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Doubled haploids of wheat from wheat × maize crosses: genotypic influence, fertility and inheritance of the 1BL-1RS chromosome

Abstract The wheat × maize cross as a technique for haploid induction in wheat was evaluated in a replicated block design comprising 18 wheat F₁ hybrids and five Zea mays L. parents. Haploid plants were regenerated at an average of 9.1 (4.4–14.7) plants per 100 florets processed. Genotypic differences for haploid production efficiency were recorded for both wheat and Zea mays L. Interaction between parents was significant for number of plants/100 florets. All 610 of the 1,703 regenerated plantlets that were analyzed by flow cytometry were haploid. At maturity, 70% (60–81%) of the colchicine-treated haploid plants were fertile, but the frequency of fertile and sterile plants was not consistent over the wheat hybrids from which they were derived. Flow cytometry performed using the first tiller which arose following colchicine treatment enabled prediction of fertility. The 1BL-1RS chromosome was found at the expected ratios in the F₂ and in the haploid progenies produced through the wheat × maize cross but deviated from the 1:1 ratio in the haploid progenies produced by anther culture.

Key words Wheat · Wheat × maize cross · Haploid · Doubled haploid · Distortion of segregation

Introduction

Unlike barley, very few wheat cultivars have been released as doubled haploids (Devaux 1992). Cultivar release is suitable to assess the efficiency of haploid production in self-pollinated crop species such as barley and wheat, for which techniques have been available for many years. Despite intensive efforts to increase anther culture response (reviewed by Henry and de Buyser 1990), its use has remained marginal in wheat breeding programs. The major limitation to a broad exploitation of anther culture has been its genotypic dependency (Lazar et al. 1984; Marsolais et al. 1984; Foroughi-Wehr and Zeller 1990). Crosses involving an anther culture-responsive parent such as a 1BL-1RS translocated line (Henry and de Buyser 1985) enabled the indirect recovery of haploid plants from recalcitrant genotypes. However, regenerated plants were skewed in the direction of the translocated parental type by the selective development of microspores or the derived embryoids (Agache et al. 1989; Devaux et al. 1990).

Wide crosses followed by elimination of the genome of one parent have been an alternate method for inducing haploid zygotic embryos and subsequent plants. The production of haploid plants from crosses between wheat and maize was first reported by Laurie and Bennett (1988). Refinements of the technique (Suenaga and Nakajima 1989; Laurie et al. 1990; Comeau et al. 1992) enabled haploid plants to be produced from many commercial wheat cultivars (Laurie and Reymondie 1991; Riera-Lizarazu et al. 1992) and hybrids with the aim of obtaining homozygous recombinant lines resistant to Russian wheat aphid (Kisana et al. 1993).

The objectives of the study presented here were (1) to assess doubled haploid (DH) production efficiency through wheat × maize or teosinte crosses over a range of wheat F₁ hybrids; this implies the regeneration of haploid plantlets and successful chromosome doubling to restore fertile DH plants; (2) to select superior maize genotypes that could increase haploid production efficiency; and (3) to compare the inheritance 1BL-1RS chromosome in haploids obtained through the maize method (MM) and by anther culture (AC) and hence to assess whether random gamete sampling takes place.
Material and methods

Plant material


The male parents consisted of the two F₁, Zea mays L. hybrids cvs ‘Saviero’ (M1) and ‘Earlibelle’ (M2); FL, an early-type population of maize (M3); and one genotype of teosinte (Zea mays ssp. mexicanum) (T4). A pollen mixture of the above four male parents (M5) were also used in this study.

Doubled haploid production

After germination, seedlings of wheat were transferred to a vernalization room held at a constant temperature of 4°C for 8 weeks, then planted out in a glasshouse with a temperature regime of approximately 20/16°C (day/night). A 16-h photoperiod was supplied by Philips SON-T400 high pressure sodium lights when necessary. Emasculations and pollinations were as described in Laurie and Bennett (1986). The 18 wheat F₁ hybrids and the five Zea mays parents were compared in a seven-replicate randomized complete-block design. Wheat spikes were considered to be replicates in cross-combinations with Zea mays. The 2.4-D tiller and floret treatment was the same as that described in Laurie and Ray mondie (1991).

