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THE EFFECTS OF GROWTH REGULATORS ON SOMACLONAL VARIATION IN RYE (*Secale cereale* L.) AND SELECTION OF SOMACLONAL VARIANTS WITH INCREASED AGRONOMIC TRAITS

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Abstract: The aim of this research was to characterize somaclonal variation in populations derived from embryos cultured on two types of induction medium (supplemented with either 2,4-D or dicamba), as well as to select and characterize several somaclonal lines.. The sexual progenies of 40 R_0 regenerants - A somaclones (derived on the medium with 2,4-D) and B somaclones (derived on the medium with dicamba) - were analysed according to the following traits: plant height, total number of tillers, number of productive tillers, spike length, number of spikelets per spike, spike compactness, number of normally developed grains per spike, weight of grains per spike, and the weight of 1000 grains. The results for twenty-two R_1 plants surpassed the variability range for the control. The transmission of positive changes to the next generation was proved in the case of 8 originally chosen R_1 plants: 7 plants selected from the A somaclones and one plant from the B somaclones. Five out of the eight created somaclonal lines proved to be stable somaclonal variants. The absolute rate of the efficiency of positive somaclonal changes was calculated as 0.64%.

Key Words: Rye, *Secale cereale*, Regeneration, Somaclonal Variation, Somaclonal Line

INTRODUCTION

Rye is one of the most important cereals in middle and eastern Europe. Recently, interest in this crop has increased because of its dietary value. However, the

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breeding value of rye is still low in comparison with other cereals such as wheat or trticale. Therefore, an intensive breeding making use of biotechnological techniques is highly justified. Somaclonal variation, a phenomenon occurring during *in vitro* culture [17], can result in new, improved forms, which can enrich breeding materials, as shown in such cereals as triticale [13], wheat [7], barley [6], maize [18], rice [1] and oats [8]. More than twenty somaclonal cultivars of different species (e.g. rice, maize, tomato, potato, red pepper, strawberry) are currently registered on the open market [26, 31]. Somaclonal variation in rye has also been observed, and has yielded some characteristics, including spike compactness, number and weight of grains per spike, 1000 grain weight, and grain yield per plant [2, 3, 28].

The main goal of this study was to characterize somaclonal variation in populations derived from embryos cultured on one of two types of induction medium, supplemented either with 2,4-D or dicamba. The secondary goal was to select and characterize several somaclonal lines which might be useful as a potential source for rye breeding. The induction of somatic embryogenesis from immature embryos of rye is much less effective than in the case of other cereals and requires a higher concentration of the auxins - 2,4-D or dicamba [27, 33] which can additionally stimulate somaclonal variation.

MATERIALS AND METHODS

The plants of a highly inbred (inbreeding degree S10 - S11) winter rye line, L318, bred in our Department were used as donor material. This completely self-compatible line was characterized as having a high breeding value because of its high grain yield, relatively high 1000 grain weight and compact, well filled spike, but it has a low number of productive stems [32]. Donor plants were grown under standard experimental field conditions [32]. For regeneration, the spikes were harvested 19 days after self-pollination, and isolated embryos cultured *in vitro* on a medium supplemented with 3 mg/dm^3 2,4-D (2,4-dichlorophenoxyacetic acid) as described previously [27] or under the same conditions, but with an inducing medium containing 2.5 mg/dm^3 dicamba (3,6-dichloro-2-methoxybenzoic acid) (DUCHEFA, Haarlem, The Netherlands) instead of 2,4-D. R_0 plants were transferred into soil in the autumn and self-pollinated the following year. The progeny of R_0 plants derived from single embryos cultured on the medium with 2,4-D were designated as A somaclones, and the plants derived from embryos cultured on the medium with dicamba - as B somaclones. Each somaclone consisted of 20-30 R_1 plants. The A and B somaclones were grown in two different vegetation seasons and compared with the control plants (30 plants of line L318) grown in the same period and under the same field conditions.

The following traits were measured: plant height (plh), total number of tillers (ttl) number of productive tillers (prtl), spike length (spl), number of spikelets per spike (spsp), spike compactness - spl/spsp x 100 (comp), number of normally developed grains per spike (grns), weight of grains per spike (grws), and weight of 1000 grains (1000grw). The values of spl, spsp, grns, grws and 1000grw were counted as a mean from three, randomly-chosen self-pollinated spikes.

