DOI: https://doi.org/10.53040/cga12.53 UDC: 57.085.2:631.527:633.11

EVALUATION OF VARIABILITY OF QUANTITATIVE CHARACTERS IN SC_4 TRITICLE SOMACLONES WITH VALUABLE AGRONOMIC PROPERTIES

Renata CIOBANU, 0009-0007-0554-6845,
renata.ciobanu@usm.md

Institute of Genetics, Physiology and Plant Protection, Moldova State University, Chișinau, Republic of Moldova

Abstract. *As a result of research on the influence of in vitro culture and gamma radiation on quantitative traits, significant variations in the coefficient of variation values were observed in SC_4 triticale somaclones. Both in vitro culture and radiation caused changes in the coefficient of variation of quantitative traits in the studied genotypes, compared to the control. The variability assessment revealed low to medium variation, indicating individual similarity across the studied traits. Radiation showed a major action on the following traits: length of the last internode, plant height and number of fertile siblings. The factors studied represent a significant source of variation, with their influence intensified by the specific characteristics of the genotype.*

Keywords: in vitro, quantitative traits, radiation, somaclones, triticale.

INTRODUCTION

Improving many agronomic traits, including cereal quality, requires the separation of their genetic variability from non-genetic influences. For many years, the main goal of breeding programs was to replace traditional species with high-yielding, disease resistant modern species, which led to a reduction in genetic diversity, especially in terms of quality traits and nutritional properties [2].

Genetic diversity can be estimated using morphological traits. Continuous development and availability of new genetic resources are essential for improvement of species, while phenotypic characterization can be used to differentiate genotypes [5].

Somaclones, obtained through *in vitro* culture, exhibit a wide range of morphological and biochemical trait variation, confirming the efficiency of callus culture in inducing variability and producing somaclones of agronomic interest [4]. The advantages of somaclonal variation and mutagenesis are well documented. In most cases, somaclonal variation in different plant species has been determined based on morphological and physiological traits [3]. The effectiveness of gamma radiation as an inducer of genetic variation in barley has been confirmed through the creation of mutant varieties with superior traits related to yield, quality, plant architecture, and disease resistance [1].

Therefore, it is of interest to evaluate the effect of *in vitro* culture alone and in combination with gamma radiation as genetic modifiers on agro-morphological traits in SC_4 triticale somaclones to identify valuable genotypes for integration into a breeding program.

MATERIALS AND METHODS

Plant material: The study material included triticale somaclones with valuable agronomic traits selected from SC_3 descendants of three triticale species (x Triticosecale): Ingen 35, Ingen 93 (standard), and 188TR 5021.

Determining somaclonal variation: SC_3 somaclone seeds were sown in randomized blocks with three replicates of 60 seeds per genotype. Each variant included three triticale genotypes: the control, gamma irradiated forms, and forms obtained via *in vitro* culture. Phenological observations were made. Plants were collected at the vegetation phase (according to the Zadocks scale): full maturity (99), and under laboratory conditions, they were analyzed based on several agro-morphological traits: plant height, number of fertile tillers, main spike length, number of internodes, number of grains per spike, grain weight, and 1000 kernel weight.

Statistical analysis: The impact of *in vitro* culture and radiation on quantitative trait variation was assessed using analysis of variance (ANOVA), influence power (IP, %), and coefficient of variation (CV, %). Mathematical data analysis was conducted using STATGRAPHICS Plus 5.0 software.

