The effect of genotype on in vitro morphogenesis of blue- and purple-grained bread wheat

A A Shkurkina¹, L P Khlebova¹, Yu V Melnikova¹, V P Vistovskaya² and S E Mityukhina¹

¹ Altai State University, 61, Lenina Ave, Barnaul, 656049, Russia
² Polzunov Altai State Technical University, 46, Lenina Ave, Barnaul, 656049, Russia

E-mail: ann7sh99@mail.ru

Abstract. Bread wheat containing anthocyanins in grain is of great interest for in vitro cell selection. These compounds are antioxidants and help the plant cope with stress. For the first time, we have evaluated the regenerative potential of eight samples with purple and blue grain using mature and immature embryos as explants. The maternal plants grew in the field conditions in the south of Western Siberia (Russia). Colored wheat had an advantage over white-grained forms in the ability to in vitro morphogenesis and adaptation to ex vivo conditions. Samples Fioletovozernaya (k-55583) and Blue A (k-43091) showed an intense proliferation of callus (94-95%), a high morphogenic activity (74-100%) and a large yield of viable regenerants (4-6 from one callus). We consider these forms promising for the selection of cell and plant lines that are resistant to adverse environmental factors and are suitable for breeding programs.

1. Introduction

Wheat (Triticum aestivum L.) is one of the most common foods in the world. Routinely, bread and baked goods are made from white and red grain cultivars, while wheat flour from purple and blue grains is rarely used [1-2]. The peculiarity of such cultivars is the synthesis of anthocyanin pigments (flavonoids) in the pericarp and aleurone layers of the caryopsis. The Pp (Purple pericarp) genes determine purple pigmentation, and the Ba (Blue aleurone) genes control blue color [3-4]. Flavonoids can be found not only in wheat, but also in other cereals (rice, barley, corn, etc.), as well as vegetables (peppers, cabbage, onions, etc.), fruits (apples, citrus fruits, etc.), berries [5-7]. They give color to flowers, stems, leaves. In addition to coloring, flavonoids have antioxidant activity [8-9]. These compounds also help the plant cope with various abiotic stresses (drought, high or low temperatures, soil salinity, soil pollution with heavy metals, UV radiation) and diseases [10].

The Altai Territory is one of the largest suppliers of wheat both in Russia and on the world market. However, due to the continental climate, crops often suffer from drought. Therefore, one of the priority tasks of breeding is the creation of stress-resistant cultivars. An important stage in breeding work is the search for new sources and donors of useful traits. Biotechnological methods have recently resulted in significant advances in crop breeding [11-14]. Cellular technologies make it possible to create new starting material and to assess the resistance of genotypes to various adverse environmental factors [15]. Evaluation and selection of stress-resistant forms in vitro on selective media involves the initiation of morphogenic callus tissue, which further regenerates plants. However, the low
morphogenic ability of individual genotypes is a serious obstacle to the widespread use of cell and tissue culture in breeding programs. Despite the great interest in colored wheat, there are no reports of in vitro initiation of cell cultures as well as of regenerants, although they can be useful in cell selection.

The aim of the study was to assess in vitro the regeneration potential of spring bread wheat with anthocyanin grain in comparison with white-grained cultivars.

2. Materials and methods
We studied seven samples of spring bread wheat with a purple grain and one sample with a blue grain. All forms were obtained from the genetic bank of N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) (St. Petersburg, Russia). Three cultivars of white-grained wheat, bred by the Research Institute of Agriculture of the South-East (Saratov, Russia), served as controls. For the induction of calli, mature and immature embryos were used as explants. The maternal plants were grown during the summer growing season of 2019 and 2020 on the experimental field of the Federal Altai Scientific Centre of Agro-BioTechnologies (53°25′ N, 83°31′ E) (Barnaul, Russia).

The caryopses were sequentially sterilized with 70% ethanol (2 min) and 2% lysoformin-3000 (15 min). The isolated embryos were aseptically placed in 100 ml flasks on a revised Murashige and Skoog’s Medium (RM-1964) [16], supplemented with 2 mg L⁻¹ 2,4-D (2,4-Dichlorophenoxyacetic acid), 0.7% agar, and 3% sucrose. The cultures were cultivated in the dark at 24±1°C for 4 weeks. To induce morphogenesis, calli were transferred to a nutrient medium of the same composition with replacement of auxin by kinetin (6-Furfurylaminopurine), a hormone of the cytokinin group, at a concentration of 0.5 mg L⁻¹. Cultures grew at 24 ± 2°C, relative humidity 55–60%, 16 h photoperiod (day) with light intensity 35–55 µmol m⁻² s⁻¹ provided by cold white fluorescent lamps.

