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Agronomic performance of lines derived from anther culture, maize pollination and single-seed descent in a spring wheat cross

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Abstract Anther culture and maize hybridization are two frequently used techniques for doubled haploid production in wheat (*Triticum aestivum* L.). Information on the field performance of lines derived from these techniques is limited. This study was conducted to compare the performance of $F_{4:6}$ lines obtained by single-seed descent with lines obtained by anther culture and maize (*Zea mays* L.) pollination from the same cross of spring wheat, 'Chris'/MN 7529. Thirty-three lines derived from each of those techniques were evaluated in six environments for grain yield, protein content, test weight, heading date, kernel weight and plant height. Mean performance of the single-seed descent lines exceeded performance of the anther culture lines for grain yield, kernel weight and plant height with no apparent differences for grain protein content, test weight and heading date. No differences between trait means for the single-seed descent and maize pollination lines were found except for plant height. The best 5 lines from each method for grain yield, protein content and test weight were similar in performance except that the protein content was higher for the maize pollination lines than for the single-seed descent lines. Acceptable levels of agronomic performance could be found among lines from each method. Wide acceptance of the doubled haploid technique for pure line production in breeding programs may, however, be limited by the often poor efficiency of doubled haploid line production, resulting in smaller population sizes for selection of desirable traits in comparison to the single-seed descent method.

Key words Wheat · Maize · Doubled haploid · Anther culture

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Introduction

The use of doubled haploid techniques in plant breeding has the potential to shorten breeding cycles by producing homozygous lines from a segregating population more rapidly than most conventional breeding methods (Khush and Virmani 1996). In wheat (*Triticum aestivum* L.), anther culture and wheat×maize (*Zea mays* L.) hybridization are two frequently used techniques for doubled haploid production (Raina 1997). Unlike in rice (*Oryzae sativum* L.) and barley (*Hordeum vulgare* L.), the application of doubled haploids for wheat improvement has been limited by the relatively low production rate of doubled haploid plants and by the genotype-specific response to green plant regeneration, especially when anther culture is employed (Ling et al. 1995). Progress has recently been made in the anther culture technique through modifying the culture medium (Ball et al. 1992; Ghaemi et al. 1994; Orshinsky and Sadasivaiah 1994; Zhou et al. 1992) and in the maize pollination method by applying growth regulators post-pollination (Matzk and Mahn 1994; Mujeeb-Kazi and Riera-Lizarazu 1996).

Few studies have compared anther culture and wheat×maize hybridization with conventional breeding methods for the selection of desirable lines. F_1 plants, rather than later generations, are often used to generate doubled haploids to maximize efficiency, but using this

generation reduces the possibility of obtaining desirable genetic recombination. Further, anther culture may cause unpredictable genetic alternations due to gametoclonal variation (Huang 1996). These factors could affect the means and genetic variances of a breeding population, thus affecting selection.

Several studies have evaluated the agronomic performance of lines derived from doubled haploid methods. Baenziger et al. (1983, 1989) and Mitchell et al. (1992) reported that wheat lines derived from anther culture were lower yielding than those derived from single-seed descent. A similar result was obtained in barley by Rossnagel et al. (1986). Generally, anther culture-derived lines were found to be later in heading (Powell et al. 1992), lower in kernel weight (Charmet and Branlard 1985; Mitchell et al. 1992) and higher in grain protein content (Bedo et al. 1996) than single-seed-descent-derived lines. Bjornstad et al. (1993) found no differences in the means of biological yield, grain yield and heading date between bulbosum and single-seed-descent-derived lines in barley, but lines from anther culture had a lower grain yield and harvest index and shorter plant height. Henry et al. (1988) found differences in yield and heading date when wheat lines derived from anther culture and wheat×maize hybridization were compared.

Our objective was to compare the performance of F_1-derived anther culture lines, maize pollination lines, and $F_{4:6}$ single-seed-descent lines of spring wheat from the same cross. Results should provide evidence as to whether doubled haploid techniques, especially maize pollination, offers the same or even better opportunity than single-seed descent for the selection of desirable wheat lines.

