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Somaclonal variations in an endangered and medicinally important cucurbit, *Citrullus colosynthis* (L.) Schrad

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ABSTRACT

Somaclonal variations were studied in *in vitro* regenerated plants of an endangered cucurbit, *Citrullus colosynthis* (L.) commonly known as 'bitter apple'. It is a medicinally important plant used as a antirheumatic, anthelminthic and its exractive 'colosynth' is a very strong laxative. Roots are used in urinary diseases, mammalitis and opthalmia. Its exract cucurbitacin glucosides inhibit growth of human breast cancer. During *in vitro* mutagenesis and regeneration studies a number of somaclones have been isolated and characterized. Regenerated plants from R_0 are scored for the identification of variant plants in R_1 and R_2 generation. During these studies a number of variations in habit, leaf and tendrillar character, floral somaclones like androecious, gynoecious, andro monoecious and andro gynoecious were isolated. Variation in fruit number, sizes, colours, and seed coat colours were also screened.

KEYWORDS

Somaclonal variations;
Citrullus colocynthis;
Cucurbit;
Androecious;
Gynoecious;
Gamma rays;
R_0 plants;
Genomic instability;
Epigenetic changes.

INTRODUCTION

In vitro regeneration usually results in high genetic and phenotypic variability in individuals been derived from plant tissue cultures or adventitious shoots which is called somatic variation. Somaclonal variation is also called tissue or culture-induced variation[1]. The term "somaclonal variation" is given by Larkin & Scowcroft[2]. Somaclonal variation is not restricted to, but is particularly common phenomenon in all plant regeneration system that involve a callus phase[3]. Somaclonal variation may vary from species to species and determining the genetic nature of the observed variation is difficult[4]. Identification of possible somaclonal variants at an early stage of development is very useful for quality control in plant tissue culture, transgenic plant production and in the introduction of variant plants carrying heritable traits. Somaclonal variability often arises in tissue culture due to epigenetic influence or changes in the genome of differentiating vegetative cells induced by tissue culture conditions[5].

Somaclonal variation often arises in tissue culture as a manifestation of epigenetic influence or changes in the genome differentiating vegetative cells induced by tissue culture conditions[5]. Somaclonal variation may arise due to any of the following events at molecular level: changes in chromosome number, structure, gene mutation, plasmagene mutation, alteration in gene ex-

pression, gene amplification and rearrangements in cytoplasmic genes[6]. Investigations have revealed that cell or tissue cultures undergo typical genetic alterations are changes in chromosome number (polyploidy and aneuploidy), chromosomal structure (deletion, translocations and duplication) and DNA sequence (base mutations). Typical epigenetic related events are : gene amplification and gene methylation[7]. Somaclonal variations are of two types: genotypic (heritable) and phenotypic (non-heritable), which in the later case can be either genetic and epigenetic changes[8,9]. The genomic instability arises due to chromosomal rearrangements, chromatid exchanges and gene amplification[10]. Showed that there are chromosomal changes that deciphered with molecular cytogenetic techniques.

Somaclonal variations are not undesirable since some may serve as novel raw material (genetic diversity) and can be beneficial in crop improvement especially on traits for which somaclonal mutants can be effectively selected for disease pathotoxins[11,12], improvement of nutritional quality, adaptation of plants to biotic or abiotic stress conditions[13] e.g., saline soils, low temperature, toxic metals, tolerance to herbicides[14,15] and to increace production of secondary metabolites of plant products used for medicinal or industrial purposes[16]. Salinity and drought are two major environmental stress that limit plant growth and productivity[17].

Evans & Sharp reported three critical variables for somaclonal variation: genotype explant origin, cultivation period and the culture condition in which culture is made[18]. Larkin reported that longer period of *in vitro* cultivation seemed to increase somaclonal variation[19]. The correlation between long-term culture of callus and the accumulation of chromosome variation was first documented in *Daucus carota*[20,21] in *Citrus grandis*. The prolonged periods of *in vitro* cultivation result in increased frequency of chromosome aberration was not supported by the results[22,23]. Another way to increase somoclonal variation is to add 2,4-D in the culture medium, as this hormone is known to introduce variations. Adventitious shoot regeneration system have been demonstrated as useful for obtaining genetically transformed plants[24]. Addition of growth regulators to culture medium is known to have influence on the frequency of the karyotype alterations in cell culture. Frequently, the auxin 2,4-D is considered to be responsible for the chromosome variation[25].

Somaclonal variation has been described for several plant species in crop plants like tobacco[26], maize[27], barley[28], potato[29], rice[30], wheat[31] and triticale[32]. There were few reports in certain cucurbitaceous plants. Few floral somaclones were isolated from *Benincasa hispida* and *Citrullus vulgaris*[33] and *Cucumis melo*[34]. There were no reports of somaclonal variations in *Citrullus colocynthis*. Both conventional breeding and transgenic approach have been used in summer squash to confer disease resistance[35]. In *Cucubita pepo*, genotypic difference in response to exogenous PGR_s and their undergoing of different callus differentiation could be useful for developing transgenic lines and somaclonal variations[36]. Adventitious shoot regeneration of diploid and tetraploid somaclonal variants has promising application in the area of genetic transformation and can be used as parental lines to breed seedless watermelon[37].

RESULTS

During our study, various somaclonal variants were isolated for a variety of traits through tissue culture. These somaclones were derived from R_0 populations. R_1 plants were obtained from self fertilized progeny of R_0 plants. Chaleff has labeled the regenerated plants from tissue culture as R_1 and R_0. Further generations are termed as R_2, R_3, R_4 etc. This terminology is consisted with historical genetics and the new breeding lines were referred as P and P_0 lines. Regenerated plants as SC_1 and further generations are referred as SC_2, SC_3 etc. (Scowcroft, 1984). The mutagenized plant is reffered and M_1 plants.

