HOW TO INCREASE THE EFFICIENCY OF CHROMOSOME DOUBLING IN ANDROGENIC REGENERANTS OF TRITICALE ?

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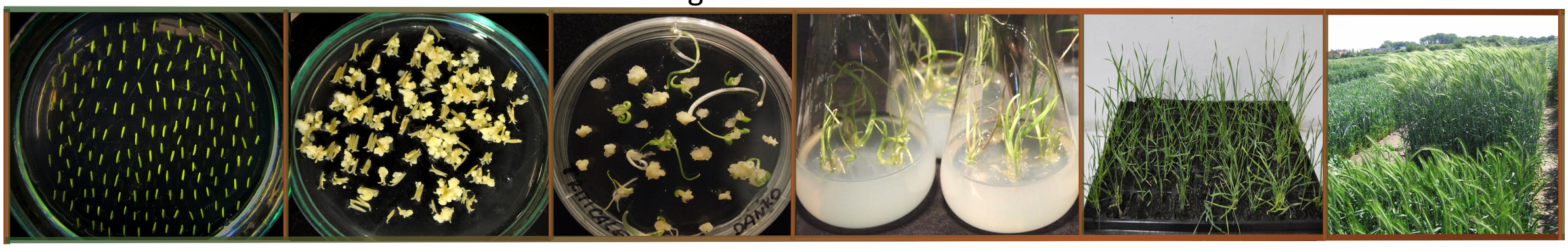
Androgenesis – anther culture



Introduction

For the doubled haploid (DH) approach to breeding to be successful, an economically effective production system is needed. Regardless of which method for the DH production is used, it has to be followed by a similarly effective and reliable method for doubling of the chromosomes number. The average rate of spontaneous chromosome doubling in triticale is ca. 20%, bringing certain urgency to the issue of chemical applications. An alternative to colchicine may be other antimitotic substances (eg. herbicides) which due to their better affinity to plant tubulin may show a similar level of chromosome doubling at lower concentrations and at low cost. Another approach to regeneration of fertile plants is to make use of meiotic restitution mechanisms and proper selection of parental lines.

Materials and methods

The experiments were performed on F1 hybrids of winter triticale from standard breeding crosses from Polish breeders (Danko and Strzelce). *In vitro* anther culture and regeneration was done using standard procedure. The survival rate of explants (immature embryos) was tested after treatments with different antimitotic agents added to regeneration media in concentrations 1 - 100µM/ 1 hour - 7 days. Additionally, the effect of doubling agents (trifluralin and aminoprophos-methyl) applied directly to the induction medium (10 µM/24h) and to 2 cm tall plantlets (5 and 10µM/2-3 days) was examined. The ability of regenerants to form caryopses was checked. A standard colchicine treatment was used as a control.

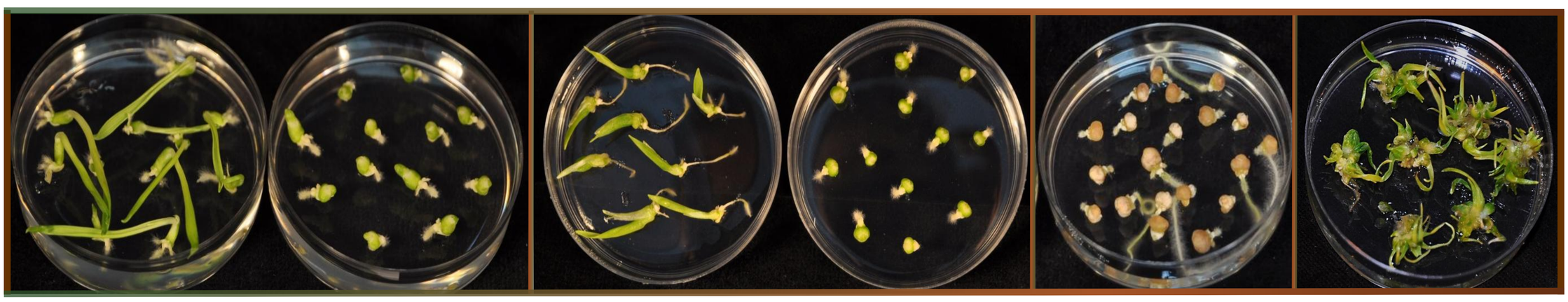


Fig.1. a, b) The response of embryos/seedlings to different antimitotic after 7 days of the culture ; c) dying, growth retardation plant and abnormal morphology .

