The Bioscan 10(1): 187-191, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECT OF CONCENTRATION AND APPLICATION DURATION OF EMS ON *IN VITRO* ROOT FORMATION OF CAMAROSA STRAWBERRY (*FRAGARIA X ANANASSA*)

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KEYWORDS
Camarosa
EMS
Fragaria chiloensis x *F. Virginiana*
In vitro
Micropropagation
Root formation

Received on :
05.12.2014

Accepted on :
17.02.2015

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ABSTRACT
In the present investigation, experiment was conducted to create variability through induced mutations in different explants of strawberry cv. Camarosa. Different EMS concentrations (0.1 %, 0.2%, 0.3% and 0.4%) along with control for various treatment durations (1.5 hr, 2.5 hr and 3.5 hr) used for the induction of variation. The concentration 0.4% was found lethal to the plants. Among different concentrations of EMS tried, 0.1% was found best followed by 0.2% and 0.3%. Among different durations treated, 1.5 hr was found best followed by 2.5 hr and 3.5 hr. While among various explants used, the runner tips explants was found best followed by shoot tips, leaf disc (abaxial) and leaf disc (adaxial). The EMS dose of 0.1% applied for 1.5 hr on runner tip explants was found best combination among all treatments in terms of *in vitro* root growth parameters recorded *viz.,* initiate early roots (45.3 days), maximum number of roots (32.5) and maximum length of roots (55.5 mm). As the increased EMS concentrations along with treatment duration there was a gradual decrease in growth and development of plants. In future, these experimental results will prove very useful for induction of variability in this fruit crops.

INTRODUCTION

The strawberry (*Fragaria xananassa* Duch.) belongs to sub family Rosoideae and family Rosaceae and it is a regular part of the diets of millions of people, known for its delicate flavour and rich vitamin content (Sharma, 2002). Among the fruits, strawberry gives the quickest returns in the shortest possible span. In India, strawberry cultivation extends from temperate to subtropical regions. Maharashtra is the leading state in the production of strawberry. It is also cultivated in Uttar Pradesh, Uttrakahand, Jammu and Kashmir, hills of Darjeeling (West Bengal), Himachal Pradesh (Sharma and Budiyala, 1980) and Haryana.

Genetic improvement of strawberry by conventional plant breeding methods has been limited somatic background because it is composed by a few nuclear and cytoplasmic germplasm (Dale and Sjulin, 1990). Mutation is the only way to induce variability within short span of time. In addition, mutation breeding combined with tissue culture has proved more effective rather than the conventional breeding and increases the efficiency of mutagenic treatments for variation induction (Predieri, 2001). Induced mutations have played an important role in crop improvement, which hardly needs any emphasis with considerable practical results obtained in many crops. In addition to the primary objective of obtaining useful mutants of direct value, mutation breeding has other advantages, mainly in the improvement of a specific character in a well-adapted and highly desirable variety, in breaking tight linkages thus helping in obtaining rare recombinants and in enlarging variability for quantitative characters.

Application of mutagen was intended to increase genetic variation and can change only one or a few specific traits of an elite cultivar that can contribute to fruit improvement (Predieri, 2001). In fruit crops, mutagenesis has already been used to introduce many useful traits affecting plant size, blooming time and fruit ripening, fruit colour, better quality, self compatibility, self-thinning, and resistance to pathogens (Maluszynski *et al.*, 1995, Kaushal *et al.*, 2004). In strawberry, wide range (5-800 Gy) of gamma rays has been applied by researchers in different plant materials such as anther calli (Kasumi, 2002), calli of leaves (Kaushal *et al.*, 2004), axillary bud (Jain, 1997) and runner (Weimin *et al.*, 2009).Treatment of EMS in high concentrations as well as the combined treatment of both the mutagens radiation and EMS was effective in increasing the variability in rice (Chakravarti *et al.,* 2012) and fatty acid content in soybean oil (Patil *et al.*, 2007) and

produced homozygous M_2 plants carrying nonsense alleles of NtabCYP82E4 that coded very low or near-null nornicotine (Julio *et al.*, 2008). Various changes in flower colour and shape were recorded after treatment by EMS in the form of chimeras in chrysanthemum (Kumari *et al.,* 2013).

