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In vitro microspore reaction of different German wheat cultivars

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Summary. The in vitro microspore androgenesis reaction of 25 commercial German spring (including 4 *Triticum durum*) and 50 winter wheat cultivars was investigated. Tremendous genotypical differences were found in microspore response. The best-responding winter wheat cultivar, 'Florida', is characterized by the presence of a 1B/1R wheat-rye translocation chromosome. The significance of this finding and other genetic systems for future use of haploids in plant breeding is discussed.

Key words: *Triticum aestivum* – Anther culture – 1B/1R translocation

Introduction

It is well known that anther culture is an extremely useful technique for the production of doubled haploids (DH) in cultivated crops. In hexaploid wheat, success has been achieved in China (Hu et al. 1983, 1986), where cultivar 'Jinghua No. 1' was selected in a breeding program using DHs. This method has also had practical application in France, where the cultivar 'Florin' was released in 1985 (De Buyser et al. 1987).

Of the various factors influencing the induction of microspore plants, two attributes of the donor plants from which the anthers are taken are important: (1) their physiological stage, i.e. the growing conditions of the donor plants, and (2) their genotype, which determines anther response and productivity in vitro.

To be successful in a breeding program using microspores for DH production, workers should include genotypes with high regeneration ability. As pointed out in different investigations, anther culture ability is a heritable trait and can be transferred into agriculturally desirable material by crossing (Bullock et al. 1982; 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.

Very little is known about the anther culture ability of commercial German wheat material. Therefore, an investigation was carried out to screen 21 hexaploid spring wheat cultivars, 4 *T. durum* cultivars and 50 hexaploid winter wheat cultivars from the National List of Wheat Cultivars of the Federal Republic of Germany (anonymous 1987), with regard to their anther culture ability. This should be of great help in selecting genotypes for breeding using DH procedures.

Materials and methods

The plant material chosen for this investigation consisted of 25 spring (including 4 *Triticum durum* varieties – 'Miradur', 'Mondur', 'Jacob' and 'Grandur') and 50 winter wheat cultivars (Fig. 1). To compensate for the seasonal influence on the physiological status and, consequently, the in vitro reaction of the donor material, anther culture was carried out over a 1-year period. For that purpose the cultivars were divided in groups of eight, and these groups were cultured alternately. Consequently, anthers of each cultivar were plated on 8–14 different dates during the whole period.

The seeds of winter cultivars were vernalized in sterile sand for 6–8 weeks at 5 °C with an 8-h photoperiod. The spring material was directly sown in 15-cm pots with peat soil in the greenhouse (Einheitserde T). The vernalized seedlings were transferred to the same soil mixture. All plants were cultured in a growth chamber with a 10-h photoperiod (15–18 klx; HQI-T, 400-W lamps) and a day/night temperature of 12°/10 °C for tillering. The plants were transferred to a greenhouse and grown under semicontrolled conditions, i.e. during winter in a 16-h photoperiod (10–12 klx) and 16°–18 °C day and 12°–14 °C night temperature; additional light was given when light intensi-

