Bangladesh J. Bot. **51**(3): 555-563, 2022 (September) DOI: https://doi.org/10.3329/bjb.v51i3.62002

HORTICULTURAL PERFORMANCE OF FIELD GROWN SOMACLONES AND IRRADIATED STRAWBERRY (*FRAGARIA* × *ANANASSA* DUCH.)VARIANTS

RUCKU GUPTA*, PARSHANT BAKSHI[1] AND RAFIQ AHMAD SHAH[1]

Division of Fruit Science SKUAST-J, Chatha, 180 009, Jammu (J&K)

Keywords: Horticultural performance**,** Field performance, Somaclones, Strawberry, Gamma irradiation

Abstract

In the present investigation horticultural performances of field grown somaclones and gamma irradiated strawberry were studied. The somaclones performed better with regard to vegetative and reproductive characters while axillary buds irradiated with lower dose of gamma rays (10 and 30 Gy) were more vigorous in terms of number of leaves and petiole length. Whereasaxillary buds irradiated with higher dose of gamma rays (50 and 60 Gy) showed abnormal fruit development, reduced growth, small leaf area and fruit size. A total of six variants were obtained on screening the somaclones and irradiated plantlets on the basis of phenotypic characters, fruiting characters and Single Sequence Repeat (SSR) markers. The frequency of variants was higher (3.22%) in soamclones than in irradiated plants (2.75%) obtained from axillary buds. These finding would be helpful in providing a powerful technological tool for the improvement of clonally propagated crops.

Introduction

The cultivated strawberry, *Fragaria* × *ananassa.*, belonging to Rosaceae is a herbaceous perennial and originated in Europe. The crop is actually the temperate beauty but its cultivation extends towards sub-tropics of the world. The major strawberry growing states in India are Maharashtra, Uttar Pradesh, Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, Uttrakhand, Rajasthan and West Bengal. With a purpose for improving fruit yield, quality characteristics and the resistance towards insect-pest and diseases in strawberry, the plant tissue culture tools have been used by earlier workers to speed up the breeding process. It is therefore**,** imperative that to improve the accessibility of the existing germplasm and the creation of new variations for crop improvement programme through micro-propagation (Podwyszynska *et al.*2010), *in vitro* selection (Rai *et al.* 2011), somaclonal variations (Krishna *et al.* 2016), and genetic transformation (Sharma *et al.* 2000) techniques have also been standardized. Somaclonal variation is a common phenomenon in plant tissue culture which regenerated *in vitro* culture (Larkin and Scrowcroft 1981) caused changes in chromosome number (polyploidy or aneuploidy), chromosomal alteration and or changes in methylation of chromatin (Peschke and Phillips1992, Phillips *et al*. 1994). Strawberry crop is commercially propagated through vegetative means. Therefore, it is very difficult to induce genetic variability through conventional method (cross hybridization). Moreover, traditional breeding in strawberry is time consuming and difficult due to its complex genome and octoploid nature (Jung *et al*. 2017). Earlier literature well cited on the selection of suitable cultivars in strawberry has also reduced genetic diversity within the cultivated strawberry (Dale and Sjulin 1990). Genetic selection carried out *via* regeneration of plants from callus (*in vitro*) has produced diverse characteristics through the somaclonal variation in strawberries. The successful use of somaclonal variation is very much dependent on its genetic

*Author for correspondence : <rucku1989@gmail.com>. [1]Division of Fruit Science, SKUAST-J, Chatha, 180 009 Jammu (J&K).

stability in the subsequent generations for which molecular markers such as RAPDs, AFLPs, SSRs and others can be helpful (Jain 2001). Contrary to this, simple sequence repeat (SSR) or microsatellite markers are well-suited for the purpose of comparative linkage mapping between diploid and octoploid strawberry (*Fragaria* spp.). Besides, the combination of mutation breeding and *in vitro* culture (also known as *in vitro* mutagenesis) has been found to induce somatic mutations more effectively (Jain 2012, Suprasanna *et al.* 2012). The present study was therefore, focused to assess somaclones and irradiated variants through SSR molecular markers for horticultural performance under field conditions. The findings of this study will also be applied for commercial scale multiplication and also to demonstrate SSR markers assisted detection of variation and irritated of strawberry plantlets.

