Induction of Polyploidy

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Polyploidy has the utmost importance in horticulture for the development of new ornamental varieties with desirable morphological traits referring to plant size and vigor, leaf thickness, larger flowers with thicker petals, intense color of leaves and flowers, long lasting flowers, compactness, dwarfness and restored fertility. Polyploidy may occur naturally due to the formation of unreduced gametes or can be artificially induced by doubling the number of chromosomes in somatic cells.

Keywords: polyploidy ; induction

1. Colchicine Induced Polyploidy/Mutation

The importance of polyploidy in plant breeding appeared to be of great interest when the mitotic inhibitor (colchicine) was first discovered in 1930s [1]. Colchicine has been used for treatment of gout disease since 1810. Colchicine is extracted from bulbs (0.1–0.5%) and seeds (0.2%–0.8%) of autumn crocus or meadow saffron (*Colchicum autumnale*) and possesses an extremely poisonous alkaloid character. It is readily soluble in cold water, chloroform or alcohol but it has low solubility in hot water [2]. Colchicine is an important mutagen that works by preventing the microtubules formation and doubles the number of chromosomes. It is commonly used to develop polyploid plants and functions as a mitotic poison by producing many mutagenic effects on plants [3]. As microtubules function in chromosome segregation, colchicine induces polyploidy by preventing the segregation of chromosomes during meiosis that results into half of the gametes (sex cells) containing double the chromosome number than usual. Whereas, half of the gametes do not contain any chromosomes and produce embryos with doubled chromosome numbers [4]. Colchicine not only helps in the doubling of chromosomes, but it also induces mutation in plants. Plants that have been mutated through colchicine are known as colchi-mutants [5]. According to the literature, a wide range of colchicine concentrations have been used for the induction of polyploidy in different plant species such as the lowest concentration of 0.00001% in campion (*Lychnic senno*) to the extremely high concentration of 1.5% in Maule's quince (*Chaenomeles japonica*), and have successfully induced polyploidy [6]. However, colchicine usually has low affinity to tubulins in plant cells, thus higher concentrations are used for effective results [7].

Colchicine is commonly applied in a form of an aqueous solution; however, it is unstable in water. Therefore, it is advisable to make a fresh aqueous solution before treatment [2]. Colchicine concentrations for seed treatment usually range from 0.1%–0.8%, but high doses cause malformation and reduce the production of tetraploids plants. Thus, it is advisable to use colchicine at concentrations that are as low as possible [8]. As colchicine is highly toxic to plants, therefore, low doses with prolonged exposure period are considered reliable to reduce its toxic effect and increase the polyploids production rate [9].

2. Methods of Application

Among different application methods, change in ploidy level of plants through chemicals has been proven to be more effective. Many chemicals work as inhibitors to block the progress of cell division cycle (mitosis) in plant cells, like colchicine that is used to block metaphase of cell cycle [9]. Methods of anti-mitotic application depend upon the plant type. One of the simplest, easiest and effective methods is to use a large number of seedlings that have small and actively growing meristematic tissues. Seedlings can be dipped/soaked or apical meristems can be submerged in anti-mitotic agent solution of different concentrations at different exposure times or frequencies. Shoots of older plants can also be used for polyploidy induction, but this method is not successful as it may yield large number of cytochimeras. However, treatment of sub-axillary and small axillary meristematic tissues is considered to be effective, whilst, on the other hand, growing buds can also be treated with chromosome doubling agents with the help of cotton, lanolin and agar or by dipping branch tips in solutions of chemicals. Wetting agents and surfactants are also sometimes used to enhance the penetration of a chemical [10]. The most successful method for tetraploidy induction is through seed treatment. Colchicine treatment of

pre-germinated seeds having emerging roots is effective as compared to dry or germinated seeds because large numbers of tetraploid plants are produced through it [8][11].

