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Effect of *In Vitro* Mutagenesis on *In Vivo* Growth Characteristics of Strawberry cv. Camarosa

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A B S T R A C T

The present study was conducted during 2013- 2014 to find out the effect of induced mutation on *in vivo* growth parameters in 'Camarosa' strawberry by following CRD factorial design. For the induction of variation explants (Runner tips, shoot tips and leaf disc) were subjected to different concentrations (0.1%, 0.2%, 0.3% and 0.4%) of EMS along with control for various treatment durations (1.5 hr, 2.5 hr and 3.5 hr). The concentration 0.4% was found lethal to the plants. The runner tip explants treated with EMS concentration 0.1% for duration 1.5 hr. gave maximum plant height (16.8 cm), plant spread (30.8 cm^2), crown height (25.3 mm), crown diameter (16.3 mm), number of leaves per plant (15.5), leaf size (31.1 mm × 30.3 mm) and was found best among all the treatment combinations. While among various explants used, the runner tips explants was found best followed by shoot tips, leaf disc (abaxial) and leaf disc (adaxial). As the increased EMS concentrations along with treatment duration there was a gradual decrease in the *in vivo* growth parameters of strawberry. In future, these experimental results will prove very useful for induction of variability in this fruit crop.

Introduction

The cultivated strawberry (*Fragaria* × *ananassa* Duch.) is one of the most delicious, refreshing and soft fruit of the world. It is the most widely distributed fruit crop in the world due to its genotypic diversity, highly heterozygous nature and broad range of environmental adaptations (Larson, 1994; Childers *et al.*, 1995). In recent past, the strawberry cultivation has been becoming popular in India due to very high returns per unit area in the shortest possible span. In India, the cultivated area under strawberry is nearly 15600 hectare and commercially grown in Himachal Pradesh, Maharashtra, Uttrakahand, Punjab, Haryana, Western Uttar Pradesh and Madhya Pradesh (Anonymous, 2011).

The cultivated strawberry is an octoploid (2n=8x=56) and the genetic background of strawberry was composed by a few nuclear and cytoplasmic germplasm (Dale and Sjulin, 1990).

The complicated genetic background presents a formidable barrier in the improvement of strawberry through conventional breeding methods. 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).

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). Another mutagen, EMS also has been applied in various plants, such as soybean (Patil *et al.,* 2007) tobacco (Julio *et al.,* 2008) and strawberry (Murti *et al.,* 2013).

he 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 vivo* growth parameters of strawberry cv. Camarosa.

Materials and Methods

The present study on "Effect of *in vitro* induced mutation on *in vivo* growth 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 2013-14 growth season. The strawberry cultivar Camarosa plants 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 and mature 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. Explants were 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±2°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.

Results and Discussion

The 0.4% concentration level of mutagen EMS being found lethal to the explants caused their complete mortality and no data could be recorded, hence, this treatment was discarded from the statistical analysis.

Differences in height of established plants on account of EMS concentrations, its treatment duration and the explants used with orientation were significant (Table 1). The interactions of concentration and treatment duration, concentration and explants and three factors of variation, except the treatment duration and explants were found significant. The concentration 0.1% resulted maximum plant height (15.9 cm) and the lowest height (14.7 cm) was note in 0.3%. The 1.5 hr duration of EMS treatment followed by 2.5 hr had tallest plants and lowest plant height was observed in 3.5 hr. The runner tip explant gave tallest plants followed by shoot tip and leaf disc (abaxial) and shortest plants height was observed in leaf disc (adaxial) explant. Thus, runner tip explant treated with EMS concentration 0.1% for duration 1.5 hr had tallest (16.8 cm) plants and was marked best among all the treatments.

