RESEARCH ARTICLE

EFFECT OF GAMMA IRRADIATION AND EMS ON *IN VITRO* SHOOT TIP CULTURES OF BANANA VARIETY *NANJANAGUDU RASABALE*

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Abstract: Banana is one of the most important crops grown in the country for domestic and export markets. Variety *Nanjanagudu Rasabale* is grown in parts of Mysuru district of Karnataka known for its unique aroma, favour and taste, is susceptible for fusarium that causes Panama wilt. Investigations on development of mutants induced resistance or tolerance using gamma rays and EMS were carried out using *in vitro* shoot tip cultures. There was no mortality with cent per cent survival and regeneration of explants when treated with gamma rays at different dosage and EMS concentrations. However, a slight change in stem colour was noticed with the use of EMS. The growth of explants was almost normal compared to untreated ones but a slight reduction in the rate of growth and proliferation was observed in both gamma and EMS treated samples. Rooting was also normal in presence of IBA. Unfortunately, all the *in vitro* grown plantlets have become susceptible when treated with fusarium inoculums, the plants turned yellow and wilted gradually over a period of time.

Keywords: Gamma rays, Banana variety, Crops

INTRODUCTION

Banana is the world's major food crops and considered as the poor man's fruit in tropical and sub-tropical countries. Banana plantations however, are subjected to various natural calamities and diseases; in particular, viral diseases constitute a major setback worldwide. Among viral infections *the Banana bunchy top nanavirus* (BBTV) (Islam *et al*., 2010) and *Banana mosaic cucumo virus* (BMV) (Swennen and Vuylsteke, 2001).

Apart from viral diseases, Fusarium wilt has a long and destructive history in the world's banana-producing regions (Ploetz *et al*., 1990; Stover, 1962). Also known as Panama disease, it is caused by a variable soil borne fungus, *Fusarium oxysporum* f. sp. *cubense*. Fusarium wilt almost destroyed the export trade during the first half of the 20th century. It has continued to damage on Gros Michel (AAA), in the eastern hemisphere. Less publicized, but potentially more destructive that the epidemics on Gros Michel are outbreaks that affects non exported, locally consumed cultivars.

In India, variety *Nanjanagudu Rasabale* (NR) having genotype AAB is grown in Mysore district of Karnataka. This popular variety known for its aroma, taste and shelf-life is now available only at a few fruit shops in the city and has been sought after by the elite. This variety is highly susceptible to wilt caused by *Fusarium oxysporum* var. *cubense* and the area under cultivation has drastically been brought down to just 5 hectares from 500 acres in the last three decades (Lakshmanan *et al*., 2007) and has been considered endangered.

Resistance can be introduced in to banana by conventional and unconventional methods (Crounch *et al*., 1998). Whereas the parthenocarpic nature of cultivated banana makes it difficult to breed for resistance to diseases (Jones, 2000), the conventional breeding of most commercially demanded banana is handicapped due to its complex genetic background, lack of seed formation, parthenocarpic nature and triplody factors that hamper the improvement of this crop with desired quality (Kumar, 2016) and limited information available on genetic and genomics (Chopra, 2005; Capdeville *et al*., 2009).

Some improved methods, such as long term breeding program, have many limitations due to sterility of cultivated banana, long growth cycles, low seed set and hybrids that are often not accepted by local consumers (Sagi, 2000). Improving disease resistance is the vital for the future survival of banana plants by tissue culture based techniques such as *in vitro* mutagenesis. Mutations for banana plants improvement can be induced by gamma radiation and by somoclonal variations. *In vitro* mutagenesis can be lead to variability in banana clones that are generated from single mother plant. Somaclonal variations also are obtained as a result of nuclear chromosomal re-arrangement, gene amplification, non-reciprocal mitotic combination, transposable element activation of silent genes (Jain, 2001; Karamural *et al.*, 2010).

