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Radiosensitivity of amla (*Emblica officinalis* Gaertn.) varieties treated with gamma rays

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ABSTRACT

Investigations were carried out at the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, during 2003-2005 to work out radiosensitivity of five varieties of amla (*Emblica officinalis* Gaertn.) exposed to different doses of gamma rays. Scions of five amla varieties, *viz.*, BSR-1, Kanchan, Krishna, NA-7 and Chakaiya, were irradiated with different doses (1.0 to 5.0 kR) and these were grafted on to rootstocks. Based on the sensitivity study, LD_{50} for 100% survival ranged from 1.0 to 2.0 kR for all the five varieties. All the amla varieties could survive upto 10-20% at lower doses (upto 2.5 kR).

Key words : Amla, sensitivity, LD_{50}

INTRODUCTION

Vegetatively propagated crops are a group of plants highly suitable for application of mutation breeding for various reasons. Continuous vegetative propagation has led to less variability in the amla plant populations. Induction of mutation is considered an important breeding tool to create new variation for economic traits. Moe and Han (1973) stated that improvement of a crop cultivar was usually accomplished by adding one or more desirable attributes to the initial, commercially grown strain and, hence, mutagenesis was the simplest means to achieve desirable breeding objectives.

Induction of mutations in vegetatively propagated plants has been investigated extensively by various authors, from Broertjes to Spiegel Roy. Induction of mutations in amla (*Emblica officinalis* Gaertn.) has been receiving increasing attention recently for crop improvement (Pathak, 2003). Adequate information on sensitivity of different varieties of amla to different doses of gamma rays is not available. The present investigation purports to assess sensitivity of amla to different gamma ray treatments in terms of survival percentage and degree of crop growth inhibition. The degree of growth inhibition in a woody plant like amla was determined by growth characteristics such as height, spread, number of buds or leaves and fresh and dry weight. The traits, *viz.*, fifty per cent bud survival and inhibition of growth, were used as biological parameters to determine sensitivity of amla varieties to different doses of gamma rays.

MATERIAL AND METHODS

The present investigation of induced mutation breeding in amla (*Emblica officinalis* Gaertn.) was undertaken in the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, from 2003-2005. Improved amla varieties, BSR –1, Krishna, Kanchan, Chakaiya and NA-7 (maintained at the Central orchard of the Horticultural College and Research Institute, TNAU), which can be readily propagated by cleft-grafting, were chosen for the study. A physical mutagen (Gamma rays) was employed in the present study. Amla scions with dormant buds were treated with gamma rays. Scions of pencil-thickness, consisting of 10 nodes (dormant buds) from seven year old mother trees, were collected and treated under a temperature range of 25± 2°C. The scions were stored by wrapping in a wet gunny-cloth at room temperature until treatment, and thereafter, till grafting on to rootstock. The treated scions were cleft-grafted the same day on one-year old amla seedling rootstocks. Both the gamma ray exposed and untreated grafts were planted in pots and these received uniform standard operations after-care.

Sensitivity studies

A preliminary study was conducted to fix the optimal dose of gamma ray irradiation on survival of grafts

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(scions treated with gamma ray). The range of gamma ray (kR) doses as 1.00, 2.00, 3.00, 4.00 and 5.00. Different criteria adopted for assessing sensitivity were:

Graft survival: The survival of the gamma-ray treated grafts was recorded at 30, 60, 90 and 120 days from grafting and expressed as percentage.

Degree of growth inhibition: The degree of growth inhibition was expressed in terms of the following parameters, measured 90 days after grafting:

1. Length of the primary shoot (cm)
2. Number of leaves (90 days from grafting)
3. Fresh weight of the primary shoot (g)

RESULTS AND DISCUSSION

The biological effect of gamma rays (sensitivity) on amla (*Emblica officinalis* Gaertn.) growth and development was studied based on four V_1M_1 growth criteria. The plant parameters studied were: survival, primary shoot length, number of leaves and fresh weight of the primary shoot.

