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INFLUENCE OF *IN VITRO* CULTURE ON SEED SIZE IN SOMACLONAL VARIANTS OF SPELT

Aim. Study the seed size of *Triticum spelta* L. regenerants obtained in the immature embryo culture R_2 generation in comparison with the original forms. ***Methods.*** Measurement of length, width and area of the grain using ImageJ software, statistical data analysis. ***Results.*** Measurements of spelt grains of five original genotypes and their 26 somaclonal variants were carried out. Significant differences in seed size were found. The Oberkulmer Rotkorn and Zorya Ukrainy spelt cultivars had the largest grains, and their somaclones showed a significant decrease in the grain size. Among the somaclonal variants of the rest spelt genotypes, a significant increase in grain size was observed with a high frequency (from 40 % to 67 % of all somaclones) and no significant decrease. Two somaclones of the *T. spelta* 4 (UK 4C/15) breeding line had significantly higher grain area values compared to all other studied genotypes. ***Conclusions.*** Somaclonal variation in seed size was revealed among biotechnological spelt plants. It was shown that the character of changes in grain size parameters depended on the initial genotype. The developed method to obtain biotechnological spelt plants makes it possible to develop high-quality starting material for the plant breeding needs.

Keywords: *Triticum spelta* L., immature embryo culture, regenerant plants, R_2 generation, somaclonal variation.

Spelt wheat (*Triticum spelta* L.) occupies a much smaller share of world production compared to the more widespread common wheat (*Triticum aestivum* L.) due to certain technological difficulties in cultivation, although it has a greater content of protein, some trace elements, in particular selenium, and other beneficial compounds. Spelt is more resistant to diseases and undemanding to growing conditions, does not require the application of significant amounts of fertilizers, that makes it an attractive object for organic farming, but has a lower yield (70–80 % common wheat yield) [1]. Plant productivity is affected by the number and weight of grains per spike. The weight of the grain directly depends on its size. Certain genes regulate the size, weight and quantity of grains in different ways. *TaGS5* and *TaGW2* are important genes affecting seed size and weight at a high level of expression [2]. High expression levels of *TaSN1*, *TaFUL1*, *TaSH1* genes affect the quantitative index of seeds per spike, control spike development, and, as a result, plant productivity [3].

Introducing useful traits to valuable species is an important element in breeding and biotechnology, where the phenomenon of somaclonal variation plays an important role. For the first time, this phenomenon was noted only in the phenotypic manifestations of *in vitro* regenerants, but today it is already known that somaclonal variation manifests itself in changes in a significant number of various traits in the genotype of *ex vitro* plants and can be used in the breeding process to improve plant productivity [4–6]. Although genomic changes (polyploidy and aneuploidy, various mitotic abnormalities) as well as mutations in DNA sequence can be the cause of phenotypic variation in plants, epigenetic regulation of gene expression at the molecular level through DNA methylation, histone modifications or RNA interference also play a major role [7]. DNA methylation can induce high levels of gene expression [8]. Changes in gene expression can also occur when mobile DNA sequences, such as transposons, are activated. Their location can influence gene stability and contribute to both gene overexpression and silencing, making those mobile DNA regions one of the explanations for somaclonal variation at the molecular level [9]. The variability and genetic changes of the obtained plants are affected by the conditions of their *in vitro* cultivation. Changes in the obtained somaclones can be reflected phenotypically and occur in the biochemical composition, or at the molecular level [8]. Therefore, somaclonal variation that occurs during the *in vitro* cultivation of cells and tissues makes it possible to obtain a wide range of phenotypically and biochemically diverse plants within the same genotype. This method was repeatedly presented as efficient in research of geneticists and breeders [5, 6, 10].

To date, there are a total of three scientific works on obtaining spelt regenerants in aseptic culture [11–13] and no study on somaclonal variation in spelt. Considering this, the aim of the work was to study somaclonal variation in seed size among spelt regenerants obtained in immature embryo culture.