Fifteen days after pollination, spikes were collected and embryos were excised and cultured in vials containing B5 medium (Gamborg et al. 1968). Embryos were incubated in the dark at 22°C for 5–10 days and then transferred to a 16-h light regime at the same temperature. Rooted seedlings were transplanted into the glasshouse. At the three to five tiller stage, plants were treated with a 0.1% aqueous solution of colchicine according to Pickering (1980), and at maturity seeds were collected from each fertile plant.

Ploidy level determination

Of the regenerated plants 610 were checked for ploidy level by flow cytometry. Briefly, 40 mg of leaf tissue was chopped with a razor blade in 2 ml of buffer (Bergouinioux et al. 1986) and 16 μl of a filter-sterilized solution of 4', 6-Diamidino-2-Phenylindole (DAP1, Sigma D-9542) at a concentration of 250 μg/ml. Samples were analyzed using a CA II flow cytometer (Partec GmbH, 4400 Miinster, Germany).

Since the CA II flow cytometer was not precise enough to detect chromosome abnormalities such as aneuploids, root-tip cell chromosome counts were carried out for 4 plants which had a peculiar growth habit, i.e. many and small tillers, narrow leaves and reduced growth speed.

To assess chromosome doubling efficiency, flow cytometry was performed on 28 colchicine-treated haploid plants. For this purpose, leaf samples were cut off from the first tiller which had arisen a few weeks following colchicine treatment. Flow cytometry profiles were analyzed using the Dpac® 2.1 software provided by Partec.

Inheritance of the 1BL-1RS translocated chromosome

Among the wheat cultivars or advanced lines which had been used as parents, only 'Rialto' possessed a homozygous 1BL-1RS translocated chromosome. 'Rialto' was the parent of the 2 F₁ hybrids 9 and 11. As demonstrated by Ainsworth and Gale (1987), the glucose phosphate isomerase (GPI) system can be used as an indicator of the presence of the 1BL-1RS translocation in wheat lines, and therefore was used in the present study. Enzyme extraction from leaves of young plantslets, gel preparation, electrophoresis and enzyme visualization were the same as described in Wendel and Weeden (1989). Inheritance of the 1BL-1RS chromosome was investigated in the haploid progenies produced by the MM and by AC and in the F₁ progeny of the 2 hybrids 9 and 11. The AC method was the same as described in DeCaux (1992). As a control, GPI analysis was carried out using the three parents, ‘Rialto’, ‘Vivant’ and ‘Trémie’, as well as the IR ‘Chinese Spring’ addition line (CS + 1R), Ditelo 1BL ‘Chinese Spring’ (D1BL) and cv ‘Gabbo’, which has a 1BL-1RS chromosome. (CS + 1R, D1BL and ‘Gabbo’ were provided by Dr. R. M. D. Koebner (IPSR Cambridge Laboratory, Norwich, UK).

Results

Haploid plant production

From the 18,716 wheat florets which were processed during this experiment, 15,342 (82%) of the ovaries enlarged after fertilization and 2,4-D treatment to reach a caryopsis size similar to that of parental selfs of the same age. However, only 3,843 (25%) of the expanded ovaries contained an embryo. Attempts to identify those caryopses having an embryo by X-ray radiography were unsuccessful. A total of 1,703 plants (44.3% of the embryos) were regenerated. The number of embryos (%EMB/FL) and plants (%PL/FL) per 100 florets ranged from 26.1 (wheat hybrid no 1: whl) to 14.4 (whlS) and from 14.7 (whl) to 4.4 (whlS), respectively (Fig. 1).

The effect of wheat genotype on the two characters was highly significant (Table 1). The Zea mays pollinator had a significant effect for %EMB/FL (highest value: 24.1 for M2, lowest: 15.2 for M3) and %PL/FL (highest value: 10.5 for M2, lowest: 6.8 for M3) (Fig. 1). The interaction between wheat and Zea mays genotypes was significant at the 0.01 level for %PL/FL (Table 1). The

Fig. 1 Effect of wheat and maize genotypes on haploid production of wheat. Horizontal lines represent homogeneous groups for number of plants per 100 florets (P < 0.05)