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Determination of mutagenic sensitivity of hardwood cuttings of grapes 'Red Globe' and 'Muscat' (*Vitis vinifera* L.) to gamma rays

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ABSTRACT

In a mutation breeding experiment, LD_{50} gives an indication of the response of different types of plant materials of a species to mutagen, so that the optimal dose(s) can be fixed to obtain higher recovery mutants with minimal population loss. In the present investigation hard wood cuttings of grape *cv*. 'Red Globe' and 'Muscat' were treated with ten different dosages of gamma rays ranging from 5 to 50 Gy and compared along with an untreated set of control. The results revealed a gradual and significant reduction in survival of cuttings, shoot length, leaf length and leaf width with increase in dosage of gamma rays. There was a complete inhibition of survival (100%) over control in the treatments above 35 Gy of gamma rays. The probit curve analysis based on the survival percentage and growth rate of treated cuttings revealed that, LD_{50} dosage of gamma rays to be 15–20 Gy for 'Red Globe' and 15–25 Gy for 'Muscat'.

1. Introduction

The grapevine (*Vitis vinifera* L., Vitaceae) is one of the most important fruit crops in the world widely distributed in the temperate regions with the ranges extending into the tropics and subtropics. Majority of the grapes produced are used for the production of wines of outstanding quality especially in the temperate regions. Exclusive varieties are also cultivated for dessert purpose. Crop improvement in grapes has been predominantly focused on *Vitis vinifera* and to certain extent on *Vitis labrusca (*Muscadine grapes*),* but other species of the genus *Vitis* have been used as well to incorporate resistance to pests and diseases. Screening of a variable population is an important procedure in selection and variations induced in the grape were mainly through hybridization techniques and to certain extent by mutation breeding. Mutation breeding has been successfully used for generating genetic variation and breeding new varieties in many crop plants during the past decades (Van Harten, 1998; Tambe and Apparao, 2009) and become ultimate source of genetic variation to provide unique germplasm and the raw material for plant breeders (Van Harten, 1998).

Spontaneous mutations in grapes, has induced all kinds of variations in characteristics including leaf colour (Boubals, 1976), yield (Woodham and Alexander, 1966), seedlessness (Nitsch et al., 1960) and change in the colour of berries (Moretti, 1983) especially in old cultivars. These have led to the release of new cultivars with economic importance after selection and testing. Induced mutagenesis in grape have been reported by many investigators (Coutinho and Corte, 1982; Das and Mukherjee, 1968; Donini, 1975, 1977; Olmo, 1960; Sharma and Mukherjee, 1972) and reported to affect all kinds of characteristics, including yield, earliness, size, number and colour of berries, hardiness, resistance to diseases (e.g. downy mildew (Golodriga and Kireeva, 1975)), and form and size of leaves.

Generally, physical mutagens such as X-rays, ^{60}Co γ-rays or thermal neutrons were employed for mutation breeding. The success of mutation breeding is greatly influenced by the main factors *viz.* the rate of mutation and the mutation efficiency. The mutation rate is affected by the total dose of the mutagen employed and can be modified by physical and biological factors. The effectiveness of a mutagenic treatment in inducing genetic variations in crop plants depends on the genetic constitution of test varieties and treatment dosage, among others (Van Harten, 1998; Mba et al., 2010). Mutagenic treatment with high doses may destroy the promoters of growth and induce the growth inhibitors along with various chromosomal aberrations. Thus high radiation doses would be lethal retaining few plants for selection which in turn limits the success of artificial selection in the subsequent mutation generations to identify useful mutants. Brown (2013) listed some negative effects of higher radiation doses such as deletions of DNA nucleotide sequences that may cause reading-frame shifts, inactive protein products, or faulty transcripts. This would subsequently lead into null mutations, in which a particular gene may be inactivated. Conversely, low radiation dose are accompanied by early emergence, increased

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percent germination and field survival with healthy and vigorous seedlings. However, this would possibly be associated with low mutation frequency with reduced selection response towards target mutants.

The first step in induced mutagenesis is an optimization of radiation dose where its predictable value, guides the researcher in the choice of the ideal dose depending on the plant materials and desired outcome. Mutation breeding is known to be a useful method for altering a particular trait without changing much the genetic background but in practicality chromosomal aberrations. The loss of pigments, decrease in protein content, and delay in development and rooting of plant and sterility are caused by irradiation treatment. Therefore, selecting the correct dose is essential to induce a higher mutation rate in a target trait with minimal effect on the remaining genetic background (Coban, 1998). The response to mutagens in plants is species specific and differs even among genotypes of the same species (Kwon and Im, 1973). The term radio sensitivity is a relative measure of the quantum of recognizable effects on the irradiated material (Owoseni et al., 2007) and it is essential to observe the growth responses after irradiation to determine optimal dosages. According to Mba et al. (2010) the better mutation frequency can be achieved by the optimum mutagenic dose. Meyer (1996) described that the LD_{50} is the dose at which death of 50% of the test material results. The optimum dose for X-rays or γ -rays for grape was reported to be approximately 20–60 Gy if dormant (rooted) cuttings are irradiated (Das and Mukherjee, 1968), although lower optimum doses of 10–20 Gy have also been reported (Shimotsuma, 1962).

