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Research Article

Regeneration of industrial sugarcane using *in-vitro* plant apical meristem

A. Ajadi [1], B.O. Ehirim [1]*, M. N. Ishaq[1], A. Isah[1], A. Isong [1]*, A. Ghali[1], O. Kester[1], and A. N. Ejizu[2]
[1]National Cereals Research Institute, P.M.B 8 Bida, Nigeria
[1]Forestry Research Institute Onne, P.M.B 006 Nchia Eleme, Nigeria
E-Mail: isongplus@yahoo.com, bernard.ehirim@gmail.com

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Abstract
A rapid micro-propagation of sugarcane, *Saccharum officinarum* variety NCS 008 using apical meristem shoots were cultured on MS medium supplement with different concentration of BAP (0.1-0.4mg/l) and NAA (0.01-0.04mg/l) for shoot proliferation and elongation. The study showed that highest number of regenerated shoots were obtained from MS medium supplemented with BAP (0.3mg/l) and NAA (0.03mg/l)giving an average of 8 shoot per meristem shoot cultured. Roots were induced using MS medium supplemented with different combinations of NAA and IBA. NAA (3.0mg/l) gave highest rooting percentage of 94.74. The meristem shoot tip demonstrated good response to plant regeneration of sugarcane variety NCS 008 through in-vitro culture.

Keywords
BAP, culture, IBA, meristem shoots, MS, NAA, sugarcane, proliferation.

Introduction

Sugarcane *Saccharum spp*. was believed to have become established as a domestic garden crop around 8,000BC by Neolithic horticulturists in New Guinea. It was first introduced in Nigeria by European sailors in 15th century (Busari, 2004) and since then it has been widely grown in all ecologies in Nigeria. There are two types of sugarcane; the industrial (Commercial) and the chewing cane. The commercial varieties got introduced in 1950 while industrial production of refined sugar stared in the early 1960s in Nigeria (Gana, 2010). It is generally propagated vegetatively by stem cutting and is also heterogeneous in varieties. It is a source of table sugar which attracts high price among food materials thus making sugarcane an important crop across the globe (Alutbi *et al*., 2006). Barba and Nickel (1969) and Heinz and Mee (1969) demonstrated that plantlets could be developed from sugarcane callus culture. Nickells (1964) made initial attempt to regenerate sugarcane through *invitro* tissue culture (TC) techniques. Protocols for in-vitroplant regeneration of sugarcane through callus culture, axillary bud and shoot tip culture have been developed by many authors (Lee 1986, 1987; Hendre *et al*., 1983; Baksha *et al.,* 2002).Therefore, it has become necessary to design a sugarcane tissue culture(TC) protocol that will encompass the general method of enhancing rapid multiplication and controlling contamination in our laboratories. Time and continuous systemic attempts to access and remove diseases of sugar-cane from field are required. Multiplication and germplasm conservation is however possible for mass production and for future use against field hazard. The main advantage of micro-propagation is the rapid multiplication of new varieties, improved plant health and its usefulness in germplasm storage. It is the best method as it produces plants phenotypically similar to the mother plant and gives much more rapid multiplication rate. It allows the production of large number of plants from small pieces of the stock plant in relatively short period of time. Depending on the species or varieties of sugar-cane in question, the original tissue piece may be taken from shoot tip, leaf, stem, root and lateral bud. In some cases, the original or mother plant is not destroyed in the process thus a factor of considerable importance to the owner of a rare or unusual plant. Shaw (1990) also reported that micro-propagation is being used in some sugarcane industries, for the development of disease free clones, mostly to facilitate their safe and speedy movement through quarantine. Lal *et al*. (1996) demonstrated that micro-propagated system exhibited a potentialto produce 75,600 shoots from a single apex ex-plant in 5 months. Once an ex-plant is placed in a tissue culture medium, proliferation of lateral buds and adventitious shoots (direct shoot regeneration) or differentiation of shoots directly from callus will results in tremendous increase in the number of plantlets available for rooting. Rooted micro cuttings or plantlets of many species have been successfully established using this method. Reports

on micro-propagation of sugar-cane meristem tips are quite enormous, most especially from Asian countries and few from Africa. Limited efforts have been made on tissue culture and in-vitro propagation for varietal development and rapid multiplication in Nigeria. Therefore, this investigation was to establish the in-vitro regeneration and rapid propagation techniques of field grown sugarcane in Nigeria. This was to investigate the use of meristem tips from sugar-cane variety NCS 008(developed by NCRI, Badeggi) to establish plant regeneration protocol.

