INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 6, p. 29-47, 2017

REVIEW PAPER OPEN ACCESS

Review of research on *Dendrobium* sonia-28, a hybrid from Orchidacea family and mutation as somaclonal variation

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Key words: *Dendrobium* sonia-28, Somaclonal variation, Gamma radiation

http://dx.doi.org/10.12692/ijb/10.6.29-47 Article published on June 16, 2017

Abstract

Dendrobium sonia-28 is an important orchid hybrid in the Malaysian flower industry for its flowering recurrence and dense inflorescences which currently facing serious production problems due fungal diseases, especially caused by *Fusarium proliferatum*. To overcome this impediment, one of the strategies being pursued is by the production of new somaclonal variants via induced mutation.

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Introduction

Orchids: Geography, morphology and importance

The flowering plant family of Orchidaceae is large in size as well as being economically significant to the international floriculture industry, primarily as cut flowers and potted plants (Arditti, 1992; Khosravi *et al*., 2009; Poobathy *et al*., 2013a; Dehgahi *et al*., 2014; Dehgahi *et al*., 2015a). Orchidacea can be considered as one of the best recorded of all angiosperm families (Chase *et al*., 2015). The orchid family is one of the largest in the flowering plant kingdom, and there were around 880 genera with recent estimation ranging from 20,000 to 35,000 species in five subfamilies (Dressler, 1981; Cribb *et al*., 2003), with extra 800 species were being recognized and added to the orchid lists yearly (Nicoletti, 2003; Bektas *et al*., 2013) and new orchid genera were being portrayed at a rate of around 13 per each every year (the average over 10 years prior to 2004) (Schuiteman, 2004; Chase *et al*., 2015).

Orchids can be found in almost every region of the world, apart from marine environments and perpetually icy regions (Sharma *et al*., 2011). About 90% of populations of the world's orchids are found in the tropical climatic regions with Asia alone between 10,000 to 15,000 species. Mostly orchids were thermophilic, but some species can be found at the lowland, montane or submontane levels (Jezek, 2003).

In tropical Asia, a total of 6,800 orchid species were discovered in which over 1,000 wild species are located in Malaysia alone (Yang and Chua, 1990; Cribb *et al*., 2003; Antony *et al*., 2010). Over 100,000 registered commercial orchid hybrids were grown as cut flowers and potted plants (Martin and Madassery, 2006; Vendrame *et al*., 2007).

Besides its aesthetic value, orchids were also known as widely favoured food, beverages, spices, flavouring, medicine, drugs, arts and religions (Arditti, 1992; Arditti and Pridgeon, 2013). Although orchids are expensive, but highly in demand in the national and international markets due to their diversity in terms of size, shape, flower colour and longevity (Saiprasad *et al*., 2004).

Both hybrids and wild orchids have the following features: bilaterally symmetrical flowers, sticky masses of pollen grains called pollinia, minute seeds containing undeveloped embryos with no nutritive materials and the ability of seeds to only germinate with the presence of a symbiotic fungus under natural conditions (Jezek, 2003; Seaton *et al*., 2010). At the present, numerous descriptions of new genera incorporate molecular analysis to exhibit their necessity, whereas in former decades, morphology has been generally accepted basis for the description of new taxa (Chase *et al*., 2015). Most of the orchids that are threatened and endangered are listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Nikishina *et al*., 2007; Swarts and Dixon, 2009).

Orchid's market

The Asia-Pacific region has the prime share of all the world area under floriculture production (FAO, 2010). Cut flowers and many other floricultural products are key export products for many countries.

These include Malaysia, where the horticultural sector has recorded phenomenal growth over the past years. In Malaysia, the floriculture industry has seen a significant increase in land under cultivation, for example, the area of land devoted to floral production from 3370 ha in 2005, has reached 7,000 ha in 2008 (Hamir *et al*., 2008). The export of orchids have increased from RM 12.8 million in 2003 to RM 14.3 million in 2005 (Fadelah, 2007).

Orchids are mostly traded as potted plants or cut flower in the flower industry globally (Japan Flower Trade Association, 2009; Supnithi *et al*., 2011). Its commercial importance, both as cut flowers and pot plants, increases globally year after year (Manners *et al*., 2013). Orchids share 8% of the global floriculture trade (Martin and Madassery, 2006; Vendrame *et al*., 2007). Export and import trading of orchids in the world is estimated more than US $150 million dollars which 80% and 20% of this are composed of cut and potted orchids respectively.