The differences in spread of plants due to concentrations of EMS, duration of EMS treatment and the explants used with the orientation were significant (Table 2). The interactions of concentration and treatment duration, concentration and explants, and explants and the treatment duration and all these factors were also found significant. The EMS concentration 0.1% gave widest plant spread the minimum plant spread was observed in 0.3%. The 1.5 hr duration gave maximum plant spread followed by 2.5 hr and the lowest plant spread was observed in 3.5 hr. The runner tip explant gave highest plant spread followed by shoot tip and leaf disc (abaxial) and the lowest plant spread was noticed in leaf disc (adaxial) explant. Concluding that the runner tip explant treated with EMS concentration 0.1% for duration 1.5 hr gave highest (30.8 cm^2) plant spread and was found best among all the treatments.

Crown height in straw berry plants was affected significantly due to concentrations of EMS, duration of its treatment and the explants used with the orientation (Table 3). The two factor interactions between concentration and treatment duration, concentration and explants, and explants and the treatment duration except all the factors of the variation were found significant. A perusal of data given in Table 3 suggested that EMS concentration 0.1% gave maximum crown height and the minimum height was observed in 0.3%. Among different durations of EMS treatment, the 1.5 hr duration resulted largest crown height followed by 2.5 hr and the shortest crowns were observed in 3.5 hr. The explant runner tip produced longest crowns followed by shoot tip and leaf disc (Abaxial) and the lowest crown height was observed in leaf disc (adaxial) explant. The explant runner tip when treated with EMS 0.1% for 1.5 hr gave maximum (25.3 mm) crown height and was found best among all the treatments. The increased EMS concentration and duration decreased the crown height. The difference in the crown height might be due to the interplay of EMS concentration, treatment duration and also the genetic make-up of the explants investigated.

Different explants used with the orientation, when treated with EMS concentrations for various durations, produced wide differences

in crown diameter (Table 4). The two way interactions between concentration and treatment duration, concentration and explants, and explants and treatment duration, and that of all these factors were found significant. Among various EMS concentrations tried, 0.1% level gave greatest crown diameter followed by 0.2% and the smallest crown diameter was observed with 0.3%. The 1.5 hr duration gave largest crown diameter followed by 2.5 hr and the lowest crown diameter was observed from 3.5 hr treatment. The runner tip explant gave largest crown diameter followed by shoot tip and leaf disc (abaxial) and the lowest crown diameter was observed in leaf disc (adaxial) explant. In conclusion, runner tip explant treated with EMS 0.1% for 1.5 hr resulted maximum (16.6 mm) crown diameter and was found best among all the treatments. The increased EMS concentrations along with treatment durations gradually decreased the crown diameter. These differences in crown height might have been caused jointly by the EMS concentration, treatment duration and also genetic make-up of the explant.

Table.1 Effect of EMS concentration and application duration on plant height (cm) 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	16.8	16.5	16.3	15.7	**16.3**
2.5 hr	16.1	16.1	15.8	15.4	**15.9**
3.5 hr	15.7	15.7	15.5	15.0	**15.5**
Mean	**16.2**	**16.1**	**15.9**	**15.4**	**15.9**
EMS 0.2%					
1.5 hr	16.2	16.0	15.7	15.0	**15.7**
2.5 hr	15.9	15.7	15.2	14.7	**15.4**
3.5 hr	15.5	15.2	15.0	14.5	**15.1**
Mean	**15.9**	**15.6**	**15.3**	**14.7**	**15.4**
EMS 0.3%					
1.5 hr	15.8	15.5	14.6	14.2	**15.0**
2.5 hr	15.6	15.3	14.3	13.8	**14.8**
3.5 hr	15.2	15.0	14.0	13.1	**14.3**
Mean	**15.5**	**15.3**	**14.3**	**13.7**	**14.7**
Control					
1.5 hr	15.6	15.3	15.0	14.5	**15.1**
2.5 hr	15.5	15.0	15.0	14.5	**15.0**
3.5 hr	15.2	15.0	15.0	14.3	**14.9**
Mean	**15.4**	**15.1**	**15.0**	**14.4**	**15.0**
Mean for treatment duration					
1.5 hr	16.0	15.8	15.3	14.8	**15.5**
2.5 hr	15.8	15.7	15.2	14.6	**15.3**
3.5 hr	15.5	15.3	14.9	14.4	**15.0**
General mean	**15.8**	**15.5**	**15.1**	**14.6**	**15.2**
CD for factor A* = 0.03 factor B* = 0.03 factor C* = 0.03					
A × B = 0.06 A × C = 0.07 B × C = N.S. A × B × C = 0.12					