Materials and methods

Development of doubled haploid lines

The cultivar 'Chris' and the breeding line MN 7529 were chosen as parents for this study. 'Chris' has been reported to be a high plant calli- and green plant-producing genotype in anther culture (Lazar et al. 1984). MN 7529, an experimental line, is a high-yielding semi-dwarf with relatively low grain protein, whereas 'Chris' is a low-yielding, tall, high-grain protein cultivar. Reciprocal crosses were made in the greenhouse in 1985, and equal amounts of F_1 seed were bulked. A proportion of the F_1 seeds was used for anther culture in the following year, and the remaining seeds were used to develop populations using maize pollination and single-seed descent. All of the F_1 plants were grown in 12.5-cm pots with 2 plants per pot in the greenhouse at 23°±2°C with a 16-h daylength.

A detailed description of the anther culture (AC) techniques used here was published by Mitchell et al. (1992). A total of 43 AC lines were generated from 1954 different anthers. Out of the 43 lines 33 produced enough seed in the greenhouse for field increase for subsequent yield trials.

Doubled haploids were produced using the maize pollination (MP) method described by Laurie and O'Donoughue (1994). At 1–2 days prior to anthesis, F_1 wheat plants grown in the greenhouse were emasculated, and 2 days later freshly collected pollen of 'Seneca 60' maize was applied with an artist brush. Twenty-four hours after the pollination, spikes were sprayed with an aqueous solution of 100 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D). About 16–18 days after the treatment, immature embryos, 1–1.5 mm in size, were excised and cultured on agar-solidified half-strength MS basal medium (Murashige and Skoog 1962) containing 20 g/l sucrose. For chromosome doubling of regenerated haploid plants, both roots and shoots of plants at the three to five tiller stage were trimmed to a length of 2–3 cm. The plants were placed with the crown submerged into a solution of 0.2% colchicine and 1% dimethyl sulfoxide for 4 h at 20°C with aeration provided by bubbling air. The treated seedlings were rinsed overnight in running tap water and then placed in pots of soil for growth to maturity. A total of 33 MP lines were produced, and seeds of each line were increased in the greenhouse for field studies.

Development of single-seed descend (SSD) population

Beginning with 150 F_2 plants, a total of 142 F_4 plants were produced by the SSD method in the greenhouse. All seeds were harvested from each of the 142 F_4 plants (F_5 seeds). Of the 142 SSD lines 50 were randomly chosen for seed increase in the same greenhouse as the AC lines. Of these 50 SSD lines, 33 lines were chosen at random for field increase to be used for this study.

Field experiments

This study was conducted in six environments (E):E1, Rosemount 1994, E2, Crookston 1994, E3, St. Paul 1995; E4, Crookston 1995; E5, St. Paul 1996; E6, Crookston 1996. Crookston is located in northwest Minnesota, representing the major spring wheat growing area of the state, and St. Paul and Rosemount are located in southeast Minnesota. Thirty-three lines from each method and the parents were planted in a sets-in-replicates complete block design with three replicates per environment. All lines from each method were randomly divided into three sets with each set containing 11 lines and parents as checks. Two-row plots were seeded with rows 0.3 m apart and 2.4 m long. Plots were separated by one spring-seeded winter wheat row to reduce possible competition effects between entries. Seeding rate was approximately 1 seed cm^{-1} of row. The recommended rate of fertilizer was incorporated into the field before seeding. Chemical and mechanical weed control were implemented as needed.

The following traits were measured on all the plots: (1) grain yield (kg/ha) of cleaned seed; (2) grain protein (%) measured by near-infrared reflectance on a whole grain basis; (3) test weight (kg/hl); and (4) heading date (days) recorded as the number of days after May 31 to the date of emergence of 50% of the main spikes in a plot. Plant height (cm), measured from the soil surface to the spike tips (excluding awn), was measured only in E1, E2, E4 and E5. Kernel weight (mg) was determined on a 250-kernel sample obtained from each of E1, E2, E3 and E4.

