Progress in Plant Polyploidization Based on Antimicrotubular Drugs

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Abstract: The results of artificial polyploidization for horticultural and agronomic plant species are presented in this review. The data on use of the classical antimitotic drug colchicine for chromosome doubling are reported. The efficacy of other compounds such as dinitroanilines and phosphorothioamidates in polyploidy induction as compared to colchicine has been summarized. The molecular basis of highly specific binding of dinitroanilines and phosphorothioamidates with plant tubulin for induction of efficient polyploidy are discussed.

INTRODUCTION

The rapid advance to genetic fixation which accompanies the doubling of a chromosome set is an attractive feature of haploid breeding, especially in those species where conventional inbreeding involves several years of manual self-pollination. Moreover, it was established that the frequency of spontaneous chromosome doubling in many plant haploids is low and extremely unreliable. To achieve a reproducable and prompt success in plant chromosome doubling there was a need to find efficient, reliable low-cost and safe agents that would promote such effects in plant cells. The experiments on polyploidization of plants were begun in 1940, when colchicine, a natural alkaloid with an antimitotic activity, obtained from the wild plant species *Colchicum autumnale*, was found as an agent with chromosome doubling activity in cells of higher plants [1]. The techniques of colchicine application have not changed much since Levan [2] soaked *Allium* (onion) roots in colchicine solutions.

COLCHICINE FOR CHROMOSOME DOUBLING

Colchicine has proven to be most useful to double the chromosome numbers of numerous crop species, including decorative flowers, medical and agricultural plants. This alkaloid, however, is very toxic to human beings and also shows undesirable mutagenic activity on plants. A comprehensive review summarizing, nevertheless, the great impact of colchicine in plant polyploidization and its roles in different plant breeding programs was written by Hancock [1]. This author summarized the data where it was shown that this compound was used for induction of polyploidy in fruit and berries, such as apples, cranberries, grapes, peaches, pears and strawberries; for chromosome doubling of several flowers, including marigolds, pinks, snapdragons, petunias, delphinium and lilies. It was also used for chromosome doubling of some agronomic crops as lettuce, chilli peppers, cotton, potato, rye, sugar beet, wheat and tobacco [1]. By 1979, the chromosome numbers of well over 150 plant species have been doubled using this compound [3]. Unfortunately, only a small number of the artificially induced crops were ever released and few of these autopolyploids ever dominated world market [1].

Colchicine is commonly used in diploidization and polyploidization of different plant species. Currently, apical meristems, secondary buds, tillers or roots are treated with colchicine [see, 4-6]. Generally, about 50% of the treated plants are responsive, but what is important, that three months can be added to the plant regeneration time to recover homozygous lines [7]. Additional drawbacks to using this approach include the regeneration of chimeras in many species [4-5], [8-12], the occurrence of aneuploids [13], abnormalities in plant development [14-15] and low seed yield. Chromosome losses or rearrangements and gene mutations caused by colchicine were found in flax, sunflower, barley and cotton [16]. Such chimaeric plants are unsuitable for breeding since the ploidy of such plants is often unstable through vegetative multiplication.

A more promising alternative is application of colchicine to cultures prior to organ formation (anthers and microspores). The use of haploid plants, generated from anther or microscope culture, has enhanced the efficiency of crop improvement programs [17]. Although haploid plants of different species can be readily regenerated *in vitro*, the haploids cannot be used directly in genetic studies and breeding programs because they are sterile [18].

In particular, colchicine techniques for microspore cultures have established more efficient ways for the production of non-chimaeric doubled haploids [19]. Colchiploids were produced successfully from corn anther-derived callus [12], [20, 21] and microspore culture [22] as well as from wheat anther culture [8, 23-25]. Colchicine diploidization at the microspore level has been applied in breeding programs with

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Tritordeum (=*Hordeum chilense x Triticum turgidum* cv. *durum*) [26], triticale [27], and with rice anther culture [28, 29]. Exposure to colchicine led to the development of diploid pollen in spikelet cultures of barley (*Hordeum vulgare* L.) and rye (*Secale cereale* L.) [30]. Colchicine-induced polyploidy has been employed widely in *Brassica* breeding and genetics [31].

But all these procedures are labour intensive [17], hazardous [8-10, 32, 33] and costly [33], because high concentrations of colchicine are needed [34]. Therefore different researchers postulated in many publications, the necessity of developing an effective alternative to colchicine [12-33].