Very little work has been done for creating variability in important strawberry cultivars being grown under North Indian conditions through chemical mutagens under *in vitro* conditions. Therefore, the present study was planned to create variability through mutation in strawberry with the objective of effect of Ethyl methane sulphonate (EMS) concentration and application duration on *in vitro* root parameters of strawberry cv. Camarosa.

MATERIALS AND METHODS

The present study on "*in vitro* induced mutations in strawberry" was conducted in the Department of Horticulture and Plant Tissue Culture Laboratory of the Centre for Plant Biotechnology, Government of Haryana, CCS HAU Campus, Hisar during 2013 to 2014 growth seasons. The strawberry cultivar Camarosaplants were selected for the present investigation as a source of explants.

The following explants were used for this investigation:

Runner tip

The runner tips measuring about 0.5 cm long were cut from healthy runners and used as explants.

Leaf disc

Healthy andmature leaves were made into sections of 2 cm and used for inoculation. Abaxial and adaxial orientation of leaf disc was used for experimentation.

Shoot tip

Healthy shoot tips of 0.5 to 1 cm were used as explants.

The explants were collected in clean polythene bags and brought to the laboratory. They were cut into convenient sizes and rinsed thoroughly in forcefully running tap water for 10 minutes. The nodal explants were surface sterilized with 3-4 drops of teepol for 10 minutes, citric acid (0.4%) and ascorbic acid (0.2%) for browning for 10 minutes, bavistin (0.4%) and streptocycline (0.3-0.4%) for 90 minutes respectively for removal of any systemic contamination. Freshly prepared chemical mutagen, Ethyl Methane Sulphonate (EMS) was used for the induction of variation. Explant was subjected to different EMS concentrations (0.1 %, 0.2%, 0.3% and 0.4%) along with control (Buffer solution, Sodium Dihydrogen Phosphate and Disodium Hydrogen Phosphate)for various treatment durations (1.5 hr, 2.5 hr and 3.5 hr) at room temperature ($27 \pm 2^{o}C$). After the mutagen treatment, the plant material was thoroughly washed in several changes of sterile distilled water. Finally, the explants were surface sterilized with mercuric chloride (0.1%) for 5 minutes inside the laminar flow cabinet. The sterilant was then washed off by rinsing in five to six changes of sterile double distilled water and cultured on MS basal medium fortified with BAP (2 mg/L).

The experimental design was Completely Randomized Design including 5 treatments, 3 durations and 3 replications (Factorial CRD). All data were subjected to OPSTAT software for analysis of variance.

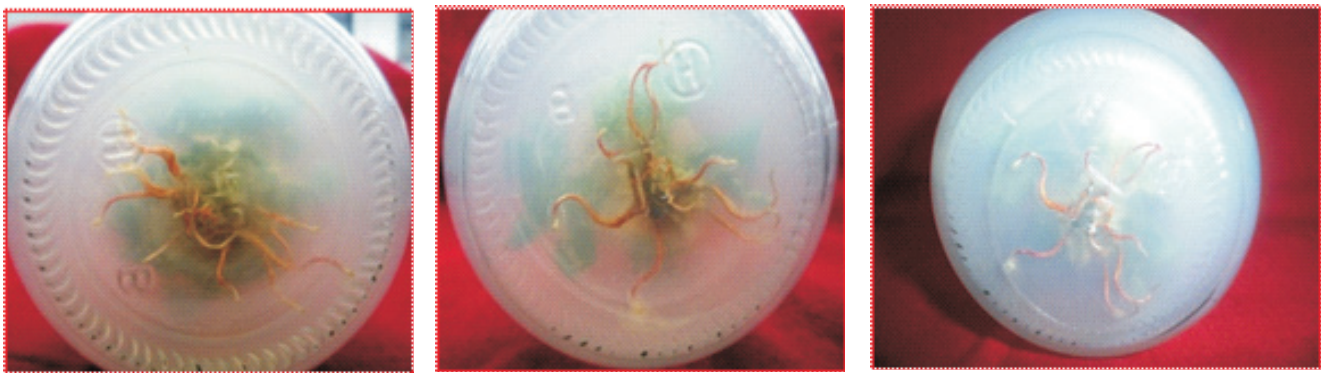
Table 1: Effect of concentration and application duration on days taken to root initiation in different explants of strawberry cv. Camarosa

