Genotype effect on wheat haploid production in wheat × maize crosses

G. Brazauskas,
I. Pašakinskienė

Lithuanian Institute of Agriculture,
LT-5051 Akademija,
Kėdainiai distr., Lithuania

The effect of wheat and maize genotypes as well as their interaction on the wheat embryo and haploid plant production efficiency in wheat × maize crosses was investigated. Wheat haploid plants in all crossing combinations (5 wheat lines × 3 maize varieties) were obtained at an average rate of 3.7% of florets pollinated. The mean numbers for wheat embryo formation and haploid plant regeneration were 10.5% and 36.1%, respectively. The embryo formation frequency (EFF) was highly ($p < 0.001$) influenced by both wheat and maize genotypes, as well as by their interaction. The influence on haploid regeneration frequency (HRF) statistically was proved only for the wheat genotype. For genotypes producing the highest EFFs, the regeneration frequency was not necessarily higher.

Key words: wheat haploids, wheat × maize cross, genotype effect

INTRODUCTION

The production of a new wheat variety is still a time-consuming process. It requires many (10–12) generations of self-pollination and selection to achieve the levels of homozygosity required for a variety to be released. Development of doubled haploid populations can shorten this process and increase the genetic stability of new varieties [1].

Production of wheat haploids from crosses with maize was first reported in 1988 [2]. It was shown that maize chromosomes are eliminated during the early divisions of hybrid zygote, thus producing a haploid wheat embryo. Since then many improvements have been made to increase the efficiency of the technique. The influence of parental genotypes at first was neglected and most attention was paid to the improvement of the crossing technique, hormonal treatment and the content of the embryo culture medium. Gradually, the genotypic specificity became evident. Haploid production efficiency was found to be variable depending on wheat line involved [3–5]. However, the importance of choice between maize varieties as pollinators is still a subject of discussion. So far, no consistency has been found weather it affects the rate of haploid embryo formation or not [3, 5].

In the present work, we have studied the influence of both wheat and maize genotype as well as their interaction on the rate of wheat embryo formation and haploid plant production through wheat × maize crosses.

MATERIALS AND METHODS

Five winter wheat F_1 hybrid lines produced at the Crop Breeding Department of Lithuanian Institute of Agriculture were used as female parents, and three maize varieties ('Early King', 'Sundance' and 'Aviriai') served as pollinators. The wheat plants were vernalised at tillering stage for 8 weeks (2 °C, 8 h a day, 2 klux) and subsequently grown in an environmentally-controlled chamber under 16 h daylength (10 klux) and 24 °C (day) / 20 °C (night) regime. Maize was grown in a glasshouse with the temperature ranging within 15–25 °C. Wheat florets were emasculated 1–2 days before anthesis. The pollination was done 2 days after emasculation with freshly collected maize pollen. The uppermost internode of pollinated tillers was filled with 2.4-D (dichlorphenoxy acetic acid, Sigma) solution immediately after pollination. Also, individual florets were given a drop of 2.4-D inbetween lema and palea 24 h after pollination. Embryo rescue was made 17 days after pollination. Excised embryos were transferred into tubes with 5 ml B5 medium (Sigma) containing 3% sucrose and 0.7% agar. Planted embryos were kept at 20 °C in the dark until germinated. Further growth was maintained at light with 16 h daylength 24 °C 4 klux. Plantlets were transferred into soil

when a third leaf appeared. The ploidy level of the regenerated plants was determined on a Partec PA flow cytometer.

The embryo formation frequency (EFF) was referred as a number of embryos formed per 100 florets pollinated and the haploid regeneration frequency (HRF) as a number of haploid plants regenerated per 100 embryos planted. Statistical analysis was performed as a two-way ANOVA for qualitative characters [6] with the 'Selekcija' software [7].

RESULTS AND DISCUSSION

A total of 6214 pollinated florets produced 648 haploid embryos (10.5% of pollinated florets), and 230 plantlets (36.1% of embryos planted) were obtained (Table 1). Flow cytometyric analysis was carried out to detect the ploidy level of the regenerants. The analysis showed that most of the plants (99.6%) were haploid and only one had a doubled haploid DNA content, which had to be caused by spontaneous autopolyploidy during the embryo culture, since selfing or crosspolination was prevented.

In all five winter wheat lines haploid plants were obtained after crossing with 3 maize varieties. To calculate the effect of parental genotypes and their interaction on the yield of embryos and haploid plants, statistical analysis was carried out (Table 2). The analysis showed that both wheat and maize genotypes, as well as their interaction had a highly significant ($p < 0.001$) effect on embryo formation frequency (EFF). The highest number of the embryos in this experiment was obtained for the wheat line No. 905 (14.1% of pollinated florets on average), and pollination by the pollen of 'Early King' was more efficient than by 'Sundance' or 'Aviriai' pollen. The best wheat × maize combination was obtained in the case of the wheat line No. 905 pollinated with the maize 'Early King' pollen (16.2% embryos of pollinated florets).