Plants chosen from both groups of somaclones were self-pollinated to obtain a second generation of somaclonal lines (SLs), which were characterized according to the same traits mentioned above. The SLs derived from the A somaclones consisted of plants which survived a winter period in the field (a long and frosty winter) and plants which were transplanted from the vernalisation room in the spring. For each SL, there were different numbers of plants in each group, so they were individually compared with the appropriate control population (line L318), made up of naturally and artificially vernalised plants in the same proportion.

The transmission of positive somaclonal changes was evaluated in subsequent progenies R_3 and R_4. Each somaclonal family (SF) of R_3 and R_4 was represented by several sublines (consisting of 30 - 60 plants) obtained after the self-pollination of plants selected from the SLs.

The following statistics were used to assess the characteristics of quantitative traits: mean, standard deviation (sd) and range (min, max). The homogenity of trait variances in the somaclones and line L318 was estimated, based on Bartlett tests. ANOVA was used for the comparison of trait means.

RESULTS AND DISCUSSION

Characteristics of R_0 regenerants

R_0 plants of both groups of regenerants survived the winter period, but they were decidedly notably weak. The differences between regenerants derived on the two media were not clearly distinguishable in this generation. Some of the A and B regenerants (6 out of 30 and 5 out of 21 respectively) failed to set any grain. The rest of the plants in both groups produced a reduced number of seeds, usually less than 5 normally developed seeds per spike. No such fertility reduction was observed for the control plants. Many factors leading to the weakness of the rye regenerants could be considered. One of them seems to be the regeneration procedure applied. This procedure allows for transplanting regenerants into the field in mid-October at the earliest, when the temperature is too low to support the proper growth of plants derived *in vitro*. This phenomenon was not reported on in previous papers concerning somaclonal variation in rye. However, the experiments described in said studies were done under completely different field conditions, in Spain [19, 20], Greece [2, 4] and Great Britain [25], where regenerants seem to adapt to a much better degree. Another possible reason could be recessive, homozygotic mutations in different groups of polygenes. Mass variation and homozygotic mutations have been mentioned as being specific for somaclonal variation [10, 14].

However, it seems rather doubtful that these two phenomena occured simultaneously. The majority of plants set few grains, while some did not set any seed. An explanation for this phenomenon could be the cytological changes emphasized by Bebeli et al. [4] and Linacero and Vazquez [19]. An additional explanation for the reduced fertility of R_0 plants could be mutations in the genes coding for self-compatibility, e.g. the epistatic gene *Sp* [29].

The characteristics of the chosen quantitative traits of R_1 A and B somaclones

Twenty-four of the A somaclones and 16 of the B somaclones were analysed according to the previously-listed quantitative traits. The mean values of most of the analysed traits in both groups of somaclones were lower than in the control (Tab. 1).

Only one trait in the A somaclones (ttl) and one trait in the B somaclones (comp) had significantly higher mean values. A significant reduction (in comparison with the A somaclones) in the mean values of the rest of the analysed traits was observed in the B somaclones. This especially concerned the traits connected with the grain yield, like grns, grws and 1000grw.

The higher reduction in the mean values of most of the traits and a low number of "better" plants in the B somaclones could be the result of a stronger mutagenic or physiological effect of dicamba in spite of the lower concentration of this growth regulator in the induction medium. It is generally considered that 2,4-D is an important factor in the induction of somaclonal variation [11, 22, 23]. However, other auxins can also induce somaclonal variation. Hitomi et al. [12] found NAA to induce somaclonal changes in eggplant more effectively than 2,4-D. Almost nothing is known about the mutagenic effect of dicamba, as no experiment similar to those presented here has been conducted till now. Only a suggestion can be found in a paper published by Bregitzer et al. [5], in which the authors put forward the idea that dicamba may be a stronger mutagenic factor than 2,4-D. However, this hypothesis was not based on experimental data.

In both groups of somaclones, single plants were found which had trait values higher than the upper value of the control variability range or control means, with regard to one or more traits (in the case of plh, the lower values were treated as "better" because the reduction of plant height is one of the goals in rye breeding). The number of such plants was significantly higher in the A somaclones (21; 3.4%) than in the B somaclones (1; 0.3%). Many other studies dealing with somaclonal variation contain an observation similar to ours; that there is a decreased value of most of the traits in the majority of the plants, and simultaneously a presence of single plants with trait values overlapping with those of the control. Among others, this has been found for rye [2, 28], wheat [30], triticale [13], barley [6], and oat [8].

