to the causal agent of clover rot.

Key words: resistance, red clover, wild ecotypes, *Trifolium pratense* L. × *Trifolium diffusum* Ehrh., *Sclerotinia trifoliorum* Erikss.

INTRODUCTION

Recently, attention has been drawn to the phenomenon of polymorphism within plant species. It has been noted that wild ecotypes of forage grasses significantly differ in terms of earliness, winter hardiness, herbage and seed yield, and feeding value parameters [1, 2]. Interspecific hybridisation is being increasingly used in forage grass breeding. This method is especially successfully employed in ryegrass and fescue breeding [3, 4]. British researchers are conducting research into quality characteristics of backcross hybrids *Trifolium repens* L. × *Trifolium ambiquum* Bieb. and *Trifolium repens* L. × *Trifolium nigrescens* Viv. [5, 6]. In Lithuania, we are currently investigating agrobiological characteristics of the hybrid populations between *Trifolium pratense* L. × *Trifolium diffusum* Ehrh. and the feasibility of their inclusion in the breeding programmes [7]. Clover survival and persistence in crop rotations are largely dependent on disease resistance. In the conditions conducive to the occurrence of diseases, as much as 80% of plants can be killed in clover crops. In the zone of moderate climate (North America, Europe), clover rot causal agent (*Sclerotinia trifoliorum* Erikss.) is one of the most devastating factors reducing sward productivity [8].

The objective of the present work was to test the variation of clover rot (*Sclerotinia trifoliorum* Erikss.) resistance in wild red clover (*Trifolium pratense* L.) ecotypes and hybrid populations (*Trifolium pratense* L. × *Trifolium diffusum* Ehrh.).

MATERIALS AND METHODS

Resistance to clover rot (*Sclerotinia trifoliorum* Erikss.) was assayed in 38 wild red clover (*Trifolium*

Table 1. **Agrobiological characteristics of wild red clover (per cent from the standard variety 'Liepsna')**

Catalogue No.	Resistance to clover rot	Over winter survival	Herbage mass per plant	Seed yield per plant	Crude protein	Digestibility
Liepsna	n	100%	100%	100%	100%	100%
2202	r	64.0	79.6	141.0	79.9	98.3
2180	mr	102.0	114.4	220.0	97.8	88.1
2199	mr	90.0	56.5	100.0	102.4	102.6
2170	mr	100.0	87.2	65.7	106.5	102.9
2208	mr	80.6	77.3	35.5	105.7	105.8
2163	mr	100.0	85.8	68.6	108.6	103.0
2136	mr	102.8	66.8	48.4	103.4	110.8
2140	mr	107.6	96.3	28.6	89.7	100.0
2138	mr	100.0	91.5	85.7	87.0	95.8
2187	mr	80.6	60.0	164.5	95.4	105.6
2143	mr	100.0	36.6	34.3	83.2	95.8
2175	mr	100.0	70.9	71.4	84.7	95.4
2144	mr	103.8	50.6	2.9	84.8	80.6
2177	mr	100.0	87.4	42.9	76.8	83.8
2129	mr	102.0	68.6	80.0	–	–
2133	mr	103.0	84.9	85.7	96.2	93.6
2204	n	64.0	63.6	90.0	104.6	100.9
2156	n	100.0	109.6	120.0	109.7	94.5
2154	n	107.6	114.4	34.3	104.3	100.0
2168	n	111.2	88.3	165.7	106.5	92.8
2178	n	100.0	94.5	128.6	88.6	98.9
2153	n	86.4	51.5	20.0	103.8	91.3
2159	n	100.0	96.3	91.4	102.1	100.0
2166	n	83.0	64.3	57.1	100.0	86.2
2179	n	100.0	86.3	71.4	100.0	87.5
2162	n	98.0	98.9	140.0	111.9	97.7
2174	n	100.0	99.1	85.7	95.1	97.8
2139	n	107.6	74.4	34.3	85.4	95.8
2142	n	80.0	45.8	22.9	–	–
2164	n	90.0	57.2	65.7	102.2	96.2
2167	n	100.0	74.6	40.0	129.2	108.1
2161	n	100.0	31.1	14.6	107.0	101.7
2128	n	100.0	68.6	78.8	106.5	98.7
2131	n	100.0	88.3	62.9	88.6	102.2
2130	n	80.0	89.2	60.0	96.7	98.3
2134	n	100.0	68.6	34.3	97.3	93.3
2200	n	77.9	66.8	48.4	103.4	111.8
2209	n	83.4	43.4	16.1	105.7	–
LSD_{05}		3.07	5.75	5.62	1.69	3.08

n – non-resistant, mr – moderately resistant, r-resistant

pratense L.) ecotypes and three hybrid populations between *Trifolium pratense* L. × *Trifolium diffusum* Ehrh.