The present study was taken up as the first step for the mutation breeding in grapes cv. 'Red Globe' and 'Muscat' which are intensively cultivated for fresh fruits in Tamil Nadu, India. Both the varieties yield marketable quality bunches having seeded berries and as much as five crops can be harvested in two years especially in cv. Muscat due to its high vigour and the favourable environmental conditions in this zone. To find out whether any useful mutant with resistance to biotic and abiotic stress could be obtained from these varieties, mutation breeding approach was planned. The present study was performed to fix the LD_{50} of gamma radiation in grapes *cv.* 'Red Globe' and 'Muscat'.

2. Materials and methods

Hard wood cuttings of 'Red Globe' and 'Muscat' cultivars with uniform size were treated with ten different doses of Gamma radiation (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 Gy) including control. Gamma irradiation was given using ^{60}Co gamma source (Gamma Chamber 1200, Board of Radiation and Isotope Technology (BRIT), Mumbai, India) at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The different dosages were fixed automatically based on the half life of the ^{60}Co (gamma source).

Fresh cuttings were collected from the University vineyard. 50 cuttings per treatment per replication was taken and irradiated with gamma rays according to assigned treatments. After exposure, treated cuttings were planted in nursery polythene bags filled with red soil: FYM: sand: (1:1:1) along with untreated cuttings as control. Planted cuttings are maintained in the mist chamber with temperature of 28–30 °C with relative humidity of 80% for 20–25 days until rooting of cuttings, then the rooted plants were shifted to 50% shade house at 45 day from planting. The percentage survival, shoot length, leaf length and width were measured after eight weeks after planting. The experiment was organized as Completely Randomized Design with three replicates and conducted for the period of twelve weeks. The LD_{50} value was calculated based on probit analysis using the survival of treated cuttings to that of control. After recording the observations plants were taken care for further observations.

2.1. Probit analysis

The LD_{50} values for gamma radiation were determined based on the Probit analysis (Finney, 1978). The probit function is the inverse cumulative distribution function (CDF) or quantile function associated with the standard normal distribution.

2.2. Analysis of variance

Data were subjected to the standard analysis of variance procedure using SPSS statistical package to identify the lethal dose (LD_{50}). The LD_{50} for each variety was estimated through the simple linear regression model by fitting the straight line equation $y = a + bx$; where y is the response variable (percent survival), x is the independent variable (irradiation dose), while a and b represent the slope and constant, respectively.

3. Results

In the present study a gradual reduction in survival rate of cuttings with increase in dose of gamma rays and complete inhibition of survival of cuttings at 35 Gy and above dosages in both the cultivars (Table 1) was observed.

LD_{50} for gamma radiation was fixed based on survival percentage and growth rate of cuttings in both varieties. Probit analysis was carried out based on survival rate of the stem cuttings after treatment with

Table 1
Effect of mutagen on survival of cuttings in Grape *cv.* 'Red Globe' and 'Muscat'.

Red Globe				Muscat			
Dose (Gy)	Survival percentage (%)	Per cent survival over control (%)	Per cent reduction over control (%)	Dose (Gy)	Survival percentage (%)	Per cent survival over control (%)	Per cent reduction over control (%)
0	90	100.00	–	**0**	85	100.00	–
5	85	94.44	5.56	**5**	75	88.23	11.77
10	75	83.33	16.67	**10**	70	82.35	17.65
15	55	61.11	38.89	**15**	60	70.58	29.42
20	50	55.55	44.45	**20**	50	58.82	41.18
25	35	38.88	61.12	**25**	35	41.17	58.83
30	15	16.66	83.34	**30**	5	5.88	94.12
35	0	0.00	100.00	**35**	0	0.00	100.00
40	0	0.00	100.00	**40**	0	0.00	100.00
45	0	0.00	100.00	**45**	0	0.00	100.00
50	0	0.00	100.00	**50**	0	0.00	100.00
SEd	**0.042**				**0.056**		
CD @ 5%	**0.092**				**0.122**		

Bold values are significant.

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