Dendrobium orchid genus

The genus *Dendrobium* belongs to one of the three largest families of Orchidaceae (Leitch *et al.*, 2010). *Dendrobium* species are widely employed in horticultural, agricultural and medicinal practices (Chattopadhyay *et al.*, 2012). *Dendrobium* sonia-28 is a commercially valuable *Dendrobium* orchid popular as a cut flower and ornamental plant because of its frequent flowering and large number of flowers for each inflorescence (Martin and Madassery, 2006; Ching *et al.*, 2012).

Since the 18th century, more than 8000 novel *Dendrobium* hybrids and cultivars have been produced in horticulture through interspecific hybridization (as been reviewed by Pongsrila *et al.*, 2014). This genus consists of more than 1100 species distributed throughout the world, ranging from Southeast Asia to New Guinea and Australia (Puchooa, 2004). Some characters of *Dendrobium* such as its floriferous flower sprays, long flowering life, year round availability and the genus's wide spectrum of shapes, colours, and sizes are reasons of *Dendrobium* expanding popularity (Kuehnle, 2007; Fadelah, 2007; Khosravi *et al.*, 2009).

Dendrobium is also mostly used as in medicinal products and cosmetic (Chang *et al.*, 2010). *Dendrobium* orchid also known *Sekkoku* in Japaneseor *Shih-hu* in Chinese was used in Chinese traditional medicine as tonic to improve digestion, eliminating heat and nourishing yin and promoting body-fluid production (Shiau *et al.*, 2005; Yin and Hong, 2009). The cane of *Dendrobium* huoshanense is also used to treat ophthalmic disorder, salivary, and stomach (Hsieh *et al.*, 2008; Yin and Hong, 2009). Dried drug of *Shih-hu* reach up to US$ 4000 Kg^{-1} (Shiau *et al.*, 2005).

Most of the *Dendrobium* hybrids produce flowers that are white, golden-yellow or lavender in colour, with some having combinations of these colours (Puchooa, 2004; Kuehnle, 2006). Rare specimens may consist of bluish, ivory, brilliant orange or scarlet flowers, with exotic markings. Most of the evergreen species of *Dendrobium* do not produce fragrance while some deciduous species may produce fresh citrus-like scents, or smell of raspberries (Puchooa, 2004). Annually, *Dendrobium* usually blooms several times as well as the flower sprays make interesting cut flowers for arrangements (Puchooa, 2004; Fadelah, 2004).

Since Japan is the biggest world's importer of cut orchids, therefore Asia dominates the world trade in orchids industry. Japan imports cut flower mostly from its surrounding ASEAN countries mainly from Malaysia, Thailand, and Singapore which 90% of its imported flowers were *Dendrobium* (Japan Flower Trade Association, 2009). In the cut flower industry, *Dendrobium* hasone of the top positions (Martin and Madassery, 2006; Fadelah, 2007). The genus of *Dendrobium* is accounted to be the main orchid cut-flower export for Malaysia and also for other Southeast Asian countries including Philippines and Thailand (Fadelah, 2007). *Dendrobium* is contributed 11.7% from Malaysia´s orchid exports almost for the past 10 years (Khosravi *et al.*, 2008; Antony *et al.*, 2010). *Dendrobium* accounts for around 80% of the total micropropagated tropical orchids usually by protocorms (Saiprasad *et al.*, 2004; da Silva, 2013).
Small seedlings and heterozygous seedlings progenies which does not result to true-to-type plants of hybrid cultivars are accounted as problems in germination of *Dendrobium* (Martin and Madassery, 2006; Poobathy *et al.*, 2013b). Small seed size, presence of reduced endosperm and the need of a symbiotic relationship between orchids and mycorrhizal fungi are other problems in *Dendrobium* germination (Saiprasad and Polisetty, 2003; Swarts and Dixon, 2009).

Dendrobium sonia-28

Dendrobium sonia-28and its other hybrid siblings are mostly popular because of their floriferous inflorescense, bright colour flowers, durability with long shelf life, free flowering characteristics and fast gowing cycle (Fadelah, 2007). *Dendrobium* sonia-28 orchid hybrid can be created by crossing two hybrids that are *Dendrobium* Caesar and *Dendrobium* Tomie

Drake it is cherished for its pink-coloured blossoms as well as the quality of the cut florae (Van Rooyen Orchids Catalogue, 2007). Good air movement and strong light are essential growth conditions for the evergreen and warm-growing hybrid (Van Rooyen Orchids Catalogue, 2007).