RESULTS AND DISCUSSION

Biometric analysis of quantitative traits in SC_4 triticale somaclones induced by gamma rays and *in vitro* culture revealed distinct traits compared to initial forms. Phenological observations, trait evaluation, and assessment of SC_4 somaclones were conducted. The vegetation period, including phases and stages, was determined using the Zadocks scale. Observations indicated that all triticale plants were at the same vegetation phase but at different developmental stages. This developmental lag persisted in the *flowering* and *grain formation* phases, as well as at the *milk ripeness* stage. The evaluation revealed variation in the developmental stages within this phase, advancing by 1-2 stages. Thus, the *in vitro* derived SC_4 somaclones and the control variant 188TR 5021 (Figure 1) were at stage 71 on the Zadocks scale, corresponding to the *watery ripe stage (first formed grains are half of their final size*), while the irradiated SC_4 188TR 5021 and its control were at stage 69 (full flowering). The Ingen 35 somaclones, both irradiated and *in vitro*-derived, were at stages 70-71 (early grain formation - watery ripe), while the SC_4 control was at stage 69, *full flowering*. For the Ingen 93 somaclones control, irradiated, and *in vitro* derived the same vegetation phase, *early grain formation*, was observed.

Plants were collected at full maturity (stage 99). Under laboratory conditions, morphometric parameters of agronomic interest were evaluated.



A **B** **C**

Figure 1. SC_4 somaclones at different vegetation phases:
A - 188TR 5021; B - Ingen 35; C - Ingen 93

Table 1. Bio-morphological trait variation in SC_4 somaclones summarized

Character/ Genotype	Number of fertile tillers	Plant height (cm)	Length of the main spike (cm)	Length of the last internode	Grains per spike	Grain weight (g)	1000 kernel weight (g)
	$\bar{x}$ ± ES CV,%	$\bar{x}$ ± ES CV,%	$\bar{x}$ ± ES CV,%	$\bar{x}$ ± ES CV,%	$\bar{x}$ ± ES CV,%	$\bar{x}$ ± ES CV,%	$\bar{x}$ ± ES CV,%
Control Ingen 35	1,86±0,11 33,68	114,6±0,98 4,70	13,58±0,18 7,35	35,41±0,40 6,29	75,03±1,98 14,46	3,54±0,09 15,34	47,26±0,67 7,86
RAD Ingen 35	2,5±0,14 ***32,80	136,6±1,99 *** 8,01	13,89±0,17 6,81	49,32±1,02 *** 11,42	**90,36±2,18 *** 13,22**	3,71±0,12 18,53	41,02±0,83 ***11,11
In vitro Ingen 35	2,43±0,17 ***38,4	120,4±0,99 *** 4,51	14,26±0,19 * 7,40	36,36±0,43 6,52	74,53±1,56 11,52	3,09±0,09 **17,46	41,39±0,90 ***11,94
Control Ingen 93	2,16±0,12 32,25	110,9±1,11 5,50	14,37±0,22 8,44	32,49±0,60 10,25	68,83±1,42 11,34	2,88±0,06 12,49	42,09±0,88 11,49
RAD Ingen 93	2,66±0,14 *30,08	121,7±1,42 ***6,46	13,68±0,20 *8,00	44,88±0,68 ***8,35	69,66±2,35 18,54	2,95±0,10 18,81	42,52±0,53 6,86
In vitro Ingen 93	2,23±0,14 34,65	**116,07±1,05 ** 4,99**	**15,58±0,19 *** 6,87**	32,88±0,77 12,86	**74,06±1,28 **9,46**	3,44±0,07 11,82	**46,52±0,57 ***6,75**
Conrol 188TR5021	2,13±0,12 31,94	114,37±1,45 6,97	14,23±0,23 9,02	39,73±0,69 9,55	88,06±2,33 14,49	3,17±0,11 19,00	36,04±0,80 12,28
RAD 188TR5021	2,37±0,09 20,75	114,39±0,97 4,59	14,35±0,17 6,48	39,24±0,50 6,93	86,68±2,18 13,57	3,01±0,09 17,78	34,72±0,59 9,19
In vitro 188TR5021	2,06±0,15 40,04	142,97±1,56 ***6,01	14,89±0,19 * 7,26	47,97±0,77 ***8,83	79,5±2,47 * 17,05	**3,85±0,14 ***20,80**	**48,40±0,81 *** 9,16**

*Note: *, **, *** - significant differences for P ≤ 0,05; 0,01; 0,001.*

To assess somaclonal variability, morphological parameters were evaluated: plant height, length of the main spike and last internode, number of fertile tillers and internodes per plant, grains per spike, grain weight, and 1000 kernel weight. Bio-morphological evaluations showed significant variation (95-99% confidence level) in agro-morphological traits of SC4 triticale somaclones depending on genotype and analyzed factor (Table 1).

