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RESEARCH ARTICLE :

Influence of *in vitro* induced mutation on reproductive growth of "Camarosa" strawberry

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SUMMARY : The present study was undertaken to find out the effect of induced mutation on reproductive growth in strawberry cv. CAMAROSA. Explants were subjected to 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).Runner tips, shoot tips, leaf disc (abaxial and adaxial) were used as explants. 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 took minimum days to bear first flower (25.5) and maximum number of flowers per plant (25.5).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 number of runners and increase days taken to initiate the runners. In future, these experimental results will prove very useful for induction of variability in this fruit crops.

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KEY WORDS :
Camarosa, *In vitro*, Induced mutation, Reproductive growth, Strawberry

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BACKGROUND AND OBJECTIVES

The modern cultivated strawberry (*Fragaria*x *ananassa* Duch.) is one of the most delicious, refreshing and soft fruit of the world. Worldwide it is the most widely distributed fruit crop due to its genotypic diversity, highly heterozygous nature and broad range of environmental adaptations (Larson, 1994 and 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, Uttarakhand, 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), auxiliary 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). 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 *invivo* growth parameters strawberry cv. CAMAROSA.

RESOURCES AND METHODS

The present study on "Effect of *In Vitro* induced mutation on reproductive 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 to 2014 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: (a) The runner tips measuring about 0.5 cm long were cut from healthy runners and used as explants. (b) 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. (c) 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 EMS solution was used for the treatments. The runner tipswere agitated with different concentrations of aqueous solution of EMS for different periods of time mentioned in the Table A 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).Regenerated plants after root formation transplanted into greenhouse in cocopeat + perlite + vermicompost (3:1:1) in PVC pots for evaluation of days bear to first flower and number of flowers per plant.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 A : Chemical mutagen used in the study

Mutagen	Dose/Concentration applied (%)	Time of treatment (hr)
Ethyl Methane Sulphonate (EMS)	0.1, 0.2, 0.3 and 0.4	1.5, 2.5 and 3.5
Control (No mutagenic treatment)	Buffer solution (Sodium Dihydrogen Phosphate and Disodium Hydrogen Phosphate)	1.5, 2.5 and 3.5

OBSERVATIONS AND ANALYSIS

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.

Days taken to bear first flower exhibited significant differences caused due to concentrations of EMS, duration of EMS treatment and the explants used with the orientation (Table 1). All two factors interactions between concentration and treatment duration, concentration and explants, treatment duration and explants and also the three factors interactions were recorded significant.The results showed that concentration 0.1% took minimum days to bear first flower followed by 0.2% and the first flowering was late in 0.3%. The 1.5 hr treatment duration took less days to

first flower and the maximum days were taken in 3.5 hr. The runner tip explant took minimum days to first flower followed by shoot tip and leaf disc (abaxial) and it was delayed maximum in leaf disc (adaxial) explant. Data suggested that the runner tip explant treated with EMS concentration 0.1% for 1.5 hr took minimum days to first flower (25.5) and was found best among all the treatments.The increased concentration and treatment duration increased delayed first flower initiation. The differences in number of days to bear first flower might be the interplay of EMS concentration, treatment duration and also the genetic make up of the explant. Early flowering might be due to the physiological changes caused by EMS. The delayed flowering and fruiting caused by mutagen at higher concentrations might be due to its inhibitory effects. The results of present study are in agreement with previous studies of George and Nayar (1973) in linseed and the early flowering reported in *Lathyrussativus* by Kumar and Dubey (1998) and Girhe and Choudhary (2002).