Mutation breeding *in vitro* is a powerful tool for the induction and selection of desirable mutants which can be utilized in banana improvement (Novak, 1993). Mutagenic treatments on banana plants have been reported and *in vivo* sucker material has proved to be less effective and yielded low mutagenized material for further screening (Novak, 1990). On the contrary, *in vitro* mutagenesis employing multiple shoot cultures has several advantages: a high shoot multiplication ratio resulting in efficient chimera separation; a drastic reduction in time and space requirements; ease of treatment with mutagens and subsequent leaching off of chemical mutagens; and optional facility for *in vitro* selection against various stresses. Therefore, an experiment on banana variety *Nanjanagudu Rasabale* was conducted to induce

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mutation and develop variants resistant or tolerant to fusarium using physical and chemical mutagens.

MATERIALS AND METHODS

In vitro mutagens treatment

Around 3-4 months old healthy and vigorously growing sword suckers of banana variety *Nanjanagudu Rasabale* free from diseases particularly fusarium infection were collected from the farmer field at Devarasanahalli, Nanjanagudu taluk, Mysuru district as a source material and established *in vitro* shoot tip cultures using MS medium with growth hormone BAP. *In vitro* plantlets of banana were trimmed to a size of 1-1.5 cm with basal rhizome tissue, raised in culture bottles and exposed to gamma rays ranging between 5 and 50 Gy (^{60}Co) from Kidwai Memorial Institute of Oncology, Bengaluru. Following irradiation, they were immediately transferred to fresh MS media with plant growth hormone BAP at 3 mg/l. In another experiment, *in vitro*-raised shoot cultures were treated with chemical mutagen Ethylmethane sulphonate (EMS) at different concentrations and time (EMS - 0.25, 0.50, 0.75 and 1.00 %, Time – 1, 2, 3 and 4 hours) under aseptic conditions in a laminar air flow cabinet. As a guide, 200 ml EMS solution can be used to treat 50 to 100 explants, whereby the volume depends on the size of the explants, but it is crucial that the explants are immersed completely in the solution.

In principle, all kinds of plant material used for mutagenic treatment, whether from explants or *in vitro* cultures are needed to undergo a series of subcultures after being mutagenized to separate chimeras and produce plants for selection of desired mutants since mutation remains undetectable by chimeras in the first generation (Banerjee, *et al*. 2015). Therefore, mutagens treated cultures were subcultured for four cycles at monthly interval to overcome the usual problem of chimera formation by mutagen treatment of shoot tips. Then individual shoots were separated from the clumps and subjected to rooting with half strength MS medium containing IBA. The cultures were incubated at 25 ± 2 ^{o}C under a 16:8-hr photo regime with white fluorescent tubes. The cultures thus established were subjected to observations on survival rate after 40 days of irradiation and shoot characteristics like length and rate of multiplication. Further, mutagens treated *in vitro* cultures were hardened off using coco peat and established for treatment of fusarium inoculums.

Inoculum preparation and inoculation procedure:

Pure isolates of *fusarium oxysporum* strain were sub cultured on to Potato Dextrose Agar (PDA) plates to serve as starter colonies. The plates were inoculated with 25-30 ^{o}C for 7 days. A 5 mm disk from each resulting colonies was used to inoculate 100 ml sterilized PD broth medium in 250 ml flasks and incubated for 15 days at 25- 30 ^{o}C for further studies. The plants were inoculated two months after planting. A suspension (200 ml/polybag) containing mycelial growth and spores (4x 10^{6} cfu/ml) of isolate was poured and covered with soil, similar to the method followed by Su, *et al*. (1986) and Sanchez-Hernandez, *et al*. (1998). The disease incidence was assessed one month after inoculation to ascertain the level of resistance, tolerance or susceptibility of the mutagens treated *in vitro* plantlets.