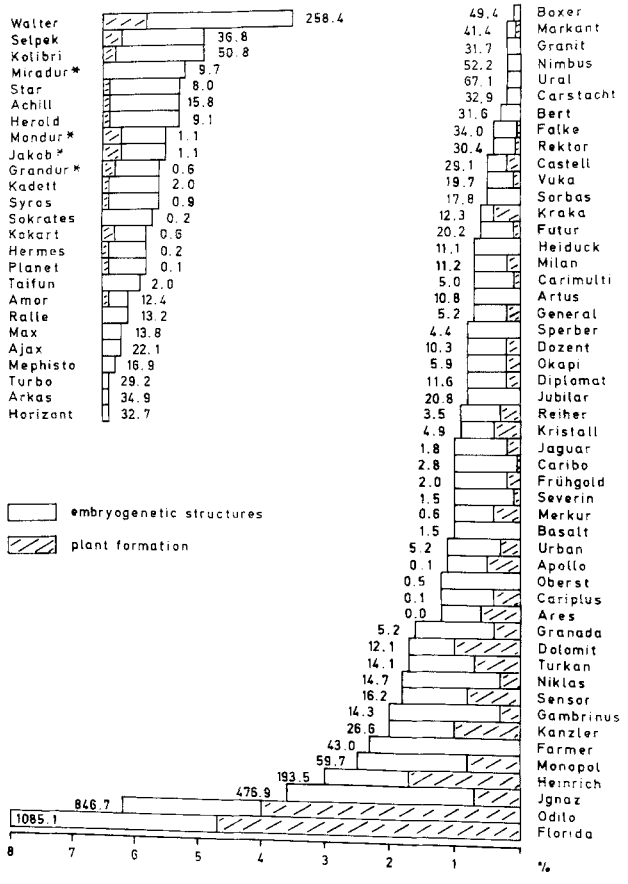

Fig. 1. In vitro microspore response of German spring (left) and winter wheat cultivars. The Chi-square values given refer to the development of embryogenic structures; values higher than 3.84 reject null-hypothesis, i. e. all varieties react in the same manner. Chi-square table value: $p=0.05$, $1df=3.84$. * *Triticum durum* ($2n=4x=28$)

ty during the day was not sufficient. During summer, the plants grew under natural light and the temperature varied between 18° and 25 °C. Spikes were collected when the microspores were in the uninucleate stage and were subjected to a cold stress for 5–12 days at 5 °C. Anthers were inoculated on the Chinese potato 2 medium (Chuang et al. 1978) with 200 mg/l glutamin (Henry and De Buyser 1981) and 90 g saccharose. The medium was solified with 90 g/l wheat starch. The anthers were kept in the dark at 28°–30 °C. After 4–6 weeks, embryos or callus were transferred to the Chinese regeneration medium (Chuang et al. 1978) with 50 mg/l glutamine and 30 g/l saccharose solified with agar. The cultures were initially maintained at 24 °C in the dark. After the regeneration of small shootlets, they were transferred to a 10-h light regime at 24 °C. The regeneration capacity was determined in in vitro culture.

Statistical analysis was carried out by Chi-square tests using the SAS-FREQ program.

Results

The callus and embryo induction frequency varied with the wheat genotype of the spring and winter cultivars,

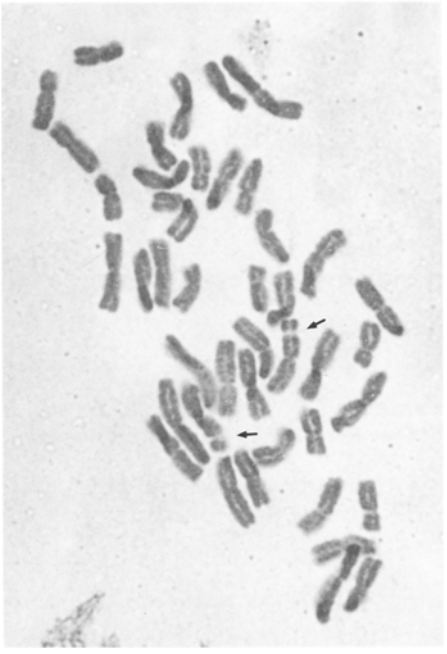

Fig. 2. A mitotic root tip cell ($2n=42$) cv Florida of a 1B/1R wheat-rye translocation line possessing two satellited chromosomes 6B (*arrowed*). The satellites of chromosome pair 1B are missing

respectively. In general, a higher frequency of responding anthers – anthers with microspores developing embryos or callus – was observed in winter cultivars. The overall regeneration rates were 0.8 and 1.2 per 100 anthers cultured for spring and winter wheat, respectively. From most of the cultivars, plants could be regenerated. In the spring material, 64% of the wheat cultivars gave rise to plants, whereas in the winter wheat cultivars, 72% reacted with plant formation (Fig. 1).

Green plants could be obtained in 15 spring and 31 winter wheat cultivars. The average of all responding cultivars was 2.3 green plants per 1,000 anthers plated.

From the presented results, it can be concluded that the hexaploid spring cultivars 'Walter' and 'Selpek' and the winter cultivars 'Florida' and 'Odilo' respond well. In 'Florida' the induction rate was 7.9% and 20 green plants could be regenerated per 1,000 anthers. The spring cultivar 'Walter' produced 3.0% embryogenic structures and 1.4 green plants per 1,000 anthers. They should be used for preferences in an anther culture breeding program.

The regeneration rate was particularly high in winter varieties which carry the 1B/1R wheat-rye translocation, such as 'Florida', 'Odilo' and 'Heinrich' (Fig. 2). On the other hand, 'Kristall' as well as 'Apollo' and 'Granada' also possess the 1B/1R translocation, but their androgenetic ability in vitro is much lower. None of the spring varieties carry a 1B/1R wheat-rye translocation, but genotypical differences are also found within these culti-

vars. The best *T. durum* variety with regard to embryogenetic response was 'Miradur', although no plants could be regenerated. Thus, for the German spring wheat material investigated here, the response was lower than in winter wheat cultivars.