Per cent survival

Percentage of survival of the scions after irradiation showed highly significant differences among different doses of gamma rays. There was progressive reduction in per cent graft-survival, with increase in dose (Table 1). The highest dose of 5.0 kR recorded 16% survival as compared to 92% in the control. The LD_{50} values for survival in the variety BSR-1 ranged from 1.00 to 2.00 kR. However, survival percentage as comparatively low in 'Kanchan' as compared to 'BSR-1' on wild amla rootstock. LD_{50} for survival was reckoned to between 1.00 and 2.00 kR. As registered in the other two varieties, a relatively low survivability of treated scions was observed in different doses of gamma rays of the variety Krishna. Percentage survival ranged from 8.69 to 58.33. Survival percentage of NA-7 amla variety was

Table 1. Survival percentage of amla varieties in V_1M_1 generation following gamma ray irradiation

Variety	Dose of gamma ray (kR)	Survival percentage (%)	Primary shoot length (cm)	Number of leaves	Fresh weight of primary shoot (g)
BSR-1	Control	92.00	21.88	22.00	5.66
	1.0	60(65.22)	26.50(121.12)	26 (118.18)	6.87(121.38)
	2.0	44(47.83)	23.23(106.17)	21(95.45)	6.76(119.40)
	3.0	36(39.13)	16.63(76.01)	19(86.36)	5.55(98.40)
	4.0	24(26.09)	12.50(57.13)	17(77.27)	4.33(76.50)
	5.0	16(17.39)	7.93(36.24)	11(50.00)	2.89(51.06)
Kanchan	Control	96.00	20.26	18.00	5.80
	1.0	56(58.33)	23.50(115.99)	20(111.11)	6.53(112.59)
	2.0	40(41.67)	22.00(108.59)	19(105.56)	6.60(113.80)
	3.0	36(37.50)	14.00(69.10)	16(88.89)	5.45(93.97)
	4.0	24(25.00)	12.00(59.23)	14(77.78)	3.89(67.07)
	5.0	12(12.50)	6.30(31.10)	11(61.11)	2.50(43.10)
Krishna	Control	92.00	21.80	18.00	7.07
	1.0	56(58.33)	21.43(98.30)	18(100.00)	6.00(84.87)
	2.0	40(43.48)	20.90(95.87)	17(94.44)	5.55(78.50)
	3.0	28(30.43)	19.47(89.31)	16(88.89)	4.80(67.89)
	4.0	16(17.39)	16.63(76.28)	12(66.67)	4.30(60.82)
	5.0	8(8.69)	6.53(29.95)	10(55.56)	3.20(45.26)
NA-7	Control	96.00	22.40	20.00	6.25
	1.0	52(54.17)	20.63(92.10)	22(110.00)	7.01(112.16)
	2.0	36(37.50)	19.50(87.05)	18(90.00)	6.45(103.20)
	3.0	28(29.17)	9.65(43.08)	15(75.00)	5.18(82.88)
	4.0	28(29.17)	5.63(25.13)	13(65.00)	5.55(88.80)
	5.0	12(12.50)	3.40(15.18)	11(55.00)	2.87(45.92)
Chakaiya	Control	88.00	23.00	22.00	6.00
	1.0	52(59.09)	22.45(97.61)	24(109.09)	6.85(114.17)
	2.0	36(40.90)	19.53(84.91)	20(90.09)	6.01(100.17)
	3.0	12(13.64)	18.43(80.13)	17(77.27)	6.00(100.00)
	4.0	8(9.09)	12.15(52.83)	13(59.09)	5.30(88.30)
	5.0	8(9.09)	9.68(42.09)	12(54.55)	3.25(53.80)

* Values in parantheses are per cent values over control

found to be inversely related to increasing doses of gamma rays. The LD_{50} sensitivity dose for survival for NA-7 variety ranged from 1.00 to 2.00 kR The highest dose of gamma rays (5.00 kR) recorded 8% survival rate in 'Chakaiya' as compared to 88.88 % in the untreated control. The LD_{50} dose for survival was 1.00 to 2.00 kR.

In general, mutagenic treatments of scions from different amla varieties in the present study resulted in lower percentage of survival. Success of the irradiated scions when grafted depends upon union of cambium layers of the stock and scion and consequent production of normal conducting tissue. Snow (1933) demonstrated that meristematic activity of cambium in the region of graft-union is stimulated by indoleacetic acid, and this view is shared by several researchers. That the level of auxin concentration in plants drops after exposure to ionizing radiation is also well-recognized. Irradiation immediately lowers free-acid auxin levels in the crop plant and the inactivation of auxin generally increases with increasing exposures (Skoog, 1935). In this regard, Gordon and Weber (1955) clearly showed that *in vivo* auxin synthesis was non- exponential with increment in gamma exposure but, that, the extent of inhibition of synthesis increases with increased dose. Moreover, mutagenic treatments cause chromosomal aberrations, which adversely affect cell-division. The lower percentaged of survival of grafts observed after treatment of the scion-wood with gamma rays may be attributed to a drop in auxin levels and to chromosomal aberrations caused by mutagenic treatments.

