

GENOTIPA UN VIDES IETEKME UZ VASARAS MIEŽU GRAUDU KVALITĀTI

Bleidere M.

Vasaras miežu (*Hordeum vulgare* L.) graudu kvalitāte būtiski nosaka to tālākās izmantošanas iespējas pārstrādē. Pētījuma mērķis bija noteikt genotipa un vides ietekmi uz miežu graudu kvalitāti. Pētījums veikts Valsts Stendes graudaugu selekcijas institūtā no 2004. līdz 2006. gadam. Piecdesmit diviem genotipiem novērtēja tilpummasu, 1000 graudu masu un graudu ķīmisko sastāvu (cieti, kopproteīnu, koptaustus, β -glikānus, kokšķiedru un koppelus). Analizēja faktoru (šķirne, gads) ietekmes īpatsvaru ($\eta\%$), kā arī meteoroloģiskos apstākļus (vidējā gaisa temperatūra un nokrišņu summa) graudu veidošanās periodā. Dispersijas analīze liecina, ka visu novērtēto graudu kvalitātes rādītāju mainību būtiski ($p \leq 0.01$) ietekmē genotips. Genotipa kā faktora ietekmes īpatsvars ($\eta\%$) virs 70% bija cietei, kopproteīnam, β -glikāniem, kokšķiedrai, 1000 graudu masai un tilpummasai. Konstatēta būtiska gada ietekme uz graudu kvalitātes mainību visām pazīmēm, izņemot β -glikāniem un tilpummasai. To nosaka starp gadiem atšķirīgie meteoroloģiskie apstākļi graudu veidošanās periodā.

THE EFFECT OF TILLER NUMBERS IN WHEAT HAPLOID PRODUCTION

Brazauskas G.

Lithuanian Institute of Agriculture, LT-58344 Akademija, Kėdainiai r., Lithuania

phone: +37034737149, e-mail: gintaras@lzi.lt

Abstract

Winter wheat doubled haploid production is widely used not only for plant breeding acceleration but also in basic research, such as genetic mapping, haploid transformation and artificial seed production. It is essential to identify all factors which determine haploid system productivity. We have studied the effect of tiller numbers on embryo development and haploid regeneration in wheat x maize haploid production system. Four winter wheat F_1 hybrid lines were pollinated with the maize variety 'Golden Bantam' pollen. Embryo formation and haploid regeneration data were collected from the first five tillers in each plant. The data showed a clear tendency of reduction in embryo formation frequency on the successive tillers. There were 17.5% and only 12.4% embryos on average formed on the main and fourth tillers, respectively. However, two-way ANOVA analysis showed these differences to be insignificant ($p > 0.05$) and only the genotype had a significant ($p < 0.01$) effect on embryo formation. There was no clear effect by both genotype and tiller numbers on haploid regeneration frequency. The overall embryo formation and haploid regeneration frequency in this experiment was 14.6% (504 embryos) and 68.1% (367 haploids), respectively. The results obtained in this study indicate that as many as five tillers per plant can be used in wheat x maize crossing systems with no significant tiller effect on the efficiency of the haploid production system.

Key words: wheat x maize, haploid, tiller number.

Introduction

Winter wheat doubled haploid production is widely used not only for plant breeding acceleration but also in basic research, such as genetic mapping, haploid transformation and artificial seed production. Several techniques exist for wheat haploid development. These include crossing with maize (Laurie and Bennet, 1988), anther culture (Last and Brettell, 1990) and isolated microspore culture (Gustafson *et al.*, 1995). Wheat x maize crossing is the system of choice in winter wheat breeding programs due to lower genotype dependency and albinism avoidance (Snape, 1998). However, wheat x maize crossing is a time consuming and costly technique. It requires manual application of maize pollen and subsequent embryo excision. Ways to determine the factors underlying the efficacy of the method are being searched for.

Various factors are related to the differences in wheat haploid formation after crossing with maize. Campbell *et al.*, (1998) determined that both temperature and light intensity influence embryo formation in the wheat x maize system. This effect was later associated with the abnormalities in

pollen tube growth at low light intensities (Campbell *et al.*, 2001). The maize genotype was also shown to have an effect on wheat haploid induction (Verma *et al.*, 1999). Several reports demonstrate the role of various hormone treatments on embryo survival (Matzk and Mahn, 1994, Ushiyama *et al.*, 2007). The effect of the spikelet position on embryo formation was shown by Martins-Lopes *et al.* (2001) where different spikelet positions (lower, middle and upper) gave highly significant differences in embryo formation. However these results contradict those reported by Bitsch *et al.* (1998), where they said that the time of pollination is not critical for the success of the crosses. Until now, only one report has shown the influence of the spike position on the plant in haploid induction in barley anther culture (Jacquard *et al.*, 2006).