Material and Methods

The experiment was conducted at the Biotechnology Laboratory of National Cereals Research Institute (NCRI), Badeggi. The experimental materials (sugarcane stalk meristem) were collected from NCRI sugar-cane research field in citric acid solution. Sterilization of explants was carried out by washing under running tap water for 20 minutes. Subsequently, the explants were washed with liquid soap (Morning fresh) for 5 minutes after which it was thoroughly rinsed with double distilled water for 3 times. The explants were then washed with two drops of Tween-20 (Industrial soap) for 5 minutes and thoroughly rinsed with DDH_2O (double distilled water). It was followed by 70% ethanol for 5minutes before transferring the explants to the laminar air flow hood for further sterilization. In the flow hood, the explants were treated with 1%savlon (vol. /vol.), then 2%NaOCl solution (vol. /vol.) for 15 minutes. The explants were thoroughly rinsed with double distilled water DDH_2O for 3 times before transferring into citric acid solution. Shoot tips 2-4mm were excised and placed on MS (Murashige and Skoog,1962) medium supplemented with different combinations of cytokinin and auxin to investigate the appropriate media combination for regeneration of sugarcane through meristem tip culture. The media was also supplemented with 3% sucrose, 0.7% agar,1% myoinositol and BAP+NAA combinations were added accordingly.P^H was adjusted to 5.8±2 before the addition of agar and activated charcoal. The solution was autoclaved at 121^0C for 15minutes. Finally, the cultured explants were incubated at 25 ± 2^0C for 16hours photoperiod regime. The experiment was replicated three times and the means and standard error of the results were calculated.

Results and Discussion

Various combinations of Benzyl amino purine **(**BAP) and Naphthalene acetic acid **(**NAA) were examined and maximum shoot on initiation was observed in explants cultured in MS Medium supplemented with 0.3mg/l BAP +0.03mg/l NAA and 0.1mg/l BAP +0.01mg/1NAA Fig (1-1c). The result showed that explants responded rapidly (5days) with this combination and 80% explants produced 2-8 shoots from a single meristem within 3-4weeks of inoculation (Table 1). Similar results have been reported by (Dhumale *et al.*1994) with BAP 3 mg/l and NAA 1 mg/l. Multiple shoot regeneration from shoot tips were remarkably influenced by combining types and concentrations of the auxin and cytokinin. High cytokinins and low auxins combination favoured the induction of multiple shoot growth (Baksha *et al.*, 2002). It has been documented that proportion of auxins to cytokinins determines the type and extent of organogenesis in plant cell cultures (Skoog and Miller,1957). Mohammad,(2014) also reported significant difference in varying hormonal concentration for meristem proliferation and elongation. Number of explants cultured and growth were recorded (Table 1). After 4weeks of shoots growth, actively growing shoots were transferred to multiplication media for further growth and proliferation. Various concentration of cytokinin (BAP and Kinetin) and auxins, (IAA, NAA) were used in different concentration and combinations for shoot regeneration. During this investigation shoot formation was highly influenced by concentrations and type of the growth regulators used in the experiment. Maximum shoot proliferation was observed in BAP 1.0mg/l + NAA 0.02mg/l combination with average dense mass of 30 shoot after 4 weeks (Table 2). However, rooting was observed in the elongated shoots in the media but they were very thin and weak. Hence, it was necessary to further incubation in a rooting medium. Shoot differentiation from shoot tips/meristem tissue has been demonstrated by Aamir *et al.* (2008).Tremendous response was observed on the average number of shoots (8.00±2.00) with concentration NAA (3.0 mg/l) and IBA (2.0mg/l) which produced 5-6 of roots per shoot with max root length of 0.75cm to 1.0cm. Highest percentage rooting 94.74 was obtained from MS medium supplemented with 3mg/l NAA. This result agrees with the finding of Heinz *et al.* (1977) who reported that preferred auxin for root initiation was NAA

In general, it has been reported that plants regenerated from shoot tips (meristem) were very similar both phenotypically and genotypically to the mother plants (Ali and Afghan, 2001).Grisham and Bourg (1989) compared other sugarcane cultivars using micro-propagation method and found that shoot tips culture was better than leaf roll culture for plant mass production. This is possible and beneficial for rapid regeneration and germplasm conservation of

elite sugarcane varieties and shoot tip will have greater potential. From the investigation, Sugarcane variety NCS 008 displayed an excellent regeneration capacity at BAP (0.3mg/l) and NAA (0.03mg/l).