DINITROANILINES AND PHOSPHOROTHIOAMIDATES AS ALTERNATIVE POLYPLOIDY INDUCERS

In the 1990s, attention has turned towards the potential use of chemicals other than colchicine which might further improve the *in vitro* chromosome doubling. Antimicrotubular herbicides may function as such alternatives because they have been reported to inhibit microtubule assembly in a way comparable to colchicine.

The effects of trifluralin on microtubule depolymerization and chromosome doubling in embryogenic microspore cultures of *Brassica napus* were examined and compared with those of colchicine [35]. The application of low concentrations of trifluralin was found to be a superior method for doubled haploid production. The embryos generated after trifluralin treatment developed normally, germinated readily and of the plants produced, close to 60% were fertile. The authors have demonstrated that the method based on trifluralin usage is simple, effective and inexpensive as compared with that based on colchicine.

In later studies, the potential of colchicine and the microtubule depolymerizing herbicides oryzalin, trifluralin and amiprophosmethyl (APM) for *in vitro* chromosome doubling during *B. napus* microspore culture was studied [36]. It was found that all three herbicides were similar to colchicines in effect, but at concentrations approximately 100 times lower. APM was less toxic than trifluralin and oryzalin, but no significant difference in chromosome doubling efficiency was detected between the compounds [36].

Oryzalin proved to be a more efficient chromosome-doubling agent than APM and colchicine in potato cell suspension culture [37]. Using colchicine, oryzalin and APM for the production of doubled-haploid embryos from cork oak (*Quercus suber* L.) anther culture it was found that oryzalin was more effective in inducing chromosome doubling that APM and colchicine, too [38].

The chromosome doubling capacity *in vitro* of colchicine and oryzalin in gynogenetic haploids was evaluated on *Gerbera* [39]. Summarizing the results obtained, the authors concluded that the lower dose of oryzalin may be considered superior to colchicine because of its lower phytotoxicity and the absence of long term effects and genetic disorders [39]. The effects of both of these antimitotic agents were tested also on kiwifruit, *Actinidia deliciosa*, where it was also found that trifluralin treatment in low concentration was more effective than colchicine in inducing chromosome doubling [40]. It was found recently that oryzalin is more efficient than colchicine in inducing polyploidy of *Rhododendron* hybrids [41].

Very successful results were obtained after oryzalin-induced chromosome doubling in *Rosa* [42]. For instance, chromosome doubling by low oryzalin concentrations modified plant morphology by increases in thickness and a darker green coloration of the leaves. Internodes were longer in tetraploids than in diploids, the number of petals per flower in the tetraploid forms was double that of the diploids, and significant increases in pollen viability of chromosome-doubled hybrids were also found [42].

Four antimitotic herbicides, APM (phosphoroamidate), pronamide (benzamide), oryzalin and trifluralin (dinitroanilines), were evaluated for their ability to induce chromosome doubling in anther-derived, haploid maize callus [43]. The results indicated that APM and pronamide at very low concentrations effectively induced chromosome doubling of maize anther culture-derived haploid callus. Moreover, they did not inhibit callus growth at these concentrations and the treated callus retained a high plant regeneration capacity. Thus, these two compounds could be used as alternatives to colchicine [43] for chromosome doubling.

The same herbicides were used for *in vitro* chromosome doubling in sugar and fodder beet (*Beta vulgaris)* ovule culture [44]. The best chromosome doubling results were obtained by treatment of the ovules with APM. The APM treatment showed relatively low toxicity on embryo formation in combination with a high doubling effect. Oryzalin and trifluralin had more severe toxic effects, which reduced embryo formation, thereby lower percentages of chromosome doubled plants were obtained. Pronamide had no significant toxic effect but it induced chromosome doubling at lower frequencies. Compared to colchicines, APM was found to be as efficient for chromosome doubling during beet ovule culture, but at molar concentrations 100 times lower than those used for chromosome doubling with colchicine [44].

The successful use of APM for chromosome doubling was also demonstrated on the forage plant, eastern gamagrass (*Tripsacum dactyloides* L.) [45]. The resulting tetraploid plants were morphologically normal and produced normal seeds [45]. In more recent studies, the testing of colchicine, trifluralin and oryzalin on polyploidization of anther culture of peace lily (*Spathiphyllum wallisii)* plants it was found that colchicine could be efficiently replaced by oryzalin or trifluralin which very effective in low micromolar concentrations [46]. Finally, a very broad spectrum of antimicrotubular herbicides was proposed as a colchicine alternative for efficient production of doubled haploids from microspore cultures [19].