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

Table.2 Effect of EMS concentration and application duration on spread of the plant (cm^2) 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	30.8	30.6	30.3	29.5	**30.3**
2.5 hr	30.2	30.0	29.5	29.0	**29.7**
3.5 hr	30.0	29.7	29.0	28.3	**29.3**
Mean	**30.3**	**30.1**	**29.6**	**28.9**	**29.7**
EMS 0.2%					
1.5 hr	30.5	29.8	29.5	28.1	**29.5**
2.5 hr	30.0	29.5	29.1	27.5	**29.0**
3.5 hr	29.7	29.0	28.6	27.0	**28.6**
Mean	**30.1**	**29.4**	**29.1**	**27.5**	**29.0**
EMS 0.3%					
1.5 hr	29.9	29.4	29.0	27.4	**28.9**
2.5 hr	29.5	29.1	28.7	26.5	**28.5**
3.5 hr	29.1	28.6	28.0	25.5	**27.8**
Mean	**29.5**	**29.0**	**28.6**	**26.5**	**28.4**
Control					
1.5 hr	30.0	29.4	29.1	28.5	**29.3**
2.5 hr	30.0	29.3	29.1	28.2	**29.2**
3.5 hr	30.0	29.0	29.0	28.0	**29.0**
Mean	**30.0**	**29.2**	**29.1**	**28.2**	**29.1**
Mean for treatment duration					
1.5 hr	30.3	29.8	29.4	28.4	**29.5**
2.5 hr	29.9	29.5	29.0	27.8	**29.0**
3.5 hr	29.7	29.1	28.7	27.2	**28.7**
General mean	**30.0**	**29.4**	**29.1**	**27.8**	**29.1**
CD for factor A* = 0.02 factor B* = 0.02 factor C* = 0.02					
A × B = 0.04 A × C = 0.05 B × C = 0.04 A × B × C = 0.09					

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

Table.3 Effect of concentration and application duration on crown height (mm) 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	25.3	23.5	21.5	19.5	**22.5**
2.5 hr	23.2	21.3	20.3	18.3	**20.8**
3.5 hr	22.3	20.3	19.3	17.3	**19.8**
Mean	**23.6**	**21.7**	**20.4**	**18.4**	**21.0**
EMS 0.2%					
1.5 hr	24.3	22.5	19.3	17.5	**20.9**
2.5 hr	21.3	20.3	18.3	16.3	**19.1**
3.5 hr	20.3	19.3	17.3	15.6	**18.1**
Mean	**22.0**	**20.7**	**18.3**	**16.5**	**19.4**
EMS 0.3%					
1.5 hr	22.3	21.3	17.2	15.6	**19.1**
2.5 hr	20.3	18.3	16.3	14.3	**17.3**
3.5 hr	19.3	17.5	15.4	12.4	**16.2**
Mean	**20.6**	**19.0**	**16.3**	**14.1**	**17.5**
Control					
1.5 hr	23.5	22.6	20.5	19.5	**21.5**
2.5 hr	23.3	21.3	20.3	18.3	**20.0**
3.5 hr	22.2	21.2	20.1	18.2	**20.4**
Mean	**23.0**	**21.7**	**20.3**	**18.7**	**20.6**
Mean for treatment duration					
1.5 hr	24.0	22.5	19.7	18.0	**21.0**
2.5 hr	22.0	20.3	18.8	16.8	**19.5**
3.5 hr	21.0	19.5	18.0	15.7	**18.5**
General mean	**22.3**	**20.8**	**18.8**	**16.9**	**19.7**
CD for factor A* = 0.28 factor B* = 0.24 factor C* = 0.28					
A × B = 0.48 A × C = 0.56 B × C = 0.48 A × B × C = NS					

