Int.J.Curr.Microbiol.App.Sci (2020) Special Issue-11: 2374-2380

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-11 pp. 2374-2380**
Journal homepage: http://www.ijcmas.com

Original Research Article

Effect of Different Growth Hormones and their Concentrations during Micropropagation on the Quality of Fruits in Plantain (*Musa paradisiaca* L.) cv. Kovvur Bontha Obtained during Field Evaluation

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A B S T R A C T

Banana (*Musa* spp.) is one of the important fruit crops of the tropics. The fruits are rich source of carbohydrate and energy**.** Plantain is a cheap source of energy like vitamins A, C, B_6 and other minerals with traces of fat. The plantlets were developed using different concentrations of thidiazuron (TDZ), benzyleaminoprine (BAP), and Kinetin were tested against the conventional planting material for quality of the fruits like TSS, acidity, TSS:acidity content, starch and sugar content. The result showed that the highest TSS (7.03 ^{O}brix), acidity (1.04%), starch (77.25%) and sugar (3.44%) when the plantlets were obtained from the T_7 (Plantlets from T_7 of micropropagation experiment where the plantlets were developed on the media added with 7.5 mgL^{-1} of Kinetin). It was concluded that quality of banana fruits was good when the plants were obtained from the media added with 7.50 mgL^{-1} of Kinetin.

Keywords

Starch, acidity, TSS, TSS:acidity, Hormone, Growth regulators

Introduction

Banana (*Musa spp*.) is an herbaceous perennial monocotyledonous plant which belongs to the family Musaceae of order Scitamineae. It is the largest produced and consumed amongst all fruits cultivated in India. It is a crop of subsistence being cultivated from prehistoric time in India with great socio-economic significance and is grown in all tropical regions. It provides well balanced diet to millions of people around the globe and also contributes to livelihood through crop production, processing (Singh, 2002) and thus, plays a key role in the economy of many developing countries.

Banana is originated from South East Asia, a region considered as the primary centre of diversification of the crop, where early domestication has occurred (Simmonds, 1962).The term "plantain" (cooking bananas) was derived from "plantano" of the Spaniards. Plantain and cooking bananas are staple food of people of many countries of Central and West Africa, the Caribbean Islands, Latin America and Kerala and Tamilu Nadu of India (Purseglove, 1975).

From the nutritional point of view, banana has a calorific value ranging from 67 to 137 calories per 100 g and is closely comparable with potatoes but digested more easily. It is relatively cheap.

According to Gopalan *et al.,* (1980), the average composition of banana fruit is moisture-70%, protein-1.2%, carbohydrate-27.0%, crude fibre-0.5%, fat-0.3%, ash-0.9%, phosphorus-290.0 ppm, calcium-80.0 ppm, iron-6.0 ppm, carotene-0.5ppm, riboflavin-0.5 ppm, niacin-7 ppm, ascorbic acid-120ppm. There are traces of potassium, copper, iodine, manganese, magnesium, sodium, zinc and cobalt as well.

India is the largest producer of banana contributing 27% of world production (FAO, 2009). In India, the total area under banana cultivation is 0.85mha with the production of 30MT and productivity is about 34.0 MT/ha (NHB, 2018).

In Odisha, the total area under banana cultivation is about24490 ha with the production of around 0.466 MT and productivity is about 19.05 MT/ha (NHB, 2018).

In plantains, fruit quality of unripe banana is the major parameter for the commercial production of cooking banana crop. In this experiment, we had investigated that the effect of different growth regulators and their different concentrations which had been used during the micropropagation to produce the plantlets for commercial cultivation, on the fruit quality of the plantain. We had investigated the major fruit quality parameter like TSS, acidity, TSS:acidity ratio, starch content, and total sugar content of the cooking banana cv. Kovvur Bontha (ABB).