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Table 1. Effects of different concentration of BAP and NAAin shoot formation of sugarcane variety NCS 008

Hormonal conc.(mg/l)	No. of ex-plant cultured	Days to shoot regenaration	Average number of initial shoots	Average length of initial shoots(cm)
BAP+NAA (0.1+0.01)	10	7	6.33±1.53	0.60±0.10
BAP+NAA (0.2+0.02)	10	9	4.66±2.08	0.76±.32
BAP+NAA (0.3+0.03)	10	5	8.00±2.00	0.76±.25
BAP+NAA (0.4+0.04)	10	10	2.33±2.52	0.53±.15

Table 2. Number of shoots formed from different combination of cytokinin and auxin

Hormonal concentration (mg/l)	Number of plantlets multiplied	Explants regenerated after 2 weeks	Explants regenerated after 4 weeks
BAP + NAA (1.0+0.02)	8	17	30
BAP+KN (0.5+1.0)	8	18	28
KN+IAA (1.0+0.5)	6	12	24
BAP +IAA (1.0+0.5)	4	8	12
S.E±	0.83	2.01	4.75
CV %	25.54	29.24	36.50

Table 3. Root formation from cultured shoot- tips using different rooting media

Hormone concentration(mg/l)	Mean number of roots	Mean root length(cm)	Mean number of rootlets	% rooting
IBA 3	7	1	10	87.5
NAA 3	9	0.5	13	94.74
NAA +IBA (2 +1)	6	0.75	11	88.89
NAA +IBA (1 +2)	10	1	10	90.1
NAA +IBA (3+2)	12	0.75	9	94.12
S.E±	2.387	0.209	1.517	40.478

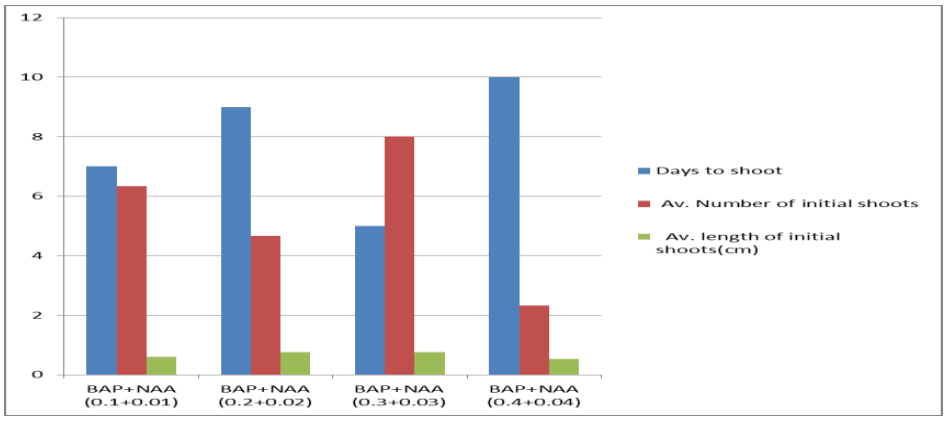

Fig. 1. Graphical representation of effects of different concentration of BAP and NAA in shoot formation of sugarcane variety NCS 008

