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Mutagenic effect of ethyl-methyl sulphonate on *in vitro* nodal segments of acid lime cv. PKM-1

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Abstract
Induced mutagenesis is an essential plant breeding tool which involves the generation of allelic variants of genes that modulate the expression of traits. To minimize the population loss due to excess dose rate of mutagens, it is necessary to optimize the dosage (LD_{50}). In a mutation breeding experiment, LD_{50} gives an indication of the response of different types of explants of a species to mutagen, so that the right dose(s) for the main field experiment can be fixed to obtain beneficial mutants. In the present investigation, axenic nodal segments of acid lime cv. PKM-1 were subjected to different dosages of chemical mutagen (Ethyl methyl sulphonate) ranging from 10 to 45 mM to find their effect on different growth related traits. The results revealed a gradual and significant reduction in survival of nodal segments, with increase in dose of the mutagens. Probit curve analysis was carried out based on the survival of nodal segments and the LD_{50} was fixed as 25 mM for chemical mutagenesis. Based on the LD_{50} the population was maintained for further study.

Keywords: *in vitro* mutagenesis; ethyl methyl sulphonate; acid lime; LD_{50}

Introduction
Acid lime (*Citrus aurantifolia* Swingle) belongs to the family Rutaceae and sub family Aurantoideae and is commercially grown in tropical and subtropical regions of India. It is the third important citrus fruit after mandarins and sweet orange. India rank fifth among major lime producing countries in the world. In India, lime occupies an area of 0.25 million hectares with an annual production 2.78 million tonnes and productivity of 10.67 metric tonnes per hectare (Anonymous, 2016)[5]. It is cultivated commercially in Andhra Pradesh, Maharashtra, Gujarat, Tamil Nadu, Karnataka and Himachal Pradesh. Because of the relatively high degree of polyembryony exhibited by this fruit, the seedlings are found true to type when seed propagated and seed propagation is still employed in most of the countries. As a consequence, very few clonal varieties have been selected through selection *viz.,* Chakradhar, Pramalini, Vikram, Tenali, Sai-Sharbati, Kagzi Lime, MangaliPattu, PDKV and PKM-1. Creation of variability is a prerequisite for crop improvement in any plant breeding programme. Spontaneous mutations have played an important role in the improvement of certain characters in some of the fruit crops. Induction of variability using physical mutagen, gamma rays has been in practice for long time by breeders. Tissue culture has a potential for improving the effectiveness of mutation induction in several aspects (Mahadevamma *et al.,* 2012)[10]. In fruit crops, mutagenesis has already been used to introduce useful mutants related to dwarfing, blooming time and fruit ripening period, fruit color, self-compatibility, self-thinning, and resistance to pathogens (Janick and Moore 1996)[13]. Chemical mutagens lead to more specific and predictable mutation, and the procedures are easier to manage without specialized, expensive equipment. *In vitro* culture techniques like direct (from explants) or indirect (from intervening callus) shoot organogenesis and somatic embryogenesis are valuable tools for conserving important indigenous and improved citrus germplasm by slow growth (medium-term) or cryopreservation (long term) protocols. Induced mutagenesis can be profitably employed as a complementary breeding procedure. Both physical and chemical mutagens have been employed to generate the desired variability.

In vitro mutagenesis is a biotechnological technique 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 (Santos *et al.,* 2004)[2]. *In vitro* mutation induction with the use of gamma rays in stem apex appeared to promote greater viability, when compared with somaclonal variation.