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

Table.4 Effect of EMS concentration and application duration on crown diameter (mm) 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	16.6	16.4	16.4	16.5	**16.5**
2.5 hr	16.2	16.1	16.0	15.7	**16.0**
3.5 hr	16.1	16.0	15.8	15.3	**15.8**
Mean	**16.3**	**16.2**	**16.1**	**15.8**	**16.1**
EMS 0.2%					
1.5 hr	16.2	16.1	15.9	15.5	**15.9**
2.5 hr	16.0	15.9	15.6	15.2	**15.7**
3.5 hr	16.0	15.8	15.2	15.0	**15.5**
Mean	**16.1**	**15.9**	**15.6**	**15.2**	**15.7**
EMS 0.3%					
1.5 hr	16.1	15.7	15.4	15.0	**15.6**
2.5 hr	15.8	15.5	15.1	14.7	**15.3**
3.5 hr	15.4	15.1	15.0	14.3	**15.0**
Mean	**15.8**	**15.4**	**15.2**	**14.7**	**15.3**
Control					
1.5 hr	16.1	16.1	15.6	15.1	**15.7**
2.5 hr	16.0	16.1	15.5	15.0	**15.7**
3.5 hr	16.0	16.0	15.0	15.0	**15.5**
Mean	**16.0**	**16.1**	**15.4**	**15.0**	**15.6**
Mean for treatment duration					
1.5 hr	16.2	16.0	15.7	15.4	**15.8**
2.5 hr	16.0	15.9	15.5	15.2	**15.6**
3.5 hr	15.9	15.7	15.2	14.9	**15.4**
Mean	**16.0**	**15.9**	**15.5**	**15.1**	**15.6**
General mean	**16.1**	**15.9**	**15.6**	**15.2**	**15.7**
CD for factor A* = 0.006 factor B* = 0.005 factor C* = 0.006					
A × B = 0.01 A × C = 0.013 B × C = 0.01 A × B × C = 0.02					

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

Table.5 Effect of EMS concentration and application duration on number of leaves/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	15.5	14.7	13.7	12.7	**14.2**
2.5 hr	13.3	13.3	11.3	11.3	**12.3**
3.5 hr	11.3	11.3	10.3	10.3	**10.8**
Mean	**13.4**	**13.1**	**11.8**	**11.4**	**12.4**
EMS 0.2%					
1.5 hr	14.3	12.3	12.3	10.3	**12.3**
2.5 hr	12.3	11.3	10.3	9.3	**10.8**
3.5 hr	10.3	10.3	9.3	8.3	**9.6**
Mean	**12.3**	**11.3**	**10.6**	**9.3**	**10.9**
EMS 0.3%					
1.5 hr	12.9	10.3	10.3	8.3	**10.5**
2.5 hr	10.3	9.3	8.3	7.3	**8.8**
3.5 hr	9.3	8.3	8.3	6.3	**8.1**
Mean	**10.8**	**9.3**	**9.0**	**7.3**	**9.1**
Control					
1.5 hr	12.3	11.3	10.3	9.3	**10.8**
2.5 hr	12.3	11.3	10.3	9.3	**10.8**
3.5 hr	11.1	10.1	10.2	9.1	**10.1**
Mean	**11.9**	**10.9**	**10.3**	**9.2**	**10.6**
Mean for treatment duration					
1.5 hr	13.5	12.2	11.7	10.2	**11.9**
2.5 hr	12.0	11.3	10.0	9.3	**10.7**
3.5 hr	10.5	10.0	9.5	8.5	**9.6**
General mean	**12.1**	**11.2**	**10.4**	**9.3**	**10.7**
CD for factor A* = 0.28 factor B* = 0.24 factor C* = 0.28					
A × B =0.48 A × C = N.S. B × C = 0.48 A × B × C = NS					

