EFFECT OF BENZYLAMINOPURINE AND NUMBER OF SPLITTING OF SHOOT-TIP EXPLANTS OF BANANA (*Musa* AAA) ON SOMACLONAL VARIATION

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ABSTRACT:

Banana (Musa spp.), is a member of the family Musaceae and it is divided into two types: dessert and cooking bananas. Banana (Musa spp.) is one of the most important of the tropical and subtropical fruits after grapes and citrus. The objective of this study is to evaluate the effect of cytokinin (BAP) concentration and number of explants splitting on induction of somaclonal variation on in vitro propagated banana cvs. Grand Nain. Plant material of banana cv. Grand Nain was selected form greenhouse in Plant Tissue Culture Laboratory at Agriculture Research Corporation (ARC), Wad Medani Sudan. The explants were inoculated on to sterilized solid basal MS medium (Murashige and Skoog's, 1962) supplemented with different concentrations of cytokinins BAP (1.0 – 10.0 mg/l) and numbers of splitting cycles (first – ten cycles). The result indicated that the highest number of shoots was produced on the second splitting. It was observed that the number of shoots per explants decreased significantly on cultivar Grand Nain except in cycles five and six. The only somaclonal variations observed was mainly dwarfism. The result indicated that the number of shoots regenerated per explants increased significantly with the increase on BAP concentration (6 – 10 mg/l) after 4, 8 and 12 weeks. Results showed that dwarf plants regenerated from the banana cv. Grand Nain restored their normal growth and plant height on MS medium supplemented with GA_3 (0.5 – 2.0 mg/l).

Keywords: Banana variety (GrandNain), Cytokinin, and Gibberellin

1. INTRODUCTION

Banana (*Musa spp*.), is a member of the family *Musaceae* and it is divided into two types: dessert and cooking bananas (Acland, 1975). Banana (*Musa spp*.) is one of the most important of the tropical and subtropical fruits after grapes and citrus (Samson, 1992). World *Musa* production is about 85.5 million tones and around 98 % of *Musa* is grown in developing countries. Most of the banana genotypes are triploids, sterile and clonally propagated by suckers. This method of propagation is slow to meet the demand for planting materials from the newly released cultivars (Cronauer and Krikorian, 1984a; Jarret *et al*., 1985a). *In vitro* culture is a great advantage for mass propagation of various vegetative propagated crops and for commercial propagation in of banana. Usually shoot multiplication is used by *in vitro* cultuer (Imelda, 1991). This technique can increase the rate of seedling

production and improve the seedlings quality, such as uniformity and true to type. The average rate of shoot formation produced by this technique was 4 – 5 shoots per subculture (Imelda, 1991; Priyono and Mawardi, 1993). The most import disadvantage of using planting materials produced by tissue culture is the induction of somaclonal variation (Scowcroft, 1985), which is the occurrence of off-type plants during *in vitro* propagation. Frequent occurrence of somaclonal variation in *Musa* has warranted investigation into its nature and extent. Rates of somaclonal variation in plants derived from shoot-tip culture vary from 0 to 70 % according to genotype. Shoot-tip culture exhibit naturally occurring variation (Smith, 1988; Vuylsteke *et al*., 1991; Israeli *et al*., 1995), conversely, somaclonal variation may provide another source of novel and useful variability (Vuylsteke, 1998). Dwarfism in Cavendish banana or inflorescence variations in plantains are often observed after micropropagation of respective mother genotype. Reuveni and Israeli (1990a) reported that the rate of somaclonal variation increases with the increase of regeneration number. Genotype explants type, culture duration and culture conditions are amongst the factors affecting somaclonal variation (Reuveni *et al*., 1986, Vuylsteke *et al*., 1988a). Therefore, this study was tentatively carried out to achieve the following objective of: to evaluate the effect of cytokinin (BAP) concentration and number of explants splitting on induction of somaclonal variation on in vitro propagated banana cvs. Grand Nain.

