ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE BIOTECHNOLOGY

RAPID IN VITRO PROPAGATION TECHNIQUE FOR SUGARCANE VARIETY 018

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ABSTRACT

Indian economy gains 1.9% of National GDP by sugarcane cultivation. Plant growth hormones such as BAP, NAA, 2, 4-D, Kn, IBA were used to initiate cultures. A successful procedure was established in the culture conditions of variety 018. MS medium supplemented with 2,4-D (3.0 mg/l) , Kn (0.2 mg/l) and 3% sucrose was the best for the initiation of callusing from leaves and MS medium supplemented with BAP (1.0 mg/l), Kn (0.5mg/l) and 3% sucrose was best for shooting in eye bud from nodal region. The best multiplication rate of shooting in case of nodal eye bud was in MS medium supplemented with BAP(0.1 mg/l), Kn (0.5) & NAA (0.25 mg/L) and 3% sucrose where as for leaves MS medium supplemented with BAP (4.0 mg/l) , IBA (0.5 mg/L) and 3% sucrose. Root system was found in ½ MS medium with NAA (3.0 mg/l) and 6% sucrose.

KEY WORDS

GDP, NAA, 2, 4-D, Kn, MS, BAP and IBA

INTRODUCTION

Sugarcane an agro-industrial crop is an important integral component of the agriculture. It has the important position in the economy by contributing nearly 1.9% of National GDP. Sugarcane is the species or hybrids of genus *Saccharum*, tall perennial tropical grass (C4 plant) that tillers at the base to produce unbranched stems, 3- 4 m or more in height with a thick ness of approximately 5 cm in diameter. The leaf has a strong midrib, white and concave on the upper surface, convex and green below. *In vitro* multiplication of sugarcane has received considerable research attention because of its economic importance as a cash crop. Micro propagation is currently the only realistic means of achieving rapid, large-scale production of disease-free quality planting material as seed canes of newly developed varieties in order to speed up the breeding and commercialization process in Sugarcane [1, 2, 3, 4 & 5]. Large scale productions of Saccharum officinarum variety 018 through multiple shoot induction by the applications of Plant Tissue Culture methodology to[Delete] produce disease resistance and improved variety is the main aim of the study.

MATERIALS AND METHODS

Collection of Explants

Plant material was collected from Tectona Biotech Resource Centre, Orissa,Bhubaneswar and also from the fields of the nearby village Nuagaan. The explants (shoot tips & eye buds from nodal region) were collected from 6-8 month old, healthy, disease free sugar canes of variety 018

Surface Sterilization of Explants

The disease free, young and healthy sugarcane tops were collected and the young leaves were removed from the top portion of plant as well as eye bud portions were cut out from above the nodal regions of the sugarcane stem. The collected explants are partially trimmed off and then washed thoroughly under running tap water for 10 minutes each for three cycles to wash off external dust/contaminant. After these initial washings, the explants were kept in an aqueous solution of Bavistin (BASF India Limited) [1% w/v] for 10 minutes. After this, tips and eye buds were again washed with a liquid detergent (Teepol) adding a few drops of Tween-20 for 15 minutes. Explants were washed again in sterile water for 10 minutes. (3 washings) .Inside the laminar hood, sterilization with 0.1% mercuric chloride was done for approximately 10 minutes and rinsed three times in double distilled water to completely remove any remaining mercuric chloride.

Medium preparation

The nutrient medium chosen for these studies were Murashige and Skoogs medium [6] and supplemented with various concentrations of growth regulators. The temperature of autoclave was maintained at a temperature of 121 º C and at 15 psi pressure for 20 mins.

Micropropagation

The explants were inoculated in solidified MS medium supplemented with different concentrations of growth regulators i.e. BAP, Kinetin and 2,4-D in initial media. After inoculation of the explants in culture bottles and tubes, the culture bottles and tubes were kept 25 ± 2 ºC for 2-3 weeks. After 2-3 weeks of duration of inoculation, the healthy and non-infected explants were transferred to callus inducing media with the same hormonal compositions. The cultures were again incubated in culture room at 25 ± 2ºC for 2-3 weeks. The culture in which callusing was

observed transferred to shooting media. The shooting medium was supplemented with MS with different concentration of BAP, IBA, kinetin and NAA. Explants calli was again incubated in culture room for 2-3 weeks and the shoot formation was observed. When shoots in shooting medium attains 3-4cm height were transferred to the rooting medium where it was supplemented with ½ MS with different concentrations of NAA & IBA. The first roots appeared after 1-2 weeks of culture. After 2-3 weeks, the root system was well developed.