The regenerants were adapted in a hydroponic installation, containing ¼ of the basic composition of macro- and microsalts according to Murashige and Skoog’s Medium [17]. The frequencies of callusogenesis, morphogenesis, and plant regeneration (%) were assessed. The experiment was performed in three repetitions, at least 60 embryos per genotype. The Microsoft Excel 2010 software was used for statistical processing of the data. The significances of differences were assessed by LSD test at p ≤ 0.05.

3. Results
During 4–7 days, the explants underwent dedifferentiation and proliferation. The frequency of callus induction in white-grained forms averaged 52 and 51% during inoculating mature and immature embryos, respectively (figure 1). However, the genotypes showed individual responses. Saratovskaya 70 initiated calli with low embryogenic activity only during culturing mature embryos (35%), which resulted in single regenerants (figure 2a). Saratovskaya 74 demonstrated a relatively high morphogenic potential when immature embryos were used, while Saratovskaya 73 induced morphogenic calli with a high frequency regardless of the type of an explant (up to 80%) (table 1).

Samples with anthocyanins, on average, were 1.5 times more responsive to in vitro cultivation compared to white-grained forms. However, they significantly differed in both callusogenic and morphogenic abilities. For example, mature embryos are preferred to obtain Purple Feed’s (k-49426) calli. The same explants of Mutant 1 (k-65585) and Mutant 2 (k-65799) began to germinate rapidly on callusogenic medium. The emergence of seedlings reduced the frequency of callusogenesis and prevented the formation of embryogenic callus. Omskaya Kormovaya (k-64472) and k-59158 actively proliferated callus from immature embryos. Blue-grained wheat (k-55583) (figure 2b), irrespective of the type of explant, formed embryogenic callus with a high frequency (74%). Among the "purple" wheats, we noted the Fioletovozernaya (k-43091) (figure 2c) sample. When immature embryos were passaged, callusogenesis reached 97%, inducing well-structured embryogenic cultures already on the initiation medium, resulting in a large number of regenerants.
An important stage in cell technologies is the preservation of regenerants after they are removed from aseptic conditions. Plants often look very weak and die in the process of adaptation. We used a hydroponic installation, which made it much easier for the regenerants to get through this critical stage. Plant survival ranged from 35 to 95%. The purple-grained forms were successfully acclimatized (figure 2d). A large waste of regenerants was observed in white-grained cultivars (more than 50%).

4. Discussion

Tissue culture-induced calli represent a promising experimental system for assessing plant responses to biotic and abiotic stresses. It is mainly determined by the similarity of morphogenesis in planta and in vitro [18-19]. In addition, the successful induction of callus tissue is the initial stage of cell selection, resulting in somaclonal variants, thereby expanding the genetic diversity of the initial and breeding material of plants for various traits happens [20-21]. The types of explants are believed to
affect significantly on callus induction and morphogenesis. In cereals, mature and immature embryos, tillering nodes, anthers, leaf discs, and root segments are used as explants. For initiation of wheat callus, mature and immature embryos are most often used [22-24]. Immature embryos are explants with a high morphogenetic potential. However, a significant drawback is the impossibility to use them throughout the year. Mature embryos are convenient for year-round cultivation, so they are often tried to be involved in the initiation of callus cultures [25, 26].

Table 1. Influence of the genotype and type of explant on the frequency of morphogenesis in blue- and purple-grained bread wheat.

| Sample           | Grain color | Frequency of morphogenic calli,% | Plant regeneration, % |
|------------------|-------------|----------------------------------|------------------------|
|                  |             | Mature embryos       | Immature embryos | Mature embryos | Immature embryos |
| Mutant 1         | purple      | 0                    | 32.8                | 0                  | 29.5                |
| Mutant 2         | purple      | 0                    | 39.4                | 0                  | 36.7                |
| Purple Feed      | purple      | 62.1                 | 0                   | 35.6               | 0                   |
| Omskaya Kormovaya | purple     | 0                    | 72.3                | 0                  | 27.6                |
| Konini           | purple      | 37.8                 | 56.9                | 23.1               | 65.5                |
| Fioletovozernaya | purple      | 47.4                 | 92.5                | 53.1               | 100.0               |
| K-59158          | purple      | 0                    | 44.8                | 0                  | 53.1                |
| Blue A           | blue        | 74.7                 | 74.1                | 42.5               | 56.1                |
| Saratovskaya 70  | white       | 35.1                 | 0                   | 10.3               | 0                   |
| Saratovskaya 73  | white       | 65.1                 | 67.4                | 51.2               | 80.0                |
| Saratovskaya 74  | white       | 15.7                 | 52.6                | 0                  | 32.8                |
| LSD$_{0.05}$     |             | 12.4                 | 11.7                | 13.2               | 12.8                |