Materials and Methods

The experiment was carried out on 'Chandler' cultivar of strawberry for two consecutive years between 2017 and 2018. The axillary buds were used as the starting material for *in vitro* process. The axillary buds were cultured on MS media supplemented with BAP at 1.00 mg/l in combination with GA_3 at 1.00 mg/lwhich was already standardized before irradiation process for the explants with different dosage of gamma rays. The same media composition was also used for the proliferation the shoots.

In vitro mutagenesis of irritated plantlets and the cultures was carried out through gamma irradiation in gamma chamber located at Punjab Agriculture University, Ludhiana. The gamma ray treatment was performed using blood Irradiator. The axillary buds were irradiated with four doses of gamma rays *viz.*, 10, 30, 50, 60 Gy for induction of mutations. The gamma ray irradiated axillary buds were then cultured on growing media for the shoot proliferation and were then subsequently, transferred to root induction MS medium supplemented with IBA at 1.5 mg/l and activated charcoal powder at 200 mg/l. The miniature explants were hardened and grown in glasshouse in pots containing soil: FYM (1:1 v/v) for 7 weeks.

The leaf explants (leaf disks) for callus culturing were excised from strawberry shoots grown under *in vitro* conditions. The leaves (*in vitro*) of 0.2 mm size without the midrib were taken. After callusing, leaf derived calli were divided into small segments of 1-1.5 cm diameter. It was further sub-cultured in MS medium for shoot initiation.

The somaclones and irradiated plantlets (variants) and the field grown plants (control) were evaluated for the variation with respect to different morphological parameters. Different horticultural parameters such as number of leaves per plant, leaf area, plant height, petiole length, and duration of flowering, number of flowers per plant, number of fruits per plant, fruit size, fruit weight and yield per plant were recorded. The frequency of occurrence of a variation was also calculated by dividing the number of variants with total number of plants produced

The ripe berries (fruits) produced during the cropping cycle were sampled. The fruits were then rapidly transferred to the laboratory for biochemical analyses using standard procedures (AOAC 1995).

To characterize the variants obtained through callus cultures and *in vitro* mutagenesis, Simple Sequence Repeat (SSR) markers were used. A representative sample size of 50 mg leaf tissues was taken for genomic DNA isolation from somaclones, mutants and the field grown plantlets according to Doyle and Doyle (1990). The total genomic DNA was extracted using the CTAB method. The quantity and quality of the DNA was checked by 0.8 per cent agarose gel electrophoresis. The 23 number of primers used for amplification were diluted to working concentration of 10 pico moles using autoclaved deionized water (Table 1). The polymerase chain reaction (PCR) was performed in a reaction volume of 20 µl containing 8.75 µl of sterile milli Q

water, 0.15 µlTaq DNA polymerase buffer, 0.5 µl, of dNTPs mixture, 2.0 µl random primer, 0.15 µlTaq polymerase and 3.0 µl genomic DNA. The PCR was carried out in a thermal cycler with a total of 35 cycles. Amplification was performed as per the following temperature profile i.e., (i) initial denaturation at 94^{o}C for 4 min, (ii) denaturation at 94^{o}C for one min, (iii) annealing temperature (varies from primer to primer) for 1 min and 40 s) extension at 72^{o}C for 40 s) the final extension at 72^{o}C for 7 min. The mixture thus obtained was held at 4^{o}C. The PCR products were resolved on non-denaturing 3% agarose gel in 1xTBE buffer and stained with ethidium bromide. DNA ladder (100 bp) was used to determine the molecular weight of PCR product. Then the gel was viewed under ultra violet light using gel documentation system.

Table 1. SSR primers and their sequences used for DNA amplification of the variants.