Wild clover seed was collected during the period 1998 – 2001 in various geographical locations of Lithuania (Ðilutë, Këdainiai, Trakai, Kaiðiadorys, Anykðèiai, Raseiniai, Birþai, Pasvalys, Molëtai, Ðvenèionys, Plungë, Jurbarkas, Ignalina) entirely unaffected by human activities (natural meadows of forest river valleys, hill and mound slopes, lakesides, etc.) or in locations undisturbed by human activities for at least 25 years (abandoned sand or gravel quarries, graveyards, etc.). Seed was collected from 30–50 plants [9, 10].

Resistance to the disease causal agent was also estimated in three hybrid clover populations (F_1C_o, B_1F_4 and B_2F_5). The hybrids were developed during 1995–2000 by conducting interspecific crossings between plants of the cultivated *Trifolium pratense* L., (2n = 14) variety 'Liepsna' and the

Table 2. **Comparison of agrobiological characteristics of resistant and non-resistant wild red clover ecotypes (per cent from the standard variety 'Liepsna')**

Ecotypes	% of ecotypes exceeding the standard according to the indicators				
	Over winter survival	Herbage mass	Seed yield	Crude protein	Digestibility
Resistant and moderately resistant	37.5	6.2	18.8	33.5	40.0
Non-resistant	13.6	9.5	18.2	50.0	25.5

Table 3. **Investigation of hybrid clover populations for clover rot resistance**

Species, populations	Plant death rate %				Resistance group
	2001	2002	2003	Average	
Trifolium pratense L. variety 'Liepsna'	86	79	58	74	n
Trifolium pratense L. variety 'Vyliai'	51	54	48	51	mr
T*rifolium diffusum* Ehrh.	10	22	56	29	r
F_1C_0 (*T. pratense* × *T. diffusum*)	17	36	70	41	mr
B_1F_4 ('Vyliai' × F_3)	85	23	44	51	mr
B_2F_5 ('Vyliai' × B_1F_4)	43	55	48	41	mr
LSD_{05}	4.60	5.19	5.60	5.48	

n – non-resistant, mr – moderately resistant, r – resistant.

plants of wild form of *Trifolium diffusum* Ehrh. (2n = 16). Fertility of interspecific hybrids was restored by using the embryo culture method and polyploidy. Fertile allotetraploids (2n = 4x = 30) were backcrossed with the tetraploid red clover variety 'Vyliai' (2n = 4x = 28) [7]. The plants used in the experiment were cultivated in a greenhouse in boxes, 50 plants per treatment. Pure culture was isolated from *Sclerotinia trifoliorum* Eriksson. Sclerotia, with which young plants (6–8 weeks old) were inoculated. After 14 days of cultivation in a moist evironment at 15 °C dead plants were counted. Resistant were considered plants with a death rate of up to 30%, medium resistant with a death rate of 31–60%, and non-resistant of 61% and more [11–13]. The tested ecotypes and hybrid populations were compared with the plants of the widely cultivated in Lithuania varieties 'Liepsna', (2n = 2x = 14) and 'Vyliai' (2n = 4x = 28). The clover plants were transferred to the experimental field and planted in the nursery at 50 × 50 cm distances, 40 plants per each ecotype. The following characteristics were assessed: overwinter survival, herbage mass, seed yield, crude protein content and digestibility.

RESULTS AND DISCUSSION

Having tested 38 ecotypes, we found 15 (38.5%) ecotypes to be of moderate resistance (36.6% of plants died after inoculation) and 1 complete resistance ecotype N.2202 (2.4% of plants died after inoculation). The latter ecotype was found in Birþai district in a natural meadow near a water body. Analysis of the relationship between the type of habitat and ecotype resistance to clover rot did not reveal any regularities. The greatest number of moderately resistant ecotypes was found in natural grasslands characterised by different conditions – forest and outskirt grass-plots, dry meadows, wet meadows near water bodies.