Nowadays, mutation using gamma rays is regularly incorporated in the banana breeding program (Smith *et al.*, 2006 and Mishra *et al.*, 2007)[1, 7]. The use of *in vitro* cultures in mutation breeding of fruit crops advantages over the conventional techniques. Generally, mutagens differ in their relative effectiveness and efficiency for the production of mutants in different genotypes (Khalil *et al.*, 2011) [14]. Mutation breeding involves the development of new varieties by generating and utilizing genetic variability through chemical and physical mutagenesis (Oladosu *et al.*, 2016) [17]. In crops in which higher efficiency to produce nucellar polyembrony exist it can be highly useful to create diversity. In perennial vegetatively propagated crops emphasis is laid on the proper way of cutting back the V_1M_1 and V_2M_1 shoots in order to stimulate outgrowth of axillary buds, thus promoting formation of stable periclinal chimeras. In any breeding programme, undesirable (mutant) genotypes must be eliminated as early as possible, especially for larger trees. This early screening requires early recognition of the desired types, which in turn requires quick production of stable mutant genotypes in mutation breeding. Somewhere between very young and very old buds, an optimum part of the bud wood can be determined, as was reported by Donini (1976) for cherries, grapes, olives and peaches. Greater emphasis is to be given to the use of combined treatment of physical mutagen and growth regulators. The combined use of this can help the breeder in creating better variability thereby better scope for selection. The use of mutation technique with *in vitro* propagation over plants to outnumbered vegetatively propogated plants is an effective technique in terms of obtaining variation, quick proliferation of mutants and obtaining mutants and subjecting them to screening for biotic and abiotic stress. Therefore the present study was undertaken to determine the sensitivity of Ethyl Methyl Sulphonate so that the mutants recovered can be used to screen against abiotic or biotic stress.

Materials 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 *in vitro* experiment was carried out in the Tissue Culture Laboratory of Horticultural College & Research Institute, TNAU, Coimbatore. Nodal segments of *in vitro* derived axenic cultures after the germination of seed with a period of one month with uniform size (50 per treatment) were used for chemical treatment using EMS. The nodal segments were soaked in filter sterlize (0.22 micron) (SigmaAldrich) (freshly prepared in phosphate buffer at pH 7.0) and EMS solution with different concentrations *viz.*, 10, 20, 25, 30, 35, 40 45 mM for 30-45 minutes. After drying, under laminar air flow and then inoculated in the test tubes, the corrected mortality was calculated based on percentage by using Abbott's formula given below.

$$\text{Corrected mortality (\%)} = \frac{M_{\text{observed}} - M_{\text{control}}}{100 - M_{\text{control}}} \text{ X } 100$$

Where,

M_{observed}- Mortality in treatment,

M_{control} – Mortality in control

Probit analysis

The shoots growth was studied under *in vitro* and the lethality was being calculated based on probit analysis by SPSS (Finney, 1971). Fifteen fully expanded leaves were taken and the experiments were repeated once. The data were subjected to statistical analysis using standard deviations of the mean, and thereafter to analysis of variance. Mean comparisons were carried out using Duncan's Multiple Range Test (a=0.05 %).

Results and Discussion

The success of mutation breeding depends upon rate of mutation, and the mutation efficiency. This experiment forms the basis for conducting the in vitro mutagenesis experiment in acid lime using EMS. The efficacy of *in vitro* mutagenesis directly depends on several variables associated with the plant, mutagen and the interactions between explant and mutagen. A total of 50 nodal segments derived through axenic culture were shaken for 30 min and then thoroughly washed with sterile distilled water followed by air drying. The lethality was observed at a period of 15 days, with lower doses treatment out of 50 nodal segments 11 were dead, similarly a number of 16, 23, 28 and 37 plants were dead at 20, 25, 30, 35 mM respectively (Table 1).A linear reduction in survival of inoculated nodal segment was obtained with increase in concentration of mutagen. The LD_{50} for gamma radiation was fixed based on survival percentage observed after the treatment of mutagen and noticed them after a period 15 day under *in vitro* condition. The survival percentage showed negative relation correlation with increased dose of mutagen.

The nodal segment showed etiolated shoots, sprouts with lacking chlorophyll, albino and stunted growth behaviour. *In vitro* techniques offer advantage over *in vivo*, where under controlled conditions plants are being monitored without any disturbance from outer environmental conditions. EMS induces a biased spectrum of G/C-to-A/T transitions, due to the alkylation at the O6 or N7 position of guanine, which leads to the replacement of cytosine with thymine base pairing. EMS also tends to produce several random point mutations and induces a low level of chromosomal breaks and lethal effects (Greene *et al.*, 2003)[12]. These effects provide a competent survival rate and allow subsequent analyses to be performed for both forward and reverse genetics. From the proposed experiment the LD_{50} calculated with the help of probit analysis which was observed at 25mM similar range of result are reported by using rough lemon seedlings (*Citrus jambhiri* Lush to observe the effect of mutagens on regeneration and growth of *in vitro* grown epicotyl segments by Sharma *et al.* (2013)[18] (Fig. 1). The lower and upper dosage mutants were and the mutants sub-cultured from chemical mutagen were subjected to subsequent generations i.e., M_1 V1, M_1V_2 and M_1V_3 to gain homogeneity as also proposed by (Jain *et al.*, 2011; Roux *et al.*, 2001)[8, 9]. Selection and keen observation of variants from the group is very important.