A number of orchid species are becoming extinct as naturally growing plants are being harvested at random and habitats of these plants are greatly disturbed. Since traditional methods used for cultivation of orchids have proved to be quite difficult, it is extremely important to develop efficient and authentic techniques for conservation of orchids in the form of germplasm conservation (Hirano *et al.*, 2005, 2009). Furthermore, endangered plant species and genetic resources may be effectively conserved using *in vitro* methods (Engelmann, 2011).

Micropropagation of orchids and protocorm-like bodies (PLBs)

The choice of explant source plays a significant role in the outcome of the micropropogation (da Silva, 2013). Different explants such as shoot tips, apical buds, stem segments, root tips, leaf segments, flower buds, mature seeds, and seed- derived rhizomes have been widely used as explants to obtain regenerative plantlets in several orchids (Kuehnle, 2006; Zhao *et al.*, 2008; Mohanty *et al.*, 2012). *Dendrobium* generally propagated by seeds or by division of off shoots which called sexually or asexually respectively (Martin and Madassery, 2006; Qiang, 2014).

Orchid regeneration through seed needs the infection of suitable myccorhiza fungus which usually supplies carbohydrates and nutrients to the orchid seeds (McKendrick *et al.*, 2000; Yoder *et al.*, 2000; Godo *et al.*, 2010). This relationship between the fungi and orchid is called “symbiosis” where the fungus provides nutrition for orchid growth, while, the orchid provides shelter to the fungi. In nature this mycorrhizal association with an orchid seed is not common and thus a high proportion of seeds fail to survive. However, the *in vitro* technique decreases mycorrhiza dependence for seed germination and excludes disease infection and reinfection to the clonal products (Razdan, 2003b, Kauth *et al.*, 2008). *In vitro* propagation of orchids has emerged as a choice for swift propagation of commercially cultivars as the conventional *in vivo* vegetative propagation presents with problems such as slow multiplication rate, high financial demand and insufficient production of clones within a short timeframe (Saiprasad and Polisetty, 2003; Martin and Madassery, 2006; Mohanty *et al.*, 2012). *In vitro* culture has also made it possible to preserve orchids, since the advent of asymbiotic seed germination (Andronova *et al.*, 2000; Nikishina *et al.*, 2001; Nikishina *et al.*, 2007; Mohanty *et al.*, 2012). However, the maintenance of *in vitro* collections requires manual labour and causes the accumulation of somaclonal variations and phenotype-based involuntary selections, which result in the homogeneity of the orchid population (Butenko, 1999; Ivannikov, 2003; Maneerattanarungroj *et al.*, 2007) and depletion of the gene pool (Nikishina *et al.*, 2007; Thorpe, 2007; Sorina *et al.*, 2013).

Micropropagation is the producing of microplants via tissue culture by which is created through initiation of meristematic material such as shoot buds, stem apices and seedlings from fully-developed plants into the culture process (Debnath *et al.*, 2006; Leva *et al.*, 2012). Protocorm-like bodies (PLBs) resemble true protocorms (germinated orchid seed) in being round shapedbut they are derived from bud explants or shoot tips (Kuehnle, 2006; Lee *et al.*, 2013).

PLBs can be used as a reliable source of potentially regenerable orchid tissues (Ishikawa *et al.*, 1997; Saiprasad and Polisetty, 2003; Yin *et al.*, 2011).

Orchid tissue culture propogation has been found almost immediate commercial trading in which placed orchids within the economic reach of the average person (Arditti, 1984; Arditti, 1990; Ahmed *et al.*, 2001; Arditti, 2010). Orchid producers have adopted the propogation technique, so that it would increase the mass and the quality of the orchids (Chugh *et al.*, 2009; Azman *et al.*, 2014).

Dendrobium sonia-28 is produced via an *in vitro* system based on regeneration of advanced plantlets through protocorms and PLBs (Saiprasad and Polisetty, 2003; Dehgahi *et al*., 2014). Stages of pro-meristematic, leaf primordial and formation of the first embryonic leaves are developmental phases for *Dendrobium* sonia-28 PLBs which happened between eight to 10 days old, 13 to 15 days old and 18 to 20 days old PLBs, respectively (Saiprasad and Polisetty, 2003).