Some time ago we reported for the first time about the comparative polyploidizing effects of colchicine, APM, and a wide spectrum of different dinitroanilines (oryzalin, ethalfluralin, trifluralin, pendimethalin, benefin), which have been assessed for production of new nep (*Nepeta* sp.) polyploid forms [47]. It was established that all dinitroanilines possessed a higher polyploidization potential than colchicine. The use of polyploidy in breeding of nep species has the potential of elevation of productivity of secondary metabolism, development of ornamental characteristics, and in

interspecific hybridization, the restoration of F_1-sterility at the tetraploid level.

MECHANISM OF COLCHICINE-TUBULIN INTERACTION

From the various studies, it has been found that the main mechanism of colchicine action is that it blocks cell division by disrupting microtubules. After summarizing the data of different researchers it was concluded that the spindle microtubules are more sensitive to colchicine than the interphase microtubules [48]. The cells blocked by colchicine at mitosis undergo an abnormal mitotic cycle, designed as "c-mitosis" or "colchicine-mitosis". C-mitosis is characterized by partial or complete absence of a spindle apparatus following the breakdown of the nuclear envelope, condensed chromosomes, and undivided centromeres [48, 49].

It was established that colchicine has a high affinity for soluble tubulin; however it does not bind to microtubules unless it first forms a tubulin-colchicine-complex (TC-complex), which attaches to the microtubule ends [48]. Kinetic analysis of the inhibition interaction suggests that the TC-complex binds to the microtubule ends and prevents the microtubule growth by sterically blocking further addition of the animal tubulin dimers to the ends [50].

Moreover, it was established that at a low TC-complex concentration, the complex incorporates into a microtubule by disturbing the formation of lateral contacts at the newly formed ends of the protofilaments while the microtubule still remain intact. On increasing the concentration of colchicine, a greater loss of lateral contacts leads to disassembly of microtubules [48].

Many attempts have been made by different investigators to define the exact colchicine binding site. To gain an insight into this the tubulin-colchicine crystal structure has been reported [51]. This complex threw light on the mechanism of tubulin-colchicine interaction. It was found that the A and C colchicine rings interact with β-subunit of tubulin and the B ring side chain interacts with the α-tubulin [48-50].

Several studies have shown that colchicine has a much lower affinity for plant tubulins than for animal tubulins [52-54]. Among the four eukaryotic families (plants, animals, fungi and protists) colchicine is known to bind most strongly to animal tubulins, with an affinity constant $\sim 10^6$ M^{-1} [55]. Plant tubulins also bind colchicine only weakly as has been found from comparative binding assays [52]. Consequently, millimolar concentrations of colchicine are usually required to inhibit plant cell division and induce chromosome doubling, which compares unfavorably with the micromolar concentrations that affect the microtubule-associated processes in animals [56].

Recently, it was shown that a unique feature of the colchicine-binding site is its striking animal-specificity [55]. To identify this specificity two working hypotheses were considered by these authors, one concerning identification of residues responsible for animal specific colchicine binding to tubulin, and the second one related to definition of primary and extended colchicine-binding sites on tubulin molecules [55]. It was proposed that if the "collective nature" of a subset of residues in animal tubulins is distinct from that of a similar subset in non-animal tubulins, then one or more of the residues in the animal tubulin subset is responsible for the animal specific colchicine binding [55]. Moreover, such a subset of residues must, directly or indirectly, be involved with colchicine binding as evident from crystal structure and experimental data.

It was found that twenty tubulin residues constituted the primary colchicine binding site (PBS). These residues in tubulin sequences across eukaryotes were then identified by multiple sequence alignment of numerous α- and β-tubulin sequences from different animals, fungi, protists and plants, and it was established that only 16 of them are considered to constitute the PBS [55]. But principal component analysis has shown that the amino acid residues comprising such sites are not sufficient to reflect the animal-specific colchicine binding trait of tubulin [55].