3. Confirmation

For crop breeding, it is important to identify the correct ploidy level at various growth stages through quick, simple and easy methods [12]. Identification or determination of correct ploidy level is very important in breeding of polyploid and asexually propagated crops as they might have several polyploid series or chimeras in their tissues [13]. For confirmation of polyploidy, different direct and indirect methods have been used. Indirect methods are easy, less time consuming and use simple instruments for screening [7]. Different morphological and physiological traits, particularly pollen diameter, number of chloroplast, stomatal size and stomatal density can be studied through these indirect methods [14]. In various species, there is a direct relationship between plant ploidy level and different physiological characteristics like stomatal length, width, density, pollen grain diameter and number of chloroplasts in guard cells. However, some disadvantages like environmental sensitivity, need for calibration or identification of mixoploids and chimeras (rather than polyploids) limit the use of these traits to identify correct ploidy level. In direct methods, techniques like chromosome counting have been examined as an effective and reliable method, but it is a laborious process, particularly for plant species with highly dense cytoplasm consisting of a large number of chromosomes [15]. Moreover, a specific protocol is required for each plant species. Thus, flow cytometry is considered to be a more reliable, rapid and simple method to analyze a large number of samples in a very short time period [16]. Flow cytometry is generally used to quantify DNA in nuclei. Quantity of DNA can be determined directly from the leaf tissues with this method. This method is also suitable for those plant species in which root tip studies are not effective due to small size and ineffective spreading of chromosome [17]. Furthermore, this method can also evaluate large number of nuclei (100–10,000 cells per second) from different cell layers and types of tissues [7].

4. Chimeras

It is important to identify the cytochimeras (plants whose ploidy level differ in different types of tissues) when polyploids are used for breeding purpose. Meristematic cells are usually divided into three histogenic layers LI, LII and LIII. Antimitotic agents might increase ploidy level in all three layers or in one or two of them. Thus, to study the reproductive behavior, it is necessary to identify the ploidy level of cortical layer or LII layer, that can be measured through pollen grain diameter and by meiotic studies of reproductive tissues (anthers). For L1 and LIII layer, guard cell studies and chromosome counting can accurately determine the ploidy level, respectively [10]. Occurrence of chimeras instead of stable tetraploid plants shows that all cells present in histogenic layers (LI, LII and LIII) of meristems tissues are not treated at the same time. The successful conversion of diploid plants into stable polyploids depends on the balance of colchicine concentration and exposure time that double the genomic content of each cell of treated tissues and ensuring that the survival and growth and development of induced plants is not severely affected [18].

5. Improvement of Ornamentals by Polyploidization

Polyploid plants are noticeably different or sometimes superior from diploid ones in terms of their phenotypic expression, which includes morphological, physiological, biochemical and cellular changes. These polyploid plants can also be taken as a new variation or a genotype that can be used in future breeding programs for crop improvement. Due to the immense importance of polyploidy, it has been artificially induced in many economically important crops, however the majority of success has been reported in ornamental industry. Chromosome doubling through colchicine by using different application methods has been obtained in many ornamental crops such as lily, salvia, phlox, gladiolus, petunia and marigold.

Polyploidy causes a wide range of effects on plants, but these effects depend upon species and range of different ploidy level within a same species, degree of heterozygosity and rely on various mechanisms that are related to gene dose effects, gene silencing, regulation of specific traits and gene interaction [10].

6. Morphology

A large number of morphological effects have been obtained from induced polyploidization. However, one of the immediate effects of polyploidy is an increase in cell size due to the increase in nuclear content which causes a reduction in a cell division during their growth and development. This “gigas effect” is mostly observed in different plant organs of commercial interests such as leaves, seeds and flowers [19]. Doubling through colchicine caused an increase in number of leaves, number of branches, plant height and stem length in salvia (*Salvia coccinea* cv Coral Nymph) [20], jasmine tobacco (*Nicotiana alata*) [21], selfheal (*Prunella vulgaris*) [22], lily (*Lilium*) [23], chaste tree (*Vitex agnus castus*) [5], orchid (*Dendrobium nobile*) [24], ornamental ginger [25], crape myrtle (*Lagerstroemia indica*) [26], calendula (*Calendula officinalis*)

[3], matted sea-lavender (*Limonium bellidifolium*) [27], white orchid tree (*Bauhinia acuminate*) [28] and London plane (*Planatus acerifolius*) [29]. Induced polyploidy also increased the leaf color in balsam (*Impatiens balsamina*), [30], self-heal (*Prunella vulgaris*) [22], wishbone flower (*Torenia fournieri*) [31], marigold (*Tagates erecta*) [32], chaste tree (*Vitex agnus castus*) [5] and chrysanthemum (*Dendranthema grandiflora*) [33], along with increasing their leaf area as well. Induced polyploidy produced ovate shaped leaves having obtuse base in gymnostachyum (*Gymnostachyum zeylanicum*) [34] and whorled arrangement of leaves in balloon flower (*Platycodon grandiflorus*) [35]. Highest variation in color and shape of leaves has been observed in tetraploid plants of pelargonium (*Pelargonium graveolens*), [36].

