

INDUCED POLYPLOIDY IN CROP IMPROVEMENT

Article Id: AL202116

Swathi Lekkala^{1*} and Saggili Ravi²

¹Dept. of Genetics and Plant Breeding, TNAU, Coimbatore, India

²Dept. of Genetics and Plant Breeding S.V. Agricultural College, Tirupathi, India

Email: swathi.lvs@gmail.com

Chromosomal doubling occurs in plants naturally, however its rate is very low and a slow process. Since time is important, chromosomal doubling/ polyploidization can be induced in a short period at a high rate by using antibiotic agents like colchicine, oryzalin etc. The concentration and duration for treatment of the explant differ with the crop and hence an efficient polyploidization protocol has to be developed for the concerned crop. The induced putative polyploids can be selected by various morphological parameters cytological and flow cytometric techniques. The exact chromosome complement can be obtained by studying the mitosis or meiosis.

Polyploidy refers to the presence of more than two complete sets of chromosomes per cells, and it plays a major role in crop evolution and diversification. Such polyploids can be obtained naturally by the interspecific or intraspecies union of unreduced gametes. But the rate is very slow, whereas artificial polyploidization is quick and can be induced by using antibiotic agents like colchicine, pronamide, oryzalin *etc.* Such genome duplications result in greater variation when compared to specific gene mutations. Polyploidization is employed in several plant species however major work is done in medicinal plants, horticultural plants and few forage crops. Polyploidization has begun after the experimental work of Eigsti. O. J with colchicines.

Methods of polyploidization

There are three major methods of polyploidization *in-vitro*, *in-vivo* and *ex-vitro*. Among those three methods, *in-vitro* is most popularly used and quicker method for polyploidy induction in limited space under aseptic conditions. However, it demands more skill. The factors effecting the *in-vitro* induction of polyploids is given in Figure 1 (Niazian and Nalouisi; 2020). In contrast, *ex-vitro* requires less skill and does not require expensive laboratory settings. It generally involves seeds or roots as explant. *In-Vivo* system of polyploidy induction can be

achieved by applying the antibiotic agents to the intact plant parts like seedling apex (cotton) or inflorescence (Jatropha).

Induction of polyploids

Synthetic polyploids production can be achieved by interfering the cell cycle by a variety of chemicals. Those chemicals which act at the end of the S-phase and before the cytokinesis are the effective candidates for polyploidization. Most of the antimitotic agents are metaphase inhibitors acting on the alpha and beta tubulin dimers. Nitrous oxide was the first putative antimitotic agent employed for genome doubling. The most commonly used antimitotic agent is colchicine, an alkaloid extracted from the bulbs and seed of *Colchicum autumnale*. However, it causes sterility, chromosomal loss and abnormal growth in many plant species. Another major disadvantage of colchicines is its high affinity to microtubules of animal cells, hence very toxic to humans. Alternatively, some of the herbicides and phosphorothioamidates have low toxicity for humans unlike colchicine. Hence these herbicides can be used as alternatives for colchicines.

The concentrations of the antimitotic agent depend on the explants used, *i.e.* Callus, buds or shoot tips, seeds, seedlings and tubers. The induction percentage is also genotype-dependent. Besides the explant, the concentration and duration of the treatment also play an important role in polyploidization. It is crop-specific, and hence standardization of the optimum concentration and duration of the treatment has to be done. Lower concentrations are not successful, while higher concentrations are lethal. The solvent used for dissolving the antimitotic agent is also very important. In many cases, anti-mitotic agent are dissolved in DMSO, which increases cell permeability allowing enhanced absorption of the chemicals. Alternatively, 70% ethanol, NaOH and Acetone can also be used.

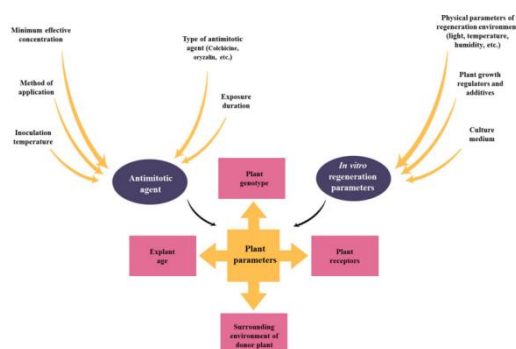


Fig 1: Factors affecting artificial polyploidization under *in vitro* conditions

Identification and confirmation of polyploids

Morphological and anatomical studies such as stomatal measurements are simple and indirect methods of polyploidy conformation, but they are often inaccurate. Stomata of polyploids are large in size when compared to their diploid counterparts. Besides the size of the stomata, the change in density can be observed. The polyploids were recorded to have a lower stomatal density (Compos *et al.*, 2009). Pollen size, similar to stomatal size, can also be used for polyploidy detection, but it is rarely used. Flow cytometry is a very common method for ploidy analysis (Dolezel *et al.*, 2007). The nuclei are extracted from the leaf tissues, and the DNA is stained using any fluorescence (mostly PI). The flow cytometry uses a light source to illuminate the stained nuclei and the emitted fluorescence is detected. The amount of fluorescence in the nucleus is proportional to the DNA content. It is a rapid method for testing ploidy and a large number of plants can be analyzed. Early-stage detection is possible and hence its time and space-saving.

