Chapter 2. PLANT GENETICS AND BIOTECHNOLOGY

II skyrius. AUGALŲ GENETIKA IR BIOTECHNOLOGIJA

ISSN 1392-3196
Žemdirbystė / Zemdirbyste / Agriculture, vol. 94, No. 4 (2007), p. 99–104
UDK (633.11+633.15):631.524

THE EFFECT OF BAP ON HAPLOID REGENERATION AFTER WHEAT × MAIZE CROSSING

Gintaras BRAZAUSKAS, Izolda PAŠAKINSKIENĖ

Lithuanian Institute of Agriculture
Akademija, Dotnuva, Kėdainiai distr., Lithuania
E-mail: gintaras@lzi.lt

Abstract

The effect of benzylaminopurine (BAP) on haploid embryo regeneration after wheat x maize crossing was investigated. BAP application of 2.5–15 mg l^{-1} showed a positive effect on embryo shoot development. Significantly more embryos formed shoots when they were planted on medium with BAP (except 10 mg l^{-1}). This result was mostly achieved by significantly reduced numbers of embryos developing only roots on medium with BAP. However, the regeneration of fully-developed plantlets was not affected by the addition of BAP and differed insignificantly among the treatments. The results of this study indicate that addition of BAP to haploid embryo regeneration media promotes shoot development. However, additional experiments are needed to determine the optimum concentration of BAP for regeneration of fully-developed plants.

Key words: benzylaminopurine (BAP), haploid, wheat x maize.

Introduction

The method of interspecific hybridization of wheat x maize was first implemented in 1988 by Laurie and Bennett. They have shown that maize chromosomes are completely eliminated shortly after hybridization and haploid embryos could be obtained. Since then wheat crossed with maize has been the method of choice for producing DHs and details for the many factors involved in DH production have been presented. Some of the factors that are important in achieving successful haploid production are growth conditions for donor plants, including temperature and light /Campbell et al., 2001/, emasculation procedures and post-pollination treatment with plant growth regulators. The genotypic influences of both donor plants, e.g. wheat and maize, have also been reported but the extent of this factor is believed to be less important than that observed for *in vitro* androgenesis.

The artificial auxin 2,4-dichlorphenoxyacetic acid (2,4-D) has been used extensively with maize pollination to sustain embryo development /Campbell et al.,

1998/. Dicamba (3,6-dichloro-o-anisinic acid) has also been used /Savaskan et al., 1997/, as have other biologically active chemical such as gibberelic acid, kinetin, silver nitrate, p-chlorphenoxyacetic acid (CPA) and 2,4,5-trichlorphenoxyacetic acid (2,4,5-T) /Matzk, Mahn, 1994/. Despite the differences in their chemical composition and mode of action the effect on haploid embryo induction were not significantly different among the chemical compounds studied.

Few studies have been conducted on the efficiency of various media components used for the *in vitro* regeneration of immature embryos resulting from wheat x maize crossing. Growth regulator concentrations in culture medium are critical for the control of growth and morphogenesis. Generally, high concentration of auxins and low cytokinins in the medium promote abundant cell proliferation with the formation of callus. Regeneration occurs either by somatic embryogenesis or adventitious bud and shoot development with subsequent rooting, while sometimes it may occur through direct organogenesis /Aionesei et al., 2005/. The use of BAP at low levels in the medium was shown to be efficient for embryogenesis and the maintenance of long-term morphogenic capacity in barley /Sharma et al., 2005/.

The objective of this study was to evaluate the effect of BAP on the regeneration of immature embryos resulting from wheat x maize crossing.

Materials and methods

The effect of synthetic cytokinin benzylaminopurine (BAP) on haploid embryo development was evaluated by adding various BAP amounts to 2/3 B5 medium (30 g l^{-1} sucrose, 7 g l^{-1} agar). The experiment consisted of control and four treatments with BAP: 2.5, 5.0, 10.0, and 15.0 mg l^{-1}. Haploid embryos for this experiment were obtained from the wheat F_1 hybrid ('Flair' × 'Asketis') pollinated with maize variety 'Sundance'. Wheat florets were not emasculated but pollinated one day before anthesis. The whole wheat inflorescence was submerged for 10 seconds in 50 mg l^{-1} 2,4-D solution 24 hours after pollination. Embryos were rescued and transferred onto 2/3 B5 medium with various BAP concentrations 17 days after pollination. 250 embryos were planted in total, 50 in each treatment and control. The embryos were incubated at 20–22 ºC in dark for 14 days and later kept at 22–25 ºC with 16 h light (8000 Lx). The regeneration efficiency was evaluated 5 weeks after planting by counting (1) fully regenerated plants (shoots and roots), (2) embryos regenerating only roots, (3) embryos regenerating only shoots, (4) embryos that did not develop at all.