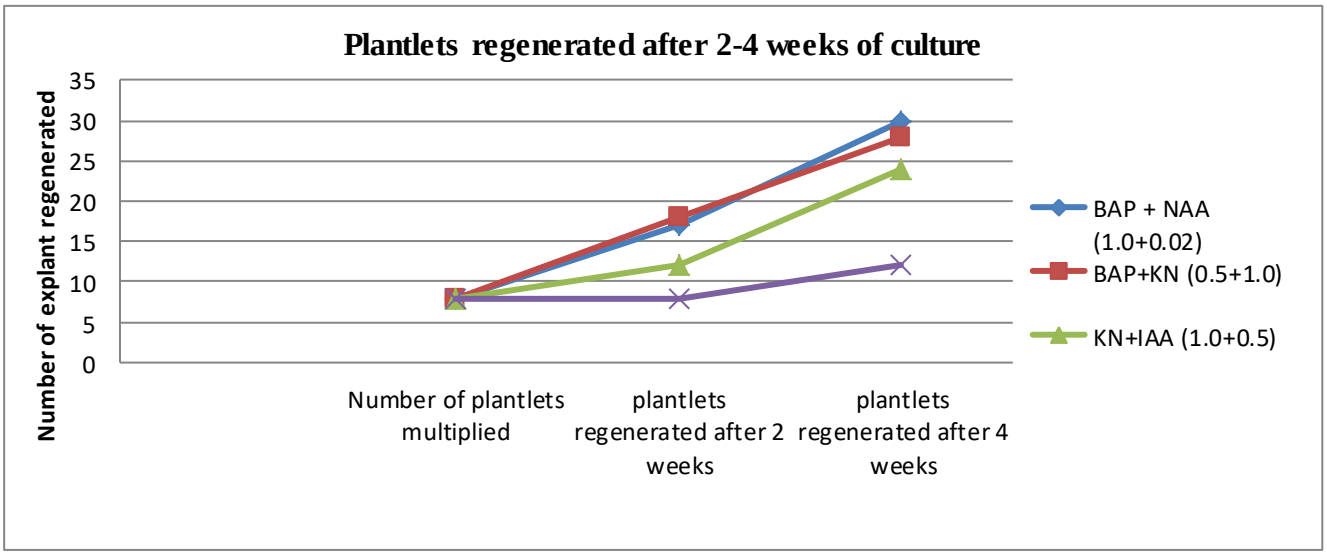

Fig. 1a. Plantlets regeneration under different concentration

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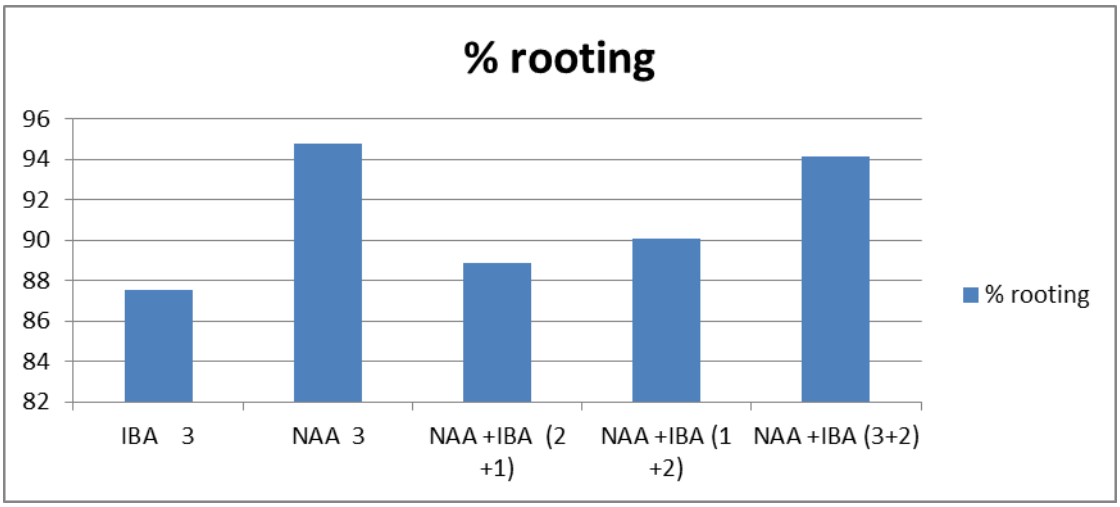

Fig. 1b. Rooting under different concentration

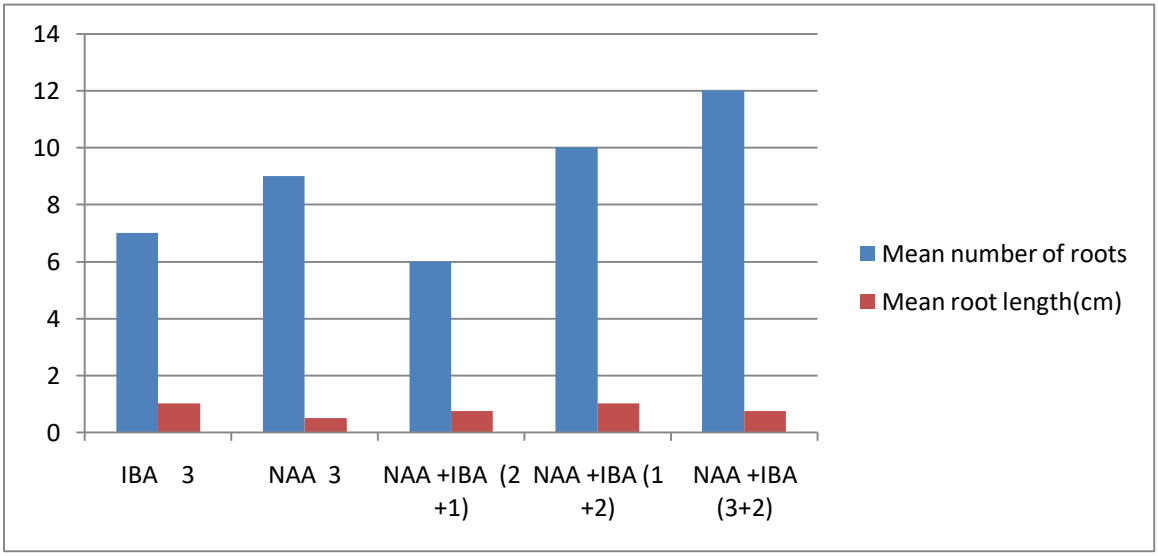

Fig. 1c. Mean number of Roots and root length