After that the principal component analysis in the extended colchicine binding sequence space was carried out across different eukaryotes. It was demonstrated that the animal tubulin extended colchicine binding site residues are distinct – suggesting that this is sufficient to explain the origin of the animal-specificity of colchicine-tubulin interaction [55]. Two sequence positions were found as animal specific: Ala250β and Pro270β, whereas Ser250β and Val/Ile270β are characteristic for plants, fungi and protists [55]. These authors also compared the sequence conservation at binding sites of two other ligands: GTP and GDP, bound to α- and β-tubulin, respectively. It was found that no residue positions show family-specific conservation.

Thus, from an analysis of abundance of α- and β-tubulin sequences and colchicine-tubulin crystal structure it have been clearly demonstrated that two residues in β-tubulin, at sequence position Pro268β and Ala248β (270β and 250β in the crystal structure 1SA0) in animal tubulin are crucial for the observed animal-specific colchicine binding to tubulin across eukaryotes [55].

STRUCTURAL PLANT TUBULIN PECULIARITIES RESPONSIBLE FOR EFFICIENT DINITROANILINE AND PHOSPHOROTHIOAMIDATE BINDING

In comparison with colchicine, such antimicrotubule compounds as dinitroanilines and phosphorothioamidates bind more specifically than this compound to plant tubulin *in vitro* [57]. Among the low-molecular weight tubulin ligands the dinitroaniline and phosphoroamidate herbicides are compounds with a higher specificity for plant tubulins than for animal tubulins [58-61]. Earlier it was proposed that these two chemically distinct classes of antimitotic herbicides bind the same receptor sites [62].

To understand the peculiarities of dinitroaniline and phosphorothioamidate interaction with plant tubulin we have modelled the spatial structure of higher plant tubulins, based upon the established three-dimensional structure of porcine tubulin (http://www.rcsb.org/pdb) [63]. The reconstruction was based on the tubulin sequences of well characterized sensitive and resistant to dinitroaniline and phosphorothioamidate herbicide biotypes of goosegrass *Eleusine indica* [64]. Earlier it was shown that this resistance is caused by a point mutation at position 239 of the α1-tubulin molecule, which results in the replacement of the amino acid Thr by Ile [58-65]. It should be noted that residue Thr239 is highly

conserved in all known fungal, plant, and animal α-tubulins. An analysis of the sequences flanking the mutation site showed that they also are conserved, though not identical in all plant α-tubulins.

To analyze the interaction specificity of dinitroanilines and phosphorothioamidates with tubulin from resistant and sensitive biotypes of *E. indica*, three-dimensional structures of both types of α-tubulins were reconstructed, including the electrostatic potential distribution on their surface [64-66]. A comparison of the spatial models of α-tubulins from resistant and sensitive biotypes with regard for the position of Thr239 made it possible to identify the dinitroaniline binding site. It is located on the surface of the α-tubulin molecule in the interdimer contact zone [64]. It was found that this area of the surface forms a clearly shaped cavity with a highly allocated positive electrostatic potential [64].

The substitution of Leu for Thr at position 239 leads to considerable changes in the distribution of electrostatic charge on the surface of the α-tubulin molecule. This redistribution of surface energy, as a result of the C to T point mutation, is apparently due to the rearrangement of side chains of neighbouring amino acid residues located on and near the surface of the tubulin molecule. This process is accompanied simultaneously by conformational changes in the predicted herbicide binding site on the α-tubulin molecule. Thus the substitution of Thr239, which is located in the vicinity of the seventh helix, can markedly alter the three-dimensional structure of the α-tubulin molecule [64]. So, α-tubulin from the resistant biotype of goosegrass lacks the interactive cavity. Spatial reorganization of the α-tubulin surface prevents ligand binding due to closing of the cavity and redistribution of electrostatic potential, when Tre239 is replaced by Ile. The comparative analysis of the surface structure of animal α-tubulin shows that animal α-tubulin, like plant mutated α-tubulin, does not possess on its surface the cavern of interaction [64].

Modelling the Thr239Ile substitution revealed a drastic decrease in the positive electrostatic potential at the herbicide interaction site [64-66]. As a result a redistribution of charge, accompanied by partial closing (narrowing) of the interaction cavity, takes place. Therefore, the affinity of this site for dinitroaniline and phosphorothioamidate herbicides appears to decrease dramatically [64-66].