Primer	Forward primer (5'-3')	Reverse primer (5'-3')
ARSFL_99	GATTAGGGAGAGGCAACGT G	CTTCAAGCAAAATGCATCA
FAC-011	GTTTTCAGGCGGTCAATTCTA	GCTTCAAGCAAAATGCATCATC
FAC-001	AAATCCTGTTCCTGCCAGTG	TGGTGACGTATTGGGTGATG
FAC-006b	ACACTGCGTTTTGTGTGCTCTA	CAGGCCGTAATCCATTTCTTAT
FAC-005a	GAT GTATTGCATTTCGTGCTAA	GCACATAATGAGAAAGCAGCAC
FAC-005b	ACTGTGGAGGTCTAGCCATTGT	GCACATAATGAGAAAGCAGCAC
FAC-008	TACTGTGCACGCAACAACAG	CTCTCCAATCCTTCATTGAT
FAC-014	GACTGAAGGAGAAGCTCAAGGA	GACCTCAGTGTCCTGCTCGTAG
FAC-004a	ATGGGAGAT GCTCTTAGTGGAA	ACAGATTCGGTTCGTTTATTGA
FAC-004b	ATCAATAAACGAACCGAATCTG	GTCCTGAAGGTGGAGAAATCAG
FAC-004c	GTCCATACTTTAAGCCGAAT GC	ACGTCCCTTCACAATAAAAT GG
ARSFL_103	CTCGAATCGACTGGAAGGAG	GAACCTCCTCACGAACTTGC
ARSFL_2	GCGAAGCGAAGCGGT GATG	GCGAACGTCGAGGAGCATTCTCAT
ARSFL_3	GCGGGT GCTTAGGTTTTCACAACT	GCGCAAGTGGTATTTAAGGGTTAG
ARSFL_9	GCGAGGCGATCAT GGAGAGA	GCGTTTCCTACGTCCCAATAAATC
ARSFL_10	GCGTCAGCCGTAGTGATGTAGCAG	GCGCCAGCCCCTCAAATATC
ARSFL_11	GCGAAGCATAACTGGCAGTATCTG	GCGGGCCTAGGTGATCTTGGA
ARSFL_17	GCGCATCACAATCGCCATAGAAAC	GCGAACACGCCTTCAACAACCAC
FAC-004d	GCCAAT GTTCGATGTTTCACTA	TCCTTGGGTCGATCACATAAAT
FAC- 007	GACGGACCGACACTAAACTTTG	CTAGCTGACCTCATTGCTCTGT
FAC-006a	ACTGGTGGAGGAGAGGACTGTA	TGTGGAGCAGAGAGAATTGAAG
FAC-013	TGTTTGAAAAGTGCT GGAC	GATATCAATATCAATACTAGATAACAG
ARSFL_23	GCGGCCGCTTGAAGAGGAG	GCGTCCCCACTGTCAAGGTAAAGA

The nomenclature of the variants was done in such a way that the name has given the maximum information generated about the variant. The first letter of the name represented the name of the cultivar such as 'C' for Chandler. The second letter denoted by the mode of obtaining the variant like 'Sc' for Somaclones derived from callus culture, and 'Mu' for *in vitro* mutagenesis. The number written in the subscript in case of callus cultures indicates the number of sub-culturing and in irradiation induced variants at the irradiation dosage.

During the months between October and November planting season, 60 selected somaclonal variants were cultivated along with the parental variety Chandler in a randomized block design with three replications. The plot size was 2 x 2 m having three rows per treatment. The row length was 2 m and distance between two rows was 0.5 m. Periodical observations were recorded on quantitative characters including plant height, leaf area, petiole length, duration of flowering, numbers of leaves, flowers, fruits, fruit size and weight and yield as well as qualitative traits like TSS, sugars (total sugars, reducing and non reducing), acidity and ascorbic acid of strawberry variants using standard methods.

Data were analyzed according to Completely Randomized Block Design as described by Panse and Sukhatme (2000). Data scored on percentage wherever necessary were subjected to arc sine transformation for the analysis of variance. The banding pattern and different allele sizes generated by SSR markers was recorded using 100 bp DNA ladder as a reference. The molecular data were scored as per allelic format as required for variant analysis with DARwin5 software (Perrier and Jacquemoud-Coleet 2006). Data were also used for the determination of dissimarlity coefficient.

Results and Discussion

Results of the field performance of the somaclonal variants with respect to the vegetative parameters are presented in Table 2. The data depicted that maximum height per plant (9.78 cm) was obtained in somaclone (Sc_{IIIa}) followed by irradiated plant with 30 Gy ($Mu_{30)}$ (9.24 cm). Maximum number of leaves per plant (9.32) was obtained in Sc_{IIIa} followed by mutant 10 Gy plants (Mu_{10}) (8.48). The minimum number of leaves (4.47) was recorded in Mutant 60 Gy plants (Mu_{60}). These results are in close conformity with the findings of Biswas *et al.* (2009) who reported that regeneration from callus culture among different somaclones showed more number of flowers, more duration of flowering and more fruit weight in strawberry. Similarly, maximum leaf area (18.51 cm^2) was obtained in Sc_{IIIa}, followed by Sc_{IIIb}(18.21) and Mu_{10}(15.89). However, it was minimum (8.88 cm^2) in plants irradiated with 60 Gy (Mu_{60}).In the present studies , the Mu_{30} plantlets also exhibited maximum petiole length (7.84 cm) followed by Sc_{IIIb} (7.72cm) andSc_{IIIa}(7.620cm).