Mitotic chromosome doubling through colchicine treatment results in production of large sized inflorescence with increased floral parts in salvia (*Salvia coccinea* cv. Coral Nymph), however flowering has been delayed up to 10–30 days [20]. In chaste tree (*Vitex agnus castus*), polyploid plants had larger flowers with longest posterior petals and unique colors. Tetraploid plants of feverfew (*Tanacetum parthenium*) had increased flower weight and diameter but produced only up to 50% of flowering as compared to its diploid plants [37]. Also, in ornamental wild ginger species (*Larsenianthus* careyanus), chromosome doubling caused an increase in leaf number, lamina length, flower size along with length of inflorescence and spike length [25]. Dense flowering with erect, compacted and short inflorescence was produced in gymnostachyum (*Gymnostachyum zeylanicum*), from 0.04% colchicine treated plants [34]. Tetraploidization in African violets (*Saintpaulia ionantha*) produced 5% white flowers with purple margin. After 7 days of flowering, flower color pattern changed from white petals with purple border to whole purple petals and this pattern has been maintained for six successive generations [38]. Tetraploid plants of pelargonium (*Pelargonium graveolens*) cv. Black Velvet Scarlet F1 produced flowers with rough edges, where flowers of cv. Gizela produced burnt margins [36]. Similarly, colchicine treatment produced more flowering with large stigma in jasmine tobacco (*Nicotiana alata*) [21], larger flower height with increased lip width in wishbone flower (*Torenia fournieri)* [31] increased the number of petals in garden balsam (*Impatiens balsamina*) [30] and increased flower diameter up to 1.2–1.3 folds in tetraploid plants of matted sea-lavender (*Limonium bellidifolium*) as compared to its diploid plants [27]. Colchi-tetraploids of chrysanthemum (*Chrysanthemum carinatum*) had larger flowers with thicker petals that helped to improve their vase life [39]. Also, in gladiolus, colchicine induced putative polyploids had larger flower size with maximum vase life. Moreover, novel variation in flower morphology like serrated margins with ruffled edges along with pointed outgrowth has been observed in flower petals of gladiolus [40]. Higher colchicine concentration in African marigold (*Tagetes erecta*) initiated early flowering (59 days) in treated plants as compared to control plants (80 days). Also, a higher number of flowers with increased diameter and weight of flowers have been produced in polyploid plants [41].

Apart from improving ornamental traits like flower or leaves in ornamental plants, polyploidy also increases the plants yield in the form of both sexual and asexual reproductive structures. After colchicine treatment, a significant increase in seed size and weight was observed in crape myrtle (*Lagerstroemia indica*) [26] and Madgascar periwinkle (*Catharanthus roseus*) [42]. Whereas, colchicine also increased seed number, seed weight and fruit setting percentage in balsam (*Impatiens balsamina*) [43]. Similarly, in vegetatively propagated crops like *Lilium,* induced chromosome doubling produced wider bulb scale [23], however, in orchid (*Dendrobium nobile*), polyploidization decreased pseudobulb diameter up to 64.9% [44].

7. Physiology

In addition to the obvious alterations in the morphology of ornamental plants, polyploidy can also show a significant impact on the number of plant physiological processes such as water relations. Larger sized stomata with lower frequency per unit area have been observed in polyploid plants of feverfew (*Tanacetum parthenium*), phlox (*Phlox drummondi)* [37][45], salvia (*Salvia hains*) [46], petunia (*Petunia hybrida*) [47], African marigold (*Tagates erecta*) [32], chrysanthemum (*Dendranthema indicum* var. Aromaticum) [48] and celosia (*Celosia argentea*) [49]. It is illustrated in the results that these changes reduced the transpiration rate (overall gaseous exchange rate) in polyploid plants. Additionally, increases in vacuole size and thicker leaves, also allows for retaining higher water content, which can be utilized during drought period conditions. Therefore, these polyploid plants could be grown in water limited areas and can also be bred with other species in order to develop drought tolerant genotypes. This approach could be helpful for domestication of some species into warmer and hot climate.