EMS concentration with duration	Explants				Mean
	Runner tip	Shoot tip	Leaf disc (Abaxial)	Leaf disc(Adaxial)	
EMS 0.1 %					
1.5 hr	45.3	47.2	48.2	50.1	47.7
2.5 hr	47.3	48.3	49.3	52.3	49.3
3.5 hr	48.3	49.3	50.3	54.3	50.6
Mean	47.0	48.3	49.3	52.2	49.2
EMS 0.2%					
1.5 hr	47.3	48.3	49.3	51.3	49.1
2.5 hr	48.3	49.3	50.3	54.3	50.6
3.5 hr	49.3	50.3	51.3	55.3	51.6
Mean	48.3	49.3	50.3	53.6	50.4
EMS 0.3%					
1.5 hr	48.3	49.3	50.3	53.3	50.3
2.5 hr	49.3	50.3	51.3	56.3	51.8
3.5 hr	51.3	52.3	52.3	57.3	53.3
Mean	49.6	50.6	51.3	55.6	51.8
Control					
1.5 hr	50.3	50.1	50.8	52.1	50.8
2.5 hr	50.3	50.5	51.2	52.3	51.1
3.5 hr	50.1	50.3	51.3	52.3	51.0
Mean	50.2	50.3	51.1	52.2	50.6
Mean for treatment duration					
1.5 hr	47.6	48.6	49.6	51.6	49.4
2.5 hr	48.8	49.5	50.5	53.8	50.7
3.5 hr	50.0	50.7	51.3	55.0	51.7
General mean	48.8	49.6	50.5	53.4	50.6
CD for factor A* = 0.28 factor B* = 0.24 factor C* = 0.28					
A x B =0.49 A x C = 0.57 B x C = 0.49 A x B x C = NS					

* Factor A = Concentrations, Factor B = Durations, Factor C = Explants, NS = not significant

RESULTS AND DISCUSSION

The days taken to root initiation were affected significantly due to concentrations of EMS, duration of EMS treatment and the explants used with the orientation. The two factor interactions between concentration and treatment duration, concentration and explants, the treatment duration and explants were found significant but three factors interaction was absent. From data shown in Table 1 it could be inferred that EMS concentration of 0.1% initiated roots early followed by 0.2% and most late with EMS 0.3%. Among different durations of EMS treatment, the root initiation was early in 1.5 hr duration followed by 2.5 hr and was delayed in 3.5 hr. The runner tip explant took minimum days for root initiation followed by shoot tip and leaf disc (abaxial) and delayed most by leaf disc (adaxial) explant. Thus, runner tip explant treated with EMS concentration 0.1% for duration 1.5 hr initiated roots early (45.3 days) and was best among all the treatments. The root initiation increased gradually with increasing concentration level of EMS and its treatment duration. The variation in the number of days to root initiation might be due to the EMS concentration, treatment duration and also genetic makeup of the explants. These results are in conformity with Dhakshanamoorthy *et al.* (2010); Giriraj *et al.* (1990) and



Runner tip **Shoot tip leaf** **disc (abaxial)**

Figure 1: Development of roots *in vitro* from the plants regenerated from the explants treated with EMS 0.1% for 1.5 hr duration

Table 2: Effect of EMS concentration and application duration on number of roots produced/plant in different explants of strawberry cv. Camarosa

EMS concentration with duration	Explants				Mean
	Runner tip	Shoot tip	Leaf disc (Abaxial)	Leaf disc(Adaxial)	
EMS 0.1 %					
1.5 hr	32.5	30.5	28.5	25.5	29.3
2.5 hr	30.3	29.3	26.3	24.3	27.6
3.5 hr	28.3	28.3	25.3	23.3	26.3
Mean	30.4	29.4	26.7	24.4	27.7
EMS 0.2%					
1.5 hr	30.3	28.3	26.3	23.3	27.1
2.5 hr	28.3	27.4	24.3	22.3	25.6
3.5 hr	26.4	26.3	22.3	20.3	23.8
Mean	28.3	27.3	24.3	22.0	25.5
EMS 0.3%					
1.5 hr	27.5	25.3	24.5	20.3	24.4
2.5 hr	25.3	24.3	22.3	19.3	22.8
3.5 hr	23.3	23.3	20.3	19.0	21.5
Mean	25.4	24.3	22.4	19.5	22.9
Control					
1.5 hr	28.5	27.7	25.5	26.4	27.0
2.5 hr	28.3	27.3	25.4	25.4	26.6
3.5 hr	28.3	27.6	25.3	24.7	26.5
Mean	28.4	27.5	25.4	25.5	25.7
Mean for treatment duration					
1.5 hr	29.6	27.8	26.3	23.8	26.9
2.5 hr	27.9	26.6	24.4	22.6	25.4
3.5 hr	26.4	26.1	23.1	21.4	24.2
General mean	28.1	27.1	24.7	22.9	25.7
CD for factor A* = 0.28 factor B* = 0.25 factor C* = 0.28					
A x B = 0.5 A x C = 0.57 B x C = 0.5 A x B x C = NS					

