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Lokesh Bora
Department of Fruit Crops,
Horticultural College & Research
Institute, Tamil Nadu
Agricultural University
Coimbatore, Tamil Nadu, India

RM Vijayakumar
Department of Fruit Crops,
Horticultural College & Research
Institute, Tamil Nadu
Agricultural University
Coimbatore, Tamil Nadu, India

N Meenakshi Ganesan
Centre for Plant Breeding and
Genetics, Tamil Nadu
Agricultural University
Coimbatore, Tamil Nadu, India

M Ganga
Horticultural Research Station
Vijayanagaram, Ooty, Tamil
Nadu Agricultural University
Coimbatore, Tamil Nadu, India

Correspondence
Lokesh Bora
Department of Fruit Crops,
Horticultural College & Research
Institute, Tamil Nadu
Agricultural University
Coimbatore, Tamil Nadu, India

In vitro rhizogenesis of acid lime cv. PKM-1 microshoots regenerated from chemical mutagen treated explants

Lokesh Bora, RM Vijayakumar, N Meenakshi Ganesan and M Ganga

Abstract

In vitro regenerated microshoots of acid lime cv. PKM-1 derived from EMS treated explants were induced to root. The best rooting treatment obtained through *in vitro* micropropagation. The chemical mutagen EMS showed an inverse trend with respect to the rooting of the microshoots. The number of days to rooting, length and number of roots showed negative correlation with the increase in dose of the mutagen. The lowest number of days 14.65 days was observed in T₁ (10 mM), followed by T₂ (20 mM) i.e., 17.81. The control (T₀) treatment exhibited longest root length of 4.98 cm, whereas gamma ray irradiation at T₁ (10 mM) registered root length of 4.78 cm followed by T₂ (20mM) with 2.64 cm of root length. Among the irradiated population, T₁ treatment (10 mM) recorded more number of roots per regenerated shoot (4.78) followed by T₂ (20 mM) with 2.64 roots per regenerated shoot. The lowest number of roots per shoot was observed in T₄ (30 mM) with 1.34 roots. The delay in rhizogenesis may be overcome by incorporation of activated charcoal @ 0.1% in the half MS media.

Keywords: rhizogenesis, microshoots, chemical mutagen

Introduction

Creation of variability is a prerequisite for crop improvement in any plant breeding programme. *In vitro* mutagenesis can be used in order to alter the agronomical characteristics governed by one or few genes in genotypes of great interest, being considered as a fine adjustment of a variety (Suprasanna *et al.*, 2014) [14]. Spontaneous mutations have played an important role in the improvement of certain characters in some of the fruit crops. The occurrence of a natural mutation takes years. Induced mutation techniques are still to be fully exploited in fruit breeding. This technology offers the possibility of altering only one or a few specific traits of an elite cultivar and can contribute to fruit improvement without upsetting the requirements of the fruit industry or of consumers (Broertjes and Van Harten, 1988) [9]. In mutagenesis, physical and chemical mutagens widely used such as ethyl methanesulphonate (EMS) and methyl methane sulphonate (MMS) are most frequently used chemical mutagens (Jain, 2005). These mutagens have different mechanisms of action. The most widely used physical mutagens are Gamma and X-rays (Mba *et al.*, 2012). Breeding of improved varieties through genetic variation has been attained through induced mutagenesis. Without altering the entire genetic makeup of a genotype, mutation may occur in a specific gene(s) responsible (point mutation) for particular character(s). This is a valuable tool not yet fully exploited in fruit breeding. Mutants of horticultural crops have been limited to only ornamentals (Bhat *et al.*, 2017) [7]. Hence, there is a need to adopt and incorporate induced mutations in combination with *in vitro* techniques much more widely to breed new varieties, their rapid release and spread to enhance horticultural production. Considering the above facts, the present investigation on *in vitro* mutagenesis in acid lime was carried out. An attempt has been made to develop an efficient protocol for micropropagation of acid lime cv PKM-1. The chemical treated explants (*in vitro* derived) were raised upto M₁V₁ to M₁V₃. The M₁V₃ generation was subjected to hardening. Therefore the objective was to study the influence of EMS on Rhizogenesis of *in vitro* derived plant microshoots.

Material and methods

Plant material

The PKM-1 variety is a selection from Kadayam type of Tirunelveli district of Tamil Nadu. Tree is vigorous and easily multiplied as seedlings and layers.

Fruits can be harvested throughout the year. The study was conducted in year 2015-16. Fruits of acid lime cv. PKM-1 were collected from plants raised in experimental Orchard at Horticultural College & Research Institute, Periyakulam, Tamil Nadu. The experiment was laid in completely randomized design. The treated explants were cultured on M_1V_1 multiplication medium supplemented with best combination of growth regulators standardized in standardization of *in vitro* protocol for acid lime cv. PKM-1 i.e., Half MS + IBA (3.0 mg l⁻¹) was used and the sub culturing was performed at an interval of 30 days, up to M_1V_3 in order to dissociate chimeras. Individual shoots of M_1V_3 cultures were transferred on to ¼ strength MS medium supplemented with 3.0 mg/L IBA and 3 g/l activated charcoal for induction of roots wherever necessary and difficult to root. The mean difference was tested by 'F' test at 5 percent level of significance (LOS). Critical difference (CD) at 5 per cent level of probability was used for comparison among treatments.