Analysis of the data on the *number of fertile tillers per plant* highlighted that genotypes Ingen 93(RAD) and Ingen 35(RAD) exhibited 23,14–34,40% higher values compared to the control, with differences significant at the 99,9% level. The coefficient of variation for this trait was medium (20,75–40,04%).

Mean *plant height* varied among *in vitro* derived somaclones and was genotype dependent. Ingen 93(*in vitro*), Ingen 35(*in vitro*), and 188TR 5021(*in vitro*) displayed higher mean values than their respective controls (by 5,06–25%), significant at the 95–99,9% level. The highest *plant height* (142,97 cm) was recorded in genotype 188TR 5021(*in vitro*). Gamma irradiated variants of Ingen 93 and Ingen 35 also showed increased values compared to the control (by 9,78–19,19%), while the lowest values were noted in 188TR 5021(RAD). The coefficient of variation was low (4,51–6,97%), indicating trait stability in SC_4 generation.

For the *main spike length*, genotype specific responses were observed. Ingen 93(*in vitro*), Ingen 35(*in vitro*), and 188TR 5021(*in vitro*) had 4,68–8,42% higher values compared to the control, significant at the 95–99.9% level, while Ingen 93(RAD) had a 5% lower mean, significant at the 95% level. The longest spike (15,58 cm) was recorded in Ingen 93(*in vitro*). The coefficient of variation ranged from 6,48–9,02%, indicating low variability.

The mean value for *last internode length* increased significantly in gamma irradiated variants (38,13–39,28%) compared to the control, with significance at the 99,9% level for Ingen 93(RAD) and Ingen 35(RAD). *In vitro* derived 188TR 5021 somaclones also had a 20,73% increase. The variability for this trait ranged from low to medium (6,29–12,86%).

For the *number of grains per spike*, Ingen 35(RAD) showed a 20,43% increase over the control, significant at the 99,9% level. The effect of *in vitro* culture showed a 7,59% increase for Ingen 93(*in vitro*) but a 1,10 fold decrease for 188TR 5021(*in vitro*) compared to their control. The variability for this trait ranged from low to medium (9,46–18,54%).

The mean *grain weight* differed significantly only for Ingen 35(*in vitro*), which had a 13% lower value compared to its control. The highest value, 21,06% above control, was recorded for 188TR 5021(*in vitro*), significant at the 99,9% level. The coefficient of variation for this trait ranged from medium (11,82–20,80%).

For the *1000 kernel weight*, *in vitro* culture led to an increase of 10,52–34,29% in Ingen 93 and 188TR 5021 genotypes, with differences significant at the 99,9% level. Gamma radiation decreased this trait by 14% in Ingen 35(RAD). The coefficient of variation ranged from 6,86–11,94%, indicating low variability.

Based on the evaluations, SC_4 somaclones of Ingen 35(RAD), Ingen 93(*in vitro*), and 188TR 5021(*in vitro*) were noteworthy for superior average values in traits such as *the number of fertile tillers, plant height, main spike length, last internode length, grain number per spike,* and *1000 kernel weight.*

Table 2. Analysis of bio-morphological indices in SC_4 (ANOVA test)