* Factor A = Concentrations, Factor B = Durations, Factor C = Explants, NS = not significant

Table 3: Effect of EMS concentration and application duration on length of roots (mm) in different explants of strawberry cv. Camarosa

EMS concentration with duration	Explants Runner tip	Shoot tip	Leaf disc (Abaxial)	Leaf disc(Adaxial)	Mean
EMS 0.1 %					
1.5 hr	55.5	50.5	50.5	45.5	50.5
2.5 hr	50.3	50.3	40.3	40.3	45.3
3.5 hr	45.3	45.3	40.3	30.3	40.3
Mean	50.4	48.7	43.7	38.7	45.4
EMS 0.2%					
1.5 hr	50.3	45.3	40.3	35.5	42.9
2.5 hr	50.3	40.3	40.3	30.3	40.3
3.5 hr	40.3	40.3	35.3	30.3	36.6
Mean	47.0	42.0	38.6	32.0	39.9
EMS 0.3%					
1.5 hr	45.3	40.4	35.3	30.3	37.8
2.5 hr	45.3	40.4	35.3	20.3	35.3
3.5 hr	35.3	35.2	30.3	15.3	29.0
Mean	42.0	38.7	33.6	22.0	34.1
Control					
1.5 hr	50.4	50.6	45.5	40.5	46.8
2.5 hr	50.4	50.4	45.3	40.3	46.6
3.5 hr	50.3	50.3	45.3	38.6	46.1
Mean	50.4	50.4	45.4	39.8	46.5
Mean for treatment duration					
1.5 hr	50.5	46.7	43.0	38.0	44.5
2.5 hr	49.2	46.7	40.5	33.0	42.3
3.5 hr	42.6	42.6	37.6	27.6	37.6
General mean	47.5	45.0	40.3	33.1	41.5
CD for factor A* = 0.31 factor B* = 0.27 factor C* = 0.31 A x B = 0.54 A x C = 0.62 B x C = 0.54 A x B x C = 1.08					

* Factor A = Concentrations, Factor B = Durations, Factor C = Explants

Runner tip **Shoot tip** **Leaf disc (abaxial)**

Figure 2: Development of roots from the plants regenerated from the explants treated with EMS 0.1% for 1.5 hr duration

Jayakumar and Selvaraj (2003).

Number of roots produced per plant differed significantly due to concentrations of EMS, duration of EMS treatment and the explants used with the orientation. Their all two factor interactions were found significant while, three factors interaction was not present (Table 2). A perusal of data presented here indicated that maximum number of roots was produced from the EMS concentration 0.1 % followed by 0.2% and the lowest from 0.3%. The 1.5 hr duration produced maximum number of roots followed by 2.5 hr and the lowest number of roots was produced in 3.5 hr. The runner tip explant produced highest number of roots followed by shoot tip and leaf disc (abaxial) and the lowest number of roots was produced by leaf disc (adaxial) explant. Concludingly, the runner tip explant treated with EMS concentration 0.1% for duration 1.5 hr produced highest (32.5) number of roots and was found best among all the treatments (Figure 2). With the increased EMS concentration along with duration, a gradual decrease in the number of roots produced per plant was observed. The stimulatory effect at a lower dose might be due to the fact that mutagens at lower concentrations stimulated the role of enzyme and growth hormone responsible for growth and yield, while the inhibitory effect was due to the fact that biological damage increased at a faster rate in higher concentrations of

mutagens. These results are in conformity with Dhakshanamoorthy *et al*. (2010); Giriraj *et al*. (1990) and Jayakumar and Selvaraj (2003).