Materials and methods

Spelt seeds of five original genotypes (the Ukrainian cultivar Zorya Ukrainy, the German cultivars Zuricher Rotkorn and Filderweiss, the ancient Swiss cultivar Oberkulmer Rotkorn and the Ukrainian breeding line *T. spelta* 4 (UK 4C/15)) and 26 somaclonal variants of these genotypes of the R_2 generation (two variants of the Zorya Ukrainy cultivar, five variants of the Zuricher Rotkorn cultivar, eleven variants of the Filderweiss cultivar, two variants of the Oberkulmer Rotkorn cultivar and six variants from the UK 4C/15 breeding line) were used in the work. Seeds of the original genotypes of the 2022 reproduction were kindly provided by the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine. Spelt regenerants were obtained in the immature embryo culture some of which, during further cultivation in a greenhouse, formed seeds of the R_1 generation. R_1 seeds of somaclonal variants and seeds of the original genotypes were sown in an experimental plot of the Institute of Cell Biology and Genetic Engineering of the NAS of Ukraine (ICBGE). The collected seeds from the 2024 reproduction experimental plot served as test material for grain size measurement.

In the study we estimated the length, width, and area of 20 randomly taken grains of each original genotype and somaclonal variant using ImageJ ver. 2 software. The grains of each genotype were scanned separately using an HP LaserJet Professional M1132 MFP scanner at a resolution of 1200 dpi. The “Distance in pixels” parameter corresponded to 25.4 (the number of millimeters per inch). Files were saved in JPEG format (Fig. 1).

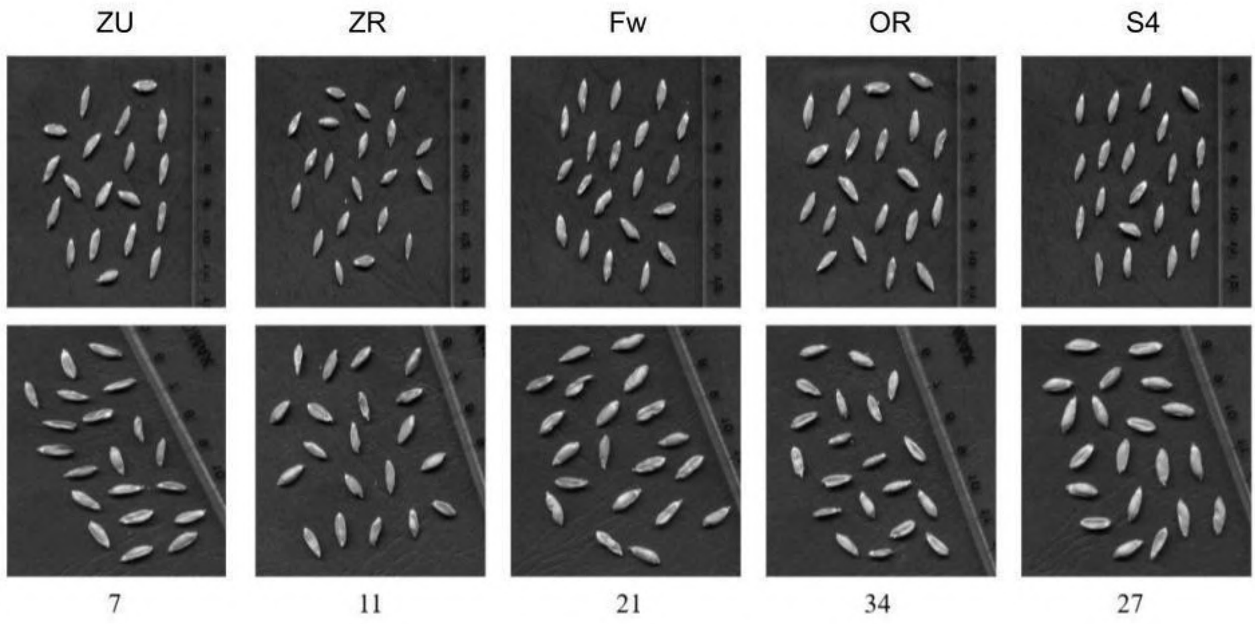

Fig. 1. Appearance of scans of 20 grains of the original spelt genotypes (ZU – cultivar Zorya Ukrainy, ZR – Zuricher Rotkorn, Fw – Filderweiss, OR – Oberkulmer Rotkorn, S4 – Ukrainian breeding line *T. spelta* 4 (UK 4C/15)) and their somaclonal variants (Nos. 7, 11, 21, 34, and 27). Scan resolution 1200 dpi.