8. Resorting Fertility in Wide Hybrids

Hybrids between different genera or species are usually sterile due to the failure of chromosome pairing under meiosis. By chromosome doubling of wide hybrid, each chromosome gets its exact copy and chromosomal homology, thus its fertility is restored. This technique has been used to restore fertility in lavandin (*Lavandula* × *intermedia*), which is grown for both oil and ornamental purpose. Along with restoration of fertility, heavier seeds have been produced in offspring with

respect to their parent seed weight. Similarly, in spurflowers (South African *Plectranthus*), polyploidy has been induced in F_1 sterile diploid hybrids (2*n* = 28) in order to obtain triploid crosses (4*n* × 2*n*) [50].

9. Overcoming Hybridization Barrier

Desirable crosses are sometimes difficult to obtain due to different ploidy level of parents. This type of interploid barrier usually occurs due to imbalance or abnormal endosperm formation and the seeds can be developed normally with a ratio of 2:1 (maternal and paternal) in the genomic makeup of the endosperms for two diploid parents. The seed that does not meet the normal development are generally aborted or may be underdeveloped. In order to break these barriers in hybridization, ploidy levels of one or both parents are manipulated to match before the hybridization [51]. Chrysanthemum, *Dendranthema indicum* var. Aromaticum is diploid (*2n=2x*=18) and *Dendranthema* × *grandiflora* is polyploid (*2n=6x=54*). Thus, to create a new scented chrysanthemum, chromosome doubling has been induced through colchicine in *D. indicum* var. Aromaticum, in order to increase the chance of cross between both the parents [48].

10. Pest Resistance and Stress Tolerance

The effect of polyploidy on various biotic and abiotic stress tolerance and adaptability to a particular environment has been studied in many crops. Polyploid plants could have a better nutrient uptake, cold tolerance and improved resistance to insect/pest and pathogens. There are a number of ways to induce polyploidy in plants that can increase their adaptability and resistance against biotic and abiotic stresses. It can also be achieved by increasing the nuclear content that ultimately increases the gene expression which results in increased production of secondary metabolites. These metabolites including chemical defenses not only enhance the plant resistance and tolerance mechanisms, but they are also valuable from a pharmaceutical point of view. They can also be used to create allopolyploids among parents having diverse endogenous secondary metabolites as compared to their parent plants. Induced triploids and tetraploids plants of swamp rosemallow (*Hibiscus moscheutos*) showed resistance to aerial *Phytophthora* disease as compared to their diploid counterparts which were severely infected [52]. Also, in garden impatiens (*Impatiens walleriana*), synthetically produced tetraploids exhibited improved resistance to downy mildew [53].

There are several plants that can be used for both ornamental as well as medicinal purposes such as the celosia flower (*Celosia argentea*), which showed an increase in biomass and pharmaceutical compounds (alkaloid, phenols, anthocyanin) in tetraploid plants [49]. Similarly, in opium poppy (*Papaver somniferum*), a 25–50% increase in alkaloid contents was observed in colchicine treated plants [54]. Whereas, increased parthenolide content was observed in polyploid plants of feverfew (*Tanacetum parthenium*) [37].