RESULTS AND DISCUSSION

After having sufficient cultures, they were treated with physical mutagen Gamma ray at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 Gray and inoculated. After 40 days of inoculation no mortality was found and 100 per cent survival and regeneration was observed. The response for the gamma ray treatments was good and plantlets were greener and healthy in all dosage of treatments. Jain (2010) has recommended for various banana cultivars based on LD_{50} values; 10-20 Gy of gamma irradiation for diploid clones Calcutta 4 (AA) and Tani (BB), 30-40 Gy of gamma irradiation for the triploids Three Hand Planty (AAB), Grande Naine (AAA), Williams (AAA) and Kamaramasenge which is a triploid and 40-50 Gy of gamma irradiation for the triploid Cachaco (ABB). Similar kind of observation was made by Abdulhafiq *et al.* (2018) when the explants treated with higher doses (40, 50, 60, and 70 Gy), showed deleterious effects of ionizing radiation. The highest survival rate among gamma treated explants recorded was 71 % in 10 Gy treatments while the lowest survivability was 15 % in 70 Gy.

However, overall rate of multiplication was reduced. At lower dosage shoot growth was good when compared to higher doses. This could be seen from the data presented in table 1. The explants treated with 20 Gy of gamma radiation showed maximum shoot length (8.30 cm) followed by 5 Gy (8.29 cm). The minimum shoot length was registered in the explants irradiated at 45 Gy (6.99 cm). Similarly, number of opened leaves was found maximum in explants irradiated with 20 Gy (3.90) and minimum with 35 Gy (2.40). However, number of shoots showed no significant differences among the treatments.

Gamma rays are the most commonly used mutagenic agent in an *in vitro* mutagenesis experiment (Roux *et al*., 2004), since it has higher degree of accuracy, sufficient reproducibility and deep penetrating power in to a biological matter that can cause a greater number of variation in physico-chemical composition (Yamaguchi, 2003). Reduction in shoot growth at higher doses of gamma rays is due to reduced mitotic activity in meristematic tissues and reduced moisture content of explants (Majeed, *et al*., 2009). A similar results were reported by several researchers (Datta and Banerjee, 1995; Fereol,1996) on the inhibitory effects of high doses of gamma radiation on shoot growth, the inhibition might be due turbulences in

physico-biochemical processes related to the action of gibberellic acid, which typically stimulates cell division and cell elongation and higher dose kills meristematic cells, or damage cells producing fewer progeny cells and causing inability of irradiated tissues cells to absorb available nutrient, which leads to reduced growth. The findings also supported by the outcomes of Abdulhafiz, *et al*. (2018) where they could see reduced shoot length with higher dose of gamma rays and vice-versa.

In another experiment on use of chemical mutagen Ethylmethane sulphonate (EMS) at varied concentrations and for a different duration, there was no mortality of *in vitro* cultures as result of EMS treatments and they were regenerated and survived similar to that of gamma rays exposure. This indicates the explants tissue potential to withstand and regenerate at all the concentrations tried. Morphologically there was not much change with shoots and leaves are concerned but for a slight stem discoloration. As anticipated from the experience of previous experiment, the response to EMS in terms of growth is normal at lower concentration (0.50, 0.25 %) and treated for short duration (1, 2 hr.). However, growth is slow or otherwise inhibited at higher concentration of EMS (0.75, 1.00 %) and treated for long periods (3, 4 hr.) as well. This is evident from the data presented in table 2, the explants treated with 0.25 % EMS for the period of 3 hour showed maximum shoot length (8.57 cm) followed by explants treated with the same concentration for 2 hour. It was shorter in the explants treated with 1.0 % EMS for 4 hour (3.55 cm). Highest number of shoots was observed in both explants treated with 0.5 % EMS for the period of 2 hour and untreated ones (5.10) and lesser number of shoots were noticed in 0.25 % EMS for 1 hour (2.30). Number of opened leaves was found maximum at 1.0 % EMS for 2 hour (3.40) and minimum with same concentration but for the period of 4 hour (1.9).