Table 2. Plant height, number of leaves, leaf area and length of petiole of field grown variants of strawberry cv. Chandler.

Variant	Height/ plant (cm)	Number of leaves/ plant	Leaf area (cm^2)	Length/ petiole (cm)
Somaclone (ScIIIa)	9.78^{c}	9.32^{d}	18.51^{c}	7.62^{b}
Somaclone (ScIIIb)	9.12^{bc}	7.67^{bcd}	18.21^{c}	7.72^{b}
Mutant 10 Gy (Mu10)	6.35^{ab}	8.48^{cd}	15.89^{bc}	4.89^{ab}
Mutant 30 Gy (Mu30)	9.24^{bc}	5.14^{ab}	11.54^{abc}	7.84^{b}
Mutant 50 Gy (Mu50)	5.78^{a}	5.28^{ab}	9.44^{ab}	3.62^{a}
Mutant 60 Gy (Mu60)	4.92^{a}	4.47^{a}	8.88^{a}	2.62^{a}
Control	7.85^{abc}	5.93^{abc}	15.38^{abc}	5.00^{ab}
SE (±)	1.41	1.38	2.97	1.57
LSD ($p \leq 0.05$)	0.18	0.46	0.26	0.16

The values represent standard error of mean (±SE) of ten plants per replicate (n=70). The values followed by the same letters within each column are not significantly different from each other according to Duncan's Multiple Range Test (DMRT, $p \leq 0.05$ growth characters).

Data on field performance of the somaclonal variants of strawberry with respect to the reproductive characteristics presented in Table 3 showed maximum duration of flowering (75 days) and the number of flowers per plant (25.63) were found in the somaclones variants, Sc_{IIIa}. Whereas, it was minimum (12.30) in irradiated plant with Mu_{60}.This finding is in agreement with findings of Karim *et al*. (2015) who observed somaclonal variations through callus culture in different morphological characters in microprogated strawberry plantlets. However, maximum number (15.23) of fruits per plant, fruit weight (15.76 g), fruit size (836.78 mm^2) and yield per plant (236.40 g) was obtained in somaclones, Sc_{IIIb} with minimum respective observation in Mu_{60} somaclone variants. These results correspond to *in vitro* mutagenesis which has shown that the lower dose of gamma irradiation resulted in better horticultural traits compared to higher dose in mandarin (Sutarto *et al*. 2009).

Table 3. Performance of the variants of strawberry cv. Chandler with respect to the duration of flowering, number of flowers, number of fruits, fruit weight, fruit size and yield.

Variant	Duration of flowering (days)	No. of flowers/plant	Number of fruits/plant	Weight/ fruit (g)	Fruit size (mm^2)	Yield/ plant (g)
Somaclone (Sc_{IIIa})	75.00^{c}	25.63^{c}	14.32^{b}	15.08^{b}	836.51^{c}	211.20^{bc}
Somaclone (Sc_{IIIb})	72.66^{c}	24.74^{c}	15.23^{b}	15.76^{b}	836.78^{c}	236.22^{c}
Mutant 10 Gy (Mu_{10})	72.00c	22.17^{bc}	11.05^{ab}	12.60^{ab}	686.00^{abc}	138.18^{abc}
Mutant 30 Gy (Mu_{30})	64.66^{bc}	17.17^{abc}	9.02^{ab}	11.66^{ab}	542.43^{ab}	104.61^{ab}
Mutant 50 Gy (Mu_{50})	50.00^{ab}	14.30^{ab}	6.07^{a}	9.57^{a}	488.35^{a}	57.29^{a}
Mutant 60 Gy (Mu_{60})	41.66^{a}	12.30^{a}	5.10^{a}	8.17^{a}	436.13^{a}	40.47^{a}
Control	71.33^{c}	18.22^{abc}	9.33^{ab}	14.49^{b}	772.62^{bc}	135.50^{abc}
SE (±)	9.60	3.79	2.84	2.12	124.37	54.01
LSD ($p \leq 0.05$)	1.49	0.81	0.24	0.16	0.17	0.46

The values represent standard error of mean (±SE) of ten plants per replicate (n=70). The values followed by the same letters within each column are not significantly different from each other according to DMRT, $p \leq 0.05$growth characters.

