Sel'skokhozyaistvennaya Biologiya [Agricultural Biology], 2011, № 5, p. 91-95

UDC 633.18:631.527:581.143.6

ESTIMATION AND SELECTION OF LINES WITH IMPROVED ECONOMIC IMPORTANT DETERMINANTS IN POPULATIONS OF RICE SOMACLONES

***V.A. Kovalevskaya*[1], *V.N. Zmeeva*[2], *S.S. Lysakovskaya*[1], *Yu.N. Zhuravlev*[2], *L.D. Seletskaya*[2], *A.V. Shubina*[2], *T.I. Muzarok*[2]**

[1] *Primorsky Research and Development Institute of Agriculture, RAAS, Primorsky territory, Ussuriisk region, Timiryazevsky settlement 692539, Russia*
e-mail: fe.smc_rf@mail.ru
[2] *Institute of Biology and Soil Science, FEB RAS, Vladivostok 690022, Russia*
e-mail: zmeeva@ibss.dvo.ru

Received August 27, 2009

S u m m a r y

In tissue culture of rice varieties of Far Eastern selection the authors isolated the somaclonal variants, estimated the variability of determinants in regenerants and selected economic important variants in population of somaclones. The high productive and dwarf lines were isolated, which can be used as initial material in subsequent selection process.

Keywords: plant breeding, rice, in vitro tissue culture, line, cultivar, somaclonal variants, somaclonal variation, productivity, shot stem.

Far East of Russia is the northern boundary of growing rice in our country and in the world (1). Rice fields occupy about 60 thousand hectares of this territory. In this region, the sum of effective temperatures during a growing season amounts to 2100-2500 °C, the probability of heat supply from year to year reaches 80-100% while a relatively favorable soil, climate and sufficient water supply. More than a half-century practice of growing rice in Primorye has confirmed it as a crop with mostly high and stable yields (60-70 t/ha). However, growing season of rice is limited by duration of warm season and insolation deficit; these conditions satisfy the biological requirements of only early-ripening varieties whose growing season is less than 112 days (2, 3). Along with it, monsoon climate contributes in some years to the spread of rise blast fungus – the most harmful disease of this crop (4).

The major direction of breeding rice in the Far East of RF is creation of early-ripening cultivars adapted to local growing conditions and combining cold resistance, stably high grain production, improved technological properties of grain, lodging resistance and immunity to blast disease. To obtain such varieties, breeders perform mainly interspecific and intraspecific hybridization of distant eco-geographic forms with further selection of valuable genotypes (5). In recent years, biotechnological approaches such as creation of somaclonal variants are widely used to obtain initial material (6-10).

Phenotypic diversity of plants regenerated from somatic cells in vitro was called a somaclonal variation (11). Somaclonal variants, as a rule, differ from the original cultivar in a number of valuable properties including such agriculturally important traits as term of booting, plant height, number of productive shoots, number of spikelets in the main panicle, grain weight per plant and shedding of spikelets (12-14). Regenerated plants often demonstrate improvement or worsening of individual traits. Most of the modified traits are reproduced in progeny, which suggests the possibility of using somaclonal variation in selection (12-14). It has been also shown the repeatedly increased efficiency of breeding programs combining classical and biotechnological techniques.

The purpose of this study was obtaining somaclonal variants in tissue culture of rice cultivars of the Far East selection along with assessing the diversity and selection of promising samples in populations of regenerated plants.

Technique. The initial material was represented by rice cultivars obtained in Primorsky Research and Development Institute of Agriculture and passing the competitive tests: Primorsky 1222, Primorsky 1461, Primorsky 390, Primorsky 1531 and Primorsky 1193. Mature grains were pre-treated: glumes were pilled off, the grains were sterilized with 0,2% diocidum solution, 3-5 times washed with distilled water and left to swell for 18 h in the last portion of water. Then the grains were put in test tubes on the surface of MS agar (15) containing 4,0 mg/l 2,4-dichlor-phenoxyacetic acid (2,4-D). Calli were formed on sterile seedling tissues. The resulting callus tissue was passaged 3 times on the medium of a similar composition with lower content of 2,4-D (3,0 mg/l) and then transferred on MS-based regeneration medium. One variant of the medium was hormone-free, three other variants contained the inducer of morphogenesis 6-benzylaminopurine (6-BAP) at contents of 2,0; 3,0 and 4,0 mg/l. Somaclones P_0 regenerated from the calli were transplanted in soil (firstly in cups, later in large vessels) and grown in a greenhouse up to the generative phase. The harvest from each regenerated plant was collected separately. The progeny of one somaclone P_0 was defined as a line. The first seed generation obtained from seeds of regenerants P_0 was grown on vegetative test sites with lysimeters. Breeding assessment was performed according to methodological guidelines (16, 17). Biometric parameters were recorded: plant height, productive tillering, height of the main panicle, number of fertile and sterile spikelets on the main panicle, weight of grain from the main panicle and grain weight per plant. Each somaclonal line was represented by 30-70 plants, the original cultivar (control) – by 30 plants.