References

1. Marzougui, N.; Boubaya, A.; Thabti, I.; Elfalleh, W.; Guasmi, F.; Ferchichi, A. Polyploidy induction of Tunisian Trigonella foenumgreaum L. populations. Afr. J. Biotechnol. 2011, 10, 8570–8577.
2. Kumar, M.K.; Rani, M.U. Colchiploidy in fruit breeding. A review. Hortic 2013, 2, 325–326.
3. El-Nashar, Y.I.; Ammar, M.H. Mutagenic influences of colchicine on phenological and molecular diversity of Calendula officinalis L. Genet. Mol. Biol. 2015, 15, 1–15.
4. Ade, R.; Rai, M.K. Review: Colchicine, current advances and future prospects. Nusantara Biosci. 2010, 2, 90–96.
5. Ari, E.; Djapo, H.; Mutlu, N.; Gurbuz, E.; Karaguzel, O. Creation of variation through gamma irradiation and polyploidization in Vitex agnus-castus L. Sci. Hortic. 2015, 195, 74–81.
6. Castro, M.; Castro, S.; Loureiro, J. Production of synthetic tetraploids as a tool for polyploid research. Web Ecol. 2018, 18, 129–141.
7. Eng, W.H.; Ho, W.S. Polyploidization using colchicine in horticultural plants: A review. Sci. Hortic. 2019, 246, 604–617.
8. Pirkoohi, M.H.; Keyvanloo, M.; Hassanpur, M. Colchicine induced polyploidy in mint by seed treatment. Int. J. Agric. Crop Sci. 2011, 3, 102–104.
9. Sajjad, Y.; Jaskani, M.J.; Mehmood, A.; Ahmad, I.; Abbas, H. Effect of colchicine on in vitro polyploidy induction in African marigold (Tagetes erecta). Pak. J. Bot. 2013, 45, 1255–1258.
10. Ranney, T.G. Polyploidy: From evolution to new plant development. Proc. Int. Plant Propagators Soc. 2006, 56, 137–142.

11. Lehrer, J.M.; Brand, M.H.; Lubell, J.D. Induction of tetraploidy in meristematically active seeds of Japanese barberry (Berberis thunbergii var. atropurpurea) through exposure to colchicine and oryzalin. Sci. Hortic. 2008, 119, 67–71.

12. Samala, S.; Te-chatoi, S. Ploidy induction through secondary somatic embryo (SSE) of oil palm by colchicine treatment. J. Agric. Technol. 2012, 8, 337–352.

13. Rêgo, M.D.; Rêgo, E.R.; Bruckner, C.H.; Finger, F.L.; Otoni, W.C. In vitro induction of autotetraploids from diploid yellow passion fruit mediated by colchicine and oryzalin. Plant Cell Tissue Organ Cult. 2011, 107, 451–459.

14. Moghbel, N.; Borujeni, M.K.; Bernard, F. Colchicine effect on the DNA content and stomata size of Glycyrrhiza glabra var. Glandulifera and Carthamus tinctorius L. cultured in vitro. J. Genet. Eng. Biotechnol. 2015, 13, 1–6.

15. Sakhanokho, H.F.; Rajasekaran, K.; Kelley, R.Y.; Islam-Faridi, N. Induced polyploidy in diploid ornamental ginger (Hedychium muluense RM Smith) using colchicine and oryzalin. HortScience 2009, 44, 1809–1814.

16. Sattler, M.C.; Carvalho, C.R.; Clarindo, W.R. The polyploidy and its key role in plant breeding. Planta 2016, 243, 281–296.

17. Xu, L.; Najeeb, U.; Naeem, M.S.; Daud, M.K.; Cao, J.S.; Gong, H.J.; Sheen, W.Q.; Zhou, W.J. Induction of tetraploidy in Juncus effusus by colchicine. Biol. Plant. 2010, 54, 659–663.

18. Harbard, J.L.; Griffin, A.R.; Foster, S.; Brooker, C.; Kha, L.D.; Koutoulis, A. Production of colchicine-induced autotetraploids as a basis for sterility breeding in Acacia mangium Willd. Forestry 2012, 85, 427–436.

19. Botelho, F.B.S.; Rodrigues, C.S.; Bruzi, A.T. Ornamental plant breeding. Ornam Hortic. 2015, 21, 9–16.

20. Kobayashi, N.; Yamashita, S.; Ohta, K.; Hosoki, T. Morphological characteristics and their inheritance in colchicine-induced salvia polyploids. J. Jpn. Soc. Hortic. Sci. 2008, 77, 186–191.

21. El-Morsy, S.I.; Dorra, M.D.M.; Abd El-Hady, E.A.A.; Hiaba, A.A.A.; Mohamed, A.Y. Comparative studies on diploid and tetraploid levels of Nicotiana alata. Acad. J. Plant Sci. 2009, 2, 182–188.