Source of variation	Sum of squares	Degrees of Freedom (DF)	Mean Square (S^2)	Fisher Test (F)	IP, %
Plant height					
Genotype	3356,89	2	1678,45	29,23***	18,50
RAD	3709,11	1	3709,11	64,60***	20,44
Genotype-RAD	2808,41	2	1404,20	24,46***	15,47
Residual	8267,93	144	57,41		
Total	18142,30	149			
Genotype	6325,34	2	3162,67	69,75***	25,76
In vitro culture	6552,49	1	6552,49	144,50***	2,66
Genotype-*in vitro*	5143,68	2	2571,84	56,72***	20,95
Residual	6529,62	144	45,34		
Total	24551,1	149			
Length of the main spike					
Genotype	8,56	2	4,28	3,66	4,54
RAD	0,61	1	0,61	0,53	0,32
Genotype-RAD	10,81	2	5,40	4,62*	5,74
Residual	168,40	144	1,17		
Total	188,40	149			
Genotype	29,47	2	14,73	11,99***	12,50
In vitro culture	26,80	1	26,80	21,82***	11,37
Genotype-*in vitro*	2,46	2	1,23	1,00	1,04
Residual	176,89	144	1,23		
Total	235,62	149			
Grains per spike					
Genotype	10151,60	2	5075,81	38,52***	**31,74**
RAD	798,11	1	798,11	6,06*	2,49
Genotype-RAD	2054,41	2	1027,21	7,79***	6,42
Residual	18977,20	144	131,786		
Total	31981,30	149			
Genotype	4847,61	2	2423,81	22,18***	22,25
In vitro culture	40,56	1	40,56	0,37	0,18
Genotype-*in vitro*	1157,08	2	578,54	5,29*	5,31
Residual	15734,20	144	109,27		
Total	21779,50	149			
Grain Weight					
Genotype	16,25	2	8,12	26,49***	26,64
RAD	0,0024	1	0,0024	0,01	-
Genotype-RAD	0,57	2	0,28	0,92	0,92
Residual	41,17	144	0,31		
Total	60,98	149			
Genotype	4,10	2	2,05	6,50**	6,53
In vitro culture	1,97	1	1,97	6,25*	3,13
Genotype-*in vitro*	11,32	2	5,66	17,94***	18,01
Residual	45,42	144	0,32		
Total	62,81	149			

Source of variation	Sum of squares	Degrees of Freedom (DF)	Mean Square (S^2)	Fisher Test (F)	IP, %
1000 kernel weight					
Genotype	2137,08	2	1068,54	65,32***	**42,11**
RAD	273,08	1	273,08	16,69***	5,38
Genotype - RAD	308,73	2	154,36	9,44***	6,08
Residual	2355,77	144	16,36		
Total	5074,65	149			
Genotype	174,57	2	87,28	4,74*	3,30
In vitro culture	335,55	1	335,55	18,24***	6,34
Genotype-*in vitro*	2129,71	2	1064,86	57,88***	**40,26**
Residual	2649,04	144	18,40		
Total	5388,87	149			

Note: * ,**, *** - significant differences for P ≤0,05; 0,01; 0,001.

Variability assessment confirmed low to medium values, suggesting uniformity among individuals for the studied traits.

The ANOVA test for quantitative traits in SC_4 triticale somaclones showed that genotype, radiation, *in vitro* culture, and their interactions had a significant impact (95–99,9% confidence) (Table 2). Radiation had a major effect on *last internode length* (40,81%), *plant height* (20,44%), and *number of fertile tillers* (7,47%), all significant at the 99,9% level. The *in vitro* culture influence ranged from 0,18–11,37%, with the highest on spike length.

Radiation exhibited a major effect on the following traits: *length of the last internode* (40,81%), *plant height* (20,44%), and the *number of fertile tillers* (7,47%), all significant at the 99,9% level. The influence power of *in vitro* culture ranged between 0,18% and 11,37%, with the highest value observed for the trait *main spike length*. The interaction between genotype and radiation, depending on the trait, showed influence values ranging from 0,92% to 23,97%. The strongest influence was found for the traits: *length of the last internode* (23,97%), *plant height* (15,47%), and the *number of grains per spike* (6,42%). The interaction between genotype and *in vitro* culture had influence values ranging from 1,04% to 40,26%, with the highest values recorded for *1000 kernel weight*, *plant height* and *grain weight*. Genotype influence in SC_4 somaclones ranged from 0,59% to 53,43%. The highest influence was recorded for the traits: *number of grains per spike* (31,74%) and *1000 kernel weight* (42,11%), both highly significant (99,9%). The most substantial genotype influence was observed for the trait *length of the last internode* (53,43%).

