Biotechnology in haploids and polyploids

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1. Introduction

Polyploidization (increasing the number of genomes) and haploidization (decreasing the number of genomes) are opposite processes. Obtaining and using both haploid and polyploid plants is the objective of many genetic investigations which employ new research methods and new technologies.

Work on polyploids developed in the nineteen thirties and forties, with the discovery of colchicine. In Poland, most intensive research in that field occurred in the fifties and sixties.

Haploids, in turn, became the subject of intense investigations when the process of artificial androgenesis (11) was discovered. The demonstration that the differentiation of young microspores into embryos and haploid plants occurs in an *in vitro* culture of young Datura anthers stimulated thousands of experiments with various plant species.

Currently, the enthusiasm accompanying the first experiments on obtaining polyploids and haploids has waned a little. The role which haploids and diploids can play in pure and applied research is now seen more realistically. At the same time, the introduction of new technologies expands the scope of research and increases the number of problems which may be addressed (12,23,32,33,38,39).

2. Haploids

New technologies may be used to obtain, characterise and select, as well as to double the chromosome number of haploids and to use them in basic research and for breeding purposes.

3. Obtaining haploids

Haploid plants contain the gametic number of chromosomes in their sporophyte. In other words, these plants have half the chromosome number of the parental plant. We distinguish between monohaploids, which contain one basic genome from a given species, and polyhaploids, which usually have half the number of a polyploid organism. A polyhaploid may thus contain several genomes. In general monohaploid organisms are sterile whereas polyhaploids may be fertile, i.e. they may have fertile progeny.

One of the most early known methods of obtaining haploids is inducing apomixis by pollination with the pollen of a foreign species, pollen of a specific clone or irradiated pollen.

A classical example is provided by obtaining dihaploids of potato (*Solanum tuberosum*) using pollination with *Solanum phureja* pollen (16). Potato is a tetraploid and the seeds obtained after such pollination contain dihaploid embryos.

Potato haploids are weaker than tetraploids but they do develop into plants and may be multiplied vegetatively relatively easily.

Greater difficulties are encountered in the case of other species, e.g. cucumber (*Cucumis sativus* L.). Pollination with irradiated pollen (0.3 kGy, 60 Co source) stimulates the development of haploid embryos from an unfertilised egg cell (28). The development of haploid embryos is delayed and often abnormal. Early embryo rescue and culture *in vitro* on a synthetic medium are indispensable. Thus, in order to obtain haploid cucumber embryos not only a source of irradiation but also the use of *in vitro* cultures is required.

Another method, called the *Hordeum bulbosum* method, also uses *in vitro* culture of young haploid embryos. This method makes use of the process of elimination of paternal chromosomes from cells of a young hybrid embryo (*H. vulgare* x *H. bulbosum*). Several days after pollination, the embryos are transferred onto a synthetic medium. This process has not only been applied to barley but also to wheat, *Triticale* (18) and other cereals.

As it has already been mentioned, in the process of androgenesis embryos or haploid callus are formed from microspores which are induced to divide. Whole anthers or isolated microspores are cultured. As a result of the androgenesis process, haploids of hundreds of species have already been obtained. However, in some species it was not possible to induce the process. A number of treatment methods are used to increase the probability of inducing the process of androgenesis. These include processes influencing the donor plant (specific culture conditions, fertilisation, illumination, temperature and gametocide treatment on e.g. wheat or maize donor plants increased haploid embryo yield.). Also, treatment of inflorescences or anthers with low or high temperatures, specific media and supplements to these media e.g. ficoll significantly increased green barley plantlet production (5). In cultures of isolated microspores in a liquid medium, various systems are used for aeration of the media and for calibrating young embryos.

The culture of ovules or ovaries is an even more difficult and highly fascinating method of obtaining haploids. The process of gynogenesis i.e. the process of sporophyte development from macrospores or young cells of the embryo sac is used here. Stimulating the induction and the development of a few haploid cells of the gametophyte in a great mass of somatic cells surrounding the embryo sac is very difficult.