Analysis of the three-dimensional structure of trifluralin and amiprophosmethyl, the most effective representatives from dinitroanilines and phosphorothioamidates, respectively, revealed a similar spatial geometry for both types of molecules as well as similarly distributed surface potentials [64]. All active compounds from these herbicide groups are characterized by the presence of common structural elements – nitro groups that are linked to the benzene ring. This basic similarity implies that the polar (electronegative) moiety of their molecules should play a key role in interaction with tubulin. Therefore, we performed spatial docking of dinitroanilines and phosphorothioamidates into the binding sites on the surface of α-tubulin molecules from sensitive and resistant *E. indica* lines.

It was found that the hydrocarbon tails of the herbicides are oriented along amino acid residues Glyl33, Asn253, and Gly256. These amino acids are located on the surface of the α-tubulin molecule, and are involved in interaction with amino acid residues of the β-tubulin molecule of the next heterodimer. A clearly distinguishable and distinct cavity, with a high electrostatic charge, is formed in this area on the interdimer surface of the α-tubulin molecule [64]. Although amino acid residue Thr239 is not exposed on the surface of α-tubulin, it is located immediately under this cavity. Interactions of negatively charged NO_2-groups, which is a common structural feature of both dinitroanilines and phosphorothioamidates, with the identified binding site on the α-tubulin molecule can have significant consequences leading to the prevention of further microtubule polymerization. Furthermore, the position of the bound herbicide ligand will be stabilized as a result of additional interaction with negatively charged groups (such as $-CF_3$ or $-SO_2$) with the NH_2-group of Arg2 [64]. Thus, these tubulin-ligand complexes acquire a sufficient level of stability so as not to dissociate under the influence of internal molecular oscillations.

Results from comparative analyses of the three-dimensional models of α-tubulins from the resistant and sensitive biotypes of *E. indica* were confirmed by a subsequent investigation of the mutant α-tubulin from *Setaria viridis* [67]. In that study it was concluded that the binding site for the dinitroanilines is located immediately adjacent to the contact zone between tubulin dimers, whereas modeling of the α-tubulin molecule from the protozoan *Toxoplasma gondii* assumed that dinitroaniline binding site is located in zone of lateral contacts between the microtubule protofilaments [68]. In the same time, the binding sites predicted for α-tubulins of higher plants and protozoans overlap partially. Therefore, it can be concluded that the binding patterns of dinitroanilines with tubulins in the two groups of eukaryotes are slightly different, likely as a result of their phylogenetic distance.

The binding of herbicides to tubulin decreases the polymerization capability of the latter, preventing the formation of microtubules and, hence, the mitotic spindle. Subsequently, the effect of herbicides on the plant cells results in a complete loss of microtubules not only during mitosis but also in the interphase and may ultimately leads to cell death.

Therefore it can be concluded that the pattern of interaction of dinitroaniline and phosphorothioamidate herbicides with the plant α-tubulin molecule is unique and has no analogues in other tubulin types from higher eukaryotes.

CONCLUSIONS

For a long period of time colchicine has been considered to be most useful as a means of producing bridge species in agronomic and floricultural crops. But postulated colchicine high toxicity for plant cells, its low binding efficiency to plant tubulin initiated screening of more potent agents for polyploidization. The present review demonstrates that such antimicrotubular compounds as dinitroanilines and phosphorothioamidates could be viable alternatives to colchicine for chromosome doubling *in vitro*. Their high polyploidization potential is based on specific interaction with plant tubulin molecule. Therefore advantages of these compounds over colchicine are that dinitroanilines and phosphorothioamidates are much less toxic for humans than colchicine, particularly so with the very low concentrations of the compounds needed for the plant treatment.

REFERENCES

[1] Hancock JF. The colchicine story. HortScience 1997; 32(6): 1011-12.

[2] Levan A. Effects of colchicine on root mitosis in *Allium*. Hereditas 1938; 24: 471-86.

[3] Dewey DR. Some applications and misapplications of polyploidy, In: Lewis W Ed. Polyploidy, Plenum Press: New York 1979; 445-70.

[4] Wong CK. A new approach to chromosome doubling for haploid rice plants. Theor Appl Genet 1989; 77: 149-51.

[5] Swanson EB, Herrgesell MJ, Arnoldo M, Sippell DW, Wong RSC. Microspore mutagenesis and selection: Canola plants with field tolerance to the imidazolinones. Theor Appl Genet 1989; 78: 525-30.