Materials and Methods

The plantlets were developed using different concentrations of thidiazuron (TDZ), benzyleaminoprine (BAP), and Kinetin *viz.* 0.05 mgL^{-1} of TDZ (T_1), 0.10 mgL^{-1} of TDZ (T_2), 0.20 $mgL^{-1}$of TDZ (T_3), 3.50 mgL^{-1} of BAP (T_4), 5.00 mgL^{-1} of BAP (T_5), 6.50 mgL^{-1} of BAP (T_6), 5.00 mgL^{-1} of Kinetin (T_7), 7.50 mgL^{-1} of Kinetin (T_8),10.00 mgL^{-1} of Kinetin (T_9), and evaluated against Sucker propagated plants (T_{10}).

Weigh 30g of pulp tissue into mortar and pestle and add 90ml of distilled water and blend for 2 mins and filter. Transfer 25ml of the filtrate into a 125ml conical flask. Add 25 ml of distilled water and 4 – 5 drops of phenolphthalein indicator. Fill a 25ml burette with 0.1N sodium hydroxide (NaOH) and adjust to the zero mark after eliminating the bubbles. Record the final value and calculate for per cent (Dadzie and Orchard, 1997).

$$\text{Titrable Acidity} = \frac{\text{Titre value X 0.1 N NaOH X Volume made up X Equivalent weight of malic acid}}{\text{Volume of sample taken for titration X weight of sample X 1000}}$$

The representative fruit samples from each replication were crushed to obtain the juice by straining through muslin cloth. The TSS of banana fruit extract was recorded with the help of digital refractometer and the results

were expressed as Degree Brix (^{o}B) (Krishnakumar and Thirupathi, 2014). Fehling's solution is made by first making two sub-solutions. Solution A is made from copper (II) sulfate pentahydrate dissolved in water and solution B contains potassium sodium tartrate tetrahydrate (Rochelle salt) and sodium hydroxide in water. The two solutions are added together in equal parts to make the final test solution.

The test is a detection method for monosaccharides, specifically aldoses and ketoses. These are detected when aldehyde oxidizes to acid and forms a cuprous oxide. Upon contact with an aldehyde group, it is reduced to cuprous ion, which forms the red precipitate and indicates the presence of reducing sugars. Total sugar present in banana fruit pulp samples were estimated, by the method outlined by Lane and Eynon (1923) and described by Ranganna (1986).

A quantity of 100 mL of lead-free filtrate was taken into a 250 mL conical flask, adds 5 mL of HCl (50% diluted) to it, mixed well and kept at ambient temperature for 24 hours. The acid was neutralized with NaOH (40%) by using a drop of phenolphthalein as an indicator till the pink colour persisted at least for few seconds. Total sugars were then estimated by taking this solution in a burette and titrated against a mixture of standard Fehling's solution A and B (1:1) by using methylene blue as an indicator until brown red colour precipitate was formed. The total sugars present in banana fruit pulp were estimated by using the following formula and were expressed in percentage.

$$\text{Total Sugar} = \frac{\text{Fehling Solution Factor (0.05)} \times \text{Vol. made up (mL)}}{\text{Titre value (mL)} \times \text{wt. of the sample taken (g)}} \times 100$$

Starch content in banana fruit pulp was estimated by using Anthrone Reagent (Sadasivam and Manickam, 1996). Sample weighing 0.5 g was taken into a mortar and it was homogenized with 80% hot ethanol to remove sugars. The sample was centrifuged at two times at 7200 rpm for 4 minutes and retained to residue.

The residue was washed repeatedly with 80% hot ethanol until the green colour and the residue was dried over a water bath. Mixture of water and perchloric acid (5.0 mL of water and 52 % Perchloric acid) were added at the rate of 6.5 mL to the residue (Don't add perchloric acid directly to residue) and continue the centrifugation at 6500 rpm for 2 minutes.

