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Creation of variation through gamma irradiation and polyploidization in *Vitex agnus-castus* L.

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ABSTRACT

The article deals with the impact of gamma irradiation or chromosome doubling treatments in *Vitex agnus-castus*. The study aimed to measure the efficiency of gamma irradiation for creating variation and chromosome doubling agents (colchicine, oryzaline and trifluralin) for polyploidization in *V. agnus-castus*. These treatments should help produce more desirable growth habit and novel characteristics would expand the use of *V. agnus-castus* L. in landscaping. The seeds were treated with seven gamma irradiation doses (10, 20, 50, 100, 200 300, 400 Gray) from a Cobalt^{60} source and six antimitotic agent concentrations (0.05 and 0.1% colchicine, and 0.005 and 0.01% oryzalin and also trifluralin). The LD_{50} was found to be 55 Gy for seed germination and 41.3 Gy for seedling survival. The desired single-stemmed plant type was obtained with mostly 50 Gy irradiation dose. Single plants survived from each of colchicine treatment. Flow cytometer analysis confirmed the plant derived from 0.05% colchicine treatment to be a polyploid. The results revealed that mutation and/or ploidy manipulation have the potential to generate much needed variation in *V. agnus-castus* for use in landscaping.

1. Introduction

Vitex agnus-castus L. (chaste tree) belongs to Lamiaceae (moved from Verbenaceae) (Judd et al., 2002; Hershberger, 2010) and is a deciduous shrub-tree species growing 2–4 m. It has palmately compound 5–7 leaves and showy panicle type inflorescence, carrying small raceme branches with the colors varying from white to lavender blue including shades of pink in June–September (Dirr, 1990). The origin of *V. agnus-castus*, is arid and semi arid regions of Mediterranean and Western Asia (Schopmeyer, 1974; Dogan et al., 2011) and distributed up to 1200 m (Boissier, 1963). It has been used as medicinal-aromatic plant for centuries (Chevallier, 1996) as well as limited garden plant. Some wild accessions of *V. agnus-castus* ($2n = 24$) (Darlington and Wylie, 1955) have some superior functional charactersistics important for landscape use such as well adaptation to hot and dry climate, resistance to drought, saline and poor soil conditions. Thus, it offers advantage in climates with hot summers. However, the general scattered growth habit of the native genotypes is the most important disadvantage of restricting its use in landscape (Arı and Karagüzel, 2011). Therefore, *V. agnus-castus* requires to be converted to more attractive plant architecture and flowering characteristics. Chemical or mechanical methods can be applied to ornamental plants for shaping, but they will be mostly tentative. However, breeding studies provide genetically more permanent solutions to change diverse plant characters.

For the breeding of ornamental plants, especially for the species, in which little or no breeding study done before, biotechnological methods such as *in vitro* somaclonal variation, haploidization, mutation and polyploidization can hasten the breeding process. Among these methods, polyploidy and mutation-assisted breeding are the most used ones especially in vegetatively propagated ornamental plants for the exploitation of genetic variability and development of new cultivars (Jain, 2006). Since the effect of mutation is very visible in the ornamentals, selection of changed flower size, shape and color is easy, and almost anything, which is novel, is of value. Thus, mutation techniques have become a major tool for breeding of ornamental plants (Maluszynski et al., 1995). According to the web page of Mutant Variety Database of The International

Abbreviations: Gy, Gray; LD, lethality dose.

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Atomic Energy Agency (IAEA) on 20 April 2015 (Iaea, 2015), of the 3218 released mutant varieties, 709 (22%) represent ornamental plants.

Mutations are induced by the nuclear energy in physical mutagens such as ultraviolet irradiation and ionizing irradiation, and in chemical mutagens from alkylating class. The mutagen treatment breaks the nuclear DNA, introducing random and heritable mutations (Jain, 2006; Lestari, 2012). Then, an efficient *in vivo* or *in vitro* plantlet regeneration system is required for leading to the occurrence of variation through the new phenotype produced (Hasbullah et al., 2012). Among the mutagens, the most commonly used is gamma irradiation. As an ionizing irradiation, it penetrates deeper into the plant tissue and can induce various types of chemical changes (Lestari, 2012). Of all the 3218 registered mutant varieties including feed, industrial, food, fruit and ornamental crops, 1690 (52%) were developed *via* gamma irradiations (Iaea, 2015).

In polyploidy, the chromosome number is folded and it is an extensively used in floriculture with number of commercial varieties (Horn, 2002). Doubling of chromosome numbers is achieved by antimitotic agents binding to tubulin dimers, a component essential for a correct polar migration of chromosomes during mitotic cell division. Among these, colchicine, a metaphase inhibitor from the class of alkaloid has been used most extensively until recently. Oryzalin and trifluralin from the class of dinitroanilines, benzoic acid, phosphoroamidates and pyridines are the other widespread used mitotic polyploidization alternatives lately (Dhooghe et al., 2011).

As a mitotic disrupter, colchicine not only serves as chromosome doubling chemical for polyploidization, but also is used for induced mutation. The mutants obtained *via* colchicines are so called colchimutants (Datta, 2009). "Datta and Gupta (1985, 1987) were the first to use colchicine to produce new flower colors in rose and chrysanthemum".

