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EFFECT OF CARBON SOURCES AND GELLING AGENTS IN *IN VITRO* MULTIPLICATION OF BANANA (*MUSA PARADISIACA L.*) VAR. ROBUSTA

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ABSTRACT
One of the factors that make micropropagation process as an expensive multiplication system was the cost of media additives. Carbohydrates and gelling agents are the two major ingredients of tissue culture media and were available at various grades and brand names. Present study was aimed to find out a suitable carbohydrate-gelling agent combination based on the hardening and establishment of the *in vitro* grown shoots of banana (*Musa paradisiaca L.*) var. Robusta. Five types of gelling agents namely Agar-agar (Himedia), Crude agar (NR Chem), Clerigel (Himedia), Gelwell (Microxpress) and Clerigel-Gelwell mixture and two types of carbon sources *viz.* LR grade sucrose (Sisco Research Laboratory) and Table sugar (Parry and Co.) were incorporated into the multiplication media and the performance of the cultures were assessed. Out of ten treatments best results were obtained (6.56 shoots/culture) from the Treatment (T8) with Clerigel (2 g/l) and Table sugar (30 g/l). Multiplication of shoots was not appreciable in Treatment (T5) with gelrite combinations (Clerigel & Gelwell) along with LR grade sucrose (SRL) (3.02 shoots/culture). Micro shoots from all the treatment groups were subjected to hardening and their survival rates were assessed. Even though 100% survival rate was obtained from all the treatments, maximum and quicker growth was achieved from the Treatment (T8) with 90% well established plantlets ready for planting in the field in 30 days.

KEY WORDS: *Gelling agents, Carbon sources, MS 230, BAP, Clerigel, Gelwell, sucrose, table sugar*, Robusta.

INTRODUCTION

Banana is one of the most important fruit crop in the world ranking second after citrus produced in tropical and sub-tropical regions. It plays a very significant role in human welfare as a staple food as well as a major export commodity for many countries. Because of its multifaceted uses and high economic returns it is referred to as "Kalpatharu" (a plant of virtues). The basic study on micropropagation of banana by Swenmen *et al.*, 1991, Kodym and Zapata-Arias, 1999, Nandwani *et al*., 2000 has led to the technological development of different banana cultivars in *in vitro* commercial production on large scale. One such banana cultivar is Robusta (AAA). It is a semi-tall variety, grown mostly in Tamil Nadu, Andra Pradesh, Karnataka and Maharastra. It is a heavy yielder and produces bunches of large size with well developed fruits. Fruits are long and green with creamy flesh, pleasantly sweet and aromatic. Each bunch weighs about 35-45 kg. It has a very good market potential. Agar and sucrose are the two major constituents which play a significant role in the cost of production for banana micropropagation. The effect of different gelling agents and carbon sources on *in vitro* shoot multiplication of banana plants was studied by few researchers (Ganapathi *et al.*, 1995; Khan *et al.*, 2001; Kodym *et al.*, 2001 and Farahani *et al.*, 2008). In all those studies banana cultivars responded differently on the same medium at different stages of multiplication. In the present study, banana cv. Robusta (AAA) was selected for studying the effect of gelling agents (Agar & Gelrite) in combination with carbon sources (sucrose and table sugar) (Table: 1) in order to find out the best medium for *in vitro* multiplication of banana cv. Robusta.

MATERIALS & METHODS

Selection of Explant

Vegetative shoot tips (apical meristem) of banana-variety Robusta is the preferred explant. This shoot tip was obtained from sword suckers collected from true-to-type, high yielding, and disease free mother plant grown in the demonstration field for tissue cultured banana of Perunthalaivar Kamaraj Krishi Vigyan Kendra, Kurumbapet, Puducherry-605 009.

Explant preparation and disinfection

The sword suckers were carefully removed from the mother plant without any damage and washed thoroughly in running tap water. Older leaves and the extraneous rhizome tissue were carefully chopped with a stainless steel knife. Trimmed suckers were then soaked in a solution of Bavistin (0.5%) for 1 hour. Shoot tips containing several sheathing leaf bases enclosing the axillary buds with subjacent rhizome tissue and measuring 4-5 cm in length were isolated. These shoot tips were soaked in a solution of cetrimide (0.1%) – a bactericidal surfactant, and surface-sterilized with distilled water for 5 minutes. Further operations were carried out under a laminar air flow chamber.