The residual was extracted and repeated the extracton using fresh perchloric acid. Pooled the supernatant liquids after each centrifugation and made up to 100 mL with distilled water. Add 4 mL of anthrone reagent and closed the test tube with alominium foil. Keep the test tubes in boiling water bath for 8 minutes add then cool to the room temperature under tap water. The density of green to dark green colour was measured at 630 nm by spectrophotometer. Glucose content in the sample was estimated by using the standard graph and the starch content was calculated by using the following formula.

Standard graph procedure

Into a series of test tubes 0.2, 0.6, 0.8 and 1.0 Ml of working standard glucose (100µl/mL) solution was taken in each tube. Then 4 mL of anthrone reagent was added and incubated at room temperature for 10 minutes. The intensity of colour developed was measured at 620 nm in spectrophotometer against blank. A standard graph with concentrated glucose on X – axis and the absorbance at 620 nm on Y- axis drawn.

$$\text{Starch (\%)} = \frac{\text{Concentration from Graph (Factor) X 100 X 0.9}}{\text{0.3 mL X 0.5 g X 1000}}$$

Results and Discussion

It was observed that the maximum total soluble solid (7.03^{o} Brix) was recorded from the fruits of treatment T_7 (Plantlets from T_7 of micropropagation experiment) which was on par with T_1(Plantlets from T_1 of micropropagation experiment) which recorded total soluble solid of 6.26^{o} Brix followed by T_4 (Plantlets from T_4 of micropropagation experiment) with 5.05^{o}Brix of total soluble solids. The lowest total soluble solid (0.82) was recorded in the fruits from T_3 (Plantlets from T_3 of micropropagations experiment) and considered as the least good treatment as per table 1.

Total soluble solids depend on many factors like environment, available soil nutrient, and varietal character. The soluble solid content of a fruit is based on soluble compounds such as sugars, acids, vitamin C, amino acid and some pectines. High TSS content was observed in the present investigation agrees with the report by Abdullah *et al.,* (1985) and Sarker *et al.,* (1997). The TSS value for different micropropagation treatments might be due to the modified physiological aspects of banana fruits, suppressed respiration and metabolic processes, lead to high TSS content. The same variation trend was also observed in some dessert and cooking bananas grown recently in Cameroon (Ngoh *et al.,* 2009). The highest amount of titratable acidity was observed in the fruits obtained from T_7 (Plantlets from T_7 of micropropagation experiment) which recorded 1.04% which was on par with T_1 (Plantlets from T_1 of micropropagation experiment) with 0.90% of titratable acidity and third-best treatment was observed in T_4 (Plantlets from T_1 of micropropagation experiment) with 0.56% of titratable acidity. The plants from T_3 (Plantlets from T_3 of micropropagation experiment) had recorded the lowest amount of percent titratable acidity (0.27%) and were considered as the least good treatment for micropropagation (Table 1).

Titratable acidity gives a measure of the amount of acid present. Assessment of titratable acidity of bananas and plantains are used primarily to estimate consumption qualities and hidden attributes.

Acids make an important contribution to the quality of the fruit, as taste is mainly a balance between the sugar and acid contents. Similar results were reported for some *Musa* cultivars in Africa (Ngalani *et al.,* 1998; Aboua 1991; Collin and Dalnic, 1991; Burdon *et al.,* 1991; Onyejegbou and Ayodele, 1995).

The highest ratio of TSS to titratable acidity for the finger was recorded in T_4 (Plantlets from T_4 of micropropagation experiment) with 9.09^{O}Brix per percentage.

The second-best treatment was considered as T_3 (Plantlets from T_3 of micropropagation experiment) which had recorded TSS to titratable acidity of 6.99^{O}Brix per percentage which was on par with the best treatment followed by T_1 (Plantlets from T_1 of micropropagation experiment) which had recorded the TSS to titratable acidity of 6.96^{O}Brix per percentage for fingers.

The plants from T_{10} (Plantlets from T_{10} of micropropagation experiment) had recorded the least TSS to titratable acidity (2.17^{O}Brix per percentage) for the fingers and were considered as the least good treatment (Table 1).