Hardening

The well developed plant with 3- 4 cm height were removed from culture vessel and thoroughly washed without causing damage to the roots. Plantlets were treated with fungicide solution (bavistin) and transferred to perforated plastic pots. The plantlets were carefully planted in the poly bags containing agro peat mixed soil, sand and compost (1:1:1) by inserting a hole in the middle of the potting mix followed transfer in to the poly house at a humidity range of 60-70%.Roots were well established and the plants were observed as acclimatized.

RESULT & DISCUSSION

Phytohormones and types of explants play a very important role in determining regeneration of sugarcane variety 018. The young leaves and eye buds from nodal regions were used as the explants out of that the leaves have shown the better response than eye buds, when treated with different concentrations of 2, 4-D, Kinetin, BAP, IBA, NAA in combination. During the development process of sugarcane variety 018, callus was observed in case of leaves which required both auxin and cytokinins. The frequency of callus induction reached 83% at a high concentration of 2, 4-D and a low concentration of Kinetin. Callus induction was observed within two weeks after incubation from the leaf sheath explants on modified MS medium containing different concentrations of 2,4-D and Kinetin(Fig.8) A large amount of greenish white and cream colored mass was formed after 4-5 weeks.

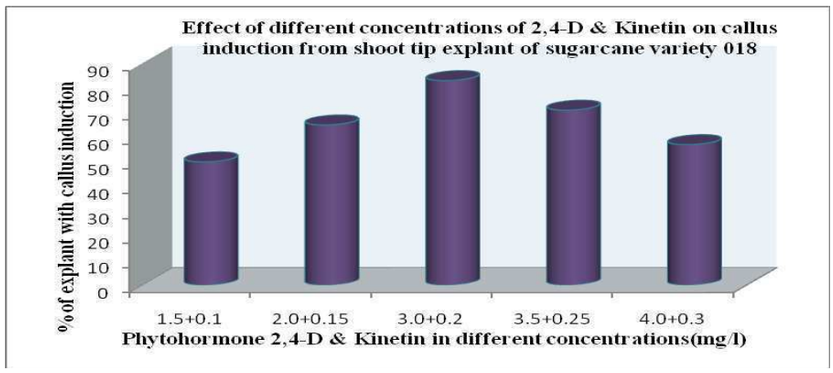

Fig.8
Effect of different concentrations of 2,4-D & Kinetin on callus induction

Though in all concentrations the callus induction was triggered but the best callus induction was observed at 3.0mg/l of 2,4-D along with 0.2 mg/l concentrations of Kinetin . The percentage of callus induction in young leaves was found 83% in duration of 13-15 days. Callus induced when transferred to different shoot formation medium to evaluate their ability of shoot development. Cut pieces of calli when transferred to regeneration medium green spots representing the shoot primordial appeared on the calli. The shoot primordial developed into shoots. During this investigation shoot formation was highly influenced by

concentrations and type of growth regulators used in the experiment. The rate of regeneration and formation of young shoots was about 82% (Fig. 9).

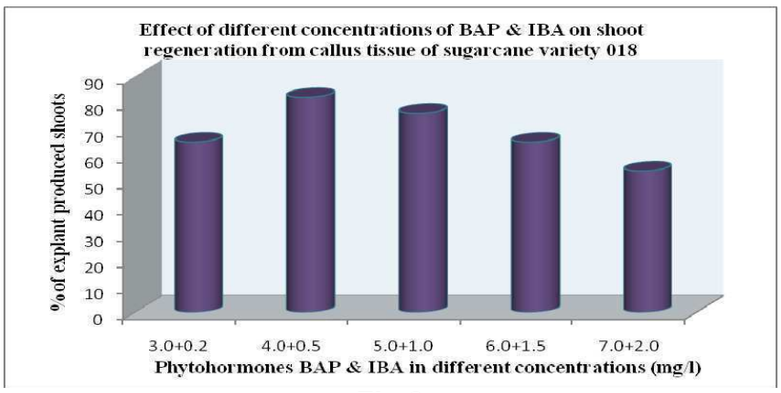

Fig.9

Effect of different concentrations of BAP & IBA on shoot regeneration from callus tissue

Shoot multiplication in case of explants from leaves the best result was observed on MS medium supplemented with BAP (4.0 mg/l) and IBA (0.5 mg/l). In these combinations the percentage of explants produced shoots was 82% .The number of shoots per explants was 12 and the average length of the usual shoot was 4.5 cm per culture. Two different types of cytokinin BAP and Kinetin were used which influenced the regeneration of shoot from the eye buds. The rates of formation of young shoots were about 85% (Fig.10).