Haploid regeneration frequency (HRF) also varied among parental genotypes. The character was significantly ($p < 0.01$) influenced by the wheat genotype and varied from 26.5 to 41.8% of embryos planted. However, nor the influence of maize genotype or a wheat × maize interaction on HRF were proved statistically. Almouslem et al. [8] also reported that maize pollinators did not have any significant influence on the germination of haploid embryo in durum wheat. Most probably wheat haploid plant regeneration is not affected by pollinator, since maize chromosomes are eliminated very early – during the first three divisions of hybrid zygote [9]. The results of this experiment indicate that the efficiency of the method is highly dependent on the wheat genotype used. In contrast, the influence of maize genotype was mild and was not proved statistically. Bitsch with colleagues [6] also detected a clear genotypic influence of wheat genotype, while the effect of maize genotype was found to be circumstantial. However, Verma with colleagues [4] pointed out that the both parental genotypes showed significant effects, but the effect of maize genotype was greater. This may be due to the high number (15) of maize genotypes investigated.

We produced haploid wheat plants in all crossing combinations at an average rate of 3.7% of florets pollinated. From a practical point of view, in this experiment the sweet corn cultivars 'Early King' and 'Sundance' turned out to be more convenient than the Lithuanian variety 'Aviriai'. Sweet corns demonstrate a good general response when used as pollinators, also they are of moderate hight, easy to cultivate in the greenhouse, grow quickily and produce large amounts of pollen. In further studies, the crossing technique, hormonal treatment and the content of embryo medium must be the main targets for the improvement of wheat haploid production.

Table 1. **Results of embryo formation frequency (EFF) and haploid regeneration frequency (HRF) in five wheat genotypes pollinated with three maize genotypes**

Wheat line	Maize variety							
	Early King		Aviriai		Sundance		Mean	
	EFF	HRF	EFF	HRF	EFF	HRF	EFF	HRF
902	14.0	35.5	9.4	45.0	11.4	44.9	11.6	41.8
903	11.7	32.0	6.5	50.0	10.2	38.2	9.5	40.1
904	11.4	27.7	10.0	26.2	4.4	42.0	8.6	32.0
905	16.2	48.5	11.3	45.7	14.9	26.0	14.1	40.1
909	6.8	30.0	4.9	33.3	14.5	16.1	8.7	26.5
Mean	12.0	34.7	8.4	40.0	11.1	33.4	10.5	36.1

Table 2. **Influence of parental genotypes and their interaction on embryo formation frequency (EFF) and haploid regeneration frequency (HRF)**

	df	EFF	HRF
Wheat	4	7.34^{***}	3.54^{**}
Maize	2	7.94^{***}	n.s.
Wheat × maize	8	4.71^{***}	n.s.

$^{***}p < 0.001$; $^{**}p = 0.01–0.001$; n.s. – not significant ($p > 0.05$).

ACKNOWLEDGEMENTS

This study was partly supported by grant No. K-049 from the Lithuanian State Science and Studies Foundation (MSF), Lithuanian Ministry of Science and Education.

References

1. Snape JW. Euphytica 1998; 100: 207–17.
2. Laurie DA, Bennett MD. Theor Appl Genet 1988; 76: 393–7.
3. Inagaki M, Tahir M. Jpn J Breed 1990; 40: 209–16.
4. Bitsch C, Groger S, Lelley T. Euphytica 1998; 103: 319–23.
5. Verma V, Bains NS, Mangat GS, Nanda GS, Gosal SS, Singh K. Crop Science 1999; 39: 1722–7.
6. Стельмах АФ. Генетический анализ количественных и качественных признаков с помощью математико-статистических методов. Москва, 1973.
7. Tarakanovas P. Selekcinių-genetinių tyrimų rezultatų apdorojimo ir įvertinimo sistema 'Selekcija'. Dotnuva–Akademija. 1996.
8. Almouslem AB, Jauhar PP, Peterson TS, Bommineni VR, Rao MB. Crop Science 1998; 38: 1080–7.
9. Laurie DA, Bennett MD. Genome 1989; 32: 953–61.

G. Brazauskas, I. Pašakinskienė

GENOTIPO ĮTAKA KVIEČIŲ HAPLOIDŲ IŠEIGAI KVIEČIŲ × KUKURŪZŲ KRYŽMINIMUOSE

Santrauka

Tirta tėvinių genotipų įtaka kviečių haploidų išeigai kviečių × kukurūzų kryžminimuose. Penkios kviečių linijos buvo apdulkintos trijų kukurūzų veislių žiedadulkėmis. Kviečių haploidiniai augalai gauti visose kryžminimo kombinacijose. Vidutinė kviečių gemalų išeiga sudarė 10,5% nuo apdulkintų žiedų. Pasodinus ant mitybinės terpės, 36,1% gemalų regeneravo į haploidinius augalus. Bendra haploidų išeiga buvo 3,7% apdulkintų žiedų skaičiaus. Gemalų formavimuisi patikimą ($p < 0,001$) įtaką darė kviečių ir kukurūzų genotipai bei jų sąveika. Gemalų regeneraciją patikimai veikė tik kviečių genotipas. Kryžminimo kombinacijos, pranašesnės pagal haploidinių gemalų užmezgimą, ne visuomet pasižymėjo geresne haploidų regeneracija.