Table.1 Comparison between the plantlets obtained from all the micropropagation treatments of experiment II and conventional planting material (sword suckers) for TSS, acidity, TSS:Acidity, and starch

Treatment	TSS* (^{O}Brix)	Acidity (%)	TSS:Acidity (^{O}Brix/%)	Starch (%)	Total sugar (%)
Plantlets from T_1 of micropropagation experiment (T_1)	6.26	0.90	6.96	77.18	3.30
Plantlets from T_2 of micropropagation experiment (T_2)	1.41	0.35	4.04	74.33	3.13
Plantlets from T_3 of micropropagation experiment (T_3)	3.48	0.50	6.99	72.11	3.20
Plantlets from T_4 of micropropagation experiment (T_4)	5.05	0.56	9.09	77.05	3.22
Plantlets from T_5 of micropropagation experiment (T_5)	1.03	0.40	2.60	71.69	3.18
Plantlets from T_6 of micropropagation experiment (T_6)	1.99	0.62	3.22	74.58	3.10
Plantlets from T_7 of micropropagation experiment (T_7)	7.03	1.04	6.77	77.25	3.44
Plantlets from T_8 of micropropagation experiment (T_8)	2.27	0.47	4.87	71.24	3.00
Plantlets from T_9 of micropropagation experiment (T_9)	1.24	0.57	3.08	76.61	3.08
Suckers as control (T_{10})	0.82	0.27	2.17	69.20	3.05
SE(m) ±	0.05	0.00	0.08	1.17	0.09
C.D.	0.14	0.01	0.25	3.48	N/A

The TSS to titratable acidity ratio provides information on fruit flavor. Fruits obtained from fourth micropropagation treatment recorded highest TSS to titratable acidity wheras Fruits obtained from fourth suckers resulted in lowest TSS to titratable acidity. Similar finding reported in sapota (Sudha *et al.*, 2007).

The starch content in the different micropropagated plantlets of banana cv. Kovvur Bontha varied from 69.20% to 77.25%. The highest amount of starch was recorded in fingers obtained fromT_7 (Plantlets fromT_7 of micropropagation experiment) with 77.25% of starch. The second-best treatment was considered as T_1 (Plantlets

fromT_1 of micropropagation experiment) where the starch percentage was 77.18% followed by T_4 (Plantlets fromT_4 of micropropagation experiment) and recorded the 77.05% of starch. Treatment T_{10} (Plantlets fromT_3 of micropropagation experiment) had recorded the least percentage of starch of 69.20% and considered as the least good treatment (Table 1).

According to Ahmad and Beg, 2001, starch is the main component of green banana and it undergoes several changes during ripening (Akihisa *et al.*, 1998) Starch, when susceptible to the action of amylase, is called digestible starch and when amylase-resistant it is referred to as resistant starch.

There were no significant differences were observed among the fruits obtained from different treatments of micropropagation. The highest amount of total sugar (3.44%) from the fruits obtained from T_7 (Plantlets fromT_7 of micropropagation experiment) followed by the second-best treatment which recorded 3.30% of total sugar in T_1 (Plantlets fromT_1 of micropropagation experiment) and the third best treatment for total sugar was obtained in T_4 (Plantlets fromT_4 of micropropagation experiment) which is 3.22%. The lowest amount of total sugar was recorded in T_{10} (Plantlets fromT_{10} of micropropagation experiment) which was 3.05% and considered as least good treatment (table 1).Presence of reducing sugar is a great importance to banana cultivators, since they are involved in reactions of non-enzymatic darkening during processing (Oetterer, 2006).

From this experiment we concluded that the T_7 (Plantlets from T_7 of micropropagation experiment) had recorded the maximum TSS* (7.03 ^{O}Brix), acidity (1.04 %), starch (77.25%), and sugar (3.44%) recommended for the mass propagation of the plants.

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