Tab. 1. The characteristics of chosen traits of the A and B somaclones and the appropriate control populations

Trait[1]	Statistics	Somaclones A	Control A	Somaclones B	Control B
plh [cm]	mean value	106.0**[a]	114.3	79.3**	95.0
	sd	8.9**	6.6	14.0**	7.59
	min	80.0	95.0	27.0	80.0
	max	131.0	125	102.0	113.0
ttl	mean value	19.6*[b]	16.7	6.2**	13.0
	sd	9.5	9.5	3.1	3.57
	min	3.0	6.0	1.0	7.0
	max	61.0	51.0	24.0	24.0
prtl	mean value	17.7	15.5	3.5**	11.47
	sd	8.6	8.3	2.5	3.10
	min	3.0	6.0	0.0	6.0
	max	56.0	46.0	13.0	21.0
spl [cm]	mean value	7.42**	8.82	6.92**	9.58
	sd	0.96**	0.83	1.42**	1.87
	min	4.50	6.70	3.50	6.00
	max	10.90	11.50	9.50	12.83
Spsp	mean value	24.74**	29.84	21.78**	26.43
	sd	2.91	2.83	4.48	4.59
	min	16.00	23.33	13.50	23.17
	max	34.00	36.00	30.00	30.67
comp [%]	mean value	33.51	33.90	31.50**	28.65
	sd	2.56*	2.30	3.07	3.89
	min	22.46	26.92	10.77	14.81
	max	45.88	38.36	46.67	48.89
Grns	mean value	21.21**	35.59	17.58**	32.56
	sd	8.57**	9.33	7.63	7.59
	min	0.00	8.67	1.00	14.67
	max	45.33	52.67	33.00	46.00
Grws [g]	mean value	0.642**	1.245	0.412**	1.001
	sd	0.284**	0.387	0.234**	0.308
	min	0.013	0.443	0.013	0.421
	max	1.657	2.219	1.094	1.856
1000grw [g]	mean value	25.073**	33.482	21.967**	30.362
	sd	6.714	6.801	5.906**	4.590
	min	7.184	18.472	6.667	19.558
	max	53.000	57.500	39.185	38.455

1) abbreviations of trait names explained in “Materials and methods”

a) ** - statistically significant differences at p=0.01

b) * - statistically significant differences at p=0.05

Characteristics of R_1 plants with positive somaclonal changes

Among 22 selected plants some were characterized as having one or two traits at a higher level than the control upper value, and several traits (usually 3-5) higher than the control mean value. Although in many cases the value of the analysed traits was not above the upper level, such an aggregation of positive traits in a single plant was rarely observed in line L318. One of the oft emphasized characteristics of somaclonal variation, particularly useful in breeding, is the mass changes of different quantitative changes [10, 14, 15, 17]. Plants A14, A16, A18, A20 and A21 seemed to be especially valuable because of their high level of yield-forming traits.

Analysis of transmission of somaclonal changes to the next generation

Twenty-two somaclonal lines obtained after the self-pollination of selected plants were compared to the control. In seven out of 21 A somaclonal lines (33.3%) - SLA6, SLA7, SLA11, SLA12, SLA14, SLA16 and SLA17 the mean value of at least one trait was significantly higher than that of the control. Three positive changes: reduced plh, increased comp, and 1000grw were transmitted in SLB1. The most stable change was increased comp, transmitted in almost all the lines.

Tab. 2. The characteristics of somaclonal lines R_2 derived from the A and B somaclones

Somaclonal line	Trait								
	plh [cm]	ttl	prtl	spl [cm]	spsp	comp [%]	grns	grws [g]	1000grw [g]
SLA7	92.59 <*[a]	10.24 >**[b]	8.71 >**	6.70	22.04	33.00 >*	19.43	0.334 >*	15.166
SLA11	91.64	15.71	13.11	6.50 <*	21.05 <*	32.51 >*	18.92	0.379	17.320
SLA12	102.60	25.20 >**	19.60 >**	7.70	27.60 >**	35.91 >**	24.94	0.604	24.272 >*
SLA14	91.37	14.30	11.77	6.52 <*	21.02	32.64 >*	20.05	0.412	15.960
SLA16	102.96	18.04	14.54	7.30	22.26	30.56	22.79	0.587	25.651 >**
SLB1	83.07 <**	6.70 <**	4.78 <**	7.14 <*	22.63 <*	31.26 >**	27.26	0.729	28.108 >*

[a] < - mean value significantly lower than in the control at 0.05 (*) and 0.01 (**)

[b] > - mean value significantly higher than in the control at 0.05 (*) and 0.01 (**)

Other positive changes selected in the rest of the plants were found to be of epigenetic nature, probably caused by the physiological effect of *in vitro* culture, or temporary changes, like DNA methylation, amplification (deamplification) or retrotransposons activity, pointed out by many authors as mechanisms of transient variation [10, 14, 24]. The activation of retrotransposons during plant regeneration was also shown in rye [21].