Results

Selection of parental lines

The frequency of spontaneous diploidization can be improved by proper choice of parental lines for crossing. It has been observed that androgenic progeny from hybrids where both parental lines had been created via standard DH production methods show significantly higher spontaneous chromosome doubling rates. The average spontaneous diploidization rate for triticale is ca. 20%; for androgenic progenies from F1 hybrids generated from androgenics themselves it was above 60% (up to 84%) (Fig.2). This all by itself may eliminate the need for the antimitotics.

Formation of unreduced gametes

Spontaneous chromosome doubling sometimes observed in wide hybrids is a consequence of formation of unreduced gametes. These are created by modified meiosis, so-called meiotic restitution. Perhaps some of spontaneous doubling among androgenic haploids is by this pathway. In meiotic restitution typical meiosis with its two consecutive of cell divisions is reduced to a single division - univalents separate sister chromatids in anaphase I generating two daughter nuclei with complete sets of somatic chromosomes and meiosis II is omitted. In the final effect diads, formed from pollen mother cells, produce standard sperm nuclei. The fusion of unreduced gametes generates from haploid plants under self-pollination fertile progeny with somatic number of chromosomes (Fig. 3).

Colchicine treatment

Colchicine used at mid-tillering stage plants growing vigorously, in concentration 0.08 - 0.1% , for ca. 7-8 hours, under aerated solutions and with 24 h rinse in water followed by recovery ca. 7-10 day at 4-7° C, does not produce ill effects while is close to 100% efficient in doubling the chromosome number. Aneuploids and plants treated too early show higher mortality than healthy material treated at the appropriate time. Chimeric effect and morphological deformities may indicative of aneuploid nature of treated plants, whereas high mortality and extensive damage may indicative of inapt application rather than colchicine itself.

Mitotic inhibitors other than colchicines

Application of antimitocis directly to anthers on the induction media significantly reduced the efficiency of the method. The highest rate of explants was obtained after use trifluralin (T), ethalfluralin (E), benfluralin (B), and aminoprophos-metyl (APM) (Fig. 1, 4) . The concentrations of mentioned above herbicides, in impact on seedling vigor and survival in vitro after 5-6 hour treatment were 3 µM, 4 µM, 5 µM and 20 µM, respectivelly. T and APM added to the regeneration media at the plantlet stage had no effect on the fertility of regenerated plants. Only in isolated case the antimitotics appeared to offer marrginal advantage above spontaneous chromosome doubling. The frequency of off-types was not related to the application of specific antimitotics. Proportions of aneuploids among regenerants appeared to be the highest among the most stable F1 hybrids and was the highest among the most recalcitrant hybrids. Tested antimitotic were not nearly as effective in chromosome doubling as well as colchicine.