The resistance of wild ecotypes to clover rot was compared with that of the standard variety 'Liepsna'. It is noteworthy that plants of the variety 'Liepsna' were found to be non-resistant by other researchers [14, 15] which was also proved by our own evidence from three experimental years. After inoculation with the pure culture of the disease causal agent up to 74% of plants were killed. As a result, it is possible to look for donors of clover rot resistance among the wild ecotypes. Investigation of some other legume varieties and populations, e.g., *Vicia faba,* showed a wide variation of resistance to clover rot [13]. In sunflower breeding, selection of parental forms for crossing is focused on wild ecotypes exhibiting increased resistance to the causal agent of clover rot [16]. Our research findings indicate that 37.5% of clover rot resistant ecotypes and 13.6% of clover rot non-resistant ecotypes have demonstrated a better overwinter survival compared with the plants of the standard variety

'Liepsna' (Tables 1 and 2). The more resistant ecotypes were also found to have a better feeding value: in terms of digestibility, 40.0% of these ecotypes surpassed the plants of the variety 'Liepsna', whereas only 25.0% of non-resistant ecotypes exceeded the standard. In terms of the seed yield, the resistant and non-resistant ecotypes were equal and exceed the standard by 18.8% and 18.2%, respectively. However, comparison of green material yield and crude protein content revealed non-resistant ecotypes to be more productive (1.4 and 1.9 times, respectively). Similar findings were obtained while estimating other plant species. Investigation of red currant varieties did not reveal any correlation between disease resistance (powdery mildew, anthracnose) and yielding capacity, and anthracnose-resistant red and white currant varieties were found to be less productive [17].

Clover rot resistance differed among the years in various populations (Table 3). Our data suggest that the most resistant was the wild clover species *T. diffusum* (29% of plants were killed). In F_1 hybrids and backcross populations, resistance to the disease causal agent remained relatively higher (on average 1.7 and 1.2 times, respectively) than that of the plants of the standard varieties 'Liepsna' and 'Vyliai'. Similar results were recorded while estimating foliar disease resistance of the intergeneric hybrids between ryegrass and fescue: the hybrids were found significantly more resistant to crown rust and leaf spots; moreover, they exhibited a better overwinter survival than the parental species [18]. Research into the polymorphism of interspecific hybrids between *Helianthus maximiliani* and clover rot *Sclerotinia sclerotiniorum* resistant wild species *H. annuus* has revealed that hybrids are generally characterised by a higher resistance [19].

CONCLUSIONS

The ecotypes of wild red clover were found to show different resistance to the clover rot causal agent (*Sclerotinia trifoliorum* Erikss.): 38.5% of the ecotypes were attributed to the moderately resistant clover type. As a result, donors for clover rot resistance can be identified among wild ecotypes. Compared with non-resistant ecotypes, moderately resistant wild ecotypes exhibited a better overwinter survival and better digestibility characteristics, but they accumulated lower contents of crude protein and herbage mass. Interspecific hybrids were characterised by an increased resistance (1.7–1.2 times) to clover rot causal agent compared with the plants of the standard varieties 'Liepsna' and 'Vyliai'.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support for this programme provided by the International Programme COST 852, the Lithuanian State Science and Studies Foundation and the Ministry of Education and Science Research Programme 'Genefund'. The author is thankful to LIA head researcher Dr. I. Gaurilèikienë for advice on isolating the pure culture of *Sclerotinia trifoliorum* Erikss.