We did not find an article on tissue culture initiation in purple- and blue-grained wheat. Comparison of the effectiveness of various explants in cultivating purple- and blue-grained wheat revealed the undoubted advantage of immature embryos both at the stage of morphogenesis induction and regeneration. We obtained 3 times more regenerants by cultivating this type of explants. In half of the samples, despite the high level of callusogenesis, we failed to obtain regenerants when working with mature embryos. During the differentiation of such calli, an active rhizogenic process was observed. The spread of roots over the entire surface of the callus blocked shoot development. Probably, intensive rhizogenesis occurred due to a change in the ratio of hormones in the cells of explants and calli [26]. Purple Feed regenerated plants only in mature embryo culture. One of the white-grained cultivars, Saratovskaya 70, showed the same reaction. However, the yield of plants was low, which makes it difficult to use them in cell selection programs.

We obtained excellent results when cultivating the Fioletovozernaya sample. The frequency of calli with a morphogenic response was 92.8%. Each morphogenic callus subsequently produced regenerants, as a rule, 4-6 pieces from one culture. We believe that this genotype, demonstrating a high morphogenetic potential, can be most useful in the future as an object of cell selection. The study carried out confirmed the opinion of a number of scientists that immature wheat embryos have a higher ability to morphogenesis. Nevertheless, the wide variability of the results, due to the genotype, resulted in identifying forms whose mature embryos are sensitive to the induction of both callusogenic and morphogenic processes (Purple Feed, Blue A).

Regenerants of colored wheat have shown good adaptability in hydroponics. The plants developed a strong root system that helped them survive when transferred to the ground. The Fioletovozernaya sample manifested a particularly high survival rate (95%). Unlike wheat with anthocyanins, white-grained forms adapted much worse. More than 50% of the regenerants died during acclimatization or transplantation into pots.
5. Conclusion

Thus, various forms of spring bread wheat with grain pigmented with anthocyanins differed in the activity of callusogenesis and the ability to form morphogenic callus when introduced into tissue culture. The Blue A (k-43091) sample with a colored aleurone layer of the caryopsis (blue wheat), regardless of the type of an explant, actively formed embryogenic callus. Among the purple wheats with the anthocyanin-colored pericarp, the sample Fioletovozernaya (k-55583) had the highest activity in tissue culture with a large yield of regenerants. Probably, these genotypes can be most effectively used as objects of cell selection for improving commercial varieties, including drought resistance. The regenerative capacity of calli from immature embryos exceeded the potential of mature embryos in both color- and white-grained genotypes.

References

[1] Usenko N I, Khlestkina E K, Asavasanti S, Gordeeva E I, Yudina R S and Otmakhova Y S 2018 Possibilities of enriching food products with anthocyanins by using new forms of cereals. *Foods Raw Materials* 6(1) 128-135

[2] Gamel T H, Wright A J, Pickard M and Abdel-Aal E S M 2020 Characterization of anthocyanin-containing purple wheat prototype products as functional foods with potential health benefits. *Cereal Chem* 97 34-38

[3] Khlestkina E K, Shoeva O Y and Gordeeva E I 2015 Flavonoid biosynthesis genes in wheat. *Russ J Genet Appl Res* 5(3) 268-278

[4] Lachman J, Martinek P, Kotikova Z, Orsák M and Šulc M 2017 Genetics and chemistry of pigments in wheat grain – a review. *J Cereal Sci* 74 145-154

[5] Francavilla A and J. Joye I J 2020 Anthocyanins in whole grain cereals and their potential effect on health. *Nutrients* 12 2922

[6] Correa R C G, Garcia J A A, Correa V G, Vieira T F, Bracht A and Peralta R M 2019 Pigments and vitamins from plants as functional ingredients: Current trends and perspectives. *Adv Food Nut Res* 90 259-303