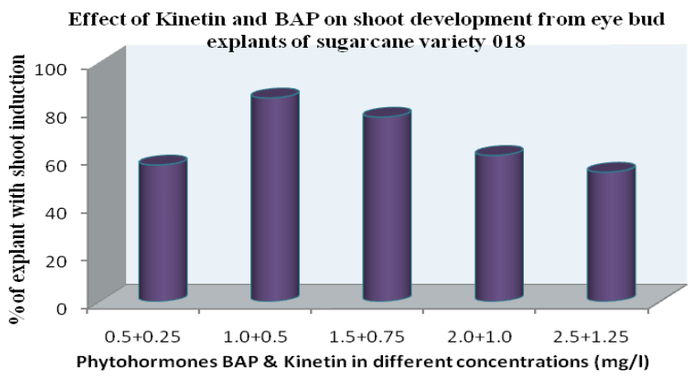

Fig.10

Effect of Kinetin & BAP on shoot development from eye bud explants

The MS medium supplemented with BAP and Kinetin was observed as the best one for the shoot formation from the eye buds of sugarcane variety 018. A high growth of *in-vitro* shoots was observed when the explants were inoculated in the media containing BAP (1.0mg/l) and Kinetin (0.5mg/l) after 13-15 days of incubation. The percentage of explants produced shoot was 85% for the above combinations. The number of explants showing shooting was 13. After the regeneration of the shoot from the nodal eye bud explants. For further multiplication it was again treated with different concentrations of auxin and cytokinin. The auxin NAA and cytokinin BAP and Kn was ideal for shoot induction (Fig.11).

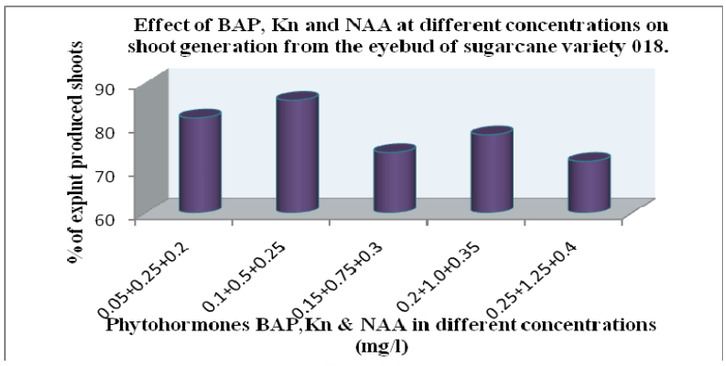

Fig.11

Effect of BAP, Kinetin & NAA at different concentration on multiple shoot generation from eye bud explants.

Medium containing BAP (0.1mg/l), Kinetin (0.5mg/l) and NAA (0.25mg/l) after 13-15 days of incubation produced shoot was 86%. The number of shoots per explant was observed 11 and the average length of shoot was observed 4.4cm. After 2-3 weeks, the shoot when separated and transferred to the same medium, multiplied more rapidly and the cycle was repeatable. Root formation occurred when the explants were cultured in different concentrations of auxins, IAA & NAA in MS media. In this case NAA has shown higher percentage of root induction than IBA in short durations (Fig.12).

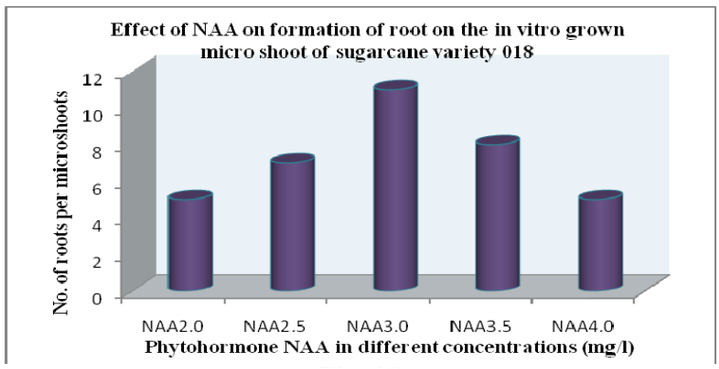

Fig.12

Effect of NAA on formation of root on in-vitro grown microshoot

The various stages of development of sugarcane variety o18 is through callus culture then multiple shoot regeneration ,followed by development of root in rooting medium finally transfer of the saplings to greenhouse for hardening as shown in (Fig 1-7)