Cut surfaces of the rhizomatous tissue and leaf bases were further removed after the treatment of Mercuric Chloride solution (0.1%) for 3-5 minutes by repeatedly washing several times with autoclaved, sterile distilled water. The

explants measuring 2-3 cm were inoculated in the test tube containing the liquid medium. After 1-2 weeks, the healthy, contamination-free explants were swollen and turned green showing morphogenetic activity. They were removed from the liquid medium and the overlapping leaf sheaths along with the discolored tissue were removed aseptically. They were then splitted into two equal halves longitudinally and transferred to semi-solid medium substituted with agar-agar as a gelling agent (0.8%).Within 4-5 weeks time, the splitted explants began to proliferate with tiny protuberances heralding the progenitors of multiple shoots. These shoots were repeatedly sub-cultured on MS-230 semi-solid medium at an interval of 4 weeks per sub-culture. Uniform shoots were collected from the sub-cultured banana variety - Robusta for conducting this study.

Media Preparation

MS basal medium (Murashige & Skoog, 1962) was used for culturing banana Growth regulators-Benzylaminopurine (BAP) - 2mg/ litre and Adenine sulphate ($Ad.So_4$) - 30mg/litre were added separately along with sucrose 3% P^H was adjusted to 5.8 before boiling. For the experiment 2½ litres of medium was prepared for 10 treatments, each with a volume of 250 ml. Treatments were categorized according to the combination of carbon sources and gelling agents (Table-1). 0.8 % agar and 0.2% gelrite was added to the media and allowed to boil for solubilization. The boiled media were poured in equal quantities to test tubes @ 15 ml / test tube and tightly closed with test tube caps. The media were then autoclaved at 121^{o}C for 20 minutes.

TABLE 1: Combination of Carbon sources and Gelling agents in MS-230

Treatment	Carbon source	Gelling agent
T1	Sucrose	Agar-agar
T2	Sucrose	Crude agar
T3	Sucrose	Clerigel
T4	Sucrose	Gelwell
T5	Sucrose	Clerigel and Gelwell
T6	Table sugar	Agar-agar
T7	Table sugar	Crude agar
T8	Table sugar	Clerigel
T9	Table sugar	Gelwell
T10	Table sugar	Clerigel and Gelwell

The mother cultures obtained by a series of sub-cultures (5th sub-culture) from the initiation stage were selected for the selection of uniform shoots for conducting the experiment. Individual shoots were excised from the mother multiplying cultures and trimmed off to have shoot segment of about 2-2.5 cm. A longitudinal incision was made keeping the corm base intact and piercing the meristem of the culture to break the apical dominance. Then the uniform shoot bits were individually inoculated in the test tubes containing treatment media by keeping the corm base completely immersed in the medium. These whole processes were carried out inside the laminar air flow chamber under aseptic conditions. A total of 10 cultures per treatment were inoculated and arranged them in the sterile stainless steel test tube stands and incubated in the incubation room with a photoperiod of 16/8 hours light/dark and a light intensity of 3000 lux at a room temperature of 26$^{+/-}$ 2^{o}C. Observations were made at weekly intervals for each treatment. Parameters such as bud formation, no. of buds/culture, no. of shoots/culture, no. of leaves/shoot and % of root development were recorded.

