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Regenration of Disease Free Banana Plants

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Abstract: Plants were regenerated from shoot tips of Banana. Shoot tips measuring 1.0 mm were Isolated from multiple shoot Cultures of banana variety "Basrai" and cultured *in vitro* on different medium compositions .The nutrient medium MS + BA + Thiamine HCL resulted increased plants regeneration percentage. The plants were successfully established in soil.

Key word: Regenration banana plants

Indrocation

In, Sindh, Banana is cultivated on 22600 hectares out of 26400 hectares in Pakistan With annual fruit production of Sindh 63600 tonnes, out of 94600 tonnes (Agricultural Statistics of Pakistan 1998 to 99). The Banana fruit has been consumed in the local market and it has been exported abroad earning in the million of rupees in foreign exchange. Recently, the "bunchy top disease" of banana caused by a virus "BBTV" has wiped out half of the banana population in Sindh.

Entire banana plantation is threatened if appropriate measures are not taken in time. It will be a catastrophe for millions of farmers who's livelihood depend on banana industry if timer rescue measures are not taken to save it. Attempts have been made to regenerate disease free banana plantlets.

The shoot tip multiplication method obviously has great potential for producing specific pathogen free planting material in quantity. Tissue culture propagation of banana using shoot tip as well as floral apex has been reported by Novak *et al.* (1986). Bapat and Rao (1988) Fitchet *et al.* (1990) Blakesley (1991). And Genapathi *et al.* (1992). Hence virus free plants have been produced *in vitro* and attempt have been made to solve the problem of bunchy top which play an important role in the economy of the country.

Materials and Methoods

Field grown plants of banana CV. "Basrai" a dwarf commercial variety of lower Sindh, were used as the experimental material. Shoot tips (1 cm) were isolated by removing the sheathing leaf bases. The pale white shoot tips were surface Sterilized with 10% Sodium hypochloride + poly oxye thyrene sor bitan mono lanrate for 10 minutes, after repeated rinsing in sterile distilled water these were cultured on Murashige and Skoog's (1962) medium with Thimmine HCL., inosital and BA. After three weeks, the shoot tips turned green and showed growth. Such explants were transferred to medium of the same composition for multiple shoot induction. This was done by making transverse cut to separate leaves. These yielded section of pseudostem approximately 3mm long including an intact vegetative bulb. For removing darkened or necrotic tissue the lower parts of explant was trimmed and making two halves of pseudostem explant by cutting in half longitunally through the apex. Each shoot produced multiple shoots and after 2 to 3 weeks the multiple shoots were separated and transferred in fresh growth media .All the experiments were conducted aseptically and under controlled conditions of light (1000 lux) temperature (25 ± 2°C) and relative humidity (50 ± 2%) for three weeks. After 3 weeks, regenerated plants transferred in the small plastic pots and placed under high humidity for weaning. The plants which survived and transferred into the field. The explant material was selected with the help of plants pathologist.

Table 1: Regeneration of plants by using different medium composition

Medium	NO of total Cultures formation	No of cultures with shoot and root	% of palnt regeneration
(A)			
MS + NAA0.5mg /1	40	19	48
MS + NAA1mg /1	40	20	50
MS + NAA1.5mg/1	40	18	45
(B)			
MS + BA 0.5mg/ 1 * Thiamine 0.5mg/1	50	31	62
MS + BA 1mg/1 *Thiamine 1mg/1	50	35	70
* Thiamin 2mg/1	50	30	60
(C)			
MS* Thiamine 1mg/1	50	16	32
MS* Thiamine 1.5mg/1	50	15	30
MS* Thiamine 2mg/1	50	14	28

Results and Disscussion

It has been proved beyond doubt that the plants propagated through the conventional methods e.g. cutting, grafting bulbs. Tubers etc are systematically infected with one or more pathogens particularly "virus" like agents unless the infection is readily detected and the plants have been freed of the pathogen by using tissue culture techniques. In case of banana how ever the number of suckers produced per plant in a year is limited in the present situation it was attempt to induce the mass multiplication of virus free banana *in vitro*.

Table1 and photographs, show the growth response of the regenerated plants in different medium compositions. It is very clear that MS + BA + thiamine gave the best results in comparison, to other compositions where 60 to 70% shoot and root formation was observed with 2 to 4 multiple shoots proliferation. Followed by MS + NAA medium which induced shoot formation by 40 to 50% . How ever, 30% plantlets were also produced by MS + Thiamine. 5 to 10% contanination was observed in all the treatments.

