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Effect of cytokinins and auxins on micropropagation of shoot tip and nodal explants of two cultivars of sweet potato (Ipomoea batatas (L) Lam)

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ABSTRACT

In vitro propagation of two selected cultivars of sweet potato (Ipomoea batatas (L) Larn), Baladi White (BW) and Nigerian (N) were investigated using shoot tips and nodal explants during the period March, 1997 to July, 1998. Shoot regeneration from both cultivars was best on Murashige and Skoog (1962) medium without plant growth regulators. Morphogenetic response varied with the different types of explants and genotypes of sweet potato. Shoot morphogenesis from the sweet potato cv. BW was better than that from cv. N. Shoot tip explant was better for in vitro propagation of sweet potato cv. N. , while nodal cuttings were. better for cv. BW. The shoot regeneration rate induced on benzylaminopurine (BAP) was higher than that on kinetin. BAP in combination with naphthalene acetic acid (NAA) resulted in shoot and root morphogenesis from nodal explants of the sweet potato cv. N. The best shoot length was found on Murashige and Skoog medium (MS) supplemented with NAA at both 0.25 and 1.0 mg/l combined with 0.5 mg/l BAP.

INTRODUCTION

Sweet potato is an extremely important staple crop worldwide due to its high yield and wide spread adaptation (Bouwkaup, 1985). It is grown in more than 100 countries of the world (FAO, 1999). Sweet potato is usually propagated vegetatively from vine cuttings in the tropics and from rooted sprouts in temperate regions. The vegetative

means have a much lower propagation rate than seed propagation (Hall and Phatak, 1993). Unless strict phytosanitary regulations are in place, these propagation methods might help in spreading diseases or insects that attack underground plant parts. The use of disease and insect free planting material is an important mean to improve the sweet potato crop. Tissue culture plays a major role in growth and development of desired cultivars of sweet potato and rapid multiplication of genetically pure and disease free material. Different techniques have been used for micropropagation of sweet potato. Somatic embryogenesis was also used by many authors (Liu and Cantliffe, 1984; Shimonishi et al., 1992). This technique can induce somaclonal variation and has been used for crop improvement rather than cloning. Organogenesis is another method for propagation of sweet potato in which plantlets were regenerated from stem, leaf and root explants (Santangelo and Sonnino, 1991; Gosukonda et ale, 1995), Meristem culture has been used to obtain virus-free plants from diseased ones for research and commercial purposes (Liao and Chung, 1979).

In this paper, the effect of two cytokinins (kinetin and benzylaminopurine) and an auxin (naphthalene acetic acid) (NAA) in a factorial combination with benzylaminopurine (BAP) were tested for in vitro cloning of two cultivars of sweet potato, Baladi Mlllite and Nigerian, using shoot tip and nodål explants.

MATERIALS AND METHODS

Shoot tip and single nodal explants (1-1.5 cm long) were taken from plants of two cultivars of sweet potato (Baladi White and Nigerian, which are widely grown in Sudan. The plants were grown in the nursery of the Horticultural Research Section, Agricultural Research Corporation, Wad Medani, Sudan, during the period March, 1997 to July, 1998. Explants were sterilized by dipping in 70 % ethanol for few seconds, followed by 15% and 20% Chlorox (commercial bleach) with 2 drops of Tween 20 as a wetting agent for shoot tip and nodal explants, respectively. The explants were then rinsed 3 times with sterilized distilled water, Nodal cuttings and shoot tip explants were then cultured on Murashige and Skoog medium (MS) supplemented with BAP or kinetin at 0, 1 and 2 mg/l.

Nodal explants of sweet potato cultivar N were also cultured on MS medium supplemented with combinations of BAP and NAA each at 0, 0.25, 0.5 and 1.0 mg/l. All growth regulators were obtained from Sigma Chemicals, England. Cultures were incubated in a growth room under 25 ^{o}c and 16 h day with light intensity of 200 lux from cooled fluorescent lamps. The growth room was in the Tissue Culture Laboratory, College of Agricultural Sciences, University of Gezira, Wad Medani, Sudan.

Number of explants with shoot, number of shoots per explant, plant height and number of nodes per regenerated shoot were observed every two weeks for a total of 6 weeks. The completely randomized design was used in all experiments. Data in percentage was transformed to arcsine before statistical analysis using Mstat-C software (MSU,1993), The Least SiY1ificant Difference test was used for means separation.

