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In vitro androgenesis in triticale

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Abstract

The technology for *in vitro* anther culturing was optimized in hexaploid triticale using combination of external factors that allowed to obtain more than 100 doubled haploid lines. Investigation of genetic variation among anther culture derived doubled haploids of triticale showed the occurrence of heterozygous plants.

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Introduction

In vitro androgenesis is the process of induction and regeneration of haploids and doubled haploids originating from male gametic cells. Due to its high effectiveness and applicability in numerous plant species, it has outstanding potential for plant breeding and commercial exploitation of doubled haploids (DH) (1). Using DH production systems, homozygosity is achieved in one generation, eliminating the need for several generations of self-pollination.

Androgenesis in triticale is performed both by the anther culture and with the microspore culture methods. However, the methods have often been described as non-cost-effective for this culture. Therefore, further improvement of methods of *in vitro* culturing is necessary.

In vitro androgenesis could be highly stressful and may lead to different genetic and epigenetic changes in derived plants (1, 2). In order to avoid heterozygosity, it is important to distinguish between desired doubled haploids and redundant heterozygous diploids. Nowadays, DNA markers are commonly used for homozygosity testing and assessment of plant origin.

Materials and Methods

The efficiency of triticale anther culture was tested. The effect of genotype, growth conditions, pretreatment, culture medium and their interaction was analyzed using 15 genotypes of donor plants. Genetic variation of 27 doubled haploids obtained through *in vitro* anther culture (5 parental DH lines and 22 derived from them) using 14 ISSR and 9 *Vrn* allele-specific molecular markers was evaluated (3-6).

Results

The anther culture protocol was elaborated including cold (+4°C) pretreatment of cut spikes within 21 days and use of liquid incubation medium C-17. Application of the given combination of external factors allows to increase the number of embryo-like structures, regenerated plantlets and doubled haploids in triticale. Upwards of 100 doubled haploid lines of triticale were developed through *in vitro* anther culture.

In order to examine genetic variation among doubled haploids 14 ISSR markers were tested and five of them (UBC811, UBC808, UBC856, UBC807, ISSR17) were shown as

high polymorphic relating to triticale genotypes. The percentage of polymorphism across the triticale lines ranged from 80.0 to 95.0 %. According to ISSR-analysis the majority of DH lines derived from one line showed high genetic similarity. In particular, 16 doubled haploids originated from DH-27-1-08-1 grouped in one cluster. However, significant differences were depicted among some lines. In particular, DH-(7-16)-1-11-1 and its "parental" line DH-50-1-08-2 (on the basis of which DH-(7-16)-1-11-1 was originated) were placed in different clusters of a phylogenetic tree.

Allelic variation in *Vrn*-genes was analyzed using allele-specific DNA markers in order to select homozygous lines and develop targeted selection programs. Two alleles (*Vrn-A1a* and *vrn-A1*) were detected at *Vrn-A1* locus and three alleles (*Vrn-B1a*, *Vrn-B1c* and *vrn-B1*) were found at *Vrn-B1* locus. All doubled haploids carried the recessive allele at the *Vrn-B3* locus. Twelve lines of spring triticale were selected, and they were characterized by an allelic composition associated with early maturity and high potential of grain yield.

Two lines DH-31-1-08-1 and DH-(7-16)-1-11-1 were not completely homozygous. Heterozygosity in the line DH-31-1-08-1 is apparently due to somatic rather than androgenetic origination. However, this explanation is not applicable to DH-(7-16)-1-11-1, because this doubled haploid carried alleles *Vrn-B1a* and *vrn-B1*, while the original line DH-50-1-08-2 contained the *vrn-B1* allele only. The occurrence of this heterozygous form may be due to somaclonal variability that takes place during in vitro cultivation.

Discussions and Conclusions

Therefore, triticale anther culture system was improved that allowed to obtain more than 100 doubled haploid lines. The results of molecular-genetic analysis showed that anther culture derived forms of triticale could have somatic origination. Besides, *in vitro* culturing could cause genetic variation in triticale.

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