This process has already been described for many species. The best results have been obtained for the sugar beet (27) and barley (3). In both previously mentioned methods, special attention is paid to culture media and genetic aspects of the induction process.

Haploids are not produced on an industrial scale. Their applications for breeding purposes are known. A well elaborated method (technology) for a given species allows to obtain hundreds or even thousands of haploid plants.

The "Bulbose" method in barley, the culture of anthers or isolated microspores in tobacco and rapeseed, and fertilisation with irradiated pollen in plants of the cucurbits may serve as good examples.

4. Characterization and selection of haploids

Haploid plants are different from diploid plants but morphological differences are not sufficient to prove the ploidy level. The most accurate method for evaluating ploidy is by estimating the chromosome number in the metaphase of a mitotic or meiotic division. This is by no means an easy task, especially for species with numerous small chromosomes or in the cases where meristematic tissue is difficult to obtain.

The introduction of flow cytometry has to a large extent made evaluation of the ploidy level in plants regenerating *in vitro* much easier. Using a flow cytometer, hundreds of plants may be analysed in a single day, which allows the assessment of large populations.

The constantly improved cytometry does not only facilitate plant selection but allows for the selection of cells or even individual chromosomes.

Selection of haploids from a plant population with different ploidy may also be facilitated by the use of marker genes. For example, it has been used for selection of tomato haploids (13). Transgenic seeds transformed with the aux 2 gene, a gene of *Agrobacterium rhizogenes* that transforms naphthalene acetamide (NAM) into naphthalene acetic acid (NAA), did not develop roots in the presence of NAM whereas wild-type tomato seeds developed a normal rooting system in its presence.

The molecular characterisation of haploids is be coming more and more popular, e.g. RAPD markers linked to genetic factors controlling the milling energy requirement of barley have been identified (4).

5. Doubled haploids (DH)

Haploid plants propagated vegetatively *in vivo* or micropropagated *in vitro* may be the object of research just by themselves. In fact, a more practical aim, is namely obtaining of fully homozygotic pure lines has been set.

After doubling of the haploid, homozygous plants are indeed obtained, although some data indicate that heritable DNA methylation changes occurr during DH production, particularly when using the anther culture method (6,29).

Molecular markers (RFLP) may be used to confirm the homozygosity of the DH lines (22).

In some cases, e.g. in the culture of rape anthers doubling occurs spontaneously to some extent. In other cases, haploid plants are treated with colchicine yielding diploids, polyploids and chimaeras (26).

Plants micropropagated *in vitro* may be treated with colchicine in the medium increasing the survival of the treated plants. Culture of haploid plants may also be used in an attempt to obtain spontaneous doubling, stimulating callus induction and inducing plant regeneration (25).

Such activities may lead to obtaining numerous doubled haploids.

Haploids, especially polyhaploids, may form unreduced gametes (24). There is then a chance of obtaining generative progeny, though not always by self-pollination.

6. Utilisation of haploids

In basic research, haploids are used for protoplast fusions, induction of mutations and transformation. The fusion of two haploid organisms usually results in a diploid organism. Thus, the characteristics of selected haploids may be combined at will.

Induction of mutations in haploid individuals is justified as the expression of a single changed gene may be observed. Work with haploid tobacco plants useful for characterisation of mobile endogenous copy-like transposable elements is a good example (8). Similarly, after the introduction of a gene into a haploid organism (transformation) it is easier to assess gene expression than in diploid plants. Transformation of maize microspore protoplasts and regeneration of haploid transgenic plants (36) is an example of utilisation of the haploid state of microspores and transformation. A similar situation occurs with the transformation of pollen embryos in *Datura innoxia* and *Nicotiana tabacum*. As a result, production of transgenic plants and fertile homozygous dihaploid plants has been achieved (30).