Statistical processing of data included the analysis of variance and Fisher's exact test with significance level 0,05 (18).

Results. Phenotypic similarities and differences of somaclonal lines and initial varieties were assessed by comparison of mean values of each studied commercially valuable trait. This comparative study revealed in cv Primorsky 1222 and its somaclonal lines the absence of significant differences in all studied traits except the number of sterile spikelets (somaclones developed was much more sterile spikelets) (Table 1). Somaclonal lines of Primorsky 1461 manifested reliably lower plant height than the cultivar (30%) as well as height of the panicle, the number of spikelets and fertile spikelets, and grain weight. Differences in other traits were not reliable. Inter-line means of most of the studied traits in somaclonal lines of the genotypes Primorsky 390, Primorsky 1531 and Primorsky 1193 were also inferior to those of the initial varieties; at the same time, significant differences were detected in several somaclonal genotypes: in Primorsky 390 - by the weight of grain from the main panicle, in Primorsky 1531 – by grain weight, the number of spikelets and number of fertile spikelets in the main panicle, and in Primorsky 1193 - by height of the panicle.

However, mean values of inter-line coefficients of variation (Cv) of all studied traits (except sterility) in four somaclonal variants the genotypes Primorsky 1461, Primorsky 1531, Primorsky 1193 and Primorsky 390 exceeded (in some cases reliably) the corresponding parameters of the initial varieties. Only in the somaclonal line of Primorsky 1222 this parameter wasn't reliably different from that in the initial cultivar, or it was lower.

1. Mean values and coefficient of variation (*Cv*) of major commercially valuable traits in rice cultivars and somaclonal lines (grown on vegetation test sites with lysimeters)

Initial cultivar and somaclonal lines derived from it	Plant height, cm		Main panicle										Productive tillering		Grain weight per plant, g	
			Length, cm		Number of spikelets, pcs.		Number of sterile spikelets, pcs.		Number of fertile spikelets, pcs.		Grain weight, g					
	X±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %
Primorsky 1222	77,9±7,0	9,0	14,5±2,1	14,6	78,1±14,0	18,0	5,9±3,3	56,2	73,3±15,0	20,4	2,3±0,5	23,5	1,5±0,5	35,1	3,0±0,9	29,7
9 lines	77,9±4,8	6,1	14,3±0,6	4,2	83,3±9,2	11,0	15,4±3,0	19,5	68,1±8,4	12,3	2,2±0,3	16,8	1,6±0,6	38,0	2,9±1,0	34,3
Primorsky 1461	91,1±3,1	3,4	16,9±1,6	9,5	101,1±11,5	11,3	5,9±2,6	44,3	95,3±11,3	11,9	2,9±0,4	12,6	1,4±0,5	35,9	3,8±1,2	32,0
10 lines	63,6±8,5	13,4	11,6±1,5	12,7	56,2±14,0	24,9	9,3±3,3	35,7	46,9±11,1	23,7	1,3±0,4	27,8	3,3±1,2	38,1	3,2±1,1	36,1
Primorsky 390	55,5±3,7	6,6	11,1±0,7	6,3	54,5±8,0	14,6	6,5±3,5	53,5	47,7±6,5	13,7	1,4±0,2	12,1	1,0±0,0	0	1,4±0,2	12,1
8 lines	50,0±5,8	11,4	9,9±0,7	7,3	47,5±8,4	17,8	10,0±3,5	34,9	37,5±7,0	18,5	1,1±0,2	20,6	1,4±0,3	18,7	1,3±0,3	23,4
Primorsky 1531	86,4±3,1	3,6	16,1±0,9	5,5	118,0±12,5	10,5	12,8±6,2	48,6	105,9±10,2	9,7	2,6±0,4	15,3	1,2±0,4	35,1	2,8±0,4	15,8
7 lines	79,0±6,3	7,9	14,5±1,5	10,0	69,5±12,5	18,0	11,6±9,1	78,3	57,8±13,9	24,0	1,8±0,5	27,7	1,4±0,5	34,7	2,2±1,0	44,1
Primorsky 1193	73,5±5,8	7,8	15,0±1,0	6,9	63,1±11,7	18,5	17,1±9,4	54,7	46,0±12,1	26,4	1,6±0,3	21,8	1,0±0,0	0	1,6±0,3	21,8
10 lines	66,0±8,8	13,4	11,6±1,1	9,6	39,7±11,7	29,4	8,0±3,9	48,5	31,8±9,9	31,0	1,0±0,3	27,7	1,2±0,2	15,6	1,1±0,3	25,6