[6] Mathias R, Robbelen G. Effective diploidization of microspore-derived haploids of rape (*Brassica napus*) by *in vitro* colchicine treatment. Plant Breed 1991; 106: 82-84.

[7] Beversdorf WD, Charne DG, Kott LS, *et al*. The utilization of microspore culture and microspore-derived doubled-haploids in a rapeseed (*Brassica napus*) breeding program. In Proc. 7th Int. Rapeseed Conf, Organizing Committee, ed, Poznan: Poland 1987; 13.

[8] Barnabas B, Pfahler PL, Kovacs G. Direct effect of colchicine on the microspore embryogenesis to produce dihaploid plants in wheat (*Triticum aestivum* L.). Theor Appl Genet 1991; 81: 675-8.

[9] de Maine MJ, Fantes JA. The results of colchicines treatment of dihaploids and their implication regarding efficiency of chromosome doubling and potato histogeny. Potato Res 1983; 26: 289-94.

[10] Hansen FL, Andersen SB, Due IK, Olesen A. Nitrous oxide as a possible alternative agent for chromosome doubling of wheat haploids. Plant Sci 1988; 54: 219-22.

[11] Honkanen J, Aapola H, Seppanen P, *et al*. Production of double haploid gerbera clones. Acta Hortic 1991; 300: 341-6.

[12] Wan Y, Petolino JF, Widholm JM. Efficient Production of doubled haploid plants through colchicine treatment of anther-derived maize cells. Theor Appl Genet 1989; 7: 889-92.

[13] Zhao JP, Davidson D. Distribution of chromosomes into discrete group in colchicine-induced C-metaphases of barley. Caryologia 1984: 37, 331-42.

[14] Hart JW, Sabnis DD. Colchicine binding activity in extracts of higher plants. J Exp Bot 1976; 27:1353-60.

[15] Loh CS, Ingram DS. The response of haploid secondary embryoids and secondary embryogeneic tissues of winter oilseed rape to treatment with colchicine. New Phytol 1983; 95: 359-66.

[16] Luckett D. Colchicine mutagenesis is associated with substantial heritable variation in cotton. Euphytica 1989, 42: 177-82.

[17] Chen JL, Beversdorf WD. Production of spontaneous diploid lines from isolated microspores following cryopreservation in spring rapeseed (*Brassica napus* L.). Plant Breed 1992; 108: 324-7.

[18] Subrahmanyam NC, Kasha KJ. Chromosome doubling of barley haploids by nitrous oxide and colchicine treatment. Can J Genet Cytol 1975; 17: 573-83.

[19] Simmonds DH, Newcomb W, Zhao J, Gervais C. Induction of embryogenesis from plant microspores. US Patent 6200808, 2001.

[20] Saisingtong S, Schmid JE, Stamp P, Büter B. Colchicine-mediated chromosome doubling during anther culture of maize (*Zea mays* L.). Theor Appl Genet 1996; 92: 1017-23.

[21] Obert B, Barnabás B. Colchicine induced embryogenesis in maize. Plant Cell Tiss Org Cult 2004; 77: 283-5.

[22] Barnabas B, Obert B, Kovacs G. Colchicine, an efficient genome-doubling agent for maize (*Zea mays* L.) microspores cultured in anthero. Plant Cell Rep 1999; 18: 858-62.

[23] Redha A, Attia T, Büter B, *et al*. Improved production of doubled haploids by colchicine application to wheat (*Triticum aestivum* L.) anther culture. Plant Cell Rep 1998. 17: 974-9.

[24] Soriano M, Cistué L, Vallés MP , Castillo AM. Effects of colchicine on anther and microspore culture of bread wheat (*Triticum aestivum* L.). Plant Cell Tissue Organ Cult 2007; 91: 225-34.

[25] Zamani I, Kovács G, Gouli-Vavdinoudi E, *et al*. Regeneration of fertile doubled haploid plants from colchicine-supplemented media in wheat anther culture. Plant Breed 2000; 119 (6): 461-5.

[26] Barcelo P, Cabrera A, Hagel C, Lörz H. Production of doubled-haploid plants from *Tritordeum* anther culture. Theor Appl Genet 1994; 87: 741-5.

[27] Arzani A, Darvey NL. The effect of colchicine on triticale anther-derived plants: Microspore pre-treatment and haploid-plant treatment using a hydroponic recovery system. Euphytica 2001; 122: 235-41.