Received 02 June 2005
Accepted 23 July 2005

References

1. Lemeþienë N, Kanapeckas J. Þemdirbystë. Mokslo darbai 2000; 72: 182–95.
2. Dabkevièienë G, Sprainaitis A, Bilis J, Paplauskienë V. Biologija 2001; 4(Suppl.): 50–3.
3. Paðakinskienë I. Plant Breeding 2000; 119: 185–7.
4. Nekrošas S, Sliesaravièius A. Þemës ûkio mokslai 2004; 3: 20–7.
5. Abberton MT, Marshall AH, Michaelson-Yeates TPT, Williams TA, Rodes I. Euphytica 2002; 127(1): 75–80.
6. Marshall AH, Abberton MT, Williams TA, Michaelson-Yeates TPT, Powell HG. Grass and Forage Science 2003; 58(3): 295–301.
7. Dabkevièienë G. Biologija 2002; 4(Suppl.): 19–21.
8. Delclos B, Mousset-Declas C, Raynal G. Euphytica 1997; 93(2): 173–9.
9. Descriptor list for forage grasses. International Board for Plant Resources. IBPGR 1987: 1–65.
10. Thorn E. Preserving plant genetic resources for food and agriculture – experiences and developments. Salaspils, 1997: 29–34.
11. Í îâîñåëîâà ÀÑ, Êàøèíîâà Î È, Çîòîâà Çß è äð. Ì åòîäè÷åñêèå óêàçàíèÿ ïî èììóíîëîãè÷åñêîé îöåíêå è ñîçäàíèþ ñåëåêöèîííîãî ìàòåðèàëà êëåâåðà ëóãîâîãî, óñòîé÷èâîãî ê ñêëåðîòèíèîçó è ôóçàðèîçó. Ì îñêâà, 1984: 59.
12. Ñëèñàðàâè÷þñ À, Áðàçàóñêåíå È è äð. Ñåëåêöèÿ è ñåìåíîâîäñòâî 1986; (6): 21–3.
13. Lithourgidis AS., Roupakias DG, Damalas ChA. Phytoprotection 2004; 85: 89–94.
14. Puodþiukas J, Grigaliûnaitë B. Horticulture and Vegetable Growing 1998; 17(30): 408–10.
15. Janèys Z. Biotechnologija augalø selekcijoje. Kaunas, 1998: 71–6.
16. Degener J, Melchinger AE, Hahn V. Plant Breeding 1999; 118(2): 157–60.
17. Þilinskaitë S, Meidus A, Meiduvienë A. Biologija 2001; 1: 14–7.
18. Paplauskienë V, Nekroðas S, Sliesaravièius A. Þemdirbystë. Mokslo darbai 1999; 65: 187–93.
19. Ronicke S, Hahn V, Hom R et al. Plant Breeding 2004; 123(2): 152.

G. Dabkevièienë, Z. Dabkevièius

LAUKINIØ RAUDONØJØ DOBILØ (*TRIFOLIUM PRATENSE* L.) EKOTIPØ IR HIBRIDINIØ (*TRIFOLIUM PRATENSE* L. × *TRIFOLIUM DIFFUSUM* EHRH.) POPULIACIJØ ATSPARUMO DOBILØ VËÞIUI (*SCLEROTINIA TRIFOLIORUM* ERIKSS.) TYRIMAI

S a n t r a u k a

Pastaruoju metu paðariniø þoliø selekcijoje intensyviai ieðkoma kokybiðkai naujos pradinës medþiagos ðaltiniø: atkreiptas dëmesys á laukiniø ekotipø polimorfizmo reiðkiná rûðies viduje, vis plaèiau taikoma tarprûðinë hibridizacija. Darbo tikslas buvo iðtirti atsparumo dobilø vëþiui (*Sclerotinia trifoliorum* Erikss.) varijavimà laukiniø raudonøjø dobilø (*Trifolium pratense* L.) ekotipuose ir hibridinëse (*Trifolium pratense* L. × *Trifolium diffusum* Ehrh.) populiacijose. Nustatyta, kad laukiniø raudonøjø dobilø ekotipai nevienodai atsparûs dobilø vëþio sukëlëjui: 38,5% ekotipø priklauso vidutiniðkai atspariø dobilø grupei, tuo tarpu standartinës veislës 'Liepsna' augalai yra neatsparûs. Vidutiniðko atsparumo laukiniai ekotipai, lyginant su neatspariaisiais, geriau þiemojo ir pasiþymëjo geresnëmis virðkinamumo savybëmis, taèiau sukaupë maþesná þaliøjø proteinø ir þolës masës kieká. Tarprûðiniai hibridai, lyginant su standartinëmis veislëmis 'Liepsna' ir 'Vyliai', buvo atsparesni (vidutiniðkai 1,4 karto) dobilø vëþio sukëlëjui.