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Research Article

EFFECT OF ETHYL METHANE SULPHONATE (EMS) ON GERMINATION BEHAVIOR AND SEEDLING SURVIVAL OF *Vinca rosea* (L.) G.Don.

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Abstract

The present investigation was carried out to study the induced mutagenesis of EMS (Ethyl methane sulphonate) on the seed germination, seedling survival and LD_{50} value of *Catharanthus roseus*. The seeds of *Vinca rosea* were treated with different concentration of EMS *viz.*, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mM. The untreated seeds were taken as control. The EMS treated and control seeds were kept in petriplates and allowed for germination, seed germination and seedling survival percentage were calculated. The germination percentage was calculated on 15^{th} day after sowing and seedling survival proportion at 30^{th} day. The 50 per cent reduction of seed germination was observed as LD_{50} (lethal dosage) value was calculated at 15^{th} day after sowing. The 50 per cent reduction of seed germination in 25KR of EMS treatment. The seedling survival also decreased with increase in the concentration of EMS.

Keywords: EMS (Ethyl methane sulphonate), Seed germination, Seedling survival, LD_{50} value and Mm (Milli Molar).

Introduction

Vinca rosea (L.) *G. Don* is an ornamental and a medicinal plant species. It belongs to the family Apocynaceae, consisting of 411 genera and 4650 species, many of them are of ornamental and medicinal values (Simpson, 2006). *V. rosea* has potent secondary metabolism responsible for production of monoterpenoid glucosides and other terpenoid compounds, steroids, phenolics, flavanoids, anthocyanins and 130 terpenoid indole alkaloids (Facchini, 2001; Van Der Heijden *et al*., 2004; Pandey-Rai *et al.,* 2003). *V.rosea* the periwinkle (Apocynaceae), native of south eastern and eastern Madagascar, has been popularly known for its considerable medicinal value. The medicinal importance of this plant has increased considerably because of the discovery of six anti-cancerous activity containing alkaloids. Among them only vincristine and vinblastine are active in human system. The credit of discovery of these two alkaloids goes to Nobel *et al*. (1958). It possesses largest number of alkaloids in plant kingdom. More than 170 alkaloids of *V.rosea* also own its importance due to the presence of antihypertensive alkaloids such as ajmalcine and serpentine in roots. The widely used anticancer drugs vinblastine and vincristine (Leveque *et al*., 1996) are semisythesized from their natural precursors vindoline and catharanthine that are obtained from *V.rosea* root and shoot organs. Ajmalicine, a cardiactonic drug is also extracted from *C. roseus* roots (Leveque *et al*., 1996). The high cost of production of pharmaceutically important is related to their low level of accumulation in *V.rosea* organs. Thus, to increase the concentrations of in plant organs is another important objective of genetic work on *V.rosea.* The annual world demand for vincristine, vinblastine and ajamalcine.

Mutation Breeding is a supplementary approach for creation of genetic variability and it is applied in improvement of *V.rosea* (L.) G. Don. mutation breeding is one of the conventional breeding methods in plant breeding (Bhattacharjee, 1998). It is relevant with

various fields like, morphology, cytogenetics, biotechnology and molecular biology etc. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement (Acharya *et al.,* 2007) and as an efficient means of supplementing existing germplasm for cultivar improvement in breeding program's (Dubinin, 1961). Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Lee *et al*., 2002). These mutations provide beneficial variation for practical plant breeding purpose. Among the conventional methods mutation breeding is a promising approach for approaches for creation of genetic variability and useful for the development of 'idiochemovars' in medicinal plants.

EMS is the mutagen we have employed, for the following reasons its effects have been well studied and it is known to generate almost exclusively G/C to A/T point mutations. These mutations may lead to a complete or partial loss of gene function or, less frequently, to some other alteration of normal gene function. Mutations are randomly distributed in the genome. A high degree of mutation saturation can be achieved with a mutagen like EMS that does not cause a lot of collateral DNA damage.