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

Table.6 Effect of EMS concentration and application duration on leaf size 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%	**Length × Breadth (leaf size in mm)**				
1.5 hr	31.1 × 30.3	29.3 × 33.3	27.3 × 27.3	25.3 × 24.3	**28.2 × 28.8**
2.5 hr	30.1 × 29.0	28.0 × 31.0	25.0 × 24.0	23.0 × 21.0	**26.5 × 26.2**
3.5 hr	29.2 × 28.0	26.0 × 30.0	24.0 × 22.0	19.0 × 17.0	**24.5 × 24.2**
Mean	**29.7 × 29.1**	**27.7 × 31.4**	**24.7 × 18.3**	**22.4 × 20.7**	**26.4 × 26.4**
EMS 0.2%					
1.5 hr	30.4 × 29.0	28.0 × 31.1	24.0 × 24.1	21.0 × 20.0	**25.8 × 26.0**
2.5 hr	29.7 × 28.0	26.0 × 28.0	22.3 × 20.0	19.0 × 16.0	**24.2 × 23.0**
3.5 hr	28.1 × 28.0	24.0 × 27.0	20.0 × 17.0	18.0 × 15.0	**22.5 × 21.7**
Mean	**29.4 × 28.3**	**26.0 × 28.7**	**22.1 × 20.3**	**19.3 × 17**	**24.1 × 23.5**
EMS 0.3%					
1.5 hr	28.7 × 27.0	26.0 × 27.0	23.0 × 21.0	18.0 × 16.0	**23.9 × 22.7**
2.5 hr	27.9 × 26.0	25.0 × 26.0	20.0 × 18.0	16.0 × 13.0	**22.2 × 20.7**
3.5hr	25.3 × 25.0	21.0 × 24.6	16.6 × 14.6	13.4 × 11.06	**19.0 × 18.8**
Mean	**27.3 × 26.0**	**24 × 25.8**	**19.8 × 17.8**	**15.8 × 13.3**	**21.7 × 20.7**
Control					
1.5 hr	29.5 × 28.2	27.3 × 30.0	25.3 × 23.0	21.0 × 18.0	**25.7 × 24.8**
2.5 hr	28.1 × 27.0	26.0 × 29.0	24.0 × 22.0	19.0 × 17.0	**24.2 × 23.7**
3.5 hr	28.0 × 26.6	25.6 × 27.6	23.6 × 20.0	17.6 × 15.6	**23.7 × 22.4**
Mean	**28.5 × 27.2**	**26.9 × 28.8**	**24.3 × 21.6**	**19.2 × 16.8**	**24.5 × 23.6**
Mean for treatment duration					
1.5 hr	29.9 × 29.2	28.9 × 28.2	27.9 × 27.2	27.8 × 30.8	**28.6 × 28.8**
2.5 hr	26.7 × 28.7	24.6 × 27.7	24.7 × 23.7	22.7 × 21.0	**24.7 × 25.3**
3.5 hr	21.2 × 18.7	21.2 × 19.5	19.2 × 16.7	17.2 × 15.0	**19.7 × 17.5**
General mean	**25.9 × 25.5**	**24.9 × 25.1**	**24.0 × 22.6**	**22.6 × 22.2**	**24.3 × 23.8**
CD for factor A* = 0.02 × 0.02, factor B* = 0.01 × 0.01, factor C*= 0.02 × 0.02					
A × B = 0.03 × 0.03, A × C = 0.04 × 0.04, B × C = 0.03, A × B × C = 0.07 × 0.07					

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

Table.7 Effect of EMS concentration and application duration on leaf shape in different explants of strawberry cv. Camarosa

EMS concentration with duration	Explants			
	Runner tip	Shoot tip	Leaf disc (Abaxial)	Leaf disc (Adaxial)
EMS 0.1 %	**Leaf shape**			
1.5 hr	Obtuse	Oval	Obtuse	Obtuse
2.5 hr	Obtuse	Oval	Obtuse	Oval
3.5 hr	Obtuse	Oval	Oval	Oval
EMS 0.2%				
1.5 hr	Obtuse	Oval	Obtuse	Obtuse
2.5 hr	Obtuse	Oval	Obtuse	Oval
3.5 hr	Obtuse	Oval	Oval	Oval
EMS 0.3%				
1.5 hr	Obtuse	Oval	Oval	Oval
2.5 hr	Obtuse	Oval	Oval	Oval
3.5hr	Obtuse	Oval	Oval	Oval
Control				
1.5 hr	Obtuse	Oval	Oval	Oval
2.5 hr	Obtuse	Oval	Oval	Oval
3.5 hr	Obtuse	Oval	Oval	Oval

