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USE OF SOMACLONAL VARIATION FOR OBTAINING OF BARLEY BREEDING SOURCE MATERIAL

Dace GRAUDA[1], Linda LEGZDIŅA[2], Isaak RASHAL[1]

[1]Institute of Biology, University of Latvia,
Salaspils, Miera Str. 3, Latvia
E-mail: dace@email.lubi.edu.lv, izaks@email.lubi.edu.lv

[2]Priekuļi State Institute of Plant Breeding
Priekuļi, Latvia
E-mail: lindaleg@navigator.lv

Abstract

Possibility to induce useful somaclonal changes for barley breeding was studied. Most widely grown Latvian barley variety 'Rūja' was used in the investigation. Regenerants were obtained from calli cultures of mature seed embryos. Somaclonal lines of R_5 and further generations were tested in field conditions for performance of agronomic traits. Lines with favourable combinations of changed traits (reduced vegetation period, better resistance to lodging and loose smut) were selected. Induced somaclonal variation has a potential for use in breeding programs as an additional source of variability.

Key words: barley, breeding, calli culture, somaclonal variation.

Introduction

Plant tissue culture is a source of new genetic variability, named as somaclonal variation /Larkin, Scowcroft, 1981/. This variability could be used for improving of some traits in well adapted crop varieties because genetic changes are obtained without hybridization and therefore adaptive gene complexes are not destroyed.

Causes of somaclonal variation are manifold and relate to both internal and external aspects of plant tissue culture (genotype, ploidy, regeneration system, time in culture, tissue source, and growth regulators). Three classes of the variation can be recognized: heritable stable, heritable unstable and non-heritable (epigenetic) variation /Kaeppler et al., 2000; Vázquez, 2001, Bednarek et al., 2007, Li et al., 2007/. From practical point of view it is important that many changes occur in agronomic traits, for example in disease resistance, heading and maturation dates, plant height, grain yield, grain quality etc. /Ahloowalia, 1987; Karp, 1994; Jain, 2001/ and rather high number of new varieties of different crops were developed based on the somaclonal variation /Jain, 2001; Li et al., 2001; Bashir, 2007/. However, the possibility to select and use somaclonal variants in breeding is depended on specific traits and on particular variety, and, therefore, unclear before concrete experiments. There is no guarantee that characters of specific interest after regeneration will vary, and even if those important characters would show variation in somaclonal progenies, whether observed changes be

in the needed direction. For example, in several reports there was described very limited or negative somaclonal variation in regenerated lines of barley /Dunwell et al., 1986; Baillie et al., 1992; Bregitzer et al., 2002/. In other publications there was found quite large variation, including favourable from the breeding point of view /Ullrich et al., 1991; Bregitzer, Poulson, 1995; Jain, 2001/.

'Rūja' is the Latvian spring barley variety, which is well adapted to the Latvian conditions having stable yield for many years and good lodging resistance. However, there are some undesirable traits of the variety: long vegetation period, susceptibility to loose smut and some leaf diseases. The aim of this work was to look for a possibility to achieve useful agronomical changes by somaclonal variation in the barley variety 'Rūja'.

Materials and Methods

Somaclonal lines were obtained from calli cultures of the variety 'Rūja' initiated from mature seed embryos. Homogenous seed material multiplied from a single plant was used. The seed sterilization was done according to previously described method /Ornicane, Rashal, 1997/. Two mm wide middle parts of mature seed embryo were used as explants. For callus initiation explants were placed on the Murashige and Skoog (MS) medium with 2 mg l^{-1} 2,4-dichlorphenoxyacetic acid (2,4-D) and 10 mg l^{-1} or 20 mg l^{-1} silver nitrate ($AgNO_3$). As a control the MS medium supplemented only by 2,4-D was used. For calli initiation 300 explants were placed on each medium. Calli were grown two-three weeks in the dark under 26 ^oC and were cultivated for 1-2 months on the same media in the light (16 h day/8 h night, 24 ^oC). For plant regeneration calli were transferred on the regeneration medium: MS medium supplemented by kinetin 1 mg l^{-1}, naphtylacetic acid (NAA) 0.5 mg l^{-1} and antioxidant fenoxan 20 mg l^{-1}. Calli and subsequent plants were grown in light conditions (16 h day/8 night 24 ^oC). Regenerated plants (R_1) at the stage of 2–3 leaves were planted in pots and grown in a climatic chamber (16 h day/8 h night, 18–22 ^oC) till harvesting.