3. Characteristics of rice cultivars and somaclonal lines with improved commercially valuable traits (grown on vegetation test sites with lysimeters)

Initial cultivar and somaclonal lines derived from it	Plant height, cm		Main panicle										Productive tillering		Grain weight per plant, g	
			Length, cm		Number of spikelets, pcs.		Number of sterile spikelets, pcs.		Number of fertile spikelets, pcs.		Grain weight, g					
	X±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %	X±tS	*Cv*, %	*X*±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %	*X*±*tS*	*Cv*, %
Primorsky 1222	77,9±7,0	9,0	14,5±2,1	14,6	78,1±14,0	18,0	5,9±3,3	56,2	73,3±15,0	20,4	2,3±0,6	25,7	1,5±0,5	35,1	3,0±0,9	29,7
line [1] 113	79,0±2,9	11,8	14,9±0,8	5,6	90,3±13,5	15,0	12,0±5,0	41,8	78,3±12,5	15,9	2,5±0,3	13,3	2,6±0,5	19,7	4,6±1,1	23,8
line [1] 121	82,4±7,2	8,7	14,1±0,8	5,9	98,7±14,4	14,6	18,1±6,2	38,3	80,5±11,6	14,4	2,8±0,6	20,7	2,6±0,8	32,2	4,4±1,7	38,0
Primorsky 1461	91,1±3,1	3,4	16,9±1,6	9,5	101,1±11,5	11,3	5,9±2,6	44,3	95,3±11,3	11,9	2,9±0,4	12,6	1,4±0,5	35,9	3,8±1,2	32,0
line [1] 181	63,1±3,3	5,2	12,3±1,4	6,3	62,8±7,5	11,9	9,3±5,6	44,6	53,5±4,9	7,9	1,4±0,1	8,8	4,9±0,1	39,0	4,9±0,1	29,9
line [1] 188	64,4±4,4	6,8	12,1±0,9	7,2	55,4±5,4	9,8	9,5±4,4	33,9	45,9±7,2	15,6	1,4±0,2	11,3	4,7±1,5	33,0	4,5±1,2	26,4
Primorsky 390	55,5±3,7	6,6	11,1±0,7	6,3	54,5±8,0	14,6	6,5±3,5	53,5	47,7±6,5	13,7	1,4±0,2	12,1	1,0±0,0	0	1,4±0,2	12,1
line [1] 212	45,6±2,9	6,3	10,3±0,6	6,2	47,7±7,6	16,0	10,9±5,2	39,9	36,8±6,9	16,8	1,0±0,2	16,1	1,8±0,9	46,3	1,5±0,7	40,7
line [1] 222	44,8±3,0	6,8	8,6±0,7	8,4	30,8±6,6	19,4	7,1±3,1	35,9	23,3±6,8	29,3	0,7±0,2	22,3	1,2±0,4	34,2	0,7±0,2	31,3
Primorsky 1531	86,4±3,1	3,6	16,1±0,9	5,5	118,0±12,5	10,5	12,8±6,2	48,6	105,9±10,2	9,7	2,6±0,4	15,3	1,2±0,4	35,1	2,8±0,4	15,8
line [1] 112	83,5±5,5	6,6	15,8±0,9	5,7	72,2±11,7	16,2	5,8±2,8	48,5	66,3±10,3	15,6	2,1±0,3	16,4	2,4±0,9	37,1	4,0±1,5	36,5