2. MATERIAL AND METHODS:

2.1. Plant materials and location of the study:

The source plants material was the banana *cvs*. Grand Nain of age 6 months old that grown that selected from green house in the Plant Tissue Culture Laboratory at the Agriculture Research Corporation, Wad Medani, Sudan.

2.2. Culture medium:

The young meristem cutting explants were inoculated on to sterilized solid basal MS medium (Murashige and Skoog's, 1962) supplemented with different concentrations of BAP.

2.2.1. Effect of splitting cycles on somaclonal variation on banana *cvs* Grand Nain:

This experiment was conducted to test the effect of propagation cycles and splitting on somaclonal variation on banana *cvs* Grand Nain. The explants from each cultivar were cultured on the initiation medium for two weeks and then the explants were splitted into two halves. The data collected after 4 weeks such splitting cycles ranging (first – ten cycles)

2.2.2. Effect of BAP concentration on somaclonal variation:

This experiment was conducted to test the effect of different concentrations of BAP (0, 1, 2, 4, 6, 8, 10 mg/l) on somaclonal variation on banana cvs, Grand Nain. The data were collected after 4, 8, 12 weeks, to evaluate the propagation number of shoots per explants.

2.2.3. Effect of GA_3 concentration on dwarf plants:

This experiment was conducted to test the effect of different concentration of GA_3 (0.0, 0.5, 1.0, 1.5, 2.0 mg/l), on the dwarf plant that appear during the subculture cycles. The data collected after 2, 4 and 8 weeks; to for elongations of shoots on dwarf banana regenerated from the cultivar Grand Nain.

3. RESULTS AND DISCUSSION:

3.1. Effect of splitting cycle on shoots morphogenesis of banana cvs. Grand Nain:

Table (3.3.1) shows the number of shoots regenerated per explant in the second splitting cycle was highly significant on both cultivars compared with all other propagation cycles. The number of shoots regenerated per explants on banana *cv.* Grand Nain was smaller as those induced on the other splitting cycles except cycles sixth and seventh. The most of the cultivars tested showed an increase in proliferation between the 3rd and 6th subcultures. Banerjee and De Langhe (1985) reported that proliferation rate was influenced by the number of subcultures and the influence of the number of subculture in somaclonal variation in micropropagation of banana *cv.* Grand Nain was also supported by Rodrigues *et al*. (1998). But Medes *et al*. (1998) showed that the multiplication rate on banana culture tended to decrease with time and after the seventh subculture new shoots may be formed at a very low rate. However, Rodrigues *et al*. (1998) found that somaclonal variation on banana *cv.* "Nanicâo" (*Musa spp*., AAA group) appeared after 5, 7, 9 and 11 subcultures at a rate of 1.3, 1.3, 2.9 and 3.8% respectively in the field. In general, all somaclonal variants produced bad quality bunches. Hence the increase in the somaclonal variation with increased numbers of subcultures is a very important factor to consider when plants are multiplied at a large scale and measures should be undertaken to minimize it.

Table (3.3.1): Effect of splitting on *in vitro* morphogenesis of shoot tip explants of banana *cvs.* Grand Nain after 4 weeks:

No of Splitting Cycles	No of shoot per explant
First	3.4 bc
Second	4.4 a
Third	3.8 b
Fourth	3.6 b
Fifth	3.8 b
Sixth	3.0 c
Seven	3.0 c
Eight	3.2 bc
Nine	3.4 bc
Ten	3.2 bc
SE±	0.03
CV %	14.9

3.2. Effect of BAP on shoots morphogenesis on banana *cv.* Grand Nain:

The percentages of explants with shoot morphogenesis on banana *cv.* Grand Nain cultured on different BAP concentrations was similar after 4, 8 and 12 weeks (Table 3.3.2). The number of shoots regenerated per explants increased significantly with the increase in BAP