Somaclonal variants isolated through cell selection are often unstable. The frequence of stable variants may range from 8-62%, perhaps depending on the species and the selection agent. Some variants phenotypes are quite stable during the cell culture as well as regenerated plant phases and exhibit transmission of the phenotypes through the sexual reproduction cycle are called mutants. Only this category of variants would find an application in crop improvement. These may represent true gene mutations.

During our study following important somaclone

variants were isolated in population (R_0).

1. Somaclonal variation in leaves.
   (a) Variations in leaf arrangement on shoot apex with cluster of leaflets (Plate I; Figure 1).
   (b) Variations in leaf sizes, texture and shapes after gamma rays treatment *In vitro* regenerated plant (Plate I; Figure 2).
   (c) Variations in branching pattern (Plate I; Figure 3).
2. Floral somaclones.
   (a) Androceious somaclone showing 3 or more male flowers at axils after gamma rays treatment (Plate I, Figure 4 and Plate II, Figure 1).
   (b) Gynoecious somaclone with female flowers in axil (Plate II, Figure 2).
   (c) Monoecious and gynoecious somaclones raised from tissue culture through regeneration (Plate II, Figure 3 & 4).
   (d) Bisexual plant with androecious and gynoecious flowers in different axils after EMS treatment (Plate II, Figure 5 & 6).
3. Somaclone variants in fruits and seeds.
   (a) Variation in number and size of fruits (Plate I, Figure 5).
   (b) Variation in seed coat colours (Plate I, Figure 6).

Plate I

Figure 1 : Variation in leaf arrangement on shoot apex with cluster of leaflets.

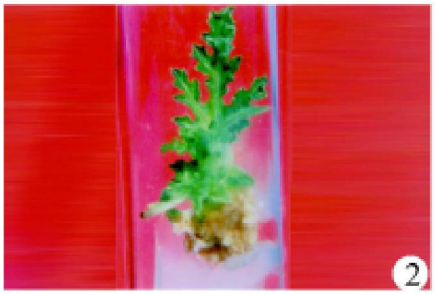

Figure 2 : Regeneration of plantles from callus on MS + 1.0 mg/l zeatin with different leaf morphology ie, very thick leaf texture and linear dissected leaf.

Figure 3 : Variation in number of leafs and branching pattern.

Figure 4 : Androceious somaclone showing 3 or more male flowers at axils after the callus exposed to gamma rays treatment.

Figure 5 : Variation in number and size of fruits.

Figure 6 : Variation in fruit colour and variation in seed coat colours.

Plate II

Figure 1 : Androceious somaclone showing 3 or more male flowers at axils after gamma rays treatment.

Figure 2 : Gynoecious somaclone with female flowers in axil.

Figure 3 : Monoecious and gynoecious somaclones raised from tissue culture through regeneration.

Figure 4 : Androecious twigs raised from tissue culture technique through regeneration.

Figure 5 : Bisexual plant with androecious and gynoecious flowers in different axils after the callus treated with EMS.

Figure 6 : Magnified female flower showing penta fid stigma after EMS treatment.

DISCUSSION

Somaclonal variation represent a useful source of introducing genetic variations that could be of value to plant breeders. Somaclones could be used to uncover new variants retaining all the favorable characters along with additional useful traits such as resistance to disease, herbicides and free from undesirable features like sterility. Various cell lines selected *in vitro* may then prove potentially applicable to agriculture and industry.

Tissue culture may lead to abnormal plants. The changes occur especially in plants produced through adventitious regeneration, multiplication from existing meristems appears to be relatively safe. The frequency of changes depends on the species (genotype), the tissue from which the adventitious plantlets have been regenerated and the medium composition. This shows that susceptibility to somaclonal variations is related to genotype as described by Roest *et al.*,[38]. Plant genotype may have important effects on somaclone regeneration and frequency. Explant source is considered the most frequent critical variable for somaclonal variation. Since explants may present dissimilar regeneration rates, selection procedures can differ among different explant types. Plant regenerated from chrysanthemum petal epidermis induced calli showed greater somaclonal variations than those from apex-induced calli[39].

Genetic changes behave as Mendelian traits in crosses. Usually expected Mendelian ratios are obtained in the R_1 progenies. But sometimes aberrant segregation ratios are encountered in R_1 possibly due to chimaeric nature of R_0 plants, the involvement of some cytological anomalies like aneuploidy and deletions etc. Such changes are known as epigenetic changes and are attributed to stable changes in gene expression[40]. Epigenetics changes do not involve changes of primary DNA sequence, but are the result of alterations in DNA that modify gene expression. The culture time-length and accumulation of chromosome variation was first documented in *Daucus carota*[20]. Hirochika *et al.*, reported an increase in the copy number of transposon *Tos 17* in rice, when submitted to long periods of incubation[41]. Addition of growth regulators to culture medium is known to have influence on the frequency of the karyotype alterations in cell cultures.

Results of present investigation, variations were observed in R_0 generation are increase in the number of fruits per plants, increased number of branches per plant, increased number of seeds per fruit and seed coat color, which were transmitted to R_1 generation. In general, R_1 progeny (regenerated from R_0 plant) are scored for the identification of variants plant and their R_2 progeny lines are evaluated for confirmation. Somaclonal variation induced *in vitro* is a wide spread phenomenon irrespective of mode of reproduction-sexual or vegetative and ploidy status of the species.

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