Somaclonal variation system

The maturation of plant cells *in* the *vitro* and their regeneration into mature plants is an asexual process that only involves mitotic division of the cells. In this context, the happening of uncontrolled and random impetous variation when culturing plant tissue is a major problem (Leva *et al*., 2012; Nwauzoma and Jaja, 2013). Gao *et al*. (2010) and Bairu *et al*. (2011) stated that occurred variation in plant micropropogation is mostly undesired.

Undirected genetic variability happening in plant tissues culture may have novel agronomic traits that might not be accomplished by conventional breeding (Jain, 2001; Piagnani *et al*., 2008). The happening of genetic variation between plants regenerated from *in vitro* culture which has been referred to as somaclonal variation (Larkin and Scowcroft, 1981; Lestari, 2006; Nwauzoma and Jaja, 2013; Bhojwani and Dantu, 2013). Somaclonal variation may yield desirable genotypes as novel cell lines or plants of agronomic and commercial advantages (Bhojwani and Dantu, 2013). Somaclonal variation can suit a very important component of the plant breeding in which variation regenerated from somatic cells can be utilised for the introduction of new tolerance, agronomic or quality traits (Jain, 2013).

Larkin and Scowcroft in 1983 have proposed the word of somaclones and have described 'Somaclonal variation' in sugarecane plants (soma=vegetative, clone=identical copy). Tissue culture regenerated variants have also been called calliclones, phenovariants, protoclones and subclones (Skirvin *et al*., 1994; Yadav *et al*., 2009).

Somaclonal variation is not limited to the plant kingdom. There have been hundreds of reports of cell line variants among animal tissue cultures (Skirvin *et al*., 1994; Bairu *et al*., 2011).

Somaclonal variation has been caused because of alterations in chromosome number, structure and point mutations, or amplification, transposition and deletion of deoxyribonucleic acid (DNA) order (Neelakandan and Wang, 2012; Landey, 2013; Jain, 2013). Cytogenetic changes such as variation in ploidy level, structural changes, and number of chromosomes represent big alterations to the genome and they are sometimes generated during *in vitro* differentiation and proliferation (Kaeppler *et al*., 2000; Neelakandan and Wang, 2012; Landey, 2013).The chromosomal changes may produced a stable alteration which transferred to the progeny (Haines, 1994; Fu *et al*., 2013).

However, the amount of somaclonal variation depends on the plant genotype, age of plant, culture medium compounds, the time of culture and the number of subculture cycles (Duncan, 1997; Sahijram *et al*., 2003; Peredo *et al*., 2006; Bairu *et al*., 2011; Landey, 2013). The true rate of somaclonal variation is difficult to ascertain because of many individual genes to examine. Many somaclones were identical which suggests a common origin.

One of the vital potential benefits of somaclonal variation is the creation of additional genetic variability in co-adapted, agronomically useful cultivars, without the need to retreat to hybridization. Somaclonal variation will be useful if *in vitro* selection method is available (Brown and Thorpe, 1995; Ketema, 1997; Roychowdhury and Tah, 2013).

It was supossed that somaclonal variants can be entensified during *in vitro* culture for some haracters, which includes resistance to disease pathotoxins, tolerance and herbicides to chemical or environmental stress (Bhojwani, 2012).

Somaclonal variation has a few disadvantages. For instance, somaclonal variation is not always resulted to the wanted plant lines (Niizeki and Lu, 2003; Semal, 2013). It is very necessary to screen a lot of materials as possible. Second, somaclonal variation usually results in changes in multiple traits. Finally, it is very crucial to point out that a big deal of effort is needed to screen the somaclones. Most of the time somaclonal variants are not novel or useful (i.e. aberrant phenotypes), the variation generated could be unstable or not reproducible (Duncan, 1997, Jain, 2001), although some variants show positive changes other traits could be altered in a negative way (Karp, 1994; Landey, 2013).

Somaclonal variants can be detected using a few techniques which are mainly categorized as morphological, leaf morphology, physiological/biochemical such as plant height, and abnormal pigmentation (Israeli *et al.*, 1995; Leva *et al.*, 2012) and molecular traits to determine somaclonal variation (Sorina *et al.*, 2013). Somaclonal variation has led to the selection of several variants with increased resistance to pests, diseases, and herbicides (Brar and Jain, 1998; Predieri, 2001; Pandey and Mukerji, 2006; Lee, 2015; Dehgahi *et al.*, 2015b).