RESULTS AND DISCUSSION

Effect of BAP and explant type on in vitro morphogenesis of sweet potato

Plantlets were regenerated from shoot tip and nodal explants of the two cultivars of sweet potato (Baladi White and Nigerian) when cultured on MS without growth regulators or on MS supplemented with l.0 or 2.0 mg/I BAP (Table 1). The percentage of explants with shoots was siY1ificantly higher when shoot tip explants of sweet potato cultivar BW were cultured on MS basic medium compared to 1.0 my/l BAP. However, there was no si81ificant difference between 100 and 2.0 mg/l BAP. The number of shoots induced per explant, shoot length and the number of nodes per regenerated shoot were comparable on all BAP concentrations. However, the percentage of nodal explants with shoot morphogenesis, shoot length and the number of nodes per shoot decreased significantly with the increase in BAP concentrations from 1.0 to 2.0 mg/l.

The number of nodes induced per nodal explants was significantly different on BAP at both 0 and 1.0 mg/l in comparison to those induced on 2.0 mg/l BAP (Table 1). The number of nodes per shoot regenerated from nodal explants was almost twice as those induced on shoot tip explants when both explants were cultured on MS medium supplemented with BAP at 0 and 1.0 mg/l. The nodal cuttings were

found to be the best explants for micropropagation of sweet potato cv. BW

Table 1. Morhpogenesis of shoot tips and nodal explants of sweet potato cvs. Baladi White and Nigerian on MS medium supplemented with different concentrations of benzylaminopurine (BAP) after 6 weeks.

BAP (mg/1)	Sweet potato cultivar	Shoot tip explants				Nodal explants			
		Explants with shoots (%)	No. of shoots per explant	Shoot length (mm)	No. of nodes per explant	Explants with shoots (%)	No. of shoots per explant	Shoot length (mm)	No. of nodes per explant
0	Baladi White	100.0 a	1.5	22.4	4.9	100.0 a	1.1	45.8 a	8.1 a
1.0		60.0 b	0.9	15.2	2.9	90.0 a	1.3	25.0 a	6.5 a
2.0		80.0 ab	1.5	22.2	5.6	62.6 b	9.6	14.1 b	2.4 b
0	Nigerian	100.0	1.8	32.8 a	6.6 a	62.5	1.3	31.4	3.0
1.0		100.0	1.7	19.7 b	5.4 b	70.0	0.9	23.1	3.5
2.0		90.0	1.2	17.2 b	3.6 b	80.0	1.2	21.8	4.0

Means within the same column followed by the same letter(s) are not significantly different at 5% level of probability using the Least Significant Difference test.

When shoot tip explants of sweet potato cultivar N were cultured on MS medium supplemented with 0, 1.0 and 2.0 mg/l BAP, shoot length and the number of nodes per regenerated shoot decreased significantly with the increase in BAP concentrations from 0 to 1.0 mg/l (Table 1). The percentage of explants with shoots and the number of shoots per explant were comparable on all BAP concentrations. The morphogenesis of nodal explants was comparable on all concentrations of BAP (Table 1).

Effect of kinetin and explant type on in vitro morphogenesis of sweet potato

Number of nodes per regenerated shoot was significantly higher when shoot tip explants of sweet potato cultivar BW were cultured on MS supplemented with 0 and 1.0 mg/l kinetin compared to MS plus 2.0 mg/l kinetin (Table 2). However, the percentage of explants with shoot morphogenesis and the number of shoots induced per explant were comparable on all kinetin concentrations. Shoot length was comparable on 0 and 1.0 mg/l kinetin, but 2.0 mg/l induced significantly shorter plantlets compared to 1.0 mg/l.

Significantly higher shoot length was obtained when nodal explants of sweet potato cultivar BW were cultured on MS medium without kinetin, compared to those induced on MS medium supplemented with 2.0 mg/l kinetin. Shoot length induced on kinetin at 0 and 1.0 mg/l was comparable (Table 2). Percentage of explants with shoots, number of shoots per explant and number of nodes per regenerated shoot induced on all kinetin concentrations were similar.

Shoot length and number of nodes per plantlets regenerated from shoot tip explants of sweet potato cultivar N decreased significantly with the increase in kinetin concentration from 1.0 to 2.0 mg/l. However, the number of shoots per explant and the percentage of explants with shoots were not significantly different on all concentrations of kinetin (Table 2). The morphogenetic response of nodal explants of sweet potato cultivar N was similar on all concentrations of kinetin. Shoot tip was the best explant for micropropagation of sweet potato cultivar N, while nodal cuttings were best for sweet potato cultivar BW.