Although morphological and flow cytometric studies can be used for primary screening; however, exact confirmation is often necessary. Sometimes polyploidization results in mixoploids (different ploidy level). Therefore, to know the exact ploidy level, cytological studies (mitosis/meiosis), involving the chromosomal counts is the perfect and ultimate method.

Applications of polyploidy in crop improvement

Approximately 40% of the cultivated species are polyploids (Simmonds 1980). Polyploidy induction is attempted in several crops like Rye, Brassica *sps*, Clover, Pearl Millet Napier Hybrids and many Ornamental and Medicinal plants. In ploidy induction, reduced fertility is a major drawback, when the reproductive organs (seeds/fruits) are of interest. However, for ornamental plants fertility reduction is not a problem as the larger flower are advantageous, and these are mostly vegetatively propagated.

Among the cultivated crops, the first allopolyploid obtained by polyploidization was Raphano brassica, but it was a failure. The first successful synthetic allopolyploid was triticale obtained by chromosome doubling of sterile F_1 by using colchicine. Several Red Clover varieties are produced by polyploidization. These varieties showed tolerance to diseases, winter hardness and high forage dry matter yield. Similarly, tetraploid ryegrass varieties were

developed, that showed tolerance to diseases, drought and improved palatability. In Pearl millet Napier grass, the F_1 (Pearl millet x Napier grass) are sterile, and the fertility restoration is done by using antimetabolic agents like colchicine, oryzalin and trifluralin. Various applications of induced polyploidy were depicted in Fig. 2 (Sattler *et al.*, 2016).

Polyploid induction is more popularly employed in ornamental plants, which results in increased flower size, deep coloured flowers and extended flower longevity. In medicinal plants increased genome content by chromosomal doubling results in overexpression of genes that are involved in biosynthetic pathways, thus increasing the productivity of desired pharma molecules.

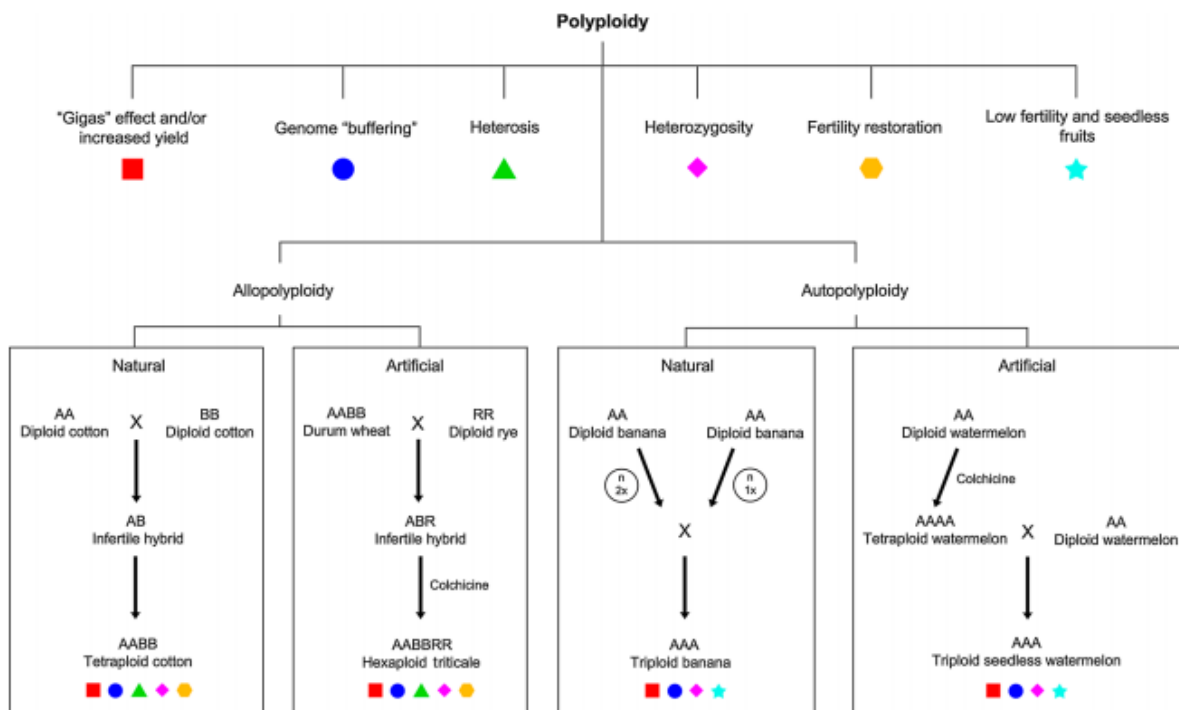


Fig 2: Schematic representation of cultivated species and the main polyploidy consequences for application in crop improvement. The symbols “n 1x” and “n 2x” refer to reduced and unreduced reproductive cells, respectively

Conclusions

Polyploidization, though a complex process has a greater impact on crop improvement. The natural polyploids have drawn a greater interest in the plant breeders, thus making them to imply artificial polyploidization for crop improvement.

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