[28] Alemanno L, Guiderdoni E. Increased doubled haploid plant regeneration from rice (*Oryza sativa* L.) anthers cultured on colchicine-supplemented media. Plant Cell Rep 1994; 13: 432-6.

[29] Chen C, Xiao H, Zhang W, *et al*. Adapting rice anther culture to gene transformation and RNA interference. Sci China C Life Sci 2006; 49: 414 -28.

[30] Szakács É, Barnabás B. Development of diploid pollen in spikelet cultures of barley (*Hordeum vulgare* L.) and rye (*Secale cereale* L.). Acta Agr Hungarica 2004; 52: 1-8.

[31] Pua E-C, Douglas CJ Eds. Brassica. Series: Biotechnology in Agriculture and Forestry 2004; 54: 344.

[32] Depaepe R, Bleton D, Gnangbe F. Basis and extent of genetic variability among doubled haploid plants obtained by pollen culture in *Nicotiana sylvestris*.Theor Appl Genet 1981; 59: 177-84.

[33] Hassawi DS, Liang GH. Antimitotic agents: Effects on doubled haploid production in wheat. Crop Sci 1991; 31: 723-6.

[34] Caperta AD, Delgado M, Ressurreição F, *et al*. Colchicine-induced polyploidization depends on tubulin polymerization in c-metaphase cells. Protoplasma 2006; 227: 147-53.

[35] Zhao J, Simmonds DH. Application of trifluralin to embryogenic microspore cultures to generate doubled haploid plants in *Brassica napus*. Physiol Plant 1995; 95: 304-9.

[36] Hansen NJP, Andersen SB. *In vitro* chromosome doubling potential of colchicine, oryzalin, trifluralin, and APM in *Brassica napus* microspore culture. Euphytica 1996; 88:159-64.

[37] Sree Ramulu K, Verhoeven HA, Dijkhuis P. Mitotic blocking, microtnucleation, and chromosome doubling by oryzalin, amiprophos-methyl, and colchicine in potato. Protoplasma 1991; 160: 65-73.

[38] Pintos B, Manzanera JA, Bueno MA. Antimitotic agents increase the production of doubled-haploid embryos from cork anther culture. J Plant Physiol 2007; 164: 1595-604.

[39] Tosca A, Pandolfi R, Citterio S, *et al*. Determination by flow cytometry of the chromosome doubling capacity of colchicines and oryzalin in gynogenetic haploids of Gerbera. Plant Cell Rep 1995; 14: 455-8.

[40] Chalak L, Legave JM. Oryzalin combained with adventitious regeneration for an efficient chromosome doubling of trihaploid kiwifruit. Plant Cell Rep 1996; 16: 97-100.

[41] Vainola A. Polyploidization and early screening of *Rhododendron* hybrids. Euphytica 2000; 112: 239-44.

[42] Kermani MJ, Sarasan V, Roberts AV, *et al*. Oryzalin-induced chromosome doubling in *Rosa* and its effect on plant morphology and pollen viability. Theor Appl Genet 2003; 107: 1195-1200.

[43] Wan Y, Duncan DR, Rayburn AL, Petolino JF, Widholm JM. The use of antimicrotubule herbicides for the production of doubled haploid plants from anther-derived maize callus. Theor Appl Genet 1991; 81: 205-11.

[44] Hansen AL, Gertz A, Joersbo M, Andersen SB. Antimicrotubule herbicides for *in vitro* chromosome doubling in *Beta vulgaris* L. ovule culture. Euphytica 1998, 101: 231-7.

[45] Salon PR, Earle ED. Chromosome doubling and mode of reproduction of induced tetraploids of eastern gamagrass (*Tripsacum dactyloides* L.). Plant Cell Rep 1998; 17: 881-5.

[46] Eeckhaut T, Werbrouck S, Leus L, *et al*. Chemically induced polyploidization in *Spathiphyllum wallisii* Regel through somatic embryogenesis. Plant Cell Tissue Organ Cult 2004; 78: 241-6.

[47] Mitrofanova IV, Zilbervarg IR, Yemets AI, *et al*. The effect of dinitroaniline and phosphorothioamidate herbicides on polyploidisation *in vitro* of *Nepeta* plants. Cell Biol Int 2003; 27(3): 229-31.