Somaclones developed the maximum diversity range of intra-line Cv in productive tillering, the number of spikelets and sterile spikelets in the main panicle, grain weight per plant, the minimum - in plant height and length of the panicle (Table 2). Along with it, somaclones demonstrated higher amplitude of fluctuations of intra-line Cv than initial varieties. The similar facts have been reported by other researchers in somaclones of rye (9). These results indicate a possible breeding value of somaclonal variants as a source of high phenotypic diversity.

2. Variability of inter-line coefficients of variation (*Cv*, %) **of major commercially valuable traits in rice somaclones of various genotypes** (grown on vegetation test sites with lysimeters)

Trait	Genotype				
	Primorsky 1222	Primorsky 1461	Primorsky 390	Primorsky 1531	Primorsky 1193
Plant height	3,6-9,7	5,0-6,9	5,2-11,7	5,6-11,9	4,6-9,3
Length of the main panicle	5,0-9,0	4,3-7,3	6,2-12,3	3,6-7,8	5,7-16,4
Number of spikelets in the main panicle	8,9-24,3	8,9-17,4	13,3-29,0	8,7-16,7	11,4-22,6
Number of sterile spikelets	30,0-59,2	26,8-44,6	29,3-46,1	23,7-78,0	24,2-56,5
Weight of grain from the main panicle	9,8-26,0	8,8-26,1	9,2-23,2	12,1-33,1	16,0-25,4
Grain weight per plant	23,1-41,1	17,4-41,1	16,6-40,7	12,9-38,3	21,1-48,8
Tillering	19,7-48,9	22,2-39,0	31,9-46,3	22,8-43,2	1,0-44,3

Mean values of each economically important trait were compared in the initial varieties and their somaclonal lines, and the most promising lines were selected. For example, somaclones obtained upon the genotype Primorsky 1222 were not reliably different from the initial variety by inter-line means of productive tillering and weight of grain from the main panicle (Table 1.). At the same time, a significant inter-line variability of these traits allowed to isolate two highly productive lines ([1] 113 and [1] 121) having the coefficient of tillering 2,6 and grain productivity of, respectively, 4,4 and 4,6 g, which significantly exceeded the corresponding parameters of the initial variety (Table 3).

In somaclones the genotype Primorsky 1461, average grain productivity of the main panicle wasn't high (1,3 g), while the tillering coefficient significantly exceeding that of the initial variety (3,3 vs. 1,4). Two somaclonal lines ([1] 181, [1] 188) with productivity exceeding the initial variety were isolated: coefficients of tillering, respectively, 4,9 and 4,7 and grain weight per plant - respectively 4,5 and 4,9 g .

Dwarfism was peculiar to somaclonal variants of the genotype Primorsky 390. Among these forms, two dwarf somaclonal lines ([1] 212, [1] 222) with plant height of 44-46 cm were isolated as a promising source material for breeding work.

One of seven somaclonal lines of the genotype Primorsky 1531 developed grain weight per plant 1,5 times exceeding the initial cultivar owing to a small number of sterile spikelets in the panicle, while the inter-line mean of this trait in somaclonal lines wasn't reliably different from the mean value in the initial variety. The number of sterile spikelets in the panicle of the studied lines varied within 3,9-28,6 pc.

Thus, the obtained somaclonal populations of rice cultivars demonstrated a wide diversity of both inter-line and intra-line coefficients of variation of major economically important traits. New highly productive and dwarf somaclonal lines of rice were selected for further use in breeding work. This study has confirmed the suitability of somaclonal variants as a promising source material in breeding work on rice.