22. Kwon, S.J.; Roy, S.K.; Cho, K.Y.; Moon, Y.J.; Woo, S.H.; Kim, H.H. Tetraploid induction approach induced by colchicine of Prunella vulgaris L. F. albiflora Nakai. Int. J. Sci. Res. Pub. 2014, 4, 1–7.

23. Balode, A. Applying colchicine and oryzaline in Lilium L polyploidization. L. J. Agron. 2008, 11, 22–28.

24. Vichiato, M.R.M.; Vichiato, M.; Pasqual, M.; Castro, D.M.D.; Dutra, L.F. Tetraploidy induction and identification in Dendrobium nobile Lindl (Orchidaceae). Rev. Cienc. Agron. 2007, 38, 385–390.

25. Prabhukumar, K.M.; Thomas, V.P.; Sabu, M.; Prasanth, M.V.; Mohanan, K.V. Induced mutation in ornamental gingers (Zingiberaceae) using chemical mutagens viz. colchicine, acridine and ethyl methane sulphonate. J. Hortic. For. Biotechnol. 2015, 19, 18–27.

26. Ye, Y.M.; Tong, J.; Shi, X.P.; Yuan, W.; Li, G.R. Morphological and cytological studies of diploid and colchicine-induced tetraploid lines of crape myrtle (Lagerstroemia indica L.). Sci. Hortic. 2010, 124, 95–101.

27. Mori, S.; Yamane, T.; Yahata, M.; Shinoda, K.; Murata, N. Chromosome doubling in Limonium bellidifolium (Gouan) Dumort. by colchicine treatment of seeds. Hortic. J. Preview 2016, 85, 366–371.

28. Basumatari, M.; Das, B.N. Karyomorphological studies in two species of Bauhinia Linn. and induction of polyploidy in Bauhinia acuminata Linn. Int. J. Life Sci. Sci. Res. 2017, 3, 1223–1229.

29. Liu, G.; Li, Z.; Bao, M. Colchicine-induced chromosome doubling in Platanus acerifolia and its effect on plant morphology. Euphytica 2007, 157, 145–154.

30. Du, X.; Sun, Y.; Yuan, S.; Li, Q.; Gong, Z. Identification of colchicines induced polyploid plants in two species of Impatiens balsamina. Acta Agric. Bor. Sin. 2011, 20, 56–59.

31. Boonbongkarn, S.; Taychasinpitak, T.; Wongchaochant, S.; Kikuchi, S. Effect of colchicine tablets on morphology of Torenia fournieri. Int. Trans. J. Eng. Manag. Sci. Technol. 2013, 4, 299–309.

32. Sadhukhan, R.; Ganguly, A.; Singh, P.K.; Sarkar, H.K. Study of Induced polyploidy in African marigold (Tagetes ecrecta L.). Environ. Ecol. 2014, 32, 1219–1222.

33. Lertsutthichawan, A.; Ruamrungsri, S.; Duangkongsan, W.; Saetiew, K. Induced mutation of chrysanthemum by colchicine. Int. J. Agric. Technol. 2017, 13, 2325–2332.

34. Khaing, T.T.; Perera, A.L.T.; Sumanasinghe, V.A.; Wijesundara, D.S.A. Improvement of Gymnostachyum species by induced mutation. Trop. Agric. Res. 2007, 19, 265–272.

35. Wu, Y.; Yang, F.; Zhao, X.; Yang, W. Identification of tetraploid mutants of Platycodon grandiflorus by colchicine induction. Caryologia 2011, 64, 343–349.