Significant differences in length of the roots due to concentrations of EMS, duration of EMS treatment and the explants used with the orientation were observed (Table 3). The pair-wise and three way interactions among these factors were found significant. The data given in Table 3 showed that among the different EMS concentrations tried, the concentration 0.1 % produced maximum root length followed by 0.2% and the minimum root length was produced in 0.3%. The 1.5 hr duration produced maximum root length followed by 2.5 hr and the minimum root length was produced in 3.5 hr. Among different explants used, the runner tip explant produced maximum root length followed by shoot tip and leaf disc (abaxial) and the minimum root length was produced by leaf disc (adaxial) explant. The runner tip explant treated for 1.5 hr duration with EMS concentration 0.1% produced maximum (55.5 mm) root length and was found best among all the treatments. The root length decreased gradually increased with EMS concentration and treatment duration. As the increase in concentration and treatment duration there was a gradual decrease in length of the roots. This might be due to the EMS concentration, treatment duration and also genetic makeup of the explants.

REFERENCES

Chakravarti, S. K. R., Kumar, H. Lal, J. P. and Vishwakarma, M. K. 2012. Induced mutation in traditional aromatic rice-frequency and spectrum of viable mutations and characterizations of economic values. *The Bioscan*. **7(4):** 739-742.

Dhakshammoorthy, D., Selvaraj, R. and Chidambaram, A. 2010. Physical and chemical mutagenesis in *Jatrophacurcas* L. To induce variability in seed germination, growth and yield traits, *Rom. J. Biol. Plant Biol*. **55:** 113-125.

Dale, A. and Sjulin, T. M. 1990. Few cytoplasms contribute to north american strawberry cultivars. *HortScience*. **25:** 1341-1342.

Giriraj K., S. R. Hiremath, and Seetharama 1990. Induced variability for flowering, seed weight and oil content in parental lines of sunflower hybrid BS-1. *Indian J. Genetics*. **50:** 1-7.

Jain, S. M. 1997. Creation of variability by mutation and tissue culture for improving plants. *Acta Horticulture*. **447:** 69-77.

Jayakumar, S. and Selvaraj, R. 2003. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in sunflower. *Madras Agriculture J.* **90(7-9):** 574-576.

Julio, E., Laporte, F., Reis, S., Rothan, C. and De Borne, F. D. 2008. Reducing the content of nicotine in tobacco via targeted mutation breeding. *Molecular Breeding*. **21:** 369-381.

Kasumi, M. 2002. The effect of gamma-ray irradiation to strawberry (*Fragaria xananassa* Duch.) calli on shoot regeneration achene formation and morphological variation of regenerant. *J. the Japanese Society for Horticultural Science*. **71(3):** 419-423.

Kaushal, K., Nath, A. K., Kaundal, P. and Sharma, D. R. 2004. Studies on somaclonal variation in strawberry (*Fragaria x ananassa*Duch.) cultivars. *Horticulture*. **662:** 269-275.

Kumari, K., Dhatt, K. K. and Kapoor, M. 2013. Induced mutagenesis in *chrysanthemum morifolium* variety 'Otome Pink' through gamma irradiation. *The Bioscan*. **8(4):** 1489-1492.

Maluszynski, M., Ahloowalia, B. S. and Sigurbjornsson, B. 1995. Application of *in vivo* and *in vitro* mutation techniques for crop improvement. *Euphytica*. **85:** 303-315.

Predieri, S. 2001. Mutation induction and tissue culture in improving fruits. *Plant Cell Tissue andOrgan Culture*. **64:** 185-210.

Patil, A., Taware, S. P., Oak, M. D., Tamhankar, S. A. and Rao, V. S. 2007. Improvement of oil quality in soybean [*Glycine max* (L.)Merrill] by mutation breeding. *J. American Oil Chemists' Society*. **84:** 1117-1124.

Sharma, R. R. 2002. Growing strawberry. Int. Book Distributing Co., India.**1:** 01-02.

Sharma, R. L. and Budiyala, S. A. 1980. A study on performance of some strawberry (*Fragaria× ananassa*) cultivars in the mid-hillregion of North India. *Prog. Hortic*. **15:** 64-68.

Weimin, W., Mizhen, Z., Zhuangei, W., Yaming, Q. andJi, Y. 2009. The study of the irradiation effect on runner plant of strawberry with 60 co-gamma ray. Proc. VI. International Strawberry Symposium. In: J. Lopes-Medina. 2008. Strawberry. *Acta Horticulture*. **842:** 597-600.