The high cost of production of pharmaceutically important is related to their low level of accumulation in *V.rosea* organs. Thus, to increase the concentrations in plant organs is another important objective of genetic work on *V.rosea.* From recent reviews relatively very little work has been done on conventional approaches for development of periwinkle varieties with high alkaloid contents. The conventional method of mutation breeding was followed in this research work. The present work has been done using EMS treatment with various concentration to the seeds of *Vinca rosea.* Thus to increase the annual world demand for vincristine, vinblastine and ajamalcine the present work has been done.

Materials and Methods

The dry and dormant seeds of variety of *Vinca rosea* were collected from J.P Laboratories, Virudhunagar District and Tamil Nadu. Two sets containing 100 well dry healthy seeds were selected for treatment. Seeds were pre soaked in distilled water for 6 hours followed by EMS at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mM concentrations. Non treated dry seeds were pre-soaked in distilled water for 6 hours and then used as control. EMS were thoroughly washed in running tap water for 8 to 10 times and then transferred to Petri dishes containing two layers of moist filter paper for germination. The control and treated seeds were then subjected to germination test. The concentrations of EMS were fixed for further studies.

Results and Discussion

Germination and seedling variations for each treatment were observed and LD_{50} for EMS and Gamma rays were determined. The LD_{50} for EMS was 20 mM and Gamma rays was 30 Kr. The morphological character was decreased in first generation in both EMS and Gamma rays treated plants with increase in concentration when compared to control, except days to first flowering, it was increased with increase in concentration. The seeds were collected from EMS and Gamma rays treated plants separately including control. The seeds were sown in the field to raise the second generation. In M2 generation morphological mutant characters was observed. Different types of mutants were tall mutant, dwarf mutant, early maturity, late maturity, leaf mutants and flower mutants were observed. The morphological yield parameters like, days to first flower, plant height, of seeds per plant, leaf and root powder weight and yield per plant all the characters observed. Tall mutant were observed in gamma rays and EMS treatment, while dwarf mutant was also observed gamma rays and EMS treatments. Early maturity was observed in lower dosages of gamma rays and EMS, late maturity was observed in higher dose of gamma rays.

Rai *et al*. (2000) reported EMS induced mutant in which embryo had only one cotyledon but upon supplementation with the natural cytokinine, kinetine it developed two cotyledons. Evident from this review, although induced mutagenesis is an efficient approach for the development of improved genotypes but it is not attempted at adequate level for the improvement of *C. roseus*. Keeping this in view, more concerted efforts on this direction are needed which may leads to development of high anticancerous and antihypertensive containing 'ideochemocultivars' which helps in decreasing extraction cost as well as market value of these alkaloids. Several mutant loci affecting the development of organs have been reported in *V.rosea* (Pandey-Rai and Kumar, 2000; Pandey-Rai and Kumar, 2001; Pandey-Rai *et al*., 2003; Saranraj *et al*., 2010).

Table - 1: Effect of induced mutagenesis on seed germination and seedling survival of M_1 generation

Treatment Dose/ con of Gamma rays and EMS	Seed germination (%) 15th day			Seedling survival (%) 30th day		
	Mean (%)	Per cent over control	Per cent reduction over control	Mean (%)	Per cent over control	Per cent reduction over control
Control	88.39±2.65	100.00	00.00	87.12±2.61	100.00	00.00
15 mM	69.66±2.08	78.80	21.20	66.90±2.00	75.91	24.09
20 mM	53.54±1.60	60.57	39.43	50.31±1.50	57.74	42.26
25 mM	47.42±1.42	53.64	46.36	43.27±1.29	49.66	50.34

Table - 2: Determination of LD_{50} value for EMS

Treatment concentration of EMS	Seed germination (%)	Per cent over control	Per cent reduction over control
Control	98.00	100.00	00.00
5 mM	87.33	89.11	10.88
10 mM	74.00	75.51	24.48
15 mM	65.33	66.66	33.33
20 mM	49.66	50.67	49.32
25 mM	42.66	43.53	56.46
30 mM	38.00	38.77	61.22
35 mM	33.75	34.43	65.56
40 mM	28.16	28.73	71.26
45 mM	25.00	25.51	74.48
50 mM	18.26	18.63	18.36

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