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ORIGINAL ARTICLE

Production of doubled haploid wheat lines (*Triticum aestivum* L.) using anther culture technique

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Abstract Anther culture response of six wheat genotypes and their F_1 crosses was investigated. Results indicated that considerable genetic variation among the tested genotypes was observed. Calli were obtained from all wheat genotypes studied. The percentage of anthers that developed calli ranged from 0.67% for Gemmeiza-9 to 18.00% for the cross (Gemmeiza-7 × Sids-4). Plants were regenerated from 19 out of 21 wheat genotypes. The highest frequencies of green plantlets were achieved from the two crosses (Giza-164 × Gemmiza-9 and Giza-164 × Line-115), while the lowest ones were obtained in the cross (Giza-164 × Sids-4). Generally, crosses showed a better response in anther culture than their parental genotypes. Significant and positive heterotic effects were observed in some crosses for callus induction and green plant regeneration. Ninety-four doubled haploid lines, which were derived from five F_1 crosses and their respective parents were evaluated under field conditions. Some doubled haploid lines that resulted from these crosses performed well and transgressed significantly the higher-yielding parent and the check variety.

Introduction

The production of haploid plants from hybrids, followed by chromosome doubling, provides the wheat breeder with a means of accelerating the process of true breeding line development (Henry and De Buyser, 1990). The doubled haploid (DH) derived from hybrid progenies can be used as recombinant line with favorable gene combinations (Bentolila et al., 1992). This technique could thus complement the conventional breeding programs to accelerate the release of new varieties.

It is known that anther culture is an extremely useful technique for the production of doubled haploid in cultivated crops. In wheat, the success has been achieved in China (Hu et al., 1983, 1988), where two new cultivars Jinghua No. 1 and No. 764 were selected in a breeding program using doubled haploid lines. This method has also had practical application in

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France and Hungary, where two new cultivars Florin (De Buyser et al., 1987) and Gk Delibab (Pauk et al., 1995) were released in 1985 and 1995.

Doubled haploids, which are developed spontaneously or by colchicine-induced chromosomal doubling leads to the direct production of completely homozygous lines from heterozygous plants in a single generation. Therefore, doubled haploid lines are considered attractive tools for both plant breeders and geneticists because many of the problems associated with the assessment of segregating populations can be overcome by their usage. Moreover, doubled haploid technique saves at least three to four generations of self-pollination for the fixation of homozygous pure lines (Hassawi et al., 2005).

The success of anther culture ability in wheat, as other crops, is found to be influenced by genotype (Andersen et al., 1987), donor plant growth conditions (Orshinsky and Sadasivaiah, 1997), the developmental stage of microspores (Haggag and El-Hennawy, 1996), pre-culture treatments, and media components (Lazaridou et al., 2005).

Almost all workers dealing with anther cultures agree that the genotype effect is the main limiting factor of in vitro androgenesis. Many wheat genotypes, as well as F_1 and F_2 breeding combinations, are incapable of morphogenesis in anther culture, which makes this method too expensive to be used for routine purposes (Yermishina et al., 2004). Therefore, one of the proposed approaches was to use anther culture only with responsive genotypes (Andersen et al., 1988). On the other hand, the responsiveness of parental genotype to anther culturing affects the responsiveness of hybrid combinations involving them (Zamani et al., 2003). Moreover, there is evidence that F_1 hybrids have a higher androgenetic capacity than the parental forms.

To be successful in a breeding program using microspores for doubled haploid production, workers should include genotypes with high regeneration ability. As pointed out in different investigations, the anther culture ability is a heritable trait and can be transferred into agriculturally desirable material by crossing (Foroughi-Wehr et al., 1982). However, as the varieties which are actually grown derive from widespread genetic sources, there is a great probability of finding good responding genotypes directly in unselected lines or crosses.

In the literature, there is limited information on transgressive segregation in wheat (Mahmood and Baenziger, 2008).

The present investigation was undertaken to study the anther culture response of some wheat genotypes and their F_1 hybrids as well as to evaluate the agronomic performance of 94 wheat doubled haploid lines and their respective parents under field conditions.

Materials and methods

The present investigation was carried out at the Cell and Tissue Culture Laboratory as well as the Experimental Farm of the Agronomy Department, Fac. of Agric., Al-Azhar Univ., Nasr City, Cairo. Six parental genotypes of bread wheat namely; Gemmeiza 7, Giza 164, Gemmeiza 9, Sids 4, Giza 168 and Line-115 representing a wide range of diversity for several traits were selected for this study. In 2007/08 season, these parents were crossed in all possible combinations excluding reciprocals, to obtain a total of 15 hybrids. The six parental genotypes and their 15 F_1 crosses were sown at the Experimental Farm in 2008/09 season to obtain the needed anthers.

Whole tillers at boot stage were collected when most microspores were at the mid- to late uninucleate stage of development, as assessed by acetocarmine staining of selected squashed anthers. Tillers with spikes at this stage were clipped off at ground level and tagged. Then, they were put in water and maintained for 6–8 days at 4 °C in the dark. After cold pretreatment, the spikes inside flag leaves were surface sterilized with 20% chlorax solution for 7 min. and rinsed 3–4 times in sterile water. Anthers were aseptically dissected out and cultures in jars containing the N6 induction medium of anther culture (Chu, 1978) supplemented with 2 mg/L 2,4-D, 1 mg/L kinetin, 90 g/L sucrose and 7 g/L agar. These jars were incubated first for 5–6 weeks in darkness at 28 °C. Completely randomized design was applied in this experiment with 21 genotypes and 10 replicates (spikes). Each replicate contained 30 anthers from each spike, which were placed in the jar.

Embryoids/callus induced from the anthers were transferred to jars containing MS regeneration medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/L NAA, 0.5 mg/L kinetin and 30 g/L sucrose. These jars were incubated for 5–6 weeks at 25–27 °C with 16 h light. The number of green and albino regenerants were counted.

Green plantlets with adequate root formation were transplanted to small pots with a mixture of soil, sand and compost, under plastic cover for 3 weeks in a growth chamber maintained at 18 °C and 16 h light per day. The regenerated plantlets were transferred to the soil in the greenhouse for growth and maturation. The chromosome number of the green plantlets was determined according to Jauhar et al. (1999). The chromosome number of haploid plants was doubled by colchicine treatment as described by Cistue et al. (2006). Seeds obtained from doubled haploid plants were harvested.

Ninety-four doubled haploid (DH) lines derived from five crosses and their respective parents were sown in randomized complete blocks design with three replications in 2009/10 season. Each experimental unit consisted of a single row with a length of 3 m for each entry. The plants were individually spaced at 15 cm within and 30 cm between rows. The commercial wheat variety Gemmeiza-9 was used as check variety. Observations and measurements were recorded on seven guarded plants in each of the DH lines and their respective parents for the following traits; days to heading, plant height, number of spikes/plant, number of grains/spike, 100-grain weight and grain yield/plant. The data recorded on all the traits were subjected to analysis of variance (Steel and Torrie, 1980) to determine the significant differences among genotypes. Heterotic effects were computed relative to better parent for callus induction and plant regeneration.

Results and discussion

Anther culture response of wheat parental genotypes and their F_1 hybrids

One of the important factors for haploid production through androgensis is the culturing of anthers at the mid to late uninucleate stage. When cultured at this stage there is shifting in the normal pathway of pollen development and after repeated mitotic divisions of the microspores, calli are formed (Haggag