In the present work we have studied the effect of tiller numbers on the plant in haploid embryo formation and regeneration after wheat x maize crossing.

Materials and Methods

Four winter wheat F₁ hybrid lines (No. 601, 602, 603 and 604) were used for pollination with the pollen of the maize variety 'Golden Bantham'. Wheat hybrid genotypes were produced through various inter-varietal crossings at the Cereal Breeding Department of the Lithuanian Institute of Agriculture. Both wheat and maize plants were grown in a heated glasshouse with a temperature range of 15 -25 °C. Wheat plants were vernalized for 8 weeks at 3 °C in dim light. The first five tillers of each wheat plant were used for pollination. The first tiller to flower was denoted as the main tiller (MT) and the subsequent tillers were numbered from 1 to 4 (T1 - T4). Seven plants of each genotype were regarded as replications. Wheat florets were not emasculated but pollinated one day before anthesis. 23-28 florets were pollinated per spike. Hormone treatment was performed by submerging the whole wheat inflorescence in 50 mg l⁻¹ 2,4-D (Duchefa Biochemie B.V.) solution for 10 seconds 24 hours after pollination. Embryo rescue was made 17 days after pollination. Aseptically excised embryos were transferred into tubes with 5 ml B5 2/3 strength medium containing 3% sucrose and 0.7 % agar (all Duchefa Biochemie B.V.). Planted embryos were kept at 20 °C in the dark until germinated. Further growth was maintained at 24 °C with a 16 h day length.

The embryo formation frequency (EFF) was referred as the number of embryos formed per 100 florets pollinated and the haploid regeneration frequency (HRF) as the number of haploid plants regenerated per 100 embryos planted. Statistical analysis was performed as a two-way ANOVA with the software 'Selekcija'.

Results and Discussion

A total of 3477 wheat florets were pollinated with maize pollen which resulted in 504 embryos. Embryos were obtained in all crossing combinations. Embryo formation frequency varied among the four wheat genotypes used. The highest EFF was recorded for the wheat line No. 604 where embryos formed in 17.6% of pollinated florets while line No. 602 had the EFF of only 9.9% (table1).

Table 1. The average values of embryo formation frequency (EFF) and haploid regeneration frequency (HRF) among wheat lines and tillers.

| Tiller number | Wheat line | | | | | | | | Mean | |
|-----------------|------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | 601 | | 602 | | 603 | | 604 | | EFF, % | HRF, % |
| | EFF, % | HRF, % | EFF, % | HRF, % | EFF, % | HRF, % | EFF, % | HRF, % | | |
| MT ¹ | 19.2 | 64.3 | 16.8 | 59.7 | 8.4 | 54.5 | 25.8 | 86.9 | 17.5 | 66.3 |
| T1 | 16.3 | 49.5 | 6.5 | 85.7 | 20.0 | 80.1 | 17.2 | 69.1 | 15.0 | 71.1 |
| T2 | 12.1 | 81.0 | 11.3 | 47.4 | 19.1 | 68.5 | 15.0 | 73.8 | 14.4 | 67.7 |
| T3 | 17.5 | 78.8 | 5.6 | 50.0 | 13.4 | 72.6 | 17.9 | 67.7 | 13.6 | 67.3 |
| T4 | 12.3 | 77.6 | 9.4 | 34.0 | 16.3 | 79.8 | 11.8 | 81.0 | 12.4 | 68.1 |
| Mean | 15.5 | 70.2 | 9.9 | 55.4 | 15.4 | 71.1 | 17.6 | 75.7 | 14.6 | 68.1 |

¹MT – main tiller, T1, T2, T3, T4 – first, second, third and fourth tillers, respectively

ANOVA analysis confirmed the genotype effect on embryo formation to be significant (P < 0.01) (table2). The data showed a clear tendency of reduction in embryo formation frequency on the

successive tillers. There were 17.5, 15.0, 14.4, 13.6 and only 12.4% embryos on average formed on the main (MT), first (T1), second (T2), third (T3) and fourth (T4) tillers, respectively. However this tendency could not be proven by ANOVA analysis which showed the differences to be insignificant ($P > 0.05$).

367 haploid plants were obtained from 504 embryos planted. The average haploid regeneration frequency varied from 55.4 to 75.7% for wheat lines No. 602 and 604, respectively (Table 1)

The same genotypes had the lowest and the highest embryo formation frequency, respectively. No obvious tendency was observed in HRF when compared with successive tillers. The highest HRF of 71.1% was recorded for T2 and the lowest HRF of 66.3% for MT. Neither the genotype nor the tiller number effect could be proven by ANOVA analysis. Both factors as well as their interaction had insignificant ($P > 0.05$) differences among variables (Table 2).