Biochemical characteristics showed no significant difference among the field grown plants (control) and variants (Table 4). This finding is in close conformity with the findings of El-Banna*et al*. (2015) who also reported no significant differences in total soluble solids and acid content in somaclones and parent in tomato. Cho and Song (2000) and Murti *et al*. (2013) observed that gamma irradiation did not induce significant loss in water soluble components such as total soluble solids, proteins, mineral content, nitrogenous constituents and total sugars content. Further, as a large number of genetic changes are based on point mutations or chromosome rearrangements. Although methods for selecting somaclones in numerous horticultural crops resistant to a variety of biotic and abiotic challenges have been developed, however, there are no *in vitro* selection procedures for complicated traits such as soluble solids, sweetness, texture, or shelf life (Evans 1989).

The frequency of occurrences of variation in the somaclone and the irradiated plants was studied. The higher occurrence of variation(3.22%) was obtained in somaclone compared to irradiated plants (2.75%). Similar results were reported by Gavazzi *et al* (1987) who also recorded somaclonal variation at a higher frequency than *in vitro* mutagenesis (Table 5).

Table 4. Evaluation of the variants with respect to TSS (°B), acidity(%) reducing sugars (%), non-reducing sugars (%) and total sugars (%) of strawberry fruits

Variant	TSS (°B)	Acidity (%)	Ascorbic acid (mg/100 g fruit)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)
Somaclone (Sc_{IIIa})	6.92^{c}	0.69^{ab}	53.66^{d}	3.36^{b}	1.04^{b}	4.47^{b}
Somaclone (Sc_{IIIb})	6.94^{c}	0.67^{a}	53.56^{cd}	3.36^{b}	1.04^{b}	4.47^{b}
Mutant 10 Gy (Mu_{10})	6.51^{abc}	0.70^{ab}	53.32^{ab}	3.31^{ab}	1.07^{c}	4.45^{b}
Mutant 30 Gy (Mu_{30})	6.33^{ab}	0.72^{bc}	53.44^{abcd}	3.27^{ab}	1.03^{ab}	4.36^{ab}
Mutant 50 Gy (Mu_{50})	6.37^{ab}	0.73^{bc}	53.42^{abc}	3.20^{a}	1.03^{ab}	4.29^{a}
Mutant 60 Gy (Mu_{60})	6.22^{a}	0.75^{c}	53.26^{a}	3.19^{a}	1.01^{a}	4.26^{a}
Control	6.78^{bc}	0.69^{ab}	53.53^{bcd}	3.38^{b}	1.07^{c}	4.51^{b}
SE (±)	0.22	0.02	0.10	0.06	0.01	0.07
LSD ($p \leq 0.05$)	0.33	0.01	0.06	0.04	0.03	NS

NS, non-significant. The values represent standard error of mean (±SE) of ten plants per replicate (n = 70). The values followed by the same letters within each column are not significantly different from each other according to DMRT, $p \leq 0.05$.

Table 5. Frequency towards morphological traits of somaclones and irradiated plants of strawberry

Treatment	Number of plants	Number of variants	Frequency (%)*
Somaclone	62	2	3.22
Irradiation (dose)			
10 Gy	42	1	2.38
30 Gy	37	1	2.70
50 Gy	34	1	2.94
60 Gy	32	1	3.12
Total	145	4	2.75

*Number of variants divided by total number.

After morphological screening of callus regenerated (somaclone) plantlets and irradiated plantlets (mutants), six variants were obtained out of which two variants were callus regenerated and four irradiated plantlets. The variant plants were characterized by using SSR markers. Out of 23 SSR markers, only six SSR markers were polymorphic. The difference in the banding pattern confirms the molecular difference of variants from field grown plant(Table 6).

Dissimilarity index was calculated considering the bands obtained by using six SSR primers showing polyphormism. Dissimilarity values were found to range from 0.16 to 0.83, the highest value was observed between somaclones ($Sc_{IIIa}$and Sc_{IIIb}) and field grown plant (control) and the least values of dissimilarity were observed between the mutant 10 Gy and field grown plant (control). The dissimilarity matrix was subjected to cluster analysis using UPGMA method producing a dendrogram (Fig. 1). Three main clusters were formed, the first cluster have two sub cluster containing Mu_{50} and $Mu_{60,}$ while, the second sub-cluster contain two somaclones (Sc_{IIIa} and Sc_{IIIb}). The second cluster consists of Mu_{10} and field grown plant (control). The Mu_{30} stands

distinctively from the rest of the plants and formed the third cluster. Mega (2009) also used SSR markers to detect the variation among gamma rays treated plants in banana. Palombi and Damiano (2002) also detected genetic variation in micro propagated plants of kiwifruit cv. Tomuri through SSR markers.