[7] Loskutov I G and Khlestkina E K 2021 Wheat, barley, and oat breeding for health benefit components in grain. *Plants* 10 86

[8] Polonskiy V, Loskutov I and Sumina A 2020 Biological role and health benefits of antioxidant compounds in cereals. *Bio Comm* 65(1) 53-67

[9] Gamel T H, Wright A J, Pickard M and Abdel-Aal E S M 2020 Characterization of anthocyanin-containing purple wheat prototype products as functional foods with potential health benefits. *Cereal Chem* 97 34-38

[10] Landi M, Tattini M and Gould K S 2015 Multiple functional roles of anthocyanins in plant-environment interactions. *Environ Exp Bot* 119 4-17

[11] Duncan D R, Waskot RM and Nabors MW 1995 In vitro screening and field evaluation of tissue culture-regenerated sorghum (*Sorghum bicolor* (L.) Moench) for soil stress tolerance. *Euphytica* 85 373-380

[12] Pykalo S, Yurchenko T and Prokopik N 2019 Cell selection of wheat (*Triticum aestivum* L.) for drought tolerance. *Biopolymers and Cell* 35 396

[13] Mahpara S S T, Hussain S T and Farooq J 2014 Drought tolerance studies in wheat (*Triticum aestivum* L.). *Cercetări Agronomice in Moldova* 4(160) 133-140

[14] Mahmood I, Razzaq A, Rasheed M, Qayyum A and Ahmad M 2014 Employment of immature embryo culture for in vitro selection of drought tolerant somaclones of wheat. *Bulgarian Journal of Agricultural Science* 20 155-161

[15] Kacema N S, Delportea F, Muhovskia Y, Djekounb A and Watillona B 2017 In vitro screening of durum wheat against water-stress mediated through polyethylene glycol. *Journal of Genetic Engineering and Biotechnology* 15(1) 239-247

[16] Linmaier E M and Skoog F 1965 Organic growth factor requirements of tobacco tissue cultures. *Physiologia Plantarum* 18 100-127
[17] Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15(2) 473-497

[18] Kruglova N N, Titova G E and Seldimirova O A 2018 Callusogenesis as an in vitro morphogenesis pathway in cereals. Russian Journal of Developmental Biology 49(5) 273-288 (In Russian)

[19] Kruglova N N, Seldimirova O A and Zinatullina A E 2019 Callus in vitro as a model system for the study of plant organogenesis. Proceedings of the RAS Ufa Scientific Centre 2 44-54 (in Russian)

[20] Khlebova L P, Sokolova G G and Titova A M 2018 Somaclonal variation in spring bread wheat. Ukrainian Journal of Ecology 8(4) 454-458

[21] Miyao A, Nakagome M, Ohnuma T, Yamagata H, Kanamori H, Katayose Y, Takahashi A, Matsumoto T. and Hirochika H 2012 Molecular spectrum of somaclonal variation in regenerated rice revealed by whole-genome sequencing. Plant Cell Physiol 53(1) 256-264

[22] Chawla H S 1989 Regeneration responses of callus from different explants and changes on isozymes during morphogenesis in wheat Biol Plantarum 31 121-125

[23] Chu Z, Chen J, Xu H, Dong Z, Chen F and Cui D 2016 Identification and comparative analysis of microRNA in wheat (Triticum aestivum L.) callus derived from mature and immature embryos during in vitro culture. Frontiers in Plant Science 7 1302

[24] Alikina O, Chernobrovkina M, Dolgov S and Miroshnichenko D 2016 Tissue culture efficiency of wheat species with different genomic formulas. Crop Breed Appl Biotechnol 16(4) 307-314

[25] Parmar S, Sainger M, Chaudhary D and Jaiwal P 2012 Plant regeneration from mature embryo of commercial Indian bread wheat (Triticum aestivum L.) cultivars. Physiol Mol Biol Plants 18(2) 177-183

[26] Biradar D., Katageri I and Shuba S 2019 Optimization of an efficient rapid regeneration of indian wheat cultivars by callus induction and multiple shoot induction using mature embryos. International Journal of Current Microbiology and Applied Sciences 8 1685-1692

[27] Seldimirova O A, Kudoyarova G R, Kruglova N N et al. 2016 Changes in distribution of cytokinins and auxins in cell during callus induction and organogenesis in vitro in immature embryo culture of wheat. In Vitro Cell Dev Biol Plant 52(3) 251-264