Another benefit of mutation and polyploidization is exploration and domestication of wild germplasm with ornamental potential. It is commonly accepted that domestication of native species has been conditioned by mutation following selection (Datta 2009). Induced mutagenesis was used for the domestication of some native plants, such as *Lantana depressa via* gamma irradiation (Datta, 1995), *Scoparia montevidiensis, Bacopa monnieri, Mecardonia tenella via in vitro* colchicine treatment (Escandon et al., 2005, 2006, 2007), *Delphinium malabaricum via* gamma rays, ethyl methane sulfonate and sodium azide (Kolar et al., 2011), *Calandrinia balonensis via* colchicine and gamma rays (Harrison et al., 2009).

The information is lacking for gamma irradiation or chromosome doubling treatments in *V. agnus–castus*. Hence, we aimed to measure the efficiency of gamma irradiation for creating variation and chromosome doubling agents (colchicine, oryzaline and trifluralin) for polyploidization in *V. agnus-castus*.

2. Materials and methods

2.1. Plant materials

The study was carried out in Batı Akdeniz Agricultural Research Institute (BATEM) in Aksu, Antalya (lat. 36°56′N, long. 30°53′E), Turkey, under Mediterranean climatic conditions with dry, hot summers and mild, wet winters in 2008–2009. The seeds of a wild *V. agnus-castus* accession collected from a well grown donor plant in Gebiz, Antalya, on 27 November 2007 were used. The treatment of physical mutagen (gamma radiation) and chromosome doubling chemicals were applied to the seeds to induce mutation and increase the ploidy level. Prior to the applications, 100 seeds for each treatment were kept in a climate chamber (Memmert, Schwabach, Germany) at 35 °C for 24 h for seed moisture equilibration. Seeds were sown as dry in both trials.

The seedlings derived from irradiated seeds were first transplanted into 12 cm plastic pots in a glasshouse on 07 August 2008, and then transplanted to 24 cm plastic pots on 22 November 2008. Later, the seedlings were transferred into soil in field on 16 December 2008, and prunned before spring season. The soil type of the field was clay loam (Paralithic Xerortent) with a 31.6% $CaNO_3$ content, 2.4% organic matter (OM) ingredient, electrical conductivity (EC) of 358 μS cm^{-1}, pH 8.2, available phosphorus (P) level of 26 mg kg^{-1}, potassium (K) 192 mg kg^{-1}, calcium (Ca) 2588 mg kg^{-1}, and magnesium (Mg) 400 mg kg^{-1}.

2.2. Treatment of gamma irradiation

The seeds were irradiated with gamma rays derived from a $Cobalt^{60}$ source in Turkish Atomic Energy Agency in Ankara, Turkey. The gamma radiation doses of 10, 20, 50, 100, 200, 300 and 400 Gray (Gy) with a 1041 Gy/h rate were tested in a completely randomized design to determine the lethality doses (LD). A hundred seeds were irradiated for each dose into glass petri dishes and 100 non-irradiated seeds were used as the control. A total of 700 seeds were sown individually by hand to each tooth of the violes containing peat and perlite mixture (3:1 v/v) in a glasshouse for the germination on 15 June 2008.

2.3. Treatments of chromosome doubling agents

Colchicine and oryzalin were dissolved in 1% dimethyl sulfoxide (Merck, Darmstadt, Germany), and trifluralin in acetone (Sigma–Aldrich, St. Louis, MO, USA). The 100 seeds were soaked in each of 0.05 and 0.1% colchicine (Sigma–Aldrich), 0.005 and 0.01% oryzalin (Supelco Bellefonte, PA, USA), 0.005 and 0.01% trifluralin (Riedel-de Haën, Seelze, Germany) solutions and control treatment in distilled water. The seeds were treated in glass bottles for 36 h at room temperature in darkness on 19 December 2008. After rinsing three times with distilled water, the seeds were sown into the mix containing the same medium mentioned above.

2.4. Data and statistical analysis

The percentages of germination and survived seedlings in each treatment were recorded as the data of gamma irradiation study. The obtained dependence of germination and survived seedlings versus delivered dose was analyzed in order to identify its functional dependence. The model applied for this analysis was the probit model where the fitted values for LD20, LD50 and LD80 were presented.

The plants derived from gamma irradiation and chromosome doubling treatments were compared against the untreated parental control plants grown under the same conditions. The plant width and height (cm), and the number of main stem branch (n) were measured on 19 June 2009 and the second plant height on 19 August 2009 among M_1V_2 plants. The flower and leaf colors were measured with the chromometer (Minolta CR-200, Minolta, Osaka, Japan) with 3 replications. According to CIELAB color system, L^* refers to color clarity, a^* indicates color green to magenta, and b^* blue to yellow (Banon et al., 2002). Chroma (color saturation) values were calculated as $(a^{*2} + b^{*2})^{1/2}$, and the hue angles as $\tan^{-1}(b^*/a^*)$.

Both an untreated parental genotype and a M_1G_2 plant derived from 0.05% colchicine were processed using CyStain UV Precise P Kit for nuclear extraction and staining, then analyzed in a flow cytometer (CyFlow Ploidy Analyzer; Partec GmbH, Münster, Germany) for ploidy determination.