Statistical analysis of data was performed using Python's statistical package Pingouin (https://pingouin-stats.org, ver. 0.4). To compare 2 means between the original form and its somaclone we used Student test. One-way ANOVA (Analysis of Variance) was used to compare the differences between the group means (initial genotypes or plants of the same genotype). Tukey-HSD test was used for multiple comparisons. Normality of data was checked with Shapiro-Wilk (omnibus) test. Homoscedasticity was analyzed using Levene test. Results were considered statistically significant at $p < 0.05$.

Results and discussion

As a result of the measurements and calculations, significant differences in seed size between the original genotypes, the original genotype and somaclonal variants, and between somaclonal variants of the same genotype were found (Table). The initial forms differed significantly in length, width and area of the seeds. The Zuricher Rotkorn and Filderweiss cultivars had shorter seeds. The Oberkulmer Rotkorn variety had a significantly larger grain area compared to the others.

Somaclonal variation in morphological parameters of seeds was observed among spelt regenerants obtained in the culture of immature embryos, both in increasing grain size compared to the original form, and decreasing depending on the plant genotype. In somaclonal variant No. 34 of the Oberkulmer Rotkorn, a decrease in the length, width and area parameters of grains was observed. The average value of the grain area was 20.3 % less than that of the original form. Also, a significant decrease in the area and width of the grains was found in the somaclonal line No. 7 of Zorya Ukrainy with a decrease in the average value of the seed area by 15.8 % (Fig. 1, Table).

Table. Analysis of the size of spelt grains of five genotypes and their somaclonal variants of the R2 generation

OG	Length, mm					Width, mm					Area, mm^2				
	ZU	ZR	Fw	OR	S4	ZU	ZR	Fw	OR	S4	ZU	ZR	Fw	OR	S4
SV	9,6[a]	8,9[b]	9,1[b]	9,7[a]	9,3[a]	3,1[ab]	3,1[b]	3,2[ab]	3,4[a]	3,1[b]	22,8[ab]	20,8[a]	21,2[a]	24,1[b]	21,2[a]
1	-	-	-	-	9,5[ad]	-	-	-	-	3,2[b]	-	-	-	-	22,6[ad]
3	9,2[a]	-	-	-	-	3,3[ab]	-	-	-	-	21,6[ab]	-	-	-	-
6	-	-	-	-	10,3[b]	-	-	-	-	3,5[a]	-	-	-	-	26,2[b]
7	9,2[a]	-	-	-	-	2,9[c]	-	-	-	-	19,2[c]	-	-	-	-
8	-	-	9,4[ac]	-	-	-	-	3,2[ab]	-	-	-	-	20,3[a]	-	-
10	-	-	9,4[bc]	-	-	-	-	3,3[ab]	-	-	-	-	22,0[ac]	-	-
11	-	9,6[a]	-	-	-	-	3,3[b]	-	-	-	-	22,8[b]	-	-	-
14	-	-	9,6[ac]	-	-	-	-	3,2[ab]	-	-	-	-	23,2[bc]	-	-
15	-	-	-	-	9,9[d]	-	-	-	-	3,2[b]	-	-	-	-	23,5[cd]
21	-	-	10,1[d]	-	-	-	-	3,6[c]	-	-	-	-	25,7[d]	-	-
22	-	-	-	-	9,6[d]	-	-	-	-	2,9[b]	-	-	-	-	21,3[a]
23	-	-	9,9[ad]	-	-	-	-	3,4[ab]	-	-	-	-	23,6[be]	-	-
24	-	-	9,9 [ad]	-	-	-	-	3,5[c]	-	-	-	-	23,2[a]	-	-
25	-	-	9,8 [ad]	-	-	-	-	3,5[c]	-	-	-	-	25,6[d]	-	-
27	-	-	-	-	10,4[b]	-	-	-	-	3,5[a]	-	-	-	-	26,4[b]
28	-	-	-	-	10,4[b]	-	-	-	-	3,1[a]	-	-	-	-	25,6[b]
29	-	-	-	10,0[a]	-	-	-	-	3,4[a]	-	-	-	-	23,6[b]	-
31	-	9,5[a]	-	-	-	-	3,3[b]	-	-	-	-	22,8[b]	-	-	-
32	-	-	9,9[ad]	-	-	-	-	3,4[ab]	-	-	-	-	25,3[d]	-	-
34	-	-	-	8,9[b]	-	-	-	-	2,9[b]	-	-	-	-	19,2[a]	-
36	-	8,6[b]	-	-	-	-	3,0[b]	-	-	-	-	19,5[a]	-	-	-
37	-	-	10,1[d]	-	-	-	-	3,5[ab]	-	-	-	-	25,7[d]	-	-
38	-	8,6[b]	-	-	-	-	3,0[b]	-	-	-	-	20,0[ab]	-	-	-
39	-	8,7[b]	-	-	-	-	3,1[b]	-	-	-	-	20,4[ab]	-	-	-
40	-	-	9,5[b]	-	-	-	-	3,2[ab]	-	-	-	-	22,4[a]	-	-
41	-	-	9,5[b]	-	-	-	-	3,4[ab]	-	-	-	-	25,2[de]	-	-