concentration. Significant higher number of shoots per explants was induced on MS medium with BAP at 8 mg/l after 8 and 12 weeks compared with BAP concentration ranging from 0-6 mg/l. After 4 weeks the number of shoots regenerated per explant cultured on 6, 8 and 10 mg/l BAP were not different. The number of shoot per explants on MS with 1 to 6 mg/l BAP was similar after 8 and 12 weeks on the banana cultivar Grand Nain (Table 3.3.2). There was no somaclonal variation observed in the culture of the banana cultivars Grand Nain was not affected by the medium composition. Rather, genotypic effects make some cultivars more prone to somaclonal variation than others. The BAP concentration at 22.2µM was optimal for micropropagation of banana *cvs*. Kibuzi and Bwara (Cronauer, and Krikorian, 1984b; Jarret *et al*., 1985a), and at 20µM as was studied by Vuylsteke (1989), the high BAP concentration level in the medium is used to maintain high multiplication rates. Rodrigues *et al*. (1998) used 5 mg/l BAP to induced shoot formation on Brazilian banana "Nanicâo" on MS medium. Khanam *et al*. (1996) found that clonal propagation of Amritsagar (Genome AAA) banana was done through meristem tip culture. Shoot proliferations were observed on MS medium supplemented with BAP at 30 µM BAP and shoot regeneration was height but stunted in growth. However, at 25µM BAP, a good number of healthy shoots were produced.

Table (3.3.2): Effect of BAP concentration on shoot morphogenesis on banana *cv.* Grand Nain shoot-tip explants cultured on MS medium after 4, 8and 12 weeks:

Treatment	**No of shoot per Explants**		
BAP (mg/l)	(4 weeks)	(8 weeks)	(12 weeks)
0	1.06 d	1.31 c	1.44 c
1	1.10 cd	1.68 b	1.79 cd
2	1.13 cd	1.60 b	1.73 d
4	1.14 bcd	1.70 b	2.01 bc
6	1.8 abc	1.73 b	1.95 cd
8	1.28 a	2.02 a	2.25 ab
10	1.24 ab	1.99 a	2.21 ab
SE±	0.01	0.04	0.04
CV	9.0	15.9	12.6

3.3. Effect of different concentration of gibberellic acid (GA_3) on dwarf plant regenerated from banana *cv.* Grand Nain:

Table (3.3.3) shows the percentage and with shoot length of dwarf plant cultured on different concentrations of gibberellic acid (GA_3) acid were comparable on banana *cv.* Grand Nain after 2, 4 and 8 weeks. The shoot length regenerated per explants increased significantly with increase in concentration of GA_3. Shoot length increase significant when cultured on GA_3 concentration ranging from 0.5 – 2 mg/l at different incubation periods 2, 4 and 8 weeks. However, the shoot length per explants induced on GA_3 from 0 mg/l and 1.5 mg/l after 8 weeks were not significantly different on cultivar Grand Nain. The majority of banana "off-types" in the *Cavendish* subgroup appear as 'dwarf' or 'giant' and it has been demonstrated that both are associated with sensitivity to gibberellic acid (GA_3) by Reuveni *et al*. (1996b).

However, Hwang and Tang (1995) isolated and characterized several gibberellic acid insensitive mutants in other plant species. Most types of the *in vitro* generated mutations in Cavendish have been resulted from a long duration in tissue culture and high number of cycles in the multiplication phase of the meristem culture (Walther *et al*., 1997).

Table (3.3.3): Effect of gibberellic acid (GA_3) on dwarf plants of banana *cv.* Grand Nain after two, four and eight weeks:

Treatment	Plant height (cm)		
Gibberellic acid (mg/l)	Two weeks	Four weeks	Eight weeks
0	6.0 b	7.5 b	9.3 b
0.5	9.0 ab	11.5 a	11.3 ab
1.0	9.8 a	11.1 a	12.1 ab
1.5	8.6 ab	10.7 ab	11.9 ab
2.0	11.3 a	12.9 a	15.2 a
SE ±	0.04	0.04	0.04
CV (%)	27.5	25.0	27.8

4. CONCLUSIONS:

Somaclonal variation on banana cultivars Grand Nain increased with the increase in the number of propagation cycles. Most of the somaclonal variation was dwarfism which can be treated by the addition of GA_3 to the medium to restore the normal growth. Further work is needed to evaluate the somaclonal variation in the field.

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