Application of *in vitro* mutagenesis especially among vegetative propagated banana has significantly improved the efficiency of mutation techniques in breeding programmes (Kulkarni *et al*., 2007). *In vitro* mutagenesis has also been applied to isolate useful variants in banana by Suprasanne *et al*. (2008). Similarly, Shirani Bidabudi *et al*. (2012) evaluated the possibility of induced variations in banana cultivars through *in vitro* mutagenesis by treating the shoot tips with EMS. EMS induced mutation of banana using a micro-cross section cultural system has been also reported to be potentially useful for banana improvement (Chen, *et al*., 2013).

Both gamma rays treated and EMS treated banana plantlets were induced to root and subjected for primary and secondary hardening off. Rooting was normal in presence of IBA at 2 mg/l. *In vitro* plantlets were successfully hardened off using coco peat under shade house. After hardening, they were treated with *Fussarium oxysporum* and kept for observation. All the plants induced with gamma rays and EMS treatments have got infected when subjected to fussarium inoculums, showing their susceptibility and have gradually wilted over a period of time. Infected plants started turning yellow and further wilted over a period of time. Similar kind of observations made by Krishna *et al*. (2013), wherein the leaf vein showed chlorosis gradually entire lamina turned yellow and resulted in the wilting of the leaves. Kishore, *et al*. (2017) screened *in vitro* derived mutants obtained from cultures treated with different chemical mutagens revealed disease development in the mutants of artificial inoculation. The chloroplasts were swollen in susceptible banana cultivars, after the injection of the culture filtrate or purified metabolic juglone (Jain and Swennen, 2004). The findings of this experiment apart from the previous studies reported above justify the statement made in the introduction; a total devastation of banana variety *Nanjanagudu Rasabale* over last three decades and regarded endangered.

CONCLUSION

In vitro mutagenesis could lead to variability in banana clones that are generated from a single mother plant. It was true to a little extent, with few morphological changes in shoot characteristics like colour, length etc. resulted from this experiment. However, it was not so easy with respect to inducing resistance or tolerance against *fusarium oxysporum*, signifies highly virulent nature of the soil-borne pathogen known to cause panama wilt in Banana.

Table 1. Effect of gamma radiations on shoot proliferation

Treatments	Shoot length (cm)	No. of shoots	Number of opened leaves
Control	7.11	3.50	3.60
5 Gy	8.29	3.80	3.30
10 Gy	7.71	3.70	3.60
15 Gy	7.95	3.00	3.10
20Gy	8.30	3.80	3.90
25 Gy	7.80	3.50	3.00
30 Gy	7.70	3.90	2.80

35 Gy	7.20	3.70	2.40
40Gy	7.83	3.50	2.90
45 Gy	6.99	3.00	2.90
50 Gy	7.40	3.80	2.50
S. Em. ±	0.065	0.086	0.822
C. D. @ 1%	0.756	NS	0.966

Table 2. Effect of EMS on shoot proliferation

Treatments	Shoot length (cm)	No. of shoots	Number of opened leaves
Control	7.00	5.10	3.20
0.25 %, 1 hr.	8.00	2.30	2.80
0.25 %, 2 hr.	8.05	2.60	3.30
0.25 %, 3 hr.	8.57	2.60	2.90
0.25 %, 4 hr.	7.93	2.80	3.00
0.50 %, 1 hr.	7.48	3.70	3.20
0.50 %, 2 hr.	6.44	5.10	3.30
0.50 %, 3 hr.	6.85	3.00	3.20
0.50 %, 4 hr.	7.00	3.50	3.20
0.75 %, 1 hr.	8.05	3.70	3.20
0.75 %, 2 hr.	5.80	3.40	2.70
0.75 %, 3 hr.	5.35	3.50	2.60
0.75 %, 4 hr.	4.64	3.70	2.30
1.00 %, 1 hr.	6.60	3.20	3.00
1.00 %, 2 hr.	6.32	3.40	3.40
1.0 0%, 3 hr.	4.70	3.40	2.20
1.00 %, 4 hr.	3.55	3.30	1.90
S. Em. ±	0.136	0.117	0.082
C. D. @ 1%	1.569	1.351	0.951

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