Number of flowers per plant :

There were significant differences in number of flowers per plant due to concentrations of EMS, its treatment duration and the explants used with the orientation (Table 2). The data also showed significant interactions between concentration and treatment duration, concentration and explants, whereas such interactions of treatment duration and explants and all the three factors of the variation tried were absent. An examination of data presented in this Table indicated that EMS concentration of 0.1% gave maximum number of flowers and the minimum number was observed in 0.3%. EMS treatment duration of 1.5 hr produced maximum

Table 1 : Effect of EMS concentration and application duration on days to bear first flower 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	70.1	73.1	75.2	78.2	74.2
2.5 hr	72.3	75.3	79.3	82.3	77.3
3.5 hr	74.3	78.3	84.3	87.3	81.1
Mean	72.2	75.6	79.6	82.6	77.5
EMS 0.2%					
1.5 hr	73.3	76.3	72.3	82.3	76.1
2.5 hr	75.3	79.3	82.3	86.3	80.8
3.5 hr	78.3	84.3	88.3	90.3	85.3
Mean	75.6	80.0	81.0	86.3	80.7
EMS 0.3%					
1.5 hr	76.3	80.3	83.3	87.2	81.8
2.5 hr	79.3	82.3	87.3	91.3	85.1
3.5 hr	81.3	88.3	90.3	95.3	88.8
Mean	79.0	83.6	87.0	91.3	85.2
Control					
1.5 hr	75.3	82.2	82.3	87.3	81.8
2.5 hr	77.3	85.3	85.3	89.3	84.3
3.5 hr	78.7	86.7	86.5	90.5	85.6
Mean	77.1	84.7	84.7	89.0	83.9
Mean for treatment duration					
1.5 hr	73.6	76.9	79.6	83.6	78.4
2.5 hr	76.0	78.8	83.5	87.3	81.4
3.5 hr	78.0	82.7	87.2	90.7	84.6
General mean	76.0	81.0	83.1	87.3	81.8
CD for factor A* = 0.25	factor B* = 0.22		factor C* = 0.25		
A x B =0.44	A x C = 0.51	B x C = 0.44	A x B x C = 0.89		

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

Runner tip Shoot tip Leaf disc (abaxial)

Plate 1 : Production of flowers 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 flowers per 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	25.5	24.5	22.5	20.5	23.3
2.5 hr	23.3	22.3	20.3	18.3	21.1
3.5 hr	21.3	20.3	18.3	16.3	19.1
Mean	23.4	22.4	20.4	18.4	21.1
EMS 0.2%					
1.5 hr	24.3	23.3	21.3	19.3	22.1
2.5 hr	22.3	21.3	19.3	17.3	20.1
3.5hr	20.3	19.3	17.3	15.3	18.1
Mean	22.3	21.3	19.3	17.3	20.1
EMS 0.3%					
1.5 hr	23.3	22.3	20.3	18.3	21.1
2.5 hr	21.3	20.3	17.3	16.3	18.8
3.5 hr	19.3	18.3	16.3	14.3	17.1
Mean	21.3	20.3	18.0	16.3	19.0
Control					
1.5 hr	22.3	21.3	20.3	18.3	20.6
2.5 hr	22.3	21.3	20.3	18.3	20.6
3.5 hr	22.2	21.2	20.2	18.3	20.5
Mean	22.3	21.3	20.3	18.3	20.5
Mean for treatment duration					
1.5 hr	24.0	23.0	21.2	19.2	21.8
2.5 hr	22.3	21.5	19.3	17.5	20.1
3.5 hr	20.7	19.7	18.0	16.0	18.6
General mean	22.3	21.3	19.5	17.6	20.2
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 = NS	A x B x C = NS		

* Factor A = Concentrations, Factor B = Durations, Factor C = Explants, NS= Non-significant

number of flowers per plant but 3.5 hr duration induced minimum number of flowers. Among different explants used, the runner tip explant had maximum number of flowers per plant followed by shoot tip and leaf disc (abaxial) and minimum flowers were noticed in explant leaf disc placed in adaxial orientation. Thus, it could be inferred that the runner tip explant treated with EMS concentration 0.1% for duration of 1.5 hr induced maximum (25.5) number of flowers per plant and was found best among all the treatments however, with increased EMS concentrations along with treatment duration the number of flowers per plant was reduced.This might be due to the EMS concentration, treatment duration and also genetic make up of the explant. These results are in agreement with various earlier investigators (Dhakshammoorthy *et al.*, 2010; George and Nayar, 1973) who reported mutagenesis in Jatrophaand linseed involving physical and chemical mutagens.

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