RESULTS & DISCUSSION

The present study revealed that the best shoot multiplication rate in banana cv. Robusta (AAA) could be achieved on the medium containing table sugar replacing the commercial grade sucrose (SRL) and gelrite (Clerigel-Himedia) as the supporting agent for growth. Promotory role of table sugar in shoot multiplication has been observed in banana cv. Basarai (AAA) (Ganapathi *et al.*, 1995), Grand Naine (AAA) (Kodym *et al.*, 2001), Amrit Sagar (AAA) (Khan *et al.*, 2001), Dwarf Cavendish (AAA) and Valery (Farahani *et al.*, 2008) by replacing 'Laboratory grade' (LR) sucrose. Low cost alternatives of gelling agent used by various workers have suffered from one or the other drawback limiting their use on commercial scale (Jain *et al.*, 2006). In the present study, the response of cultures in Gelrite (Clerigel-Himedia) evoked similar response as that of control (AR grade Agar-Himedia) in standard multiplication medium (MS-230) at the same concentration. However, the shoots/plantlets developed in Gelrite and table sugar treatment were bigger and healthier than those derived from control (AR grade Agar and Sucrose). Incorporation of Gelrite and table sugar (T8) in standard banana multiplication medium (MS-230) in the present experiment has significantly increased the rate of shoot multiplication, leaf development and root formation compared to other treatments (T1-T10), (Table: 2). The objective of this investigation was to develop an in vitro method for the micro propagation of banana – var. Robusta by substituting sucrose and agar with table sugar and gelrite respectively, and minimizing the use of chemical components (Table:1). From the results obtained, it is evident that cultures grown on table sugar as carbon source showed remarkable difference in growth than those on commercial grade sucrose (LR) treatments (Table:3). 266 shoots were obtained at various development stages with table sugar as carbon source (T6-T10), with commercial grade sucrose produced 238 shoots at the end of 4th week (Table:3) (Fig:1).

TABLE 2: Response of cultures for various treatments at the end of 4th week

Treatment	% of Shoot Development	No. of shoots/Culture	No. of leaves/shoot	% of cultures with roots
T1	100	5.79	2.98	3
T2	90	4.58	2.94	3
T3	85	4.37	2.66	2
T4	90	5.75	2.47	4
T5	75	3.02	2.79	5
T6	70	4.38	2.45	6
T7	95	4.41	2.32	5
T8	100	6.56	3.01	7
T9	80	5.57	2.43	6
T10	70	5.69	2.17	2

TABLE 3: Shoots collected from cultures at the end of 4th Week

Treatments	WDS	YS	DS/B	TOTAL
T1	32	10	18	60
T2	20	16	10	46
T3	19	10	15	44
T4	24	15	19	58
T5	18	5	7	30
SUCROSE GROUP- T1>T4>T2>T3>T5				238
T6	32	8	4	44
T7	19	8	17	44
T8	35	14	16	65
T9	26	16	14	56
T10	25	23	9	57
TABLE SUGAR GROUP- T8>T10>T9>T7>T6				266

WDS-Well Developed Shoots, **YS**-Young Shoots, **DS/B**-Developing Shoots/Buds

In the case of the combined effect of various gelling agents with different carbon sources, the best result was obtained (T8) containing Gelrite (Clerigel-Himedia) and table sugar with a maximum number of shoots (65) than that of (T5) with Clerigel and Gelwell medium containing commercial grade sucrose (30) (Table:3).

WLS - Well Developed Shoots YS – Young shoots DS – Developing shoots

FIGURE 1: Showing shoots development

Based on the observations during hardening and establishment of *in vitro* grown shoots it is evident that the plantlets derived from Gelrite-table sugar based medium performed better than those obtained from Agar-Sucrose medium (Table : 4) (Fig:2).