Morphogenesis of nodal explants on BAP in combination with NAA on sweet potato cv. Baladi White

Callus was induced at cut ends touching the medium on almost all nodal cuttings cultured on BAP in a factorial combination with NAA

Table 2. Morhpogenesis of shoot tips and nodal explants of sweet potato cvs. Baladi White and Nigerian on MS medium supplemented with different concentrations of kinetin after 6 weeks.

Kinetin (mg/1)	Sweet potato cultivar	Shoot tips				Nodes			
		Explants with shoots (%)	No. of shoots per explant	Shoot length (mm)	No. of nodes per explant	Explants with shoots (%)	No. of shoots per explant	Shoot length (mm)	No. of nodes
0	Baladi	70.0	0.7	11.5 ab	3.4 a	80.0	0.8	36.7 a	6.4 a
1.0		87.5	0.9	50.5 a	4.4 a	62.5	0.6	17.9 ab	3.5 a
2.0		83.3	0.8	5.8 b	1.3 b	70.0	0.7	12.2 b	2.6 b
0	Nigerian	90.0	1.0	19.9 a	4.8 a	75.0	0.8	31.3	4.3
1.0		90.0	0.9	13.4 a	3.1 b	87.5	0.9	23.8	4.5
2.0		62.5	0.6	4.5 b	1.1 c	83.3	0.8	23.0	4.0

Means within the same column followed by the same letter(s) are not significantly different at 5% level of probability using the Least Significant Difference test.

each at O, 0.25, 0.5 and 1.0 mg/l, except those cultured on MS without growth regulators. Shoots and roots were formed on all treatments. Explants with shoot morphogenesis (%), number of shoots per explant and number of nodes per regenerated shoot were comparable on all combinations of BAP and NAA. However, the combination of BAP and NAA significantly affected shoot length (Table 3). The highest shoot length was induced on either NAA at 0025 and 1.0 mg/l or BAP at 0.5 mg/l combined with 1.0 mg/l NAA.

Table 3, Length of shoots (cm) regenerated on nodal explants of sweet potato cv. Baladi White cultured on MS medium supplemented with different concentrations of BAP and NAA in factorial combinations.

NAA	BAP(mg/l)			
(mg/l)	0.0	0.25	0.5	1.0
0.0	34.1	20.4	18.6	32.5
0025	38.0	32.4	33.5	35.4
0.5	26.0	24.3	28.9	25.4
1.0	37.0	28.5	38.0	5.3

These results are in agreement with those of Kriuk et aL (1988), who found that axillary buds were the best explants for organogenesis for some species of sweet potato. In contrast with these results, they reported that BAP was the best for shoot and root formation in comparison to other plant growth substances. However, Santangelo and Sonnino (1991) reported that MS medium was superior for micropropagation of five cultivars of sweet potato compared to that supplemented with 0.3 mg/I BAP. Yamaguchi and Nakajima (1972) were able to induce regeneration of adventitious shoots on leaf discs of sweet potato when cytokinins were replaced by abscisic acid (ABA)g It was found that ABA antagonized indigenous BAP.

In this study, regeneration of plantlets was affected by genotype of sweet potato, the type of explant and plant growth regulator. The genotypic differences in morphogenesis of sweet potato were reported by Alconero et al. (1975) and Santangelo and Sonnino (1991). Litz and Conover (1978) successfully propagated two cultivars of sweet potato (White Star and PL 315343) using shoot tip and axillary buds as explants on MS medium supplemented with BAP, IAA and kinetin.

The best propagation medium was MS without growth regulators due to the production of high number of nodes per explant and •the relatively low cost of this treatment. Kinetin was used to induce shoot regeneration from shoot tip explants of sweet potato (Liao and Chung, 1979; Alconero et al., 1975). In agreement with this study, complete plants were also regenerated from shoot tip and axillary buds of sweet potato on MS supplemented with NAA or combination of IAA and kinetin (Alconero et al., 1975). The poor morphogenetic response in this study was possibly caused by genotypic effect of the two cultivars of sweet potato, which have a single axillary bud in each node producing one plant. Testing of more growth regulators may improve the cloning rates of these cultivars of sweet potato.

In conclusion, both sweet potato cultivars can be propagated by both shoot tip and nodal explants using MS medium without growth regulators which is relatively cheaper. Nodal explant of cv. BW had higher regeneration rate compared with shoot tip explant. While the shoot tip explant of cv. N was more responsive when cultured on MS medium without growth regulators. Number of nodes per explant induced on BAP was higher than that induced on kinetin for both cultivars of sweet potato.

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