Evaluation of morphological characters and valuable agronomic traits of common timothy (*Phleum pratense* L.) R_0 and R_1 regenerants

N. Lemežienė,
E. Lemežis

Lithuanian Institute of Agriculture, LT-5051 Dotnuva-Akademija, Kėdainiai distr., Lithuania.
E-mail: nijole@lzi.lt

The efficiency of breeding work is determined by the genetic diversity of initial material, which can be enhanced by the use of non-traditional breeding techniques such as cell culture. The objective of the present work was to evaluate the morphological characters and valuable agronomic properties of common timothy plants-regenerants in the R_0 and R_1 generations.

Common timothy regenerants in the R_0 generation were very luxuriant, had wider and longer flag leaves, taller stems, longer inflorescenses, etc. Also, plants of R_0 regenerants were characterised by later maturity and a lower susceptibility to leaf spots.

However, in R_1 generation we did not find any increase in the values of the morphological characters and agronomic properties. Regenerants differed from the R_0 control only in later maturity and lower susceptibility to leaf spots.

Key words: regeneration, morphological characters, agronomic properties

INTRODUCTION

The efficiency of breeding work is determined by the genetic diversity of initial material, which can be enhanced by the use of non-traditional breeding techniques such as cell culture [1, 2]. The cell culture technique's uniqueness lies in the fact that somatic cells cultured on special nutrient media produce a mass of non-differentiated cells, *i.e.* callus, from which plants-regenerants are regenerated later [3]. Quite often, which is extremely important in plant breeding, regenerants surpass the initial form by agronomically valuable characteristics [1].

There are data in the literature on common timothy regeneration from callus and somatoclonal variation [4, 5]. Regenerants of perennial ryegrass [6], meadow fescue [7], cocksfoot [8] produced at the Lithuanian Institute of Agriculture had a varied chromosome number, differed among themselves and from the initial forms in value for cultivation, morphological and biochemical properties. In 1993 were obtained the first 78 regenerants of smooth-stalked meadow grass, of which two differed from the parental forms [9]. However, the rest of the smooth-stalked meadow grass regenerants did not differ from the initial forms, *i.e.* demonstrated genetic stability.

The objective of this work was to compare some morphological and agronomically valuable characteristics of R_0 and R_1 regenerants and control plants in field conditions.

EXPERIMENTAL METHODS

In our experiments we investigated five clones of common timothy: from the variety 'Gintaras II' and from the breeding numbers 1456, 1514, 1513, 437. Growth points at II–III organogenesis stages were used for callus induction. Callus culturing, plant regeneration and other callus-related manipulations were carried out in accordance with the methodological directions issued at our institute [10].

Proliferation and regeneration of common timothy callus was performed without the use of any extra selective media. Plant regeneration from callus was carried out in a temperature-controlled growth room at a temperature of +26 °C, 18 h photoperiod and a light intensity of 10000 lux. The regenerated plants were removed from the test tubes, agar was cleaned off their roots, the leaves were shortened, and the plants were transplanted into pots for vegetative propagation in a greenhouse. The vegetatively propagated regenerants were transferred to the breeding nurseries.

Table 1. **Comparison of some morphological characters and agronomically valuable properties of common timothy R_0 plants-regenerants with the control plants**

Dotnuva, 1998–1999

Characteristics	LSD_{05}	Clone (No. 437) originated from the breeding material		Clone originated from the variety 'Gintaras II'	
		R_0	control	R_0	control
Time of 50% inflorescence emergence, points	1.1	7	5	7	5
Habitus at ear emergence, points	1.1	5	5	3	3
Length of longest stem (including inflorescence), cm	12.9	79.9	68.1	88.5	63.0
Length of flag leaf, cm	2.2	9.9	9.2	10.2	8.8
Width of flag leaf, cm	0.02	0.65	0.57	0.59	0.55
Length of upper internode of stem, cm	1.1	6.5	5.5	5.9	5.5
Number of stems per bush	112	235	265	279	238
Herbage yield, points	1.4	9	7	9	7
Length of inflorescence, cm	1.2	12.8	10.2	11.1	9.5
Susceptibility to leaf spots, points	1.1	3	5	3	5

Establishment of breeding nurseries. 16–32 plants of each number were transplanted in the field at 50×50 cm interwals. Reference plants were planted every 10th plot, with no replications. The following morphological characters or agronomically useful traits were assessed: regrowth in spring, herbage yield of the 1st and 2nd cuts, plant diameter and density, colour of inflorescences, leaf length and width, susceptibility to causal agents of mildew, rust and leaf spots etc. A 1–9 or 3–7 score system was used for assessment, where 1–3 means very low and low value of the character, 5 – moderate, 7–9 – very high or high value of the character [11].