T1 **T2**

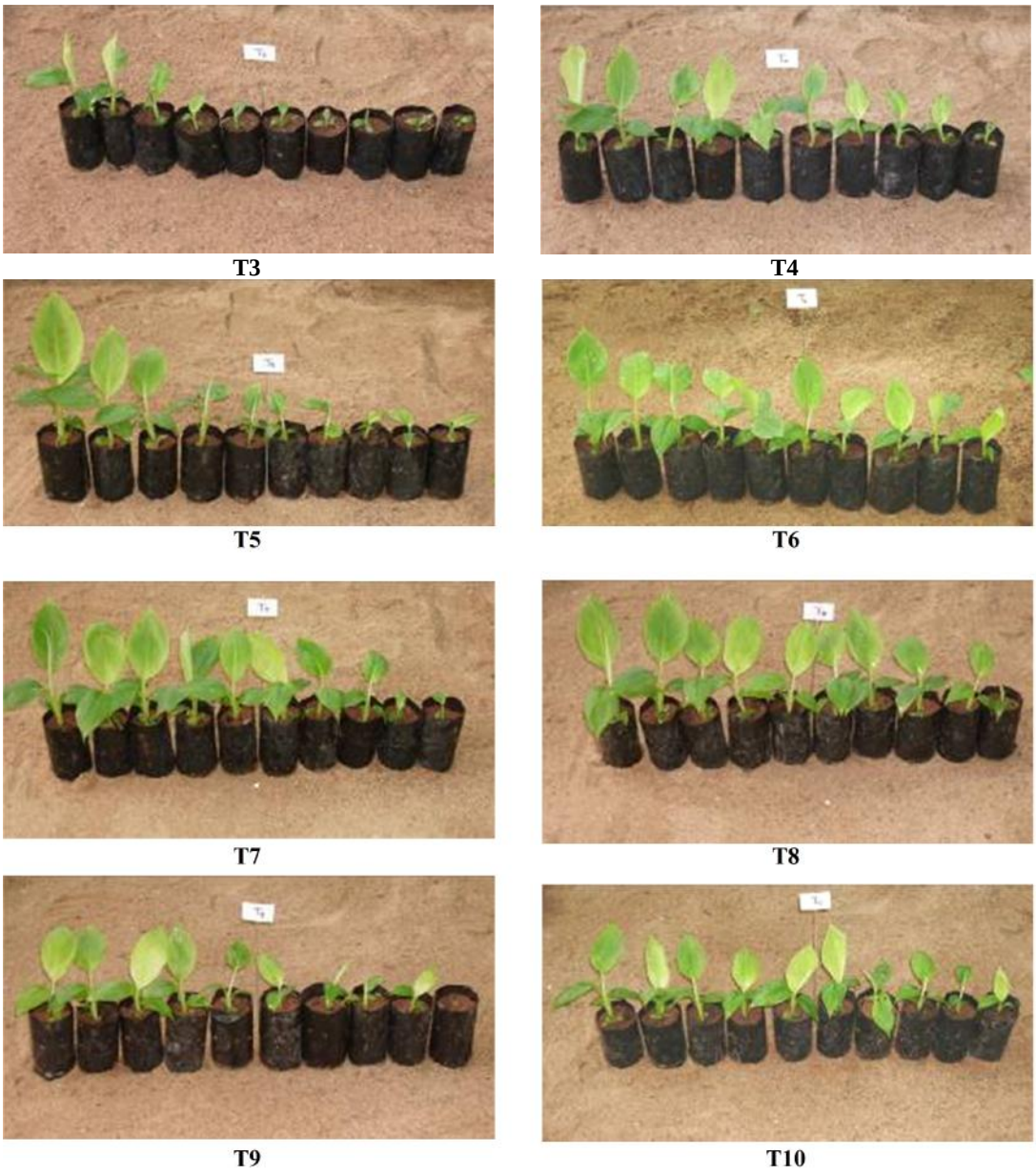

FIGURE 2: Response of Banana plantlets for Treatments (T1-T10) at the end of 30th day of Hardening

TABLE 4: Growth response of the plantlets at the end of 30th day of Hardening

Treatment	% of WEP ready for planting	No. of Expanded leaves	No. of Furled leaves	No. of leaves / plantlet	Length of plantlet (cm)
T1	30	41	6	4.7	6.8
T2	50	49	3	5.2	7.9
T3	30	44	1	4.5	5.8
T4	60	53	4	5.7	9.7
T5	70	51	5	5.6	10.3
T6	60	47	5	5.2	10.2
T7	80	52	5	5.7	11.3
T8	90	57	3	6.0	12.1
T9	50	44	2	4.6	9.3
T10	80	51	3	5.4	9.5

WEP – Well Established Plantlets.

CONCLUSION

The present study has revealed that the best shoot multiplication rate could be achieved for the banana var. Robusta on the medium MS 230 containing Gelrite (Clerigel-Himedia) and table sugar (Parry and Co.,) which enhances the growth both in vitro and ex vitro thus contributing to the mass production of healthy and superior banana plantlets.

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