Some minor leaf variations were observed such as leaf shape, leaf length and serration in the or also called as chimeral effect (Albina, Chlorina, Viridis and Xantha). Variations in the morphological and physiological characters can be effectively generated and utilized for meaning full purposes in

future crop improvement program. Usage of chemical mutagen for Induced mutagenesis is an effective tool to break the limits of variability and to create new alterations in a short period of time period (Kumar and Kumar, 2007) [6]. Under *in vitro* condition in addition, there is no loss of the mutants, as micropropagules are sub-cultured under sterile conditions (Ling *et al.*, 2008) [3].

Mutation breeding has been employed with varying degrees of success in fruit crops for crop improvement. Mutation breeding combined with tissue culture offered an impetuous and excellent chance for improving the genetic variation of citrus by improving the selection technology and by accelerating the breeding time (Novak *et al*., 1985) [4]. Differential response to chemical mutagen by citrus was noticed. The morphological mutants based on the visual characters were subjected to generations second and third by means of sub culturing.

Table 1: Effect of mutagen on survival of nodal segments in acid lime cv. PKM-1

Concentration (mM)	Number of treated	No. of dead	No. of survive	Probability	corrected mortality	LD_{50}
Absolute control	50	6	44	0.059	-	
10	50	11	39	0.180	5.32	
20	50	16	34	0.396	10.64	
25	50	23	27	0.525	18.09	
30	50	28	22	0.651	23.40	
35	50	37	13	0.762	32.98	24.04
40	50	42	8	0.851	38.30	
45	50	49	1	0.914	45.74	

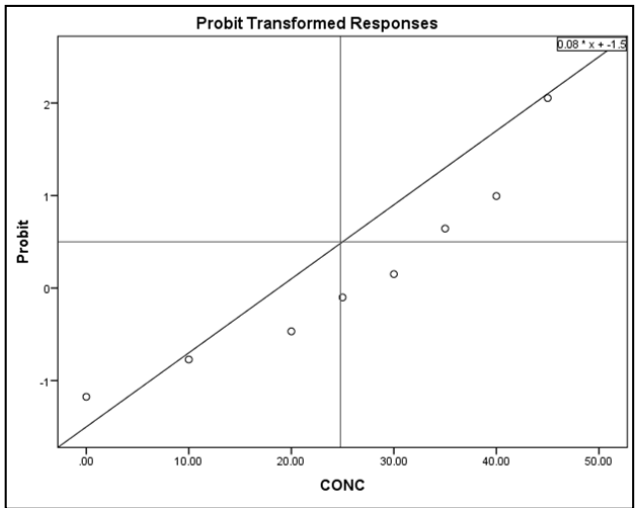

Fig 1: Probit curve for calculation of LD_{50} in nodal segments of acid lime against ethyl methysulphonate

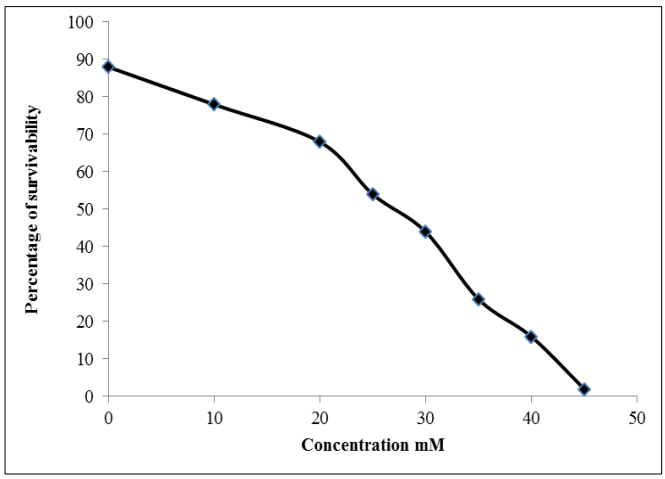

Fig 2: Survival percentage of nodal segments treated with different concentration of Ethyl methyl sulphonate.