36. Jadrná, P.; Plavcová, O.; Kobza, F. Morphological changes in colchicine treated Pelargonium×Hortorum LH bailey greenhouse plants. HortScience 2010, 37, 27–33.
37. Majdi, M.; Karimzadeh, G.; Malboobi, M.A.; Omidbaigi, R.; Mirzaghaderi, G. Induction of tetraploidy to feverfew (Tanacetum parthenium Schulz-Bip.): Morphological, physiological, cytological, and phytochemical changes. HortScience 2010, 45, 16–21.
38. Seneviratne, K.A.C.N.; Wijesundara, D.S.A. First African violets (Saintpaulia ionantha, H. Wendl.) with a changing colour pattern induced by mutation. Am. J. Plant Physiol. 2007, 2, 233–236.
39. Kushwah, K.S.; Verma, R.C.; Patel, S.; Jain, N.K. Colchicine induced polyploidy in Chrysanthemum carinatum L. J. Phylogenet. Evol. Biol. 2018, 6, 2–4.
40. Manzoor, A.; Ahmad, T.; Bashir, M.A.; Baig, M.M.Q.; Quresh, A.A.; Shah, M.K.N.; Hafiz, I.A. Induction and identification of colchicine induced polyploidy in Gladiolus grandiflorus 'White Prosperity'. Folia Hortic. 2018, 30, 307–319.
41. Rathod, A.D.; Patil, S.R.; Taksande, P.N.; Karad, G.W.; Kalamkar, V.B.; Jayade, V.S. Effect of colchicine on morphological and biometrical traits in African marigold. J. Soils Crops 2018, 28, 72–80.
42. Hosseini, H.; Chehrazi, M.; Sorestani, M.M.; Ahmadi, D. Polyploidy and comparison of diploid and autotetraploid seedling of Madagascar periwinkle (Catharanthus roseus cv. Alba). Int. Res. J. Appl. Basic Sci. 2013, 4, 402–406.
43. Anurita, D.; Girjesh, K. Morphogenetic analysis of colchitetraploids in Impatiens balsamina L. Caryologia 2007, 60, 199–202.
44. Vichiato, M.R.D.M.; Vichiato, M.; Pasqual, M.; Rodrigues, F.A.; Castro, D.M.D. Morphological effects of induced polyploidy in Dendrobium nobile Lindl. (Orchidaceae). Crop Breed. Appl. Biot. 2014, 14, 154–159.
45. Tiwari, A.K.; Mishra, S.K. Effect of colchicine on mitotic polyploidization and morphological characteristics of Phlox drummondi. Afr. J. Biotechnol. 2012, 11, 9336–9342.
46. Grouh, M.S.H.; Meftahizade, H.; Lotfi, N.; Rahimi, V.; Baniasadi, B. Doubling the chromosome number of Salvia hains using colchicine: Evaluation of morphological traits of recovered plants. J. Med. Plants Res. 2011, 5, 4892–4898.
47. Ning, G.G.; Shi, X.P.; Hu, H.R.; Yan, Y.; Bao, M.Z. Development of a range of polyploid lines in Petunia hybrida and the relationship of ploidy with the single-double-flower trait. HortScience 2009, 44, 250–255.
48. He, M.; Gao, W.; Gao, Y.; Liu, Y.; Yang, X.; Jiao, H.; Zhou, Y. Polyploidy induced by colchicine in Dendranthema indicum var. Aromaticum, a scented chrysanthemum. Eur. J. Hortic. Sci. 2016, 81, 219–226.
49. Mostafa, G.G.; Alhamd, M.F.A. Detection and evaluation the tetraploid plants of Celosia argentea induced by colchicine. Int. J. Plant Breed. Genet. 2016, 10, 110–115.
50. Brits, G.J.; Ling, L. Polyploid breeding of wild South African Plectranthus (spurflowers) as new flowering pot plants. Acta Hortic. 2008, 774, 437–442.
51. Bharadwaj, D.N. Plant Biology and Biotechnology: Polyploidy in Crop Improvement and Evolution; Springer: New Dehli, India, 2015; pp. 619–638.
52. Li, Z.; Ruter, J.M. Development and Evaluation of diploid and polyploid Hibiscus moscheutos. HortScience 2017, 52, 676–681.
53. Wang, W.; He, Y.; Cao, Z.; Deng, Z. Induction of tetraploids in impatiens (Impatiens walleriana) and characterization of their changes in morphology and resistance to downy mildew. HortScience 2018, 53, 925–931.
54. Mishra, B.K.; Pathak, S.; Sharma, A.; Trivedi, P.K.; Shukla, S. Modulated gene expression in newly synthesized auto-tetraploid of Papaver somniferum L. S. Afr. J. Bot. 2010, 76, 447–452.