Statistical analysis

Single and combined environment analyses of variance were performed for each trait measured. Methods were tested for significance using environment×method interaction as the error.

The set effect was non-significant for all traits measured except for plant height in the combined analysis. The set effect was due to set three having a significantly higher mean height than the other two sets even though the check mean across sets did not differ for plant height. The lines from the SSD method were taller in set three than expected, which may have been caused by sampling error when lines were assigned to sets rather than by environmental factors. Therefore, plant height was not adjusted for sets by the check for analysis.

Genetic variances of lines in each method were estimated for each trait using expected mean squares. Means of the highest 5 lines of each method were compared for yield, protein and test weight using the *t*-test.

Table 1 Means of traits measured for lines of wheat derived from anther culture (AC), maize pollination (MP) and single-seed descent (SSD) methods across environments

Method	Grain yield[a] (×1000 kg/ha)	Grain protein (%)	Test weight (kg/hl)	Heading date[b] (days from 31 May)	Kernel weight[c] (mg)	Plant height[d] (cm)
AC	3.33 b[e]	14.4 a	69.3 a	29.0 b	29.8 a	85.0 a
MP	3.50 a	14.7 a	70.5 b	28.0 a	31.4 a, b	85.9 a
SSD	3.58 a	14.3 a	69.9 a, b	28.6 a, b	32.5 b	88.9 b
Midparent	3.75	14.4	70.6	28.3	32.4	90.4
MN 7529	4.52	13.5	70.3	27.2	35.3	86.6
Chris	2.97	15.4	71.0	29.3	29.4	94.3

[a] Data from low scab environments (E1, E3, E5 and E6)
[b] Five environments (E1, E3, E4, E5 and E6) only
[c] Four environments (E1, E2, E3 and E4) only
[d] Four environments (E1, E2, E4 and E5) only
[e] Means followed by different letters in the same column differ significantly at P=0.05 (LSD)

Results and discussion

The two parents differed in plant height with 'Chris' being a tall line and MN 7529 being a semi-dwarf line with one semi-dwarf gene. As a test for randomness of lines derived from each method, lines of each method at each of the environments were categorized as either short (≤MN 7529±SE) or tall, and then tested for goodness-of-fit using chi-square analysis. A ratio of 9 tall to 7 semi-dwarf was expected for one-gene segregation in the $F_{4:6}$ generation for the SSD method and a ratio of 1 tall to 1 semidwarf was expected for the AC and MP methods. Unlike the AC lines, both the SSD (P>0.975) and the MP lines (P>0.10) fit the expected ratio in all environments. However, when the same AC lines were tested for segregation of sensitivity to gibberellic acid, they fit a 1 sensitive (tall): 1 insensitive (semi-dwarf) ratio (Mitchell et al. 1992). The lines used in each of these methods therefore appeared to be a random sample for plant height, and thus are likely to be a random sample for other traits as well.

Significant method×environment interaction was found for grain yield only. The interaction for grain yield was not surprising because Fusarium Head Blight (FHB) or scab (caused by *Fusarium graminearum* Schwabe) was epidemic during the 1994 and 1995 crop seasons in Crookston (E2 and E4). The FHB pressure was low to non-existent in 1994 at Rosemount (E1) in 1995 at St. Paul (E3) and in 1996 at all locations (E5, E6). Data analyses for grain yield were conducted only over low FHB environments because both parents, 'Chris' and MN 7529, were moderately to highly susceptible to FHB, and all lines derived from this cross were moderately to highly susceptible. No method × environment interaction was detected for test weight, protein content, heading date, plant height and kernel weight. Apparently FHB did not significantly affect method ranking across environments for these traits.