These results highlight the potential of the studied factors to expand genetic diversity and variability in triticale. This data supports the necessity of using somaclones in subsequent generations for selecting genotypes with superior agronomic traits.

CONCLUSION

1. Following the evaluations, the SC_4 somaclones of genotypes Ingen 35(RAD)**,** Ingen 93(*in vitro*), and 188TR 5021(*in vitro*) stood out compared to the control. The most determinative traits were: *number of fertile tillers per plant, plant height, main spike length, last internode length, number of grains per spike,* and *1000 kernel weight*.

2.The variability assessment revealed low to medium variation, indicating individual similarity across the studied traits.

3. Radiation had a major effect on the traits: *last internode length* (40,81%), *plant height* (20,44%), and *number of fertile tillers* (7,47%), all statistically significant at 99,9%. The genotype × radiation interaction had a maximal influence for the traits: *last internode length* (23,97%), *plant height* (15,47%), and *number of grains per spike* (6,42%). The genotype × *in vitro* culture interaction reached values between 1,04% and 40,26%, with the highest for *1000 kernel weight, plant height,* and *grain weight*. Genotypic influence was greatest for the traits: *number of grains per spike* (31,74%) and *1000 kernel weight* (42,11%), both significant at 99,9%. The maximum influence from genotype was on *last internode length* (53,43%).

4. Productivity related indices *plant height, main spike length, last internode length, number of grains per spike,* and *1000 kernel weight* can be used as key indicators for evaluating somaclonal diversity induced by gamma radiation and *in vitro* culture. Therefore, they should be given top priority in the selection process for triticale breeding.

Acknowledgments: The research was carried out within the Subprogramme 011102 Expansion and conservation of genetic diversity, improvement of agricultural crop genotypes in the context of climate change, financed by the Ministry of Education and Research.

REFERENCES

1. GRIGOROV, T., SMEREA, S., ANDRONIC, L. Variabilitatea caracterelor agromorfologice la somaclonele de orz (SC_0) indusă de radiație gama și infecție virală. In: Realizări științifice in ameliorare și tehnologii inovative la culturile cerealiere în contextul schimbărilor climaterice: conferința șt.- pract. cu participare intern., 4-5 sept. 2020. Chișinău, 2020, pp. 153-161. ISBN 978-9975-56-177-8.
2. KENZHEBAYEVA, S., DOKTYRBAY, G., SARSU, F., OMIRBEKOVA, N., ABEKOVA, A., TASHENEV, D. Mutant resources of spring wheat to improve grain quality and morphology. In: Global Wheat Production. 2018, p. 250. 10.5772/intechopen.75706.
3. MUSHTAQ, A., RAKESH, C. Assessment of the effects of gamma radiations on various morphological and agronomic traits of common wheat (*Triticum aestivum* L.) var. WH - 147. In: European Journal of Experimental Biology. 2015, vol. 5(7), pp. 6-11. ISSN 2248-9215.
4. ROGHAYEH, Ah., NASSER Z., ASGHARI-ZAKARTA, R., SHEIKHZADEH, P. Efficient in vitro somatic embryogenesis and plant regeneration from mature and immature embryos of wheat (*Triticum aestivum* L.). In: Brazilian Archives of Biology and Technology. 2016, vol. 59. e160288. ISSN 1516-8913.
5. ZULFIQAR, S., ISHFAQ, S., IKRAM, M., NAWAZ, M., RAHMAN, M. Characterization of gamma – rays – induced spring wheat mutants for morphological and quality traits through multivariate and GT Bi - Plot analysis. In: Agronomy. 2021, vol. 11(11), p. 2288. ISSN 2073-4395.