Result and Discussion

Influence of chemical mutagen on Days to rooting

The results showed that significant differences existed among the different concentration of chemical mutagen on days to taken to root initiation. The T_0 treatment required 12.43 days for initiation. Among the chemical treated population with continuous sub-culturing upto third vegetative growth generation recorded an increasing trend for the number of days taken for rooting with increase in doses of EMS. The lowest number of days 14.65 days was observed in T_1 (10 mM), followed by T_2 (20 mM) i.e., 17.81 (Table 1). The T_4 (30 mM) required 21.99 days for root initiation. Also in some plants of higher dosage was no rooting was observed. However activated charcoal was used and rooting was obtained.

Influence of chemical mutagen on length of roots

Among the treatments, there existed significant inhibitory effect on mean length of roots due to increase in irradiation dosage and ranged from 1.34 to 4.98 cm. The control (T_0) treatment exhibited longest root length of 4.98 cm, whereas gamma ray irradiation at T_1 (10 mM) registered root length of 4.78 cm followed by T_2 (20mM) with 2.64 cm of root length. The shortest mean root length of 1.34 cm was observed in T_4 (30 mM).

Influence of chemical mutagen on Number of roots per shoot at hardening stage

The data on number of roots for M_1V_3 generation regenerated shoot as influenced by different doses of gamma irradiation showed significant difference and ranged between 1.34 and 4.98 roots (Fig.). Increase in chemical mutagen dosage showed proportional reduction in number of roots per regenerated shoot (Fig 1). The untreated control treatment (T_0) recorded highest number of roots (4.98). Among the irradiated population, T_1 treatment (10 mM) recorded more number of roots per regenerated shoot (4.78) followed by T_2 (20 mM) with 2.64 roots per regenerated shoot. The lowest number of roots per shoot was observed in T_4 (30 mM) with 1.34 roots.

Gamma ray treatment exhibited a negative trend in terms of number of roots produced and length of roots as the dosage increased. The delay may also be due to residual effect of chemical mutagen on the subsequent vegetative generations. Inhibition of synthesis and activity of auxins might be possible reason for the retarded response to rhizogenesis (Smith and Kersten, 1942 and Gaur and Notani, 1960) [3, 11]. The variability in the response to rhizogenesis indicates that the radiation doses might have caused mutation. For irradiation by gamma rays, the occurrence of insertion or inversion changes could explain the occurrence of mutation. However, the mutation frequency may be influenced by number of factors such as the mechanism of mutagen action, target gene size, nucleotide composition, genomic location, chromatin structure, replication timing, efficiency of DNA repair and transcriptional activation (Puchooa, 2005) [8].

This might be due to the EMS concentration, treatment duration and also genetic make up of the explants used. Increase EMS concentration and treatment duration decreased the number of roots produced per plant. 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) [6] and Jayakumar and Selvaraj (2003) [10] Bhat *et al.*, 2017 [13].

The retarded rate of rhizogenesis obtained in the present investigation is in agreement with the earlier work of Kalimuthu *et al.* (2007) [7] in red banana. In contrary to these reports, Charbaji and Nabulsi (1999) [2] reported significant increase in number of roots produced and length of roots in irradiated (5 Gy) shoot tip explants of grapevine rootstock, R99.

Table 1: Effect of ethyl methylsulphonate on days to rooting, number of roots and length of roots for *in vitro* derived nodal segments of acid lime cv. PKM-1.

Treatment	Concentration	Days to root	Number of roots	Length of roots
T_0	Control	12.43	4.98	4.98
T_1	10mM	14.65	4.78	4.78
T_2	20mM	17.81	2.63	2.64
T_3	25mM	20.21	1.83	1.83
T_4	30mM	21.99	1.34	1.34
SEd		0.58	0.76	0.98
CD (p=0.05)		1.32	1.65	2.21

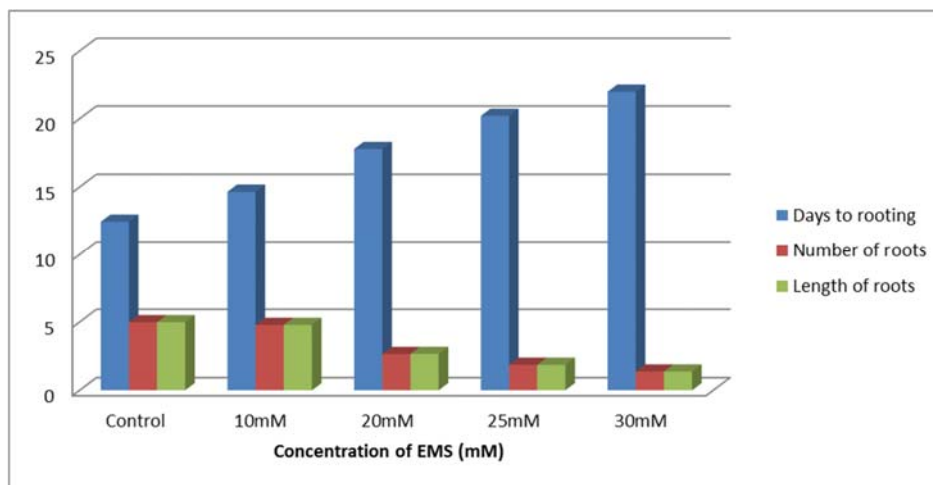


Fig 1: Effect of EMS on Rhizogenesis of microshoots of third vegetative generation M₁V₃

Conclusion

The mutants subjected to subsequent generation may be multiplied easily with modifying the rooting media and then screening may be conducted for biotic or abiotic stress under in vitro condition.

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