The SSD and MP lines had similar grain yield, which was higher (P<0.05) than that of the AC lines (Table 1). This result is similar to that of Bjornstadt et al. (1993) in barley when he compared lines derived from the SSD, *H. bulbosum* and AC methods for grain yield.

The AC lines had the greatest genetic variance (P<0.05) because of several low-yielding lines (≤'Chris'±SE). This resulted in a lower mean grain yield for AC than for the other two methods (Fig. 1). In contrast, the SSD lines had the lowest genetic variance with a high proportion of average yielding lines (Table 2). However, differences were not detected among methods when the highest 5 yielding lines of each method were compared (Table 3).

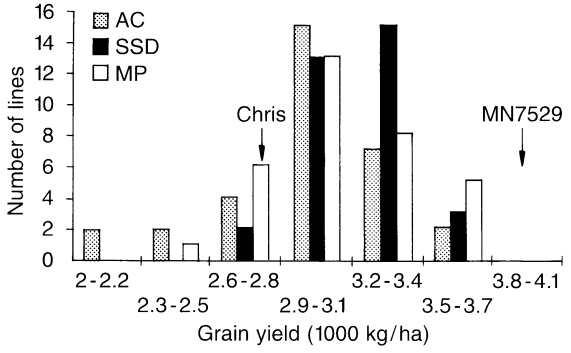

Fig. 1 Frequency distributions based on mean grain yield of wheat lines derived from anther culture (*AC*), single-seed descent (*SSD*) and maize pollination (*MP*) methods

SSD populations have been hypothesized to differ from doubled haploid populations because of the more opportunities for genetic recombination (Snape 1976; Riggs and Snape 1977). However, this does not appear to be the main reason for the higher grain yield observed for SSD than for AC because there were no differences between the MP and SSD lines. If genetic recombination was the main cause of the difference, the MP and AC methods should be similar, not those of MP and SSD. The higher proportion of low-yielding lines associated with the AC method found in this study and in the study of Baenziger et al. (1989) were likely due to either the occurrence of deleterious gametoclonal variation in AC-derived lines (Huang 1996; Marburger and Jauhar 1989) or the possible genetic association of a low-yielding trait with high green plant regeneration ability as noticed in barley (Zivy et al. 1992; Thompson et al. 1991). On the other hand, Snape et al. (1992) found that genotypic selection for fitness traits (e.g. biomass, grain yield and grain weight) occurred using SSD that results in a higher mean population performance when compared to AC because weak genotypes were eliminated.

Table 2 Genetic variance (±SE) of lines derived from anther culture (AC), maize pollination (MP) and single-seed descent (SSD) from combined analysis

Method	Grain yield[a]	Grain protein	Test weight	Heading date[b]	Kernel weight[c]	Plant height[d]
AC	130.8±39.3[e]	0.4±0.1	4.6±1.5	3.0±0.8	7.5±2.1	51.4±13.4
MP	65.6±23.0	0.5±0.1	2.8±0.8	3.3±0.9	4.7±1.6	73.1±18.5
SSD	11.8±11.2	0.1±0.04	1.8±0.6	2.8±0.7	2.9±2.2	81.6±20.8

[a] Reported values=actual values ×1000
[b] Five environments (E1, E3, E4, E5 and E6) only
[c] Four environments (E1, E2, E3 and E4) only
[d] Four environments (E1, E2, E4 and E5) only
[e] Standard error

Table 3 Ranges and means of the best five lines from anther culture (AC), maize pollination (MP), and single-seed descend (SSD) for grain yield, protein content and test weight

Method	Grain yield[a] (×1000, kg/ha)	Protein content (%)	Test weight (kg/hl)
AC	3.6 a[b] 3.5–3.7	15.6 a, b 15.2–15.8	72.1 a 71.6–73.2
MP	3.6 a 3.5–3.7	15.8 b 15.4–16.8	73.1 a 72.3–74.5
SSD	3.5 a 3.4–3.6	15.1 a 14.9–15.2	72.1 a 71.9–72.2