46 seeds of R_2 generation were obtained from R_1 plants. Theeir progenies were multiplied as a bulk (lines) next two generations (R_3 and R_4). Starting R_5 lines were tested in breeding plots in field conditions for agronomic traits performance. Testing was conducted during 2003 (R_5 – 46 lines), 2004 (R_6 – 20 lines) and 2005 (R_7 – 6 lines). Each year the best lines were selected for the testing next year.

In 2003 the lines were grown in 0.2–2 m^2 plots depending on available amount of seeds, in 2004 plot size was 2 m^2, in 2005 plot size was 6.4 m^2. Experiments were arranged in the random block design with two (2004) or three (2005) replications. Elite seed material of the variety 'Ruja' was used as a control. Grain yield, plant height, length of period from sowing to flowering, 1000 grain weight, grain quality (volume weight, content of crude protein and starch) and disease infection were evaluated. Content of crude protein and starch was determined by the express method (Infratec 1275 Analyser). Infection with powdery mildew (*Blumeria graminis* f.sp. *hordei*) and net blotch (*Drechslera teres*) was scored according to scale 0–4 (0 – no infection, 4 – very high infection). Spikes infected by loose smut (*Ustilago nuda*) were counted after flowering. Duration of vegetation period, plant height, ear length and number of grains per spike were recorded.

The growing conditions of plants in field experiments were: sod-podzolic loamy sand, pH_{KCl} 5.3–5.9, plant available P_2O_5 and K_2O was high, organic matter 14–19 g kg^{-1}, previous crop potato, fertilizers applied N 90–96 kg ha^{-1}, P_2O_5 45–60 kg ha^{-1} and K_2O 45–90 kg ha^{-1}.

Data were analysed according to the standard statistical methods including one or two way ANOVA. Somaclonal lines were compared with the control variety by the Least Significant Difference (LSD) at the 0.05 level.

Results and Discussion

The variety 'Ruja' had a rather low (10.1 %) calli initiation capacity (Table 1) in comparison with the variety 'Abava' previously used in our investigations (21 % of explants on control media) /Ornicane, Rashal, 1997/.

Table 1. Calli iniation and regeneration capacity of the variety 'Rūja' depending on the concentration of silver nitrate in calli induction media
1 lentelė. *Veislės 'Rūja' kaliaus iniciacijos ir regeneracijos priklausomumas nuo sidabro nitrato koncentracijos kaliaus indukcijos terpėse*

Calli initiation media *Kaliaus iniciacijos terpė*	Calli, % from explants *Kalius % nuo eksplantų*	Plants-regenerants, % from calli *Augalai-regenerantai % nuo kaliaus*
MS	10.1	0
MS + $AgNO_3$ (10 mg l^{-1})	41.0	20.5
MS + $AgNO_3$ (20 mg l^{-1})	53.9	0

The use of silver nitrate substantially increased the frequency of obtained calli and had also influence on calli regeneration. Only calli cultivated on media with 10 mg l^{-1} $AgNO_3$ developed plants-regenerants. Addition of 20 mg l^{-1} $AgNO_3$ to the calli induction media had negative influence on regeneration process: calli grown on this media formed only roots. Supplementation of the regeneration media with antioxidant fenoxan, which was found earlier as an effective substance for obtaining plants-regenerants /Ornicāne, Rashal, 1998/, was effective only in the case of addition of 10 mg/l of silver nitrate to the calli induction media. This combination of the calli induction and regeneration media gave a possibility to obtain fertile somaclonal plants-regenerants of the variety 'Rūja'.

Results of the field evaluation of somaclonal lines are presented in Tables 2 and 3. Considerable variation among the somaclonal lines in some economically important traits was found in R_5 generation. Some of lines differed from the variety 'Rūja' in one or several tested traits. Main attention was paid to changes in the duration of vegetation period, powdery mildew resistance, yield and lodging resistance (Table 2). Six lines had a higher yield than the variety 'Rūja' and four lines lower. Two lines had both reduced vegetation period and higher resistance to powdery mildew, one line had reduced vegetation period, higher resistance to powdery mildew and better lodging resistance. These lines were most interesting from the breeding point of view.