Epigenetic variation is also known as physiological variation or developmental. It involves nonpermanent changes which may be unstable and non-heritable and potentially reversible (Kaeppler *et al.*, 2000; Leva *et al.*, 2012). In disparity, enduring changes are heritable and sometimes represent expression of preexisting variation in the source of plant or are an effect of induced variation (Larkin and Scowcroft, 1981; Leva *et al.*, 2012). Epigenetic modifications are mostly found in DNA methylation and histones and are associated with changes in the gene expression (Kaeppler *et al.*, 2000; Zhang and Meaney, 2010; Ahmad *et al.*, 2010; Vanyushin and Ashapkin, 2011). In general, genetic stability is high in shoot tips than from explants that have no preformed shoot meristems, such as leaves, roots, or protoplasts (Skirvin *et al.*, 1994; Kaur and Sandhu, 2015).

Plant advancement through somaclonal variation and *in vitro* selection are a few techniques of *in vitro* culture to procure plant genotype tolerance to the abiotic or biotic stresses (Ahmed *et al.*, 1996; Yusnita *et al.*, 2005; Xu *et al.*, 2012).

Mutation breeding

Somaclonal variation may be one of the most advantageous sources when reliable early selection methods for the trait of interest are available (Kumar and Arya, 2009; Gupta, 2011). Mutagenesis is a skill which is being utilized by both human beings and nature in order to upgrade the quantitative and qualitative traits in plants against diverse abiotic and biotic stresses (Maluszynski *et al.*, 1995; Ahloowalia and Maluszynski, 2001; Wu *et al.*, 2012; Yunus *et al.*, 2013; Perera *et al.*, 2015). Mutagenic agents are more helpful than harmful and without them evolution of species would have been arrested at a very primitive stage (Fishbein, 2012).

Plants in their natural habitat are persistently exposed to insect herbivores, fungi, viruses and bacteria. In response to attack by these organisms, plants have developed certain defence mechanisms such as induction of structural and biochemical changes (Agrios, 2005). Changes of the external environment and the inherent instability of the genetic structure in plants under natural conditions can be resulted to induced spontaneous genetic mutations. However, frequency of such mutations differs between plant species and genes and is extremely low (Drake *et al.*, 1998). In plant breeding, induced mutation is an alternative and complementary technique for genetic modification and establishment of new genetic resources.

For modern and industrialized horticulture, there is always a demand and necessity for new cultivars. Induced mutations have played an important role in the improvement of plants and more than 3200 mutant cultivars have been developed through this technology (Yunus*et al.*, 2013). The successful outcome of a mutation depends on the efficient induction of mutation as well as the effective recognition and recovery of the desired mutant plants through subculture (Puchooa, 2005; Yunus*et al.*, 2013).

In vitro mutagenesis is a combination of *in vitro* culture and mutation induction, which provides the opportunity to increase variability of an economically important cultivar or used on plants in developing varieties that are agriculturally and economically have high productivity potential (Jain *et al.*, 1998; El-Beltagi *et al.*, 2011). Advantage of mutation is the ability to change one or a few characters of an outstanding cultivar without altering the remaining genotype and main disadvantage are the formation of chimeras (Broertjes and Van Harten, 2013).

Plant water content is significant in its radiosensitivity, since most of the frequent main quarry of ionizing radiation is the water molecule (Predieri, 2001; Miguel and Marum, 2011; Draganic, 2012). Mutation affects cells and mutated cells have to grow out into group and layer of cells. Various layers have different radiosensitivity, maybe because of differential mitotic activity and organogenic properties (Broertjes and Van Harten, 2013). If more than one cell is present at the moment of mutagenic event, chimerism will be occurred. Chimeras will be transformed into the plant progenies by repeated multiplication (Yang and Schmidt, 1994; Mba, 2013). Consequently, it is mostly advantageous to dissociate chimeras by following subcultures up to M1V3–M1V4 generations (Jain *et al.*, 1998; Mandal *et al.*, 2000; Yunus *et al.*, 2013). Subculture will maintain and secure the stability of mutant traits and guarantee that the chosen mutants are secure from chimeras (Yunus *et al.*, 2013). Induced mutation needs screening of very large population, since, induced mutation lays in the low recovery frequencies (10^{-4} to 10^{-6}) of specific single gene mutants in M2 populations (Esmaiel *et al.*, 2012). Nevertheless, somaclonal variation frequently happens at very high frequencies (up to 10% per cycle of regeneration) than radiation or chemical persuade mutation, making it a feasible alternative to mutagenesis and a preciouse tool for the plant geneticist to initiate dissimilarity into breeding programme (Skirvin *et al.*, 2000; Esmaiel *et al.*, 2012).