Advances have been made with doubled haploids by the construction of a genetic linkage map to mark agriculturally significant traits and to aid breeding programmes. An RFLP map based on doubled haploids from anther cultured indica/japonica F1 rice (37) as well as the mapping of quantitative

powdery mildew resistance (15) and ym4 virus resistance of barley (9) are under construction. Potato dihaploids are also utilised for genetic analysis of resistance traits. An example is provided by the work on segregation analysis and RFLP mapping of R1 and R3 alleles conferring race-specific resistance to *Phytophtora infestans* in progeny of dihaploid potato parents (17).

Moreover, doubled haploid lines derived from a cross between wheat cultivars were used as the plant material (RFLP analysis) for identification of alleles of the Pm3 locus conferring powdery mildew resistance (14).

In a culture doubled hybrids and their generative progeny may be used to obtain new varieties (23). Numerous of investigations have shown that doubled haploids are comparable to conventionally bred material.

7. Polyploids

Polyploids occur in the world of plants which surrounds us. Many cultivated species are auto- or allopolyploids. Polyploids obtained by human effort are most often colchiploids, i.e. individuals obtained after colchicine treatment. More and more polyploids are being obtained during *in vitro* cultures of e.g. a long-term callus culture, cell suspensions, etc.

We often deal with polyploids when we observe somaclonal variation (1, 20). Polyploids are also formed in cultures of anthers or embryos, besides haploids and diploids. Biotechnological methods are often applied when we want to use wild germplasm to create allopolyploids.

Analyses like RAPD and PCR as well as flow cytometry are very helpful in polyploid selection. Molecular techniques (such as the use of RFLP and RAPD markers) have provided a wealth of data which have facilitated the resolution of several controversial questions in polyploid evolution in plants (33).

Mapping and application of RFLPs in polyploids has lagged behind that of diploids due to technical problems (large number of segregating genotypes, poorly characterised genome constitution, chromosome behaviour, multiple fragments). Linkage maps of haploid and doubled haploid lines facilitate mapping in polyploids (35). The polymerase chain reaction (PCR) with selected primers has been shown to be an efficient method to generate fingerprints which are useful in genetic mapping and genomic fingerprinting. Some markers can be placed into linkage groups (34). Multicolour fluorescence *in situ* hybridization (FISH) can also help in physical mapping and chromosome painting and has been used with very good results for instance in wheat (21). Genomic *in situ* hybridization (GISH) offers opportunities for testing genome relationships (2).

In specific cases, such as introduction of the CMS trait from one species of *Brassica* to another protoplast, fusion has proved useful (31). Joining diploid protoplasts in order to obtain valuable polyploids has also proved helpful, for example in joining diploid varieties from the genus *Citrus* (10).

Polyploids, especially those which are valuable for cultivation, are subject

to transformation. For example, the Bt gene carrying resistance to some *Lepidoptera* has been introduced into cotton and *Brassica* plants.

Some polyploids, e.g. those with odd numbers of genomes, cannot be propagated generatively. In such cases, micropropagation and embryo rescue are helpful (7).

Multiplication of polyploids on an industrial scale may take place using biofermentors, e.g. formation of minibulbs in potato.

Biofermentors are also utilised for the formation of somatic embryos (19). The thus produced embryos are calibrated, appropriately dried and encapsulated to form artificial seeds. These procedures require further development.

The review presented here summarises the advances made so far in haploid and polyploid biotechnology and suggests some of the directions that it might take in the future.

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Summary

The progress made by new biotechnologies in haploid and polyploid developments is outlined. There are many applications for biotechnology in haploid production via anther or ovule culture or embryo rescue, clonal propagation, doubling, protoplast fusion, mutant induction and transformation.

In several species, transformed germplasm derived from somatic fusion or gene transfer is already being used in field trials. Meiotic mutants that form unreduced gametes have improved results of crosses between species with different levels of ploidy.

Genomic maps based on RFLP technique and doubled haploids as well as physical maps (FISH technique) are under construction. New molecular (RFLP, RAPD) and immunological markers are being used for diagnostic assays. The priorities for biotechnology research in plants are emphasised.

Key words:
biotechnology, haploid, polyploid, progress.

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