The ploidy status of regenerated plants was evaluated by flow cytometry (Partec PA). Approximately 20 mm^2 samples of young leaves were used for flow cytometric analysis. These samples were chopped with a razor blade in 250 µl buffer solution (0.1 M citric acid and 0.5 % Tween 20, pH 2.5). The chopped sample was passed through a nylon filter of 40 µm mesh size and 500 µl of the staining buffer (0.4 M Na_2HPO_4 and 2 mg l^{-1} DAPI, pH 8.5) was added. The voltage of the photomultiplier for external standard (winter wheat variety 'Širvinta 1') was adjusted to channel position 100, so the haploid samples produced peaks at channel position 50.

The significance of differences between treatments was evaluated by contingency χ^2 test with software STATISTICA v. 6.0.

Results and discussion

Two hundred fifty haploid embryos were transferred to B5 culture medium with various concentrations of BAP (0–15 mg l^{-1}) to study the effect of BAP on haploid embryo regeneration (Table). Sixty five embryos regenerated fully-developed plantlets with both roots and shoots, another 24 embryos developed only roots while 49 had only shoots. Finally, 112 of the planted embryos did not develop at all and were lost subsequently.

The results obtained in this study showed a positive effect of BAP on embryo shoot development. Significantly more embryos developed shoots when they were planted on B5 medium with BAP (2.5–15 mg l^{-1}, except 10 mg l^{-1}) compared with B5 medium without BAP (Table). Significantly fewer embryos formed only roots on medium with BAP as well. However, more fully developed plants (developing both shoots and roots) were obtained on medium without BAP.

The number of only-root-forming embryos decreased from 30.0 % to 2.0–8.0 % of embryos planted when BAP was added to B5 medium. This indicates the role of BAP in suppression of the root formation versus promoted shoot development. 40.0 % to 58.0 % of embryos planted on B5 medium formed shoots (only shoots or both shoots and roots) while on the medium without BAP 34.0 % of embryos planted developed fully (formed shoots and roots) and not only-shoot-forming embryos were obtained.

Table. The effect of BAP on embryo regeneration
Lentelė. *BAP įtaka gemalų regeneracijai*

BAP concen-tration *BAP koncent-racija*	Embryos planted *Pasodinta gemalų*	Developed shoots and roots *Išsivystė stiebai ir šaknys*		Developed only roots *Išsivystė tik šaknys*		Developed only shoots *Išsivystė tik stiebai*		Embryos did not develop *Gemalai nesivystė*	
mg l^{-1}	No. *Vnt.*	No. *Vnt.*	%	No. *Vnt.*	%	No. *Vnt.*	%	No. *Vnt.*	%
0	50	17	34.0	15	30.0	0	0.0	18	36.0
2.5	50	12	24.0 n.s.	3	6.0*	11	22.0*	24	48.0 n.s.
5.0	50	12	24.0 n.s.	1	2.0*	13	26.0***	24	48.0 n.s.
10.0	50	12	24.0 n.s.	4	8.0*	8	16.0 n.s.	26	52.0 n.s.
15.0	50	12	24.0 n.s.	1	2.0*	17	34.0***	20	40.0 n.s.
Total *Iš viso*	250	65	26.0 n.s.	24	9.6*	49	19.6*	112	44.8 n.s.