Experimental results were processed by dispersion and statistical analysis methods [12].

Table 2. **Comparison of morohological and agronomically valuable characteristics of common timothy R_1 plants-regenerants with the control plants**

Dotnuva, 2000–2001

Characteristics	LSD_{05}	Clone (No. 437) originated from the breeding material		Clone originated from the variety 'Gintaras II'	
		R_1	control	R_1	control
Time of 50% inflorescence emergence, points	1.1	7	5	7	5
Habitus at ear emergence, points	1.1	5	5	3	3
Length of longest stem (including inflorescence), cm	12.9	60.9	65.2	55.5	53.0
Length of flag leaf, cm	2.2	8.9	9.2	8.2	8.0
Width of flag leaf, cm	0.02	0.57	0.56	0.54	0.55
Length of upper internode of stem, cm	1.1	5.6	5.5	5.1	5.5
Number of stems per bush	112	247	278	283	268
Herbage yield, points	1.4	7	7	7	7
Length of inflorescence, cm	1.2	9.8	10.2	10.1	9.5
Susceptibility to leaf spots, points	1.1	3	5	3	5

RESULTS AND DISCUSSION

Plants-regenerants from variety 'Gintaras II' and breeding material (number 437) were chosen for a more comprehensive testing in field conditions. Some morphological characters and agronomically valuable properties of R_0 plants-regenerants of common timothy were investigated (Table 1). Our investigations showed that R_0 regenerants markedly surpassed the control plants by such morphological characters as plant height, herbage yield, length and width of flag leaf, length of inflorescences and length of upper internode of stem. These findings obtained in our studies coincide with the investigations carried out

at our institute, in which the plants regenerated from somatic cells via tissue culture surpassed the donor cock's-foot plants by green grass yield and other agronomically valuable properties in R_0 generation [8]. Moreover, R_0 regenerants of common timothy had a longer growth season and were less susceptible to causal agents of leaf spots. Such characters as bush shape, number of stems per bush remained unchanged.

In order to check whether an increase in morphological characters and changes in agronomically valuable properties persist in other generations, we harvested seed from the regenerants and in the following year planted it in the breeding nursery. A comparison of morphological characters and agronomically valuable properties of R_1 plants-regenerants with the control plants is provided in Table 2. One can see that there was no increase in the qualitative morphological characters in R_1 plants-regenerants. The instability of some valuable qualitative morphological characters (plant height, herbage yield, length and width of flag leaf, length of inflorescences and length of the upper internode of stem) were observed in R_1 generation. An increase in the values of the characters of common timothy R_0 regenerants, in our understanding, was most likely influenced by growth stimulators, present in the regeneration medium.

The length of the growing season of R_1 plants-regenerants remained unchanged (the same as in R_0 generation), *i.e*. common timothy regenerants were later-maturing than the control plants, they were also less susceptible to the causal agents of leaf spots. Nevertheless, we suppose that the somatoclonal variants are a valuable source of genetic diversity in timothy breeding, because they enable in a relatively short time to develop individual plants with improved characters.

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N. Lemežienė, E. Lemežis

PAŠARINIŲ MOTIEJUKŲ R_0 IR R_1 KARTOS AUGALŲ-REGENERANTŲ MORFOLOGINIŲ IR ŪKIŠKAI NAUDINGŲ POŽYMIŲ TYRIMAS

S a n t r a u k a

Selekcinio darbo efektyvumą nulemia pradinės medžiagos genetinė įvairovė, kurią padidinti galima naudojant netradicinius selekcinius metodus, tokius kaip ląstelių kultūra. Šio darbo tikslas – palyginti R_0 bei R_1 kartos regenerantų bei kontrolės kai kuriuos morfologinius ir ūkiškai naudingus požymius lauke.

R_0 kartos regenerantai gerokai pralenkė kontrolę šiais morfologiniais požymiais: augalų aukščiu, žolės derliumi, vėliavinio lapo ilgiu, vėliavinio lapo pločiu, žiedynų ilgiu, stiebo viršutinio tarpubamblio ilgiu. Be to, R_0 kartos pašarinių motiejukų regenerantai pasižymėjo ilgesniu vegetacijos periodu, buvo mažiau jautrūs dėmėtligių sukėlėjų pažeidimams. R_1 kartoje augalų-regenerantų kiekybiniai morfologiniai požymiai padidėję nebuvo, tai yra R_1 kartoje augalai-regenerantai nesiskyrė nuo kontrolės. Tačiau R_1 augalų kartoje išliko nepakitusi (tokia pati kaip ir R_0 kartoje) augalų-regenerantų vegetacijos periodo trukmė – pašarinių motiejukų regenerantai buvo vėlyvesni už kontrolę, taip pat jie buvo mažiau jautrūs dėmėtligių sukėlėjams.