Table 2. The results of ANOVA analysis for genotype, tiller number and their interaction

| Factor | df | EFF | HRF |
|--------------------------|----|---------------------|-----------|
| Genotype | 3 | 5.28** ¹ | 1.96 n.s. |
| Tiller number | 4 | 1.52 n.s. | 0.05 n.s. |
| Genotype x tiller number | 12 | 1.74 n.s. | 1.44 n.s. |

¹** $P < 0.01$, n.s. – not significant ($P > 0.05$)

The wheat x maize crossing technique involves several key steps. These include: synchronization of wheat and maize flowering, manual wheat florets' pollination with maize pollen, embryo excision/culture *in vitro* and haploid treatment with colchicine. All these steps are labor intensive and raise the costs of the haploid production. Improvements are needed to increase the efficiency of the method.

The number of florets as potential haploid producers could be increased per plant by pollination (more florets per spike) or by using more spikes per plant. Previous studies have shown that different spikelet positions (lower, middle and upper) gave different success ratios for embryo initiation (Martins-Lopes *et al.*, 2001). However Bitsch *et al.* (1998) earlier reported that embryo initiation was found to be dispersed evenly all over the wheat spike. In a conventional wheat x maize crossing technique 25-30 florets are usually pollinated per spike. It would be difficult to increase this number due to the lag in flowering synchronization. However the number of the crossed spikes per plant could be easily increased by growing spaced plants but this would be feasible only if there is no considerable loss in method efficiency parameters on the subsequent tillers.

The results of our study indicate the tendency of influence of the tiller number on embryo formation. This tendency is supported by Jacquard *et al.* (2006) findings in barley anther culture. They have shown that the position of the tiller on the plate of tillering has an impact on both the number of regenerated plantlets and the percentage of green plants. The anther response decreased significantly when the donor spike originates from the main shoot or the fourth tiller. However our results could not be proven statistically. The tiller number effect on barley anther culture was explained by tiller competition for carbon assimilate or nitrogen compounds. Regular application of complex fertilizers to wheat plants could minimize the starvation effect in our experiment.

Conclusions

The results obtained in this study indicate that as many as five tillers per plant can be used in wheat x maize crossing systems with no significant tiller effect on the efficiency of the haploid production system.

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STIEBRU KĀRTAS SKAITA IETEKME UZ KVIEŠU HAPLOĪDU VEIDOŠANOS

Brazauskas G.

Ziemas kviešu dubultotie haploīdi tiek plaši izmantoti ne tikai augu selekcijas procesa paātrināšanai, bet arī tādos fundamentālos pētījumos, kā, piemēram, ģenētiskā kartēšana, haploīdu transformācija un mākslīgo sēklu ražošana. Ļoti svarīgi ir noteikt visus faktoros, kas nosaka haploīdu iegūšanas efektivitāti. Izmēģinājumā pētīta dzinumu skaita ietekme uz embriju attīstību un haploīdu reģenerāciju iegūstot kviešu haploīdus ar kviešu x kukurūzas haploīdu iegūšanas shēmu. Četras ziemas kviešu F₁ hibrīdās līnijas tika apputeksnētas ar kukurūzas 'Golden Bantham' putekšņiem. Tika vērtēti embriju veidošanās un haploīdu reģenerācijas dati no katra auga pirmajām piecām vārpām. Iegūtie rezultāti parādīja tendenci, ka visvairāk embriju veidojās no galvenās vārpas, bet no pārējām to skaits secīgi samazinājās. No galvenās vārpas tika iegūti 17.5% embriju, bet no ceturtais – tikai 12.4%. Tomēr divu faktoru dispersijas analīze parādīja, ka starpība starp iegūto embriju skaitu no pirmās un sekojošām vārpām nav būtiska ($p > 0.05$); embriju veidošanos būtiski ietekmēja tikai genotips ($p < 0.01$). Netika konstatēta skaidra genotipa un stiebru skaita ietekme uz haploīdu reģenerācijas biežumu. Kopumā izmēģinājumā tika iegūti 14.6% (504) embriji un 68.1% (367) haploīdi. Izmēģinājuma rezultāti liecina, ka pirmās piecas vārpas no katra auga var tikt izmantotas kviešu x kukurūzas haploīdu ražošanas shēmā bez būtiskas dzinuma ietekmes uz iegūto haploīdu skaitu.