Further, it was also observed, in the present study, that the survival percentage of amla grafts decreased gradually as the dose of gamma rays increased, but, the decrease was rather sharp at 4 and 5 kR for all the five amla varieties. This was further exemplified by the sensitivity of LD_{50} doses required to cause 50% lethality. According to Viswanathan *et al* (1992), reduced survival per cent at higher doses of gamma radiation may be mainly due to cell death and higher rate of ionization in the nuclei. The drastic decrease in survival percentage under different doses of irradiation may be due to physiological imbalance and damages caused at the molecular level, which results in chromosomal aberrations causing considerable cytological changes.

Primary shoot length

The primary-shoot length on 90th day from grafting of the irradiated scion of BSR-1 variety was lower than that in the control, particularly, at higher doses (3.00, 4.00 and 5.00 kR), but at lower doses (1.00 and 2.00 kR), there was a slight increase in the primary shoot length as compared to the untreated control. LD_{50} values for this trait ranged from 4.00 to 5.00 kR. In the variety 'Kanchan', an increase of 15.99 and 8.59% over the control was recorded in 1.00 and 2.00 kR treatments, respectively whereas, the primary-shoot length at different doses showed a decreasing trend with 3.00, 4.00 and 5.00 kR of gamma rays. The LD_{50} dose of gamma rays for this trait was noticed to be between 4.00 and 5.00 kR. Reduction in primary-shoot length of the treated plants of 'Krishna' showed linearity with the increasing dose of gamma rays. Reduction in the primary-shoot length ranged from 98.3 to 29.95% of control, indicating a drastic reduction for this character at higher doses. Fifty per cent reduction in lethality was obtained between doses of 4.00 and 5.00 kR. Gamma ray irradiated NA-7 amla grafts registered a reduction in primary-shoot length showing an inverse relationship to increase in dose of gamma rays and the reduction ranged from 20.63 to 3.4 cm as dosage increased from 1.00 kR to 5.00 kR. The LD_{50} value for this trait ranged between 3.00 and 4.00 kR. The percentage of reduction ranged from 97.61 to 42.09 in the variety Chakaya. The LD_{50} sensitivity value was observed in the dose range of 4.00 and 5.00 kR.

It is seen clearly that length of the primary-shoot gets gradually reduced in proportion to increase in dose of gamma rays. This reduction in shoot length of amla is considered to be a combined effect of mortality of a few cell initials, delay in sprouting and slow growth-rate. Reduction in growth of mutagen-treated meristems of the shoot is a cumulative expression of at least three different types of cytologically identifiable effects (Evans, 1965).

Positive explanations for the reduction in plant height have been offered for reduced crop growth at different stages following mutagenic treatments, such as auxin destruction (Skoog, 1935), inhibition of auxin synthesis (Gordon, 1954), failure of the assimilatory mechanism (Quastler and Baer, 1950), production of diffusible growth-retarding substances (Mackey, 1951), changes in specific activity of enzymes (Haskins and Chapman, 1956) and inhibition of DNA synthesis (Mikaelson, 1968).

Growth indices of the physiological effects, viz. number of leaves and fresh weight of shoot were also studied.

Number of leaves and fresh weight of primary shoot

Gamma ray treatments in the present study recorded marked inhibitory effect in respect of number of leaves. Fifty per cent reduction in the number of leaves per plant was observed between doses of 4.00 to 5.00 kR in all the varieties. Inhibition occurred at 3.00 kR for BSR-1, Kanchan and Krishna, and, 3.00 and 5.00 kR for NA-7 and Chakaiya. This may be due to direct effect of the mutagens on the growing points of amla varieties. Depending upon the physiological and developmental stage these might have been killed or inactivated by various doses of the toxic mutagen and, hence, the reduction in number of leaves. However, stimulatory effects of ionizing radiation were obtained for fresh weight of shoot and production of leaf at lower doses of gamma rays. The increase was significant in some cases, but was less in magnitude indicating that such physiological stimulation are is not likely to be exploited on a commercial scale for crop improvement. The basis for stimulatory responses obtained, though small in magnitude, is of significant interest.

In general, there was a clear and perceptible variation in susceptibility of amla varieties to injury by gamma ray. There is a considerable variation in the LD_{50} values and the differences exhibited were greater between levels of the mutagen and between varieties. In most of the cases, no exposure produced those exact levels of survival and hence LD_{50} values were determined by interpolation from the survival-curve. A possible explanation for this differential sensitivity could be that frequency of cells involved in the different treatments may be higher.

Thus, the present study clearly indicated that survival percentage is a reliable criterion for arriving at the optimum dose of irradiation in amla (*Emblica officinalis* Gaertn.). Better survival percentage of plants seen at lower doses may be due to radiation resistant-nature of the biological material upto a certain dose. This is evident from the result that higher doses of the mutagen resulted in poor survival percentage.

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