Table 2. Number of R_5 lines with substantial deviations in powdery mildew resistance, vegetation period and yield in plus ("+") and minus ("–") directions in comparison with the variety 'Rūja'

2 lentelė. *R_5 linijų skaičius su dideliais nukrypimais pagal atsparumą miltligei, vegetacijos periodą ir derlių plius ("+") ir minus ("–") kryptimis, palyginti su veisle 'Rūja'*

Number of lines *Linijų skaičius*	Yield *Derlius*	Vegetation period *Vegetacijos periodas*	Lodging resistance *Atsparumas išgulimui*	Powdery mildew resistance *Atsparumas miltligei*
2		-		
2		-		+
4			+	
1		-	+	+
7				+
6	+			
4	-			
Total / *Iš viso*	26			

20 R_5 lines with favourable changes of agronomically important traits were selected for the evaluation in R_6, six – in R_7 generation. Three lines showed inheritance of reduced number of days till heading and resistance to loose smut till R_7 (Table 3). Other lines did not retain significant differences from the variety 'Rūja' in the traits of interest.

Table 3. Quantitative traits of some somaclonal lines, derived from the variety 'Rūja', in 2003–2005

3 lentelė. *Kai kurių somakloninių linijų, gautų iš veislės 'Rūja', kiekybinės savybės*

Variety, lines *Veislė, linija*	Days till heading *Dienos iki plaukėjimo*			Infection severity of loose smut (infected spikes per m²) *Užsikrėtimo dulkančiosiomis kūlėmis intensyvumas (užsikrėtusių varpučių m²)*			Yield t ha^{-1} *Derlius t ha^{-1}*		
	2003	2004	2005	2003	2004	2005	2003	2004	2005
Rūja	59	69	67	7	32	6	6.00	6.15	4.06
Sm99.1.8	53	65*	60*	0	21*	22*	6.50	6.84*	3.91
Sm99.1.9	52	65*	60*	0	16*	16*	6.47	5.98	3.80
Sm99.7.2	59	69	67	0	1*	4*	6.60	5.77	3.33*

* Significant difference from the variety 'Rūja' at $P \leq 0.05$

** Esminiai skirtumai nuo veislės 'Rūja' esant $P \leq 0,05$*

Nevertheless, that there was high level of variation of investigated traits due to different environmental conditions among the growing seasons, all years we found several lines had significant differences from the initial variety 'Rūja'. Decreasing number of such lines in consecutive generations suggests that not all of them are true somaclons. For three lines – Sm99.1.8, Sm99.1.9 and Sm99.7.2, which differed significantly from the initial variety till R_7 generation (Table 3), there is a rather high

probability that they are the result of stable genetic changes initiated during the cultivation in callus culture. The lines Sm99.1.8 and Sm99.1.9 are derived from the same R_1 plant that can suggest the one somaclonal event during the calli cultivation.

Conclusions

1. Combination of addition in the optimal concentrations of silver nitrate and antioxidant fenoxan to calli induction and regeneration media, respectively, gave a possibility to produce fertile plants from mature embryos calli cultures of barley variety 'Rūja'.

2. Considerable variation in some important agronomic traits has been observed among the lines originated from plants regenerated from calli cultures.

3. Lines with favourable changes, for example with reduced number of days till heading and higher resistance to loose smut, were selected. This fact proves that there is a potential to exploit somaclonal variation in applied breeding programs as an additional source of different trait variability.

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SOMAKLONINĖS VARIACIJOS NAUDOJIMAS MIEŽIŲ SELEKCINEI MEDŽIAGAI GAUTI

D. Grauda, L. Legzdina, I. Rashal

Santrauka

Tirta galimybė indukuoti naudingus somakloninius pokyčius miežių selekcijai. Naudota plačiausiai auginama latviška miežių veislė ‘Rūja’. Regenerantai buvo gauti iš subrendusių sėklų gemalų kaliaus kultūrų. R_5 ir tolesnių kartų somakloninių linijų agronominės savybės buvo tiriamos lauko sąlygomis. Buvo atrinktos linijos su pakeistų savybių palankiomis kombinacijomis (sutrumpėjęs vegetacijos periodas, geresnis atsparumas išgulimui ir dulkančiosioms kūlėms). Indukuota somakloninė variacija gali būti naudojama selekcinėse programose kaip papildomas įvairovės šaltinis.

Reikšminiai žodžiai: miežiai, selekcija, kaliaus kultūra, somakloninė variacija.