Conclusion

The lethal dosage of the experiment was useful to set the mutation experiment and to generate variability. The mutants subjected to subsequent generation may be multiplied and then screen for biotic or abiotic stress under *in vitro* condition.

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References

1. Smith MK, Hamill SD, Langdon PW, Giles JE, Doogan VJ, Pegg KG. Towards the development of a Cavendish banana resistant to race 4 of Fusarium wilt: gamma irradiation of micropropagated Dwarf Parfitt (*Musa* spp., AAA group, Cavendish subgroup). Australian Journal of Experimental Agriculture. 2006; 46(1):107-113.
2. Santos, SJA, Silva SO, Dias DL. Musa mutants induced by gamma radiation. In: XVI Reunión Internacional ACORBAT, Oaxaca Acorbat, 2004, 263-263.
3. Ling, APK, Chia, JY, Hussein S, Harun AR. Physiological responses of Citrus sinensis to gamma irradiation. World Applied Sciences Journal. 2008; 5(1):12-19.
4. Novak FJ, Domini B, Hermelin J, Micke A. Potential of banana and plantain improvement through invitro mutation breeding. In: Resumenes VII Reunion. ACORBAT, 1985, 75-79.
5. Anonymous, Horticultural Statistics at a Glance, National Horticulture Board, Gurgaon, Haryana. Ministry of Agriculture, Govt. of India. 2016, 385.
6. Kumar G, Kumar PR. EMS Induced Karyomorphological Variations in Maize (Zea mays L.) Inbreds. Turk. J Biol. 2007; 31:187-195.
7. Mishra PJ, Ganapathi TR, Suprasanna P, Bapat VA. Effect of Single and Recurrent Gamma Irradiation on in vitro Shoot Cultures of Banana. International J. Fruit Science.2007; 7:45-57.
8. Jain SM, Till B, Suprasanna P, Roux N. Mutations and cultivar development of banana. In: Pillay M, Tenkouano A, editors. Banana Breeding, Progress and Challenges. Boca Raton, FL: CRC Press. 2011, 203-217.
9. Roux N, Dolezel J, Swennen R, Zapata-Arias FJ. Effectiveness of three micropropagation techniques to dissociate cytochimeras in Musa spp. Plant Cell Tiss Org. 2001; 66:189-197.

10. Mahadevamma M, Dinesh MR, Kumari VR, Shankarappa TH. Evaluation of induced variability in papaya (*Carica papaya* L.) by physical mutagenesis. CIBTech Journal of Biotechnology. 2012; 1(1):66-71.
11. Khalil SA, Sattar A, Zamir R. Development of sparse-seeded mutant kinnow (*Citrus reticulata* Blanco) through budwood irradiation. African Journal of Biotechnology. 2012; 10(65):14562-14565.
12. Greene EA, Codomo CA, Taylor NE, Henikoff JG, Till BJ. Spectrum of chemically induced mutations from a large-scale reverse genetic screen in Arabidopsis. Genetics.2003; 164:731-740.
13. Janick J, Moore JN. Fruit breeding. Tree and tropical fruits. John Wiley & Sons, New York, 1996.
14. Khalil SA, Sattar A, Zamir R. Development of sparese seeded mutant kinnow *Citrus reticulata* Blanco through budwood irradiation. African Journal of Biotechnology. 2011; 10(65):14562-14565.
15. Domini B, Breeding methods and applied mutagenesis in fruit plants. In: proc workshop, Eur. Comm-Isreal, Use of Ionizing radiation, Wageningen, 1976. Assoc. Euratom-ITAL, Wageningen, 1976, 445-478.
16. Finney DJ. Probit analysis, 3rd (Ed.), Cambridge University Press, London, 1971, 333.
17. Oladosu Y, Rafii MY, Abdullah N, Hussin G, Ramli A, Rahim HA *et al*. Principle and application of plant mutagenesis in crop improvement: A review, Biotechnology and Biotechnological Equipment. 2016; 30(1):1-16.
18. Sharma LK., Kaushal M, Gill MIS, Bali SK. Germination and Survival of Citrus Jambhiri Seeds and Epicotyls after Treating with Different Mutagens under *in vitro* Conditions. Middle-East Journal of Scientific Research; 2013. 16 (2): 250-255.