Table 6. SSR markers amplified products from field grown plants (control) and variants of strawberry.

Primer	Control (bp)	$Sc_{(III)a}$ (bp)	$Sc_{(III)b}$ (bp)	$Mu_{(10)}$ (bp)	$Mu_{(30)}$ (bp)	$Mu_{(50)}$ (bp)	$Mu_{(60)}$ (bp)
ARSFL_99	230	240	240	230	240	230	240
ARSFL_10	250	260	260	250	240	230	230
FAC-011	290	292	292	294	294	280	280
FAC-004b	280	282	282	280	274	270	270
FAC-005b	210	210	210	210	215	215	210
FAC-014	250	264	264	250	250	264	264

bp, base pair.

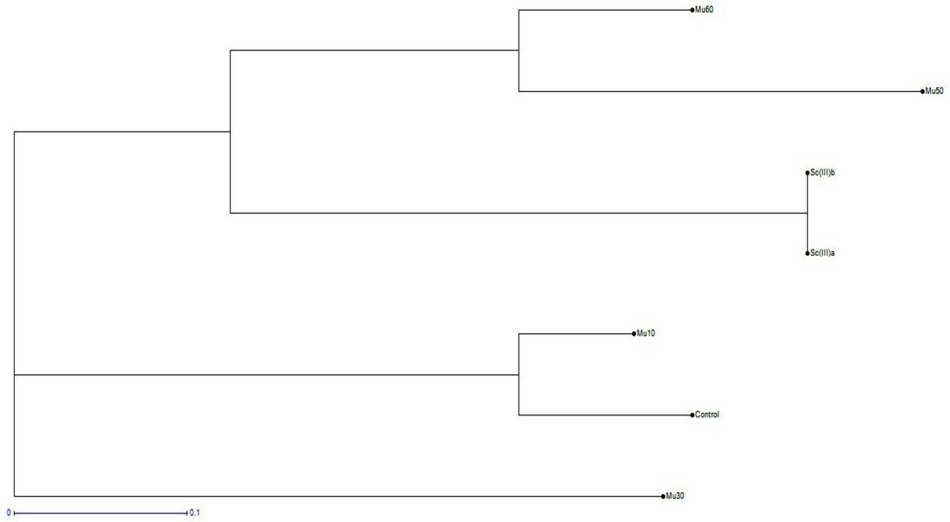

Fig. 1. Dendrogram of variants and cluster analysis using DARwin software.

From the present studyit may be concluded that the usefulness of SSR markers in the molecular differentiation of the individual variants and field grown plant through the amount of polymorphism is obtained from SSR analysis. The somaclones and irradiated plantlets under field conditions for various morphological characters revealed that there is drastic reduction with increase in irradiation doses without showing any significant changes in the bio-chemical characters. The leaf derived callus regenerated plants and gamma rays irradiated plants consisting of 10 and 30 Gy were found to induce better vegetative and reproductive characters in strawberry cv. Chandler. Both somaclonal variation as well as conventional mutagenesis is complimentary to each other to create variability for improvement through selection of variants for improved traits of strawberry.

References

AOAC 1995.Official Methods of Analysis of Association of Official Analytical Chemists. International Association of Official Analytical Chemists, Gaithersburg, USA.16th edn.

Biswas MK, Dutt M, Roy UK, Islam, R and Hossain M 2009.Development and evaluation of in vitro somaclonal variation in strawberry for improved horticultural traits.ScientiaHortic. **122**(3): 409-416.

Cho Y and Song KB 2000.Effect of γ-irradiation on the molecular properties of BSA and β-lactoglobulin. J. Steroid Biochem. Mole. Bio. **33**:133-137.

Dale A and Sjulin T M 1990. Few cytoplasm contribute to North American strawberry cultivars. Hort. Sci. **25**: 1341-1342.

Doyle JJ and Doyle JL 1990.Isolation of plant DNA from fresh tissue. Focus **12**(1): 13-15.