Notes: the means are presented in the table; means of the same parameter with the same letter when compared separately between the original genotypes and separately between the original genotype and its somaclonal variants are not significantly different at the 0.05 significance level; OG – original genotypes; SV – somaclonal variants; ZU – cultivar Zorya Ukrainy; ZR – Zuricher Rotkorn; Fw – Filderweiss; OR – Oberkulmer Rotkorn; S4 – Ukrainian breeding line *T. spelta* 4 (UK 4C/15).

Among the rest of genotypes, an increase in grain size and no significant decrease were found. In somaclones of Zuricher Rotkorn, the means of the morphological parameters of the grains were both larger and smaller than the original form; however, we found a significant difference only in cases of increasing seed size. An increase in the morphological parameters of the grains was observed in two somaclones (Nos. 11 and 31) of Zuricher Rotkorn with a frequency of 40 % of the total number, seven somaclones (Nos. 14, 21, 23, 25, 32, 37, and 41) of Filderweiss with a frequency of 63.6 % and four (Nos. 6, 15, 27, and 28) somaclones of the Ukrainian breeding line *T. spelta* 4 with a frequency of 67.7 %. In a study by other authors, only 10 % of somaclonal variants of wheat had higher yield than the original forms [5].

In somaclones derived from Filderweiss and *T. spelta* 4, greater deviations of the length, width and grain area values of the initial forms were observed compared to Zuricher Rotkorn somaclones (Table). On average, the increase in seed area among somaclones was 19.9 % compared to the initial genotype *T. spelta* 4, by 17.5 % from seeds of Filderweiss and by 9.6 % for the Zuricher Rotkorn genotype. This indicates a wider reaction norm by the trait of grain size in the first two genotypes compared to the last one. In general, the trait of seed size is quite stable and does not depend strongly on cultivation conditions such as soil composition, insolation, humidity, infections, etc. For example, the morphological parameters of the seeds of the original genotypes of the 2022 reproduction (Hlevakha village, Kyiv region) did not differ significantly from such of seeds of the 2024 reproduction (Kyiv, ICBGI). Therefore, somaclonal variation in spelt seed size is a powerful source of biodiversity to address breeding needs to improve spelt wheat productivity.