Chemical mutagen

There are two types of chemical and physical mutagens, the chemical mutagens most useful for mutation induction in plants belong to the class of alkylating agents [ethyl methanesulphonate (EMS); diethyl sulphate (dES); ethyleneimine (EI); ethyl nitroso urethane (ENU), ethyl nitroso urea (ENH), methyl nitroso urea (MNH) and azides (Predieri *et al.*, 2001; Jain, 2010).

For inducing mutation in vegetatively propagated plants, chemical mutagens are not usually considerd, mainly because the number of cases in which they have been applied successfully (and in which they were better than radiation) has been small (Broertjes and Van Harten, 2013). Othervise, some authors stated that chemical mutagens have a higher efficiency and this perspective has doubtlessly been assisted by the perspective simplicity for acquiring chemical mutagens as well as undemanding set-up demands. It should also be pointed out that chemical mutagens are very poisonous toxic that generally need elaborate detoxification of laboratory ware and discarding of used reagents. Comparitively, the imperils to health constituted by physical mutagens are far less (Mba, 2013). In general, most mutation breeders prefer ionizing radiation (easily applicable, clean, good penetration and reproducibility, high mutation frequency). On the other hand, the incrising possibilities of *in vitro* techniques (which may give better penetration of chemicals) may tip the balance towards the use of chemical agents (Broertjes and Van Harten, 2013; Sarada *et al.*, 2015).

Physical mutagen

Muler in 1927 introduced induced physical mutation which produced mutant fruit fly (*Drosophila melanogaster*) by x-rays (as been cited by Shu *et al.*, 2012). Plant radiosensitivity, propagation and selection methods are some characters which affect the physical mutagenesis most suitable dosage to apply (Broertjes and Van Harten, 2013; Mba, 2013). Normally, effects of irradiation are devided into primary effects (physiological damages) and heritable changes or mutation (Broertjes and Van Harten, 2013).

There are a lot of physical and chemical mutagens currently used in mutation breeding (Ahloowalia and Maluszynski, 2001; Medina *et al*., 2005; Jain *et al*., 2007; Jain, 2012). X-, β- and γ-rays, neutrons and protons are some of the several energy rays that are widely used in mutation breeding.

Gamma irradiation

X-ray, ultraviolet, gamma ray, fast neutron, beta radiations and thermal neutron were frequently used physical mutagens (Yaqoob and Rashid, 2001; Kumar and Srivastava, 2011). In past decades, gamma irradiation has been haggared the notice as a new and fast technique to enhance the quantitative and qualitative characters of numerous plants (El-Beltagi *et al*., 2011; Fulzele *et al*., 2015).

The most common physical mutagen applied in plants is gamma-ray radiation (Jain *et al*., 2013). Gamma rays are one of the most efficient sources of ionizing radiation, which induces a high frequency of mutations in plants. It has been reported that gamma rays can induce about 70 % of the world's mutant varieties (Nagatomi and Degi, 2009). Radiation rays can enhance mutation rate ranges more than a thousand-fold in the plants (Kovács and Keresztes, 2002). Cobalt-60 as the usual radiation source for induced-mutation, is widely used in agriculture and forestry, especially in ornamental and economically-valuable plants (Thapa, 2004; Borzouei *et al*., 2010). Gamma rays are an ionizing radiation that interacts with atoms or molecules to generate free radicals in cells. These free radicals can destroy important components of plant cells and differentially affect the morphology, anatomy, biochemistry and physiology of plants depending on the level of irradiation. Gamma radiation can affect plant photosynthesis, depending on the irradiation dosage (Kovacs and Keresztes, 2002; Kim *et al*., 2004; Wi *et al*., 2007). Recently, mutation breeding has been used for some important ornamental plants including orchids (Kikuchi, 2000).

Generally, gamma irradiation breaks DNA into compact particles and then the DNA commences a restore mechanism.

