

## USING ANDROGENESIS IN STUDIES ON SINGLE CELL-DERIVED EMBRYO DEVELOPMENT

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**Abstract.** The method for regeneration of plants from microspores was being developed for the last 50 years. Through the use of this method a tremendous progress in breeding of many important crop plants was achieved. This applies to cereals too. We have developed microspore culture techniques for many cereals and grasses, and here we recommend androgenesis as a tool to study morphological and biochemical changes during the plant embryo development.

**Key words:** androgenesis, cereals, microspore, haploid plants, embryo development

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Already in the 1800's the scientists dreamt of regenerating whole plants from single cells. Plant regeneration from somatic cells has proven to be possible in the 1950's. Later, a system of regeneration from anthers and isolated microspores – androgenesis, has been developed. Androgenesis was observed for the first time by Guha and Maheshwari in the sixties, in *in vitro* cultures of *Datura* (GUHA & MAHESHWARI 1964).

Each such regenerant is different, which enables selection of lines with unique combinations of genes and allows estimating the characteristics of the new homozygous genotype. Pure lines that exhibit high yielding, yield stability, resistance to diseases and adverse environmental conditions are the basis for obtaining new varieties of crops. Such lines in conventional breeding can be obtained by time consuming repeated self-fertilization. The use of techniques based on *in vitro* cultures such as anther and isolated microspore culture helps to increase the number of recombinants with desirable qualities and thereby improve the profitability of agricultural production. For plant breeders it is necessary to obtain a large number of haploid and doubled haploid (DH) plants (BERNARD *et al.* 1986). Anther culture is the most common method of double haploid regeneration. In many studies stress

and genotype were shown to be of crucial importance for the performance of androgenesis induction and plant regeneration (GONZÁLEZ & JOUVE 2000; BALLA *et al.* 2012). Although the induction process requires the use of an external treatment to change the way of microspore development to switch from generative to sporophyte pathway (GONZÁLEZ & JOUVE 2005), there are genotypes resistant to stress-induction (TOURAEV *et al.* 1996; IMMONEN & ROBINSON 2000).

Generally, plants regenerated from cells of gametophytic pathway should be sterile, as microspores after meiosis contain the number of chromosomes reduced by half, but certain amount of regenerants appear to be fertile due to the early, spontaneous chromosome doubling. The percentage of spontaneous double haploid lines ranged for different cereals from 10% to 60% (CHARMET *et al.* 1986; ŚLUSARKIEWICZ-JARZINA & PONITKA 2003). As a result of induced androgenesis we can obtain not only doubled haploids, but also aneuploids and mixoploids (LUKJANJUK & IGNATOVA 1986; OLESZCZUK *et al.* 2011).

A more “sophisticated” but, in some cases, also more efficient way is to regenerate haploid plants from *in vitro* cultured isolated microspores. First reports on the use of isolated microspore culture techniques in cereals have

been published in the eighties and nineties. The advantage of the isolated microspore cultures is a direct development of embryos, the ability to avoid the regeneration from somatic anther tissue and often higher regeneration efficiency (JÄHNE & LÖRZ 1995). Although numerous studies on cereals' androgenesis allow understanding the process better, there is still a lot of problems to solve, such as increasing the yield of DH lines, a large number of albino regenerants and a low percentage of spontaneous doubled haploids (LAURIE & SNAPE 1990).

Androgenesis has become a well-established method of double haploid production for commercially important cereals such as barley, rice wheat and triticale, but it is also used for recalcitrant cereals like rye and oat, as well as for other species from Poaceae family (MORDHOST & LÖRZ 1993; IMMONEN & ATILLA 1999; KONIECZNY *et al.* 2003; OLESZCZUK *et al.* 2004, 2006; EUDES & AMUNDSEN 2005; KIVIHARJU *et al.* 2005; ŽUR *et al.* 2008). It has to be noticed that homozygous progeny of double haploids can be used in heterosis breeding in order to establish new high yielding varieties (e.g. in rye breeding) and become at the moment often an important element in modern breeding programs such as the use of reverse breeding, tilling, genetically modified plants etc.

Genetic and morphological instability among microspore derived regenerants and their progeny are undesirable and such variations can disqualify material obtained in various stages of research and breeding. While genetic alterations can be easily recognized at the morphological level, non-genetic changes usually are difficult to observe. Such epigenetic source of variation can be beneficial for selection and can be identified with the help of modern methods of molecular biology and bioinformatics (OLESZCZUK *et al.* 2002; BEDNAREK *et al.* 2007).

### Culture development and morphological observations

Androgenesis (Fig. 1) allows for tracking the early stages of embryo development starting from a single cell and later stages of development to a fully formed embryo, and

therefore is a convenient tool used in modern embryology. *In vitro* culture of anthers or isolated microspores is possible due to induced change in developmental pathway of microspores from gametophytic to sporophytic one. The process begins with a microspore's nucleus division phase. Some nuclei fuse with each other giving rise to diploid cells. During the next stage of microspore embryogenesis repeated divisions, inside of the former microspores wall – sporoderm, occur. After bursting of the microspores' wall, proembryos develop from cell clusters. Proembryos subsequently become fully developed coleoptilar embryos. They contain all organs typical for cereal embryos and that is why they can subsequently germinate into normal plantlets. This regeneration system makes it possible to study the developmental pathway of plant embryo at morphological as well as at biochemical level. Development of androgenic embryos is similar to the normal zygotic embryogenesis in monocot species.