Survival problems were oncountored during weaning which were mainly due to inadequate facilities. while Rowe and

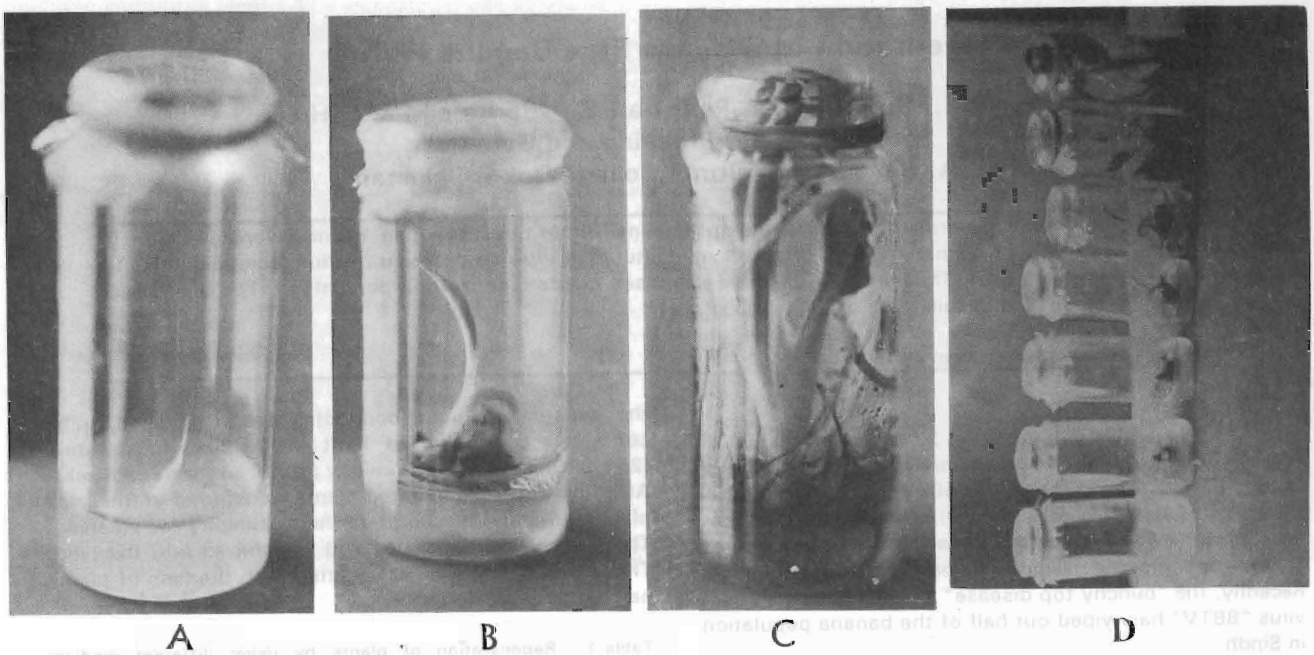

Fig. 1a: Banana shoot tip excised from desired plant

Fig. 1b: Regeneration of healthy shoot after two years

Fig. 1c: Regeneration of rooted shoot (Plantlet after four weeks)

Fig. 1d: Successive devlopment stages of banana, starts from vegetative shoot apieces (L) upto plantlet (R)

Richardson(1975) and Bapat and Rao (1988) regenerated banana shoot tip cultures with low contamination, many other research workers like, Ko *et al.* (1991) worked on meristem tissue of banana and produced the plantlets sucessfully,Wu and Su (1990) and Blakesly (1991) conducted the experiments on production of "Bunchy top" virus free plants and have recommended the tissue culture techniques for development of healthy plants . These workers used several media compositions and found that MS + BA gave the satisfactory results .

The main objective of this research was to assess the posibility on production of disease free plantlets through tissue culture in the area, where the situation of banana is very critical.

Suggestion: It is suggested that a package of technology may be developed to comabat the banana courage successfully. Some of the remedial steps are suggested here.

1. Entries banana plantation located in disease areas may be destroyed by burning.
2. Un-infected areas of banana may be separated from infected areas by creating buffer zone of 6-10 km.
3. Health banana plants may be protected through efficient pest control measures against insects specially with sucking complex.
4. Healthy cultural conditions may be provided to the banana plantations e.g. Regular manuring, weeding and mulching and removal of dried leaves.
5. The plantation may be replaced with disease free plants, which may be involving-huge foreign exchange, or proper incentives may be offered to the scientists to regenerate locally through tissue culture technology.

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