Plate.1 Plants regenerated from the explants treated with EMS 0.1% for 1.5 hr duration

Runner tip

Shoot tip

Leaf disc (abaxial)

Number of leaves per plant differed significantly on account of EMS concentrations, duration of its treatment and the explants used with the orientation (Table 5). The interaction between concentration and treatment duration and that of three factors interaction of the variation were significant, while interactions of concentration and explants and explants and treatment duration were absent. The data mentioned here revealed that the concentration 0.1% produced maximum number of leaves per plant followed by 0.2% and the minimum number of leaves was observed in 0.3%. The 1.5 hr duration gave maximum number of leaves per plant followed by 2.5 hr while minimum number of leaves was recorded in 3.5 hr. The runner tip explant gave maximum number of leaves per plant followed by shoot tip and leaf disc (abaxial) and the minimum number of leaves was observed in leaf disc (adaxial) explant. From data presented in

Table 6 shows that it could be inferred that the runner tip explant treated with EMS concentration 0.1% for 1.5 hr gave maximum (15.5) number of leaves per plant and was found best among all the treatments. The increased EMS concentration along with treatment duration gradually decreased the number of leaves per plant. The variation in leaf number might be due to the EMS concentration, treatment duration and also the genetic make-up of the explants involved in study. The finding of this present investigation supports the results of Murti *et al.,* (2013).

Leaf size differed significantly due to concentrations of EMS, duration of its application and explants used with orientation (Table 6). The interactions between EMS concentration and treatment duration, concentration and explants, treatment duration and explants and that of all the factors of the variation were found significant. Among the different EMS concentrations tried, the concentration 0.1% gave maximum leaf size and the size was found minimum in EMS 0.3% followed by 2.5 hr and the minimum fruit size was found in 3.5 hr. Among different explants used, the runner tip explants gave maximum leaf size followed by shoot tip and leaf disc (abaxial) and the minimum fruit size was found in leaf disc (adaxial) explants. Conclusively, the runner tip explants treated with EMS 0.1% for 1.5 hr produced maximum sized leaves (31.1mm × 30.3mm) and was found best among all the treatment combinations investigated. The increased EMS concentration along with duration gradually decreased the leaf size and thus exhibited a negative relationship. These results are in conformity with the findings of Murti *et al.,* (2013).

Based on the leaf size obtained under various treatments, the leaf shape was designated accordingly as it appeared in botanical terms (Table 7). The leaf shape differed according to the concentrations of EMS, duration of its application and explants used in special orientation.

Two different leaf shapes, obtuse and oval were noticed, of which former shape was present in runner tip and at lower concentrations of EMS in which leaf disc explants were tested whereas the oval shape was observed in shoot tip explants and leaf disc explants at highest EMS concentration with longer treatment duration.

The increased EMS concentration along with duration caused a gradual reduction in plant height. This might also be due to genetic make-up of the explants used.

The results are in conformity with various earlier authors Jabeen and Mirza (2002) who observed the variability in plant height through EMS treatments in *Capsicum annum,* Dhakshanamoorthy *et al.,* (2010) in *Jatropha curcas* and Giriraj *et al.,* (1990) and Jayakumar and Selvaraj (2003) in Sunflower.

The increasing levels of EMS concentration along with increasing duration gradually reduced the plant spread. This might be due to the interaction of EMS concentration, treatment duration and also genetic makeup of the explant.

Encouraging results obtained from the present investigation proved that strawberry is amenable for inducing variations *in vitro* and emphasized good efficiency of *in vitro* mutation induction for the improvement of this important fruit crop.

The runner tip explants treated with EMS concentration 0.1% for duration 1.5 hr. was found best among all the treatment combinations for better growth. While among various explants used, the runner tips explants was found best followed by shoot tips, leaf disc (abaxial) and leaf disc (adaxial).

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