We found significant variations in grain size parameters among somaclones of Filderweiss and *T. spelta* 4 genotypes. Thus, somaclonal lines Nos. 21, 25, 32, and 37 of the Filderweiss cultivar had a significantly stronger increase in grain size compared to lines Nos. 14, 23, and 41 (Fig. 2, A). Somaclones Nos. 6, 27, and 28 of the *T. spelta* 4 had a larger grain size compared to somaclone 15, which exceeded the original genotype in this trait (Fig. 2, B). Somaclonal lines No. 6 and 27, which originated from the initial form *T. spelta* 4, had significantly larger grain area compared to all other genotypes, as well as grain length and width values, which makes them promising starting material for spelt breeding to increase productivity (Fig. 3). Genotype No. 28, also derived from *T. spelta* 4, had significantly superior area values compared to other somaclones of this group (Fig. 2, B) and the Zorya Ukrainy cultivar (Fig. 3).

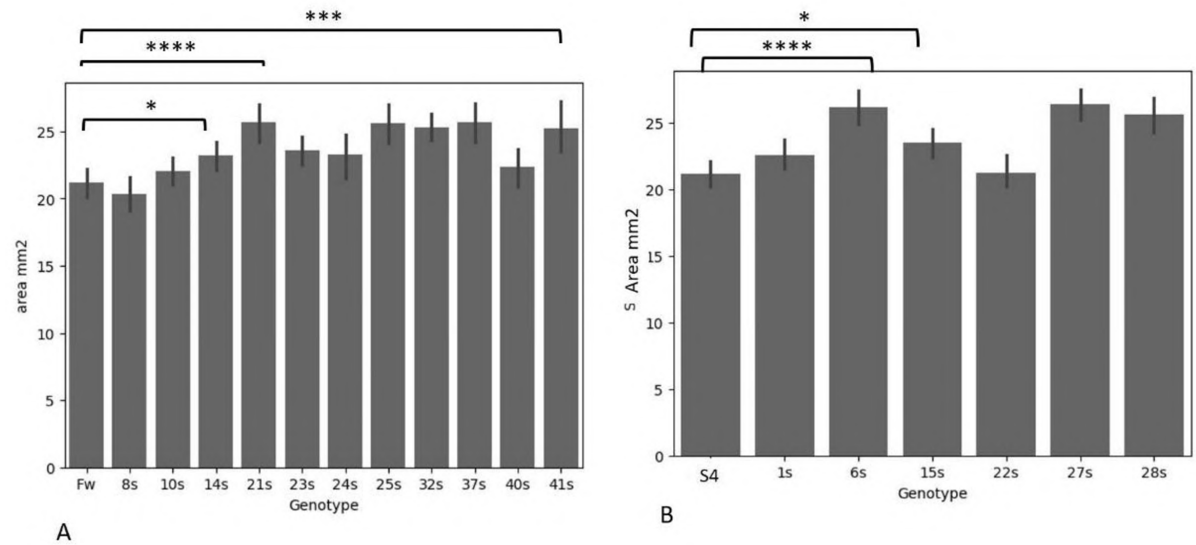

Fig. 2. Illustration of significant differences between the means in the grain area of Filderweiss (Fw, A) and the breeding line *T. spelta* 4 (S4, B) genotypes and their somaclones. The means are given with confidence intervals at a significance level of 0.05. *: 1.00e-02 < p <= 5.00e-02, ***: 1.00e-04 < p <= 1.00e-03.****: p <= 1.00e-04.