Evans.D.A. 1989.Somaclonal variation—genetic basis and breeding applications. Trends Genet. 5:46-50.

El-Banna AN, Dora SA, Aboshosha AA and El-Morsy NA 2015. Horticultural and Genetical Characteristics of Tomato Somaclones under Salt and Heat Stresses.Int. J. Curr. Res. Bios.Pl. Bio. **2**(**4**): 128-142.

Gavazzi G, Tonelli C, Todesco G, Arreghini E, Raffaldi F, Vecchi F, Barbuzzi G, Biasini MG and Sala F 1987. Somaclonal variation versus chemically induced mutagenesis in *tomato* (*Lycopersicon esculentum* L.). Theor. Appl. Gen. **74**: 733-738 .

Jain SM 2001.Tissue culture-derived variation in crop improvement.Euphytica.**118**: 153-1662.

Jain SM 2012.*In vitro* mutagenesis for improving date palm (*Phoenix dactylifera* L.). Emirates J. Food Agri. **24**(**5**): 386-399.

Jung C, Capistrano-Gossmann G, Braatz J, Sashidhar N andMelzer S 2017.Recent developments in genome editing and applications in plant breeding. Pl. Breed. **137**: 1–9.

Karim R, Ahmed F, Krishna U, Roy, AT, Islam R and Hossain M 2015.Varietal improvement of strawberry (*Fragaria* x *ananassa* Duch.) through somaclonal variation using *in vitro* techniques. J. Agri. Sci. Tech. 17: 977-986.

Krishna H, Alizadeh M, Singh D, Singh U, Chauhan N, Eftekhari M and Sadh RK 2016. Somaclonal variations and their applications in horticultural crops improvement.Biotech.**6**: 54.

Larkin PS and Scrowcroft WR 1981.Somaclonal variation – a novel source of variability from the cell cultures for plant improvement. Theor. Appl. Gen. **60**: 197-214.

Mega S 2009.*In vitro* Induction of Genetic Variation through Gamma Irradiation in Banana (*Musa* spp.). M.Sc. Thesis. Submitted to Punjab Agricultural University, Ludhiana.56-58.

Murti HR, Kim HY and Yeoung YR 2013.Effectiveness of gamma ray irradiation and ethyl methane sulphonate on in vitro mutagenesis of strawberry. African J. Biotech. **12**(30): 4803-4812.

Palombi MA and Damiano C 2002.Comparison between RAPD and SSR molecular markers in detecting genetic variation in kiwifruit (*Actinidia deliciosa* A Chev).Pl. Cell Reports. **20**: 1061-1066.

Panse VG and Sukhatme PV 2000. Statistical Methods for Agricultural Workers.Publication and Information Division of ICAR, New Delhi.

Perrier X and Jacquemoud JP 2006.DARwin software. http://www. darwin.cirad.fr/darwin.

Peschke VM and Phillips RL 1992. Genetic implications of somaclonal variation in plants: Adv. Genet. **30**: 41-75.

Phillips RL, Kaeppler SM and Olhoft P 1994. Genetic instability of plant tissue cultures: breakdown of normal controls. Proc. Natl. Acad. Sci. **91**(12): 5222-5226.

Podwyszynska M, Kuras A andKorbin M 2010.Somaclonal variation in micropropagated tulips as a source of novel genotypes-field and molecular characteristic. ActaHortic.**855**: 225–232.

Rai MK, Kaliaa RK, Singh R, Gangola MP and Dhawana AK 2011.Developing stress tolerant plants through in vitro selection - An overview of the recent progress. Environ. Exp. Bot. **71**: 89-98.

Sharma HC, Sharma KK, Seetharama N and Ortiz R 2000.Prospects for using transgenic resistance to insects in crop improvement. Elec. J. Biotech. **3**(2): 76-95.

Sutarto I, Agisimanto D and Supriyanto A 2009. Development of Promising Seedless Citrus Mutants through Gamma Irradiation. Q.Y. Shu (ed.), Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations, Rome.306-308.

Suprasanna P, Jain SM, Ochatt SJ, Kulkarni, VM and Predieri S 2012. Applications of in vitro techniques in mutation breeding of vegetatively propagated crops'. *In:* Shu, Q.Y., Forster, B.P. and Nakagawa, H. (eds.) Plant mutation breeding and biotechnology. CABI Publishing, Wallingford, UK.371-385.

(Manuscript received on 20 May, 2021; revised on 27 August, 2022)