[48] Bhattacharyya B, Panda D, Gupta S, Banerjee M. Anti-mitotic activity of colchicine and the structural basis for its interaction with tubulin. Med Res Rev 2008; 28 (1): 155-83.

[49] Frankhauser G, Humphrey RR. The rare occurrence of mitosis without spindle apparatus ("colchicine mitosis") producing endopolyploidy in embryos of the axolotl. Proc Natl Acad Sci USA 1952; 38: 1073-82.

[50] Brossi A, Yeh HJ, Chranowska M, *et al*. Colchicine and its analogues. Recent finding. Med Res Rev 1988; 8: 77-94.

[51] Ravelli RB, Gigant B, Curmi PA, *et al*. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. Nature 2004; 428: 198-202.

[52] Morejohn LC, Bureau TE, Tocchi LP, Fosket DE. Tubulins from different higher plant species are immunologically nonidentical and bind colchicine differentially. Proc Natl Acad Sci USA 1984; 81: 1440-4.

[53] Morejohn LC, Fosket DE. Taxol-induced rose microtubule polymerization *in vitro* and its inhibition by colchicine. J Cell Biol 1984; 99: 141-7.

[54] Morejohn LC, Bureau TE, Tocchi LP, Fosket DE. Resistance of *Rosa* microtubule polymerization to colchicine results from a low-affinity interaction of colchicine and tubulin. Planta 1987; 170: 230-41.

[55] Banerjee M, Roy D, Bhattacharyya B, Basu G. Differential colchicine-binding across eukaryotic families: the role of highly conservative Pro268β and Ala248β residues in animal tubulin. FEBS Lett 2007; 581: 5019-23.

[56] Hart JW, Sabnis DD. Colchicine and plant microtubules: A critical evaluation. In: Smith H Ed. Commentaries in Plant Science Pergamon Press, Oxford, NY 1976; 1095-1104.

[57] Bajer AS, Mole-Bajer J. Drugs with colchicine-like effects that specifically disassemble plant but not animal microtubules. In: Sorfer D Ed. Dynamic aspects of microtubule biology. Ann N Y Acad Sci 1986; 767-84.

[58] Antony RG, Hussey P. Dinitroaniline herbicide resistance and microtubule cytoskeleton. Trends Plant Sci 1999; 4: 112-6.

[59] Baird WV, Blume YaB, Wick S. Microtubular and cytoskeletal mutants. In: Nick P Ed. Plant microtubules: Potential for biotechnology, Springer, Berlin 2000; 159-91.

[60] Yemets AI, Blume YaB. Resistance to herbicides with antimicrotubular activity: From natural mutants to transgenic plants. Russ J Plant Physiol 1999; 46(6): 899-907.

[61] Yemets AI, Blume YaB. Mutant plant tubulin genes as selective marker genes for genetic engineering. Cytol Genet 2007; 41(3): 29-43.

[62] Ellis JR, Taylor R, Hussey P. Molecular modeling indicates that two chemically distinct classes of antimitotic herbicides bind to the same receptor site(s). Plant Physiol 1994; 105: 15-8.

[63] Nogales E, Wolf SG, Downing KH. Structure of the αß tubuin dimer by electron crystallography. Nature 1998; 391: 199-203.

[64] Blume YaB, Yemets AI, Nyporko AYu, Baird WV. Structural modelling of plant β-tubulin interaction with dinitroanilines and phosphoroamidates. Cell Biol Int 2003; 27: 171-4.

[65] Yamamoto E, Zeng L, Baird WV. α-Tubulin missense mutations correlate with antimicrotubule drug resistance in *Eleusine indica*. Plant Cell 1998; 10: 297-308.

[66] Nyporko AY, Yemets AI, Klimkina LA, Blume YB. Relationship between the sensitivity of *Eleusine indica* to trifluralin and amiprophosmethyl and characteristics of interaction of these compounds with tubulin. Russ J Plant Physiol 2002; 49(3): 459-66.

[67] Delye C, Menchari Y, Michel S, Darmency H. Molecular basis for sensitivity to tubulin-binding herbicides in green foxtail. Plant Physiol 2004; 136: 3920-32.

[68] Morissette NS, Mitra A, Sept D, Sibley LD. Dinitroanilines bind α-tubulin to disrupt microtubules. Mol Biol Cell 2004; 15: 1960-8.

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