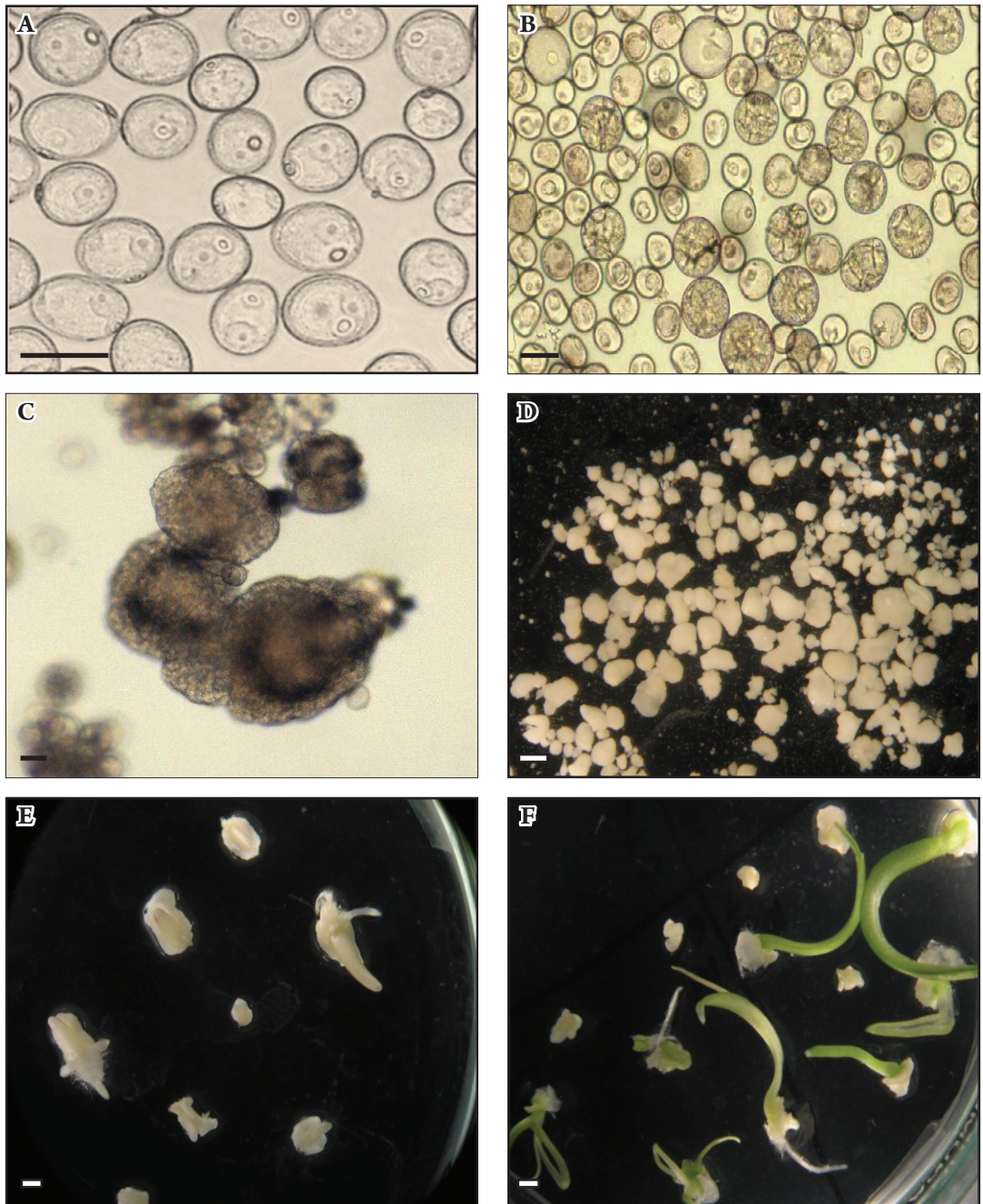
### Final remarks

Due to its unique properties, *in vitro* cultures of microspores have enormous potential for various biotechnological applications such as the *in vitro* selection and mutagenesis. They may also be used to generate transgenic plants with desirable properties and to stabilize transformed lines (KUMLEHN *et al.* 2006) and to create genetic maps for plant species and particular agronomic traits (TANHUANPÄÄ *et al.* 2012). Moreover, microspore cultures can be a source of protoplasts, cell suspensions and "nurse" cultures for other cells.

In addition to practical applications in breeding and embryology, which was already mentioned, we want to recommend here, homozygous lines for use in any type of research, as they provide genetic and phenotypic uniformity of plant populations.

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**Fig. 1** Androgenesis induced from isolated microspores of triticale: **A** – isolated microspores; **B** – first divisions of microspores; **C** – proembryonic clusters of cells; **D** – proembryos; **E** – germinating embryos on regeneration medium; **F** – microspore derived plantlets. For **A-C** bar represents 20  $\mu\text{m}$ , for **D-F** – 1000  $\mu\text{m}$ .

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