Five A Sls and one SLB were selected for further experiments. Their characteristics are given in Tab. 2. Three lines - SLA11 SLA12 and SLA16 were chosen because they had single traits with high values (comp, 1000grw), and the rest of the SLs as a potential source for improvement of other rye characteristics. SLA12 seemed to be the most promising, because it displayed increased values for five of its traits, among them such important traits as productive shoot number and 1000grw. These two characteristics are usually negatively correlated in rye [32].

Tab. 3. The characteristics of somaclonal families (SFs) R_3 and R_4 derived from the A and B somaclones

Maternal SL	Number of sublines analysed in each SF		Mean values of the traits used as selection criterion	
	SFs R_3	SFs R_4	SFs R_3	SFs R_4
SLA7	9	12	plh - 90.13 cm <**[a]	plh - 98.19 <*
			ttl - 10.67 <*	ttl - 7.4 <*
			prtl - 8.63 <*	prtl - 5.4
			comp - 33.13 % >*[b]	comp - 29.99 % >*
			grws - 33.73 g	grws - 32.84 g
SLA11	7	11	comp - 35.77 >**	comp - 34.35% >**
SLA12	7	12	ttl - 12.93	ttl - 18.20
			prtl - 10.67	prtl - 14.00
			spsp - 26.72	spsp - 28.44
			comp - 34.46 % >**	comp - 31.38 % >*
			1000grw - 32.227 g	1000grw - 34.008g >*
SLA14	9	10	comp - 33.49 % >*	comp - % 35.35>*
SLA16	11	10	1000grw - 32.585 g >*	1000grw - 34.872g >*
SLB1	11	13	plh - 92.50 cm <*	plh - 92.35 cm <*
			comp - 42.33 % >**	comp - 35.74 % >*
			1000grw - 35.676 g >*	1000grw - 35.292 g >**

[a] < - mean value significantly lower than in the control at 0.05 (*) and 0.01 (**)

[b] > - mean value significantly higher than in the control at 0.05 (*) and 0.01 (**)

Somaclonal variation was monitored over the two next sexual generations, R_3 and R_4; the results of these experiments are summarised in Tab. 3. Such a long period is necessary as it was shown in several cases that potential somaclonal changes can disappear over several subsequent progenies [24].

The experiments on somaclonal variation in rye presented here have resulted in the production of several potentially valuable somaclonal lines. The stability of somaclonal changes has been demonstrated through four subsequent sexual generations. At least six out of the 942 R_1 individuals proved to be somaclonal variants with stable positive changes, so the rate of efficiency of positive somaclonal changes (PSCR) could be calculated as 0.64%. Of course, it is not an absolute somaclonal variation frequency, as only the positively changed plants were of interest to us. Although this rate seems rather low, it is higher than that in some other works on the selection of positive somaclonal variants. In maize, Lazanyi et al. [18] began with 13850 R_1 plants: 62 were characterized as having reduced plant height, 13 - earlier flowering time and 37 - later flowering time. These changes proved to be sexually inherited in the progenies of six plants with reduced height and two plants with earlier flowering time. Eight lines selected from 13850 R_1 plants gives a PSCR of 0.06%. A similarly low efficiency of positive somaclonal change inheritance was found in rice [1], oats [8], wheat [7] and *Lotus corniculatus* [9].

Obviously it is possible to generate new forms of rye using traditional methods, nor biotechnological ones. Although many years of searching for chemically-induced mutagenesis in rye led to the obtaining of many mutants, no forms with increased agronomic value were found [16].

We expect new somaclonal lines to enrich our genetic variability resources. In addition, it appears that while dicamba may be a better growth regulator to induce plant regeneration, it seems to cause more negative changes, probably the result of physiological effects, in derived populations than when 2,4-D is applied.

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