*** P < 0.001; ** P = 0.001-0.01; * P = 0.01-0.05; n.s. (non significant) P > 0.05 according to contingency χ^2 test
*** *P < 0,001; ** P = 0,001-0,01; * P = 0,01-0,05; n.r.(neesminis) P > 0,05 pagal dvireikšmių lentelių* χ^2

Synthetic auxins, e. g. 2,4-D, were shown to be useful in overcoming crossability barriers in wide crossings. When *Triticum* species are pollinated with maize, the ovaries remain small and gradually dry out on the plants. However, application of 2,4-D promotes ovary swelling and thus supports embryo formation.

Several authors have shown that rather high 2,4-D concentrations of 50–100 mg l^{-1} should be applied to obtain maximum embryo formation /Inagaki, 2003/. On the other hand high auxin concentrations applied disturb endogenous hormone balance and lead to increased callus formation from immature embryos when transferred to culture medium. The results of our study show that application of 2,4-D at a concentration of 50 mg l^{-1} one day after pollination promoted embryo formation but subsequently induced callusogenesis in 30 % of the embryos planted. These embryos formed only roots which is also an indication of excess auxin treatment. Cytokinins were shown to counteract the effect of auxins and endogenous auxin/cytokinin balance is needed to promote embryo regeneration /Kyte, Kleyn, 2005/.

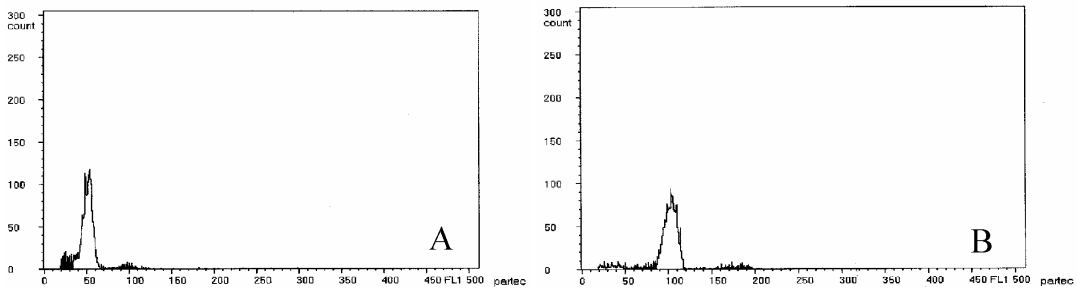

Figure. Ploidy evaluation of wheat haploids with Partec ploidy analyser. A – haploid wheat (DNA fluorescence intensity of 50 relative units). B – hexaploid wheat (DNA fluorescence intensity of 100 relative units).

Paveikslas. *Kviečių haploidų ploidiškumo įvertinimas „Partec" ploidiškumo analizatoriumi. A – haploidinis kvietys (DNR fluorescencijos intensyvumas – 50 santykinių vienetų). B – heksaploidinis kvietys (DNR fluorescencijos intensyvumas – 100 santykinių vienetų)*

Benzylaminopurine (BAP) being cytokinin in tissue culture media is incorporated mainly for cell division and differentiation of adventitious shoots from callus and organs. BAP has been successfully applied in barley for regeneration from mature embryos in vitro. The incorporation of BAP at low levels in the medium was found to be most effective for embryogenesis and the maintenance of long term morphogenic capacity /Sharma et al., 2005/.

However, in our study more fully developed plants were obtained on the medium without BAP than on the medium with BAP, respectively 34.0 % and 24.0 %. Probably rather high (2.5–15 mg l^{-1}) concentrations of BAP applied in this experiment had too strong an effect on embryo rhizogenesis, and therefore part of the embryos formed only shoots. Such an effect was also observed in the development of interspecific clover hybrids on B5 medium with 10 mg l^{-1} BAP added /Dabkevičienė, 2000/. The

application of lower (< 2.5 mg l^{-1}) BAP concentrations could probably enhance development of fully regenerated plants from haploid embryos *in vitro*.

Extensive spontaneous chromosome doubling of up to 70 % have been reported in barley isolated microspore culture /Davies, 2003/. These spontaneous doubled haploids do not require application of polyploidization agents and are desired in haploid production systems. We have screened 65 fully regenerated plants in our experiment with Partec Ploidy Analyser against external standard of hexaploid wheat variety 'Sirvinta1'. All 65 regenerated plants had DNA fluorescence intensity of 50 relative units as compared to that of 100 relative units in standard hexaploid (Figure). Our results show that spontaneous chromosome doubling is unlikely to occur in wheat crossings with maize and application of polyploidization agents is necessary to restore fertility of doubled haploids.