[a] Low scab environments (E1, E3, E5 and E6) data only
[b] Means followed by different letters in the same column differ significantly at P=0.05 (LSD)

In contrast to the AC method, doubled haploid lines derived from the MP method have been recognized to have a lower frequency of somaclonal variation (Raina 1997). In an independent study, 18 MP-derived doubled haploid lines from a spring wheat cultivar, 'Vance', were evaluated in the same six environments as described here for grain yield, protein content and plant height. The doubled haploid lines did not differ for these traits nor from the 'Vance' control (data not presented). Apparently, minimum genetic alternations occurred when the MP method was employed to produce doubled haploids. These results agree with the findings obtained by Laurie and Snape (1990) in a similar study evaluating several quantitative traits in wheat. However, deleterious somaclonal variation for doubled haploid lines produced through the MP method was reported by Suenaga and Nakajima (1993). They attributed the variation to the colchicine treatment rather than to the 2,4-D treatment or in vitro culture.

The methods did not differ for grain protein in the combined analysis (Table 1), but the genetic variances of the AC lines and the MP lines were greater than that of the SSD lines (Table 2). When the means of the highest 5 lines for protein content from each method were compared, the MP lines were similar to the AC lines, and higher than the SSD lines (P<0.05). Of the lines derived from MP and AC lines, 24% and 18% respectively, had a mean protein content either higher than or equal to 'Chris', the high-protein parent, while with SSD only 6% met this criterium. Our results, indicating that doubled haploid techniques are suitable for generating lines with higher protein content, are similar to those obtained by Mitchell et al. (1992) and Bedo et al. (1996). However, the greater proportions of high-protein lines in doubled haploid populations did not result in method differences for protein content.

The mean test weight of MP lines was similar to that of SSD lines, and greater than that of AC lines (Table 1). The genetic variance was largest for AC lines with a higher proportion of low test weight lines (≤MN 7529±SE) than the other two methods. However, when the means of the 5 lines with the highest test weight from each method were compared, differences among methods were not found.

Significant differences in mean heading date were detected among methods (Table 1). AC lines were the latest heading, and MP lines were the earliest. Genetic variance did not differ among methods (Table 2). The same set of AC lines was tested by Mitchell et al. (1992) in 1988 and 1989, but no differences were detected between AC and SSD methods. However, 1988 was a drought year, and plants under drought stress tend to head earlier and more uniformly than those under more favorable conditions. Because the heading dates for both AC and SSD lines were normally and symmetrically distributed, unconscious selection against late plants did not likely occur in either one of the methods. Surprisingly, the distribution of MP lines was asymmetric and skewed toward early heading, resulting in the population mean heading date being earlier than that of the other two methods. Choo et al. (1982) found no differences in frequency distributions for heading dates in barley when comparing bulbosum-derived doubled haploids, a technique similar to the maize-derived method, and SSD lines.

SSD and MP lines were similar for mean kernel weight, but AC lines were lower. SSD lines were taller than MP and AC lines. However, lines with an acceptable range for these traits were found within each method.

With this limited sample of lines evaluated, the doubled haploid techniques provided a similar chance of selecting desirable wheat lines as the SSD method. The MP method many be even better than the SSD method for the selection of lines with high-grain protein content and early heading; however, the results need to be extended to a wider array of germplasm for confirmation.

The wheat×maize system has emerged as a reliable approach for producing doubled haploids in wheat due

largely to less dependence on genotypes and fewer occurrences of plants with deleterious gametoclonal variation in comparison to the anther culture method (Raina 1997). Several wheat programs in North America have used the wheat×maize system as a supplementary approach for generating pure lines. However, careful consideration must be given to determine if it is a cost-effective approach. Besides the known technical difficulties associated with particular methods, factors including the growth habits of wheat (e.g. spring or winter), the overall breeding scheme (breeding method, greenhouse facilities and winter nursey for off-season planting), core germplasm, expertise and costs need to be evaluated before doubled haploid techniques should be used.

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