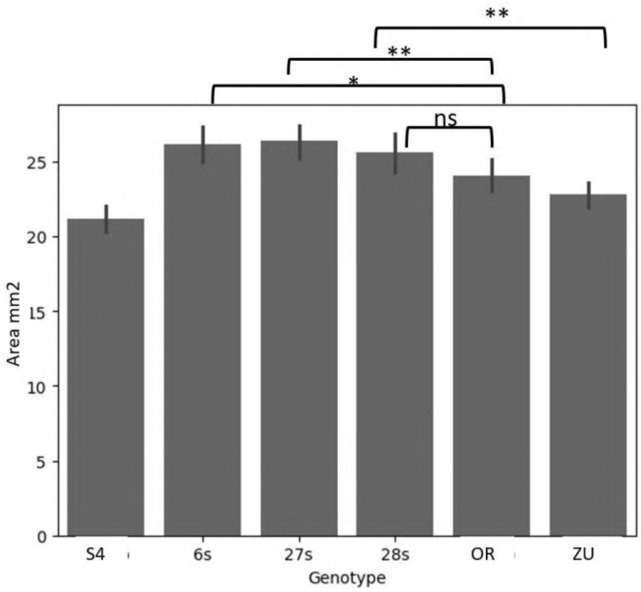

Fig. 3. Illustration of significant differences between the means in the grain area of spelt somaclonal lines Nos. 6, 27, and 28 of the *T. spelta* 4 (S4) breeding line, Zorya Ukrainy (ZU), and Oberkulmer Rotkorn (OR) cultivars. The means are given with confidence intervals at the 0.05 significance level. *: 1.00e-02 < p <= 5.00e-02, **: 1.00e-03 < p <= 1.00e-02, ns: no significant difference.

The studied spelt wheat genotypes differed in the manifestation of somaclonal variation in grain size. For some genotypes (Zorya Ukrainy and Oberkulmer Rotkorn), which had the largest seed size among the others, we observed an exceptionally significant decrease in grain size in somaclonal variants. Among somaclones of other genotypes we determined a significant increase in grain size, and no significant reduction in grain size was observed at all, although the number of somaclones of these genotypes studied was several times greater. This may be due to the fact that grain size parameters have the maximum expression in the Zorya Ukrainy and Oberkulmer Rotkorn genotypes, and their change leads only to a grain size reduction. Genotypes Filderweiss and *T. spelta* 4 are genetically highly plastic, since most somaclonal variants showed a significant increase in grain size compared to the original genotypes with a wide reaction norm for this trait. Other researchers have also observed the dependence of somaclonal variation on plant species or cultivar [4–7].

Conclusions

As a result of the conducted research, somaclonal variation in spelt regenerants was revealed by the trait of grain size. It was shown that the character of the changes in seed size in somaclonal variants depended on the plant genotype. Among somaclonal variants of some genotypes, an exceptionally significant decrease in grain size was observed, while in somaclones of other genotypes an increase in grain size had place. The technology to obtain spelt regenerants in immature embryo culture is efficient to develop somaclonal variation and biodiversity. Genotypes Filderweiss and *T. spelta* 4 are promising for further research on the development of initial breeding material due to high genetic plasticity. The obtained somaclones of these genotypes can be involved in the breeding process to produce new spelt cultivars.

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ВПЛИВ КУЛЬТУРИ *IN VITRO* НА РОЗМІР НАСІННЯ СОМАКЛОНАЛЬНИХ ВАРІАНТІВ СПЕЛЬТИ

Мета. Дослідити розміри зернівок рослин-регенерантів спельти (*Triticum spelta* L.) покоління R_2, отриманих у культурі незрілих зародків, у порівнянні з вихідними генотипами. ***Методи.*** Вимірювання довжини, ширини і площі зернівки за допомогою програмного забезпечення ImageJ, статистичний аналіз даних. ***Результати.*** Проведено виміри зернівок спельти п'яти вихідних генотипів та їх 26 сомаклональних варіантів. Виявлено значущу різницю у розмірах між генотипами та їх сомаклонами. Рослини спельти сортів Oberkulmer Rotkorn та Зоря України мали найкрупніше зерно серед вихідних генотипів, а у їх сомаклонів спостерігали значуще зменшення зернівок у розмірі. У сомаклональних варіантів решти генотипів спостерігали суттєве збільшення розміру зернівок порівняно зі вихідними рослинами з високою частотою (від 40 % до 67 % всіх сомаклонів) та відсутність його зменшення. Два сомаклони селекційної лінії *T. spelta* 4 (УК 4С/15) мали значущо більші показники площі зерна у порівнянні з усіма іншими досліджуваними генотипами. ***Висновки.*** Виявлено сомаклональну мінливість за розміром насіння серед біотехнологічних рослин спельти. Показано, що характер змінення показників розміру зерна залежав від вихідного генотипу. Розроблений метод отримання біотехнологічних рослин спельти, дає можливість створювати якісний вихідний матеріал для потреб вітчизняної селекції.

Ключові слова: *Triticum spelta* L., культура незрілих зародків, рослини-регенеранти, покоління R_2, сомаклональна мінливість.