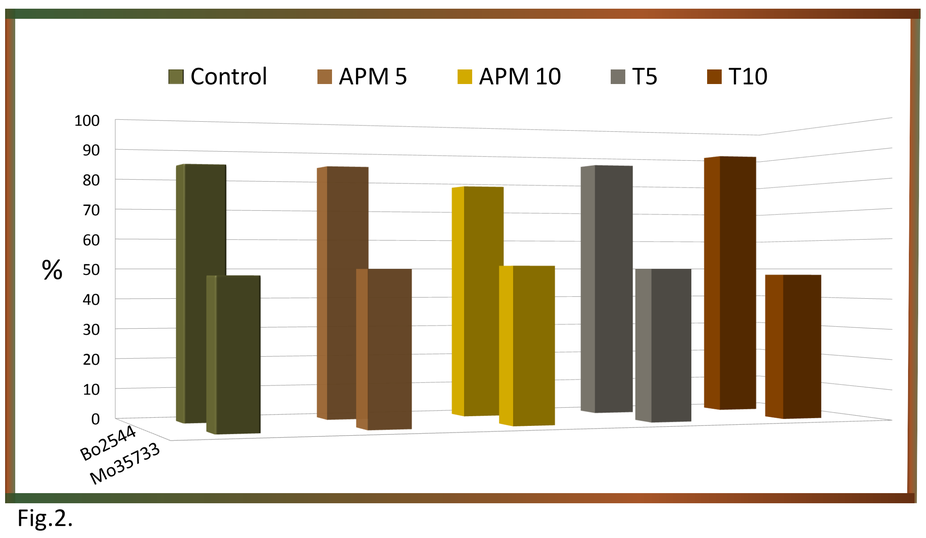


Fig.2.

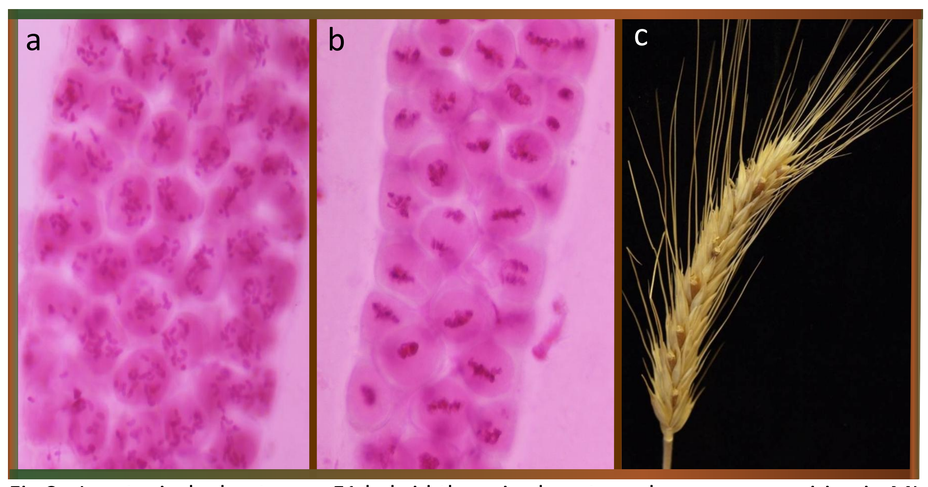


Fig.3. In a typical wheat x rye F1 hybrid there is almost no chromosome pairing in MI of meiosis and univalents randomly migrate to poles. The resulting diads are chromosomally unbalanced and no functional gametes are produced. The hybrid is sterile. In a hybrid with meiotic restitution univalents line up on the MI plate and in AI separate sister chromatids to the poles (b). The resulting diads have somatic chromosome numbers. The second meiotic division is omitted and the diad nuclei form gametes. These gametes are functional and the plant is fertile (c).

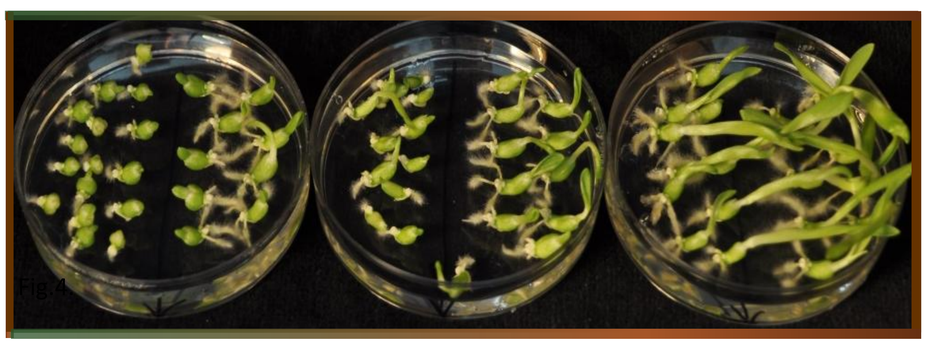


Fig.4. The response of embryos/seedlings to different antimitotic after 7 days of the culture