As was reported for barley (Foroughi-Wehr et al. 1982), embryo or callus production in wheat is correlated in most of the cultivars with the total plant regeneration frequency ($r = 0.81$ ***). A high plant production presupposed a high embryo or callus formation. On the other hand, a high callus rate did not always yield a sufficient frequency of plant regenerants. Although the spring wheat variety 'Miradur' and the winter variety 'Farmer' responded with a relatively high number of embryogenic structures, no plants could be regenerated (Fig. 1). In general the anther response was low in comparison to the results of Andersen et al. (1987), who obtained a frequency of 3.3% responding anthers from 215 different winter-type cultivars from various regions. But Henry et al. (1982) also obtained 1.1 green plants per 1,000 anthers plated from 70 different crosses.

Besides the genotypical determination, the anther response is also influenced by the growing conditions of the donor material. Field-grown material gave better results than plants grown in the greenhouse (Ouyang et al. 1987; Bjornstad et al. 1989). The results obtained in this investigation are from greenhouse-grown material, which might not have always been at an optimal physiological condition, because the anthers were taken from plants throughout the year. For this reason a higher number of plants was not expected.

For practical breeding purposes it is necessary to select common varieties with good in vitro response. The heritable nature of in vitro responsiveness is known from different species (Foroughi-Wehr et al. 1982; Bullock et al. 1982; Petolino et al. 1988), so that these characters can be transferred to other genotypes by crossing.

Discussion

Henry and de Buyser (1985) postulated three different and independently inherited traits for androgenetic plant production in wheat: induction rate of embryogenic structures, regeneration capacity and the appearance of albinos. Henry and de Buyser (1985) and Agache et al. (1989) found a high regeneration rate in cultivars carrying the 1B/1R translocation. Furthermore, Müller et al. (1989) described 45 wheat cultivars, F_1 hybrids or F_2 populations also possessing the 1B/1R translocation chromosome. The relatively high androgenetic response of these lines has been verified in the present work. The best-responding winter wheat cultivars, such as 'Florida', 'Odilo', 'Heinrich', 'Niklas', 'Sensor' and 'Granada', all carry the 1B/1R wheat-rye chromosome translocation.

The spring wheat varieties investigated in these experiments do not carry a 1B/1R translocation; in general their response was lower than in the winter wheat material. But genotypical differences could also be found among them. It is known that some spring cultivars, e.g. 'Atys' (a French cultivar), which lack this translocation, respond as well as the best winter varieties (De Buyser and Henry 1980; Foroughi 1985; Datta and Wenzel 1987).

It is remarkable that several wheat cultivars possessing the 1B/1R translocation induce in vivo haploids at a high frequency when their nucleus is introduced into cytoplasms of *Aegilops caudata, Ae. kotschyi* or *Ae. variabilis* (Kihara and Tsunewaki 1962; Kobayashi and Tsunewaki 1980; Mukai 1983; F. J. Zeller unpublished results). The gene controlling haploid induction in alloplasmic wheat lines has been located on the rye segment of the 1B/1R translocation chromosome. However, there are other cultivars with the 1B/1R translocation which have a much lower androgenetic ability, i.e. 'Apollo' and 'Kristall'. Genotypical differences were also found among the 1B/1R lines investigated by Müller et al. (1989). It can be assumed that in addition to the 1B/1R translocation, there are other genetic systems which influence processes for microspore embryogenesis. Lazar et al. (1987) found that rye chromosome 4R also carries genes which significantly increase callus formation and plant regeneration from anther cultures of wheat (Chinese Spring)-rye chromosome addition lines. The effect of the rye chromosomes also seems dependent on the genetic background; in rye microspores there is no in vitro response in most of the populations. But rye chromosomes 6R and 7R in hexaploid wheat background carry positive genes which affect the regeneration response in immature embryo culture. Also Higgins and Mathias (1987) postulated an increase in morphogenesis and plant regeneration from callus derived from immature embryos of Chinese Spring in which chromosome 4B has been substituted from different cultivars. However, it appears that the increased regeneration frequencies among the 4B substitution lines may result from a shift in hormone metabolism, which reduces the sensitivity of the cells in these lines to exogenous hormones (Mathias et al. 1988). Several Rht (reduced height) genes which are known to disturb hormone metabolism (Gale and Youseffian 1988) have been localized on wheat chromosomes of homoeologous group 4 (McIntosh 1988). Since different major genetic effects seem to control tissue culture response, it should be possible to manipulate culture behaviour by simple selection strategies in the future.

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