Conclusion

The results of our study show that benzylaminopurine (BAP) applied in vitro enhances shoot formation of haploid embryos after wheat x maize crossings. However, additional experiments are needed to determine the optimum concentration of BAP for regeneration of fully-developed plants.

Received 23 08 2007
Aaccepted 08 10 2007

REFERENCES

1. Aionesei T., Touraev A., Heberle-Bors E. Pathways to microspore embryogenesis // In Haploids in crop improvement II / Ed. C. E. Palmer, W. A. Keller and K. J. Kasha. – Germany, 2005, p. 11–34

2. Campbell A. W., Griffin W. B., Burritt D. J., Conner A. J. The importance of light intensity for the pollen tube growth and embryo survival in wheat × maize crosses // Annals of Botany. – 2001, vol. 87, p. 517–522

3. Campbell A. W., Griffin W. B., Conner A. J. et al. The effects of temperature and light intensity on embryo numbers in wheat doubled haploid production through wheat × maize crosses // Annals of Botany. – 1998, vol. 82, p. 29–33

4. Dabkevičienė G. Embryo culture and micropropagation in clover allopolyploidy // Horticulture and Vegetable Growing. – 2000, vol. 19, iss. 3, p. 375–383

5. Davies P. A. Barley isolated microspore culture (IMC) method // In Doubled haploid production in crop plants / Ed. M. Maluszynski et al. – Netherlands, 2003, p. 49–52

6. Inagaki M. N. Doubled haploid production in wheat through wide hybridization // In Doubled haploid production in crop plants / Ed. M. Maluszynski et al. – Netherlands, 2003, p. 53–58

7. Kyte L., Kleyn J. Plants from test tubes // An introduction to micropropagation. – US, 2005. – 69 p.

8. Laurie D. A., Bennett M. D. The production of haploid wheat plants from wheat x maize crosses // Theoretical and Applied Genetics. – 1988, vol. 76, p. 393–397

9. Matzk F., Mahn A. Improved techniques for haploid production in wheat using chromosome elimination // Plant Breeding. – 1994, vol. 113, p. 125–129

10. Savaskan C., Ellerbrook C., Fish L. J., Snape J. W. Doubled haploid production in Turkish durum wheats using crosses with maize // Plant Breeding. – 1997, vol. 116, p. 299–301

11. Sharma V. K., Hansch R., Mendel R. R., Schultze J. Mature embryo axis-based high frequency somatic embryogenesis and plant regeneration from multiple cultivars of barley (*Hordeum vulgare* L.) // Journal of Experimental Botany. – 2005, vol. 56, iss. 417, p. 1913–1922

ISSN 1392-3196
Žemdirbystė, t. 94, Nr. 4 (2007), p. 99–104
UDK (633.11+633.15):631.524

BAP ĮTAKA HAPLOIDŲ REGENERACIJAI KVIEČIO IR KUKURŪZO KRYŽMINIMUOSE

G. Brazauskas, I. Pašakinskienė

Santrauka

Siekiant pagerinti haploidinių augalų išeigą, tirtas citokinino BAP poveikis haploidinių gemalų regeneracijai kviečio ir kukurūzo kryžminimuose. Maitinamąją terpę papildžius 2,5–15 mg l^{-1} BAP, pastebėta, kad gemalai suformavo iš esmės daugiau stiebų (išskyrus 10 mg l^{-1} BAP). Taip pat, panaudojus terpę su BAP, iš esmės mažiau gauta gemalų, formuojančių tik šaknis. Vis dėlto visiškai regeneravusių augalų skaičiui BAP įtakos nedarė ir skirtumai tarp eksperimento variantų nebuvo esminiai. Gauti duomenys rodo, kad BAP skatina haploidinių gemalų stiebų vystymąsi, tačiau būtini papildomi tyrimai, siekiant nustatyti optimalią BAP koncentraciją visiškai išsivysčiusių haploidų regeneracijai skatinti.

Reikšminiai žodžiai: benzilaminopurinas (BAP), haploidas, kvietys x kukurūzas.