Throughout this second step, mutations occur or new variations develop (Jain, 2013). Mutations generally referred to changes in protein-coding genes rather than to chromosomal changes (Nei, 2013). The ionizing irradiation causes substantial cell nuclear harm and is accountable for the lethality. When biological materials absorb gamma ray, it will connect with atoms or molecules (particularly water) to make free radicals in cells, and targeted accessible cells. These radicals can modify disparate fundamental compounds of the plant cells, creating harm to plant cells. This effect of irradiation is scathing for vegetative cells with cytoplasm eccommodates about 80% water content (Kovacs and Keresztes, 2002; Yunus *et al*., 2013).

Gamma rays are commonly known to impact on the plant's growth and development by persuading physiological, cytological, morphogenetic, biochemical, and genetical changes in tissues and cells (Kumar and Srivastava, 2011; Yunus *et al*., 2013; Mujar *et al*., 2014).

Gamma rays in an energetic form of electromagnetic radiations which is known to be the most favoured mutagens for their straight forward application with high mutation frequency, good penetration, reproducibility and less disposal troubles (Chahal and Ghosal, 2002; Sarada *et al*., 2015). Gamma radiation treatment of multicellular tissues is frequently leads to chimeras, relying on the phenomenon of mutations in the L1, L2, and L3 meristematic layers (Jain, 2010).

There are two streams of gamma irradiation methods wich are acute and chronic irradiations. The acute irradiation provisions are for example gamma cell and gamma room have been extensively utilised handled with compact materials in less time. And in the chronic irradiation, provisons such as gamma greenhouse, gamma field and gamma phytotron have been used with a high amount of plant substance under stubby dose rates in the natural state for long lasting durations (Nagatomi, 2001; Nagatomi and Degi, 2009; Geras' kin *et al*., 2013). Exposure to chronic and acute radiation has different effects (Wickliffe *et al*., 2003; Møller and Mousseau, 2011).

Chronic exposure has largely been used, but it does not appear to have any advantages over acute irradiation, which is more suitable for induced mutagenesis in tissue cultures (Predieri, 2001).

Gamma application is preferred since it is not a threat for humankind and environment (Ulukapi and Nasircilar, 2015). Gamma application could be resulted for development of new hybrids in coriander, tomato, anthurium and mungbean (Ulukapi and Nasircilar, 2015). Gamma irradiation has been widely used in the biological study of plants including in plant tissue culture system.

Mutation frequency enhances with increasing irradiation dose (linearly with gamma rays), but survival and capacity to regenerate decrease with increasing dose. At high doses, too many mutational events per cell may be induced, with increased risk that a favourable mutation is accompanied by one or more unfavourable genetic changes (Broertjes and Van Harten, 2013).

Physiological disturbances and chromosome aberrations are able to increase the number of such abnormal treated plants (Datta, 1997).Irradiation affects cell division, which is the most sensitive part to irradiation and is able to inhibit plantlets growth (Vazquez-Tello *et al*., 1996).

In chinese cabbage, red pepper, onion, spinach, bottle gourd, pumpkin and soybean, low-dose gamma-irradiation stimulated germination and early seedling growth (Kim *et al*., 2004). They also reported that changing of hormonal signaling network or anti-oxidative capacity in the plant cells because of low dose radiation simplify overcome to daily stress factors like variations in light intensity and temperature in the growing environment will stimulate plant growth.

The results obtained by Kiong *et al.* (2008) indicated that survival of plants to maturity depends on the nature and extent of chromosomal damage.

Increasing frequency of chromosomal damage with increasing radiation dose may be responsible for less germinability and reduction in plant growth and survival.

Higher doses of gamma radiation reduce the amount of endogenous growth regulators like cytokinins (Afrasiab and Iqbal, 2010). Lower doses of gamma radiation resulted to increasing of plant final fresh weight (Al-Oudat 1990; Afrasiab and Iqbal, 2010). Higher doses of gamma rays significantly postpone germination of plants (Majeed and Muhammad, 2010). In general, ionizing radiation influences plant growth and development in living plants. Low doses of ionizing radiation are known to have stimulatory effects on plant growth, whereas high radiation levels induce increasingly harmful effects on vegetative growth, as well as pronounced reductions in reproductive fitness and yields (Kovalchuk *et al*., 2003 & 2004).

Conclusion

In conclusion, Somaclonal variation has led to the selection of *Dendrobium* sonia-28 with increased resistance to *Fusarium Proliferatum*. To obtain Fusarium-resistant *Dendrobium* sonia-28 plants, mutation induction can be applied. Mutagens including chemical or physical agents provide the opportunity to increase plant genetic variability to pursue plant development.

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