REFERENCES

1. Neunylov B.A. and Krivolapov I.E., Problems of Growing Rice in the Russian Far East, in *Tr. Dal'nevostochnoi risovoi opytnoi stantsii* (Sci. Works of Far Eastern Research and Experimental Station of Rice), Vladivostok, 1970, no. 1, pp. 3-22.
2. Chaika A.K. and Kovalevakaya V.A., State and Prospects of Rice Growing in the Russian Far East, *Risovodstvo,* 2008, no. 13, pp. 3-6.
3. Kovalevskaya V.A., Introduction of New Cultivars of Rice in the Russian Far East, *Vest. RASKhN,* 2010, no. 3, pp. 39-41.
4. Kovalevskaya V.A., Piriculariosis of Rice in Primorsky Krai, in *Selektsiya, semenovodstvo i tekhnologii vozdelyvaniya sel'skokhozyaistvennykh kul'tur v Primor'ye* (Crop Selection, Seed Growing and Agricultural Techniques in Primor'ye: Compilation of Sci. Works), Novosibirsk, 1990, pp. 69-72.
5. Kovalevskaya V.A., Selection of Rice in the Far Eastern Zone of Rice Growing, *Dostizheniya nauki i tekhniki APK,* 2008, no. 6, pp. 8-10.
6. Butenko R.G., In Vitro Techniques in Agriculture, *S.-kh. biol.,* 1983, no. 5, pp. 3-7.
7. Kucherenko L.A., Induced Morphogenesis in Tissue Culture of Rice and Its Use for Creation of Initial Selection Material, in *Kul'tura kletok rastenii i biotekhnologiya* (Plant Cell Culture and Biotechnology: Compilation of Sci. Works), Moscow, 1986, pp. 211-213.
8. Evans D.A., Somaclonal Variation — Its Genetics Basis and Breeding Applications, *Trends Genet.,* 1989, vol. 5, no. 2, pp. 46-50.
9. Rakoczy-Trojanowska M., The Effects of Growth Regulators on Somaclonal Variation in Rye (*Secale cereale* L.) and Selection of Somaclonal Variants with Increased Agronomic Traits, *Cell. Mol. Biol. Lett.,* 2002, vol. 7, no. 4, pp. 1111-1120.
10. Arun B., Joshi A.K., Chand R. and Singh B.D., Wheat Somaclonal Variants Showing Earliness, Improved Spot Blotch Resistance and Higher Yield. *Euphytica*, 2003, vol. 132, no. 3, 235-241.
11. Larkin P.J. and Scowcroft W.R., Somaclonal Variation — a Novel Source of Variability from Cell Cultures for Plant Improvement, *Theor. Appl. Genet.,* 1981, vol. 60, no. 4, pp. 197-214.
12. Sun Z.X., Zhao C.Z., Zheng K.L., Qi X.F. and Fu Y.P., Somaclonal Genetics of Rice, *Oryza sativa* L., *Theor. Appl. Genet.,* 1983, vol. 67, no.1, pp. 67-73.
13. Zmeeva V.N., Zhuravlev Yu.N., Pisetskaya N.F. and Bakhmut N.A., Somaclonal Variants of Far Eastern Rice Cultivars, *Izv. SO AN SSSR, ser. biol. nauk,* 1989, no. 1, pp. 12-17.
14. Zhuravlev Yu.N. and Zmeeva V.N., Biotechnological Approaches to Selection of Rice in Primorsky Krai, in *Nauchnoe obespechenie APK Dal'nego Vostoka* (Scientific Support for Agriculture in the Russian Far East: Compilation of Sci. Works), Novosibirsk, 1995, pp. 132-136.
15. Murashige T. and Skoog F.A., A Revised Medium for Rapid Growth and Bioassay with Tobacco Tissue Cultures, *Physiol. Plant.,* 1962, vol. 15, no. 3, pp. 474-497.
16. Smetanin A.P., Dzuba V.N. and Aprod A.I., *Metodiki opytnykh rabot po selektsii, semenovodstvu, semenovedeniyu i kontrolyu za kachestvom semyan risa* (Guidelines for Experimental Works on Rice Selection, Seed Examination, Growing and Quality Control), Krasnodar, 1972.
17. Lyakhovsky L.G., *Metodicheskie ukazaniya po izucheniyu mirovoi kollektsii risa i klassificator roda Oryza L.* (Methodological Instruction on the Study of World Collection of Rice and Classifier of Genus *Oryza* L.), Leningrad, 1982.
18. Dospekhov B.A., *Metodika polevogo opyta* (Field Experimentation), Moscow, 1968, pp. 213-320.