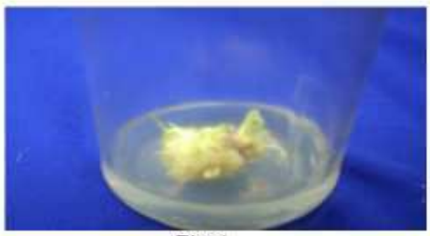
Fig.1

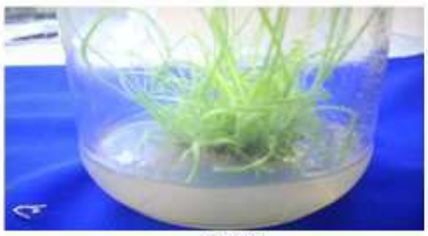
Fig.2

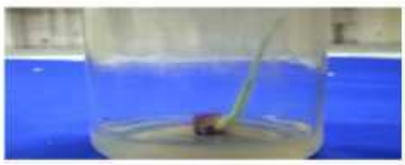
Fig.3

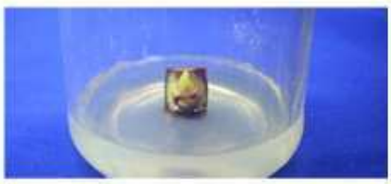
Fig.3

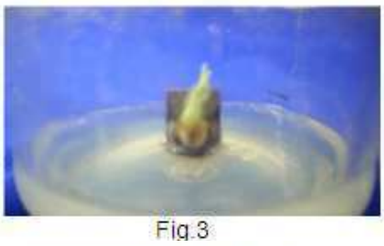
Fig.3

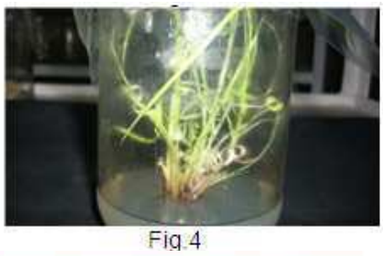
Fig.4


Fig.5


Fig.6 (a)


Fig. 6 (b)


Fig.6 (b)


Fig.7

Figure 1-*Callus induction in case of leaves in sugarcane variety 018*
Figure 2-*Shoot generation from callus in case of leaves in sugarcane variety 018.*
Figure 3-*Different stages of shoot initiation from eye buds*
Figure 4-*Multiple shoot regeneration from eye bud explants*
Figure 5-*Root generation from sugarcane variety 018.*
Figure 6 *(a) Plantlets ready to transfer to soil (b) Potted young sugarcane plant.*
Figure 7 *sugarcane plants after the hardening process*

During the present in-*vitro* cultivation of sugarcane variety 018, the two different explants i.e young leaves and nodal region eye buds depended upon the culture media and plant growth regulators. The first step towards de novo regeneration was to establish callus or cell suspension culture. Formation of callus was initiated after 12-16 days of explanting of leaves in MS medium. A large amount of whitish and cream colored mass was formed when the explants was inoculated in MS media containing 2, 4-D (3.0mg/l) and Kinetin (0.2mg/l).This practice of maintaining the calli through serial dilution in basal media has also been reported [7] The percentage of callus induction was 83% in a duration of 13-15 days. Studies have been made that high percentage of callus induction at varying concentrations of 2, 4-D in Bangladeshi sugarcane varieties .[8] when the hormone NAA was used in different concentrations, a small amount of callusing was observed. All these studies indicate that sugarcane explants requires higher concentrations of 2, 4-D for callus induction. Various concentrations of cytokinins & auxins were used in different concentrations for shoot regenerations. During this investigations shoot formation was highly influenced by concentration & type of growth regulators used in this experiment. On the transfer of cut pieces of calli to regeneration medium, green spots representing the shoot primordial appeared on the calli. The shoot primordial developed into shoot which started bearing young leaves after 12-13 days of culturing in regeneration medium .In various concentrations and combinations for shoot multiplications , best performance was showed on MS medium supplemented with BAP (4.0mg/l) & IBA (0.5mg/l).The percentage of explants produced shoot was 82% for the sugarcane 018 variety. The number of usable shoots, average, length of shoots was 12 & 4.5 cm respectively of the variety 018. There are reports[9] that the positive effects of BAP and IBA combination on shoot formation in sugarcane variety 018. Best rooting was observed in ½ strength of MS medium supplemented with 3.0 mg/l NAA in a duration of 9-12 days. The highest number of roots per micro shoots was observed 11 and the average length of the root was found 2.8cm in this case (Fig.12).

In case of auxin IBA, the best rooting was observed in ½ strength of MS media supplemented with IBA (3.5mg/l) in a duration of 12-14 days (Figure 6). In this case the highest number of roots per microshoot was 10 and the average length of root was found 1.8 cm. After 3 weeks of culture, when the height of the roots reached 2-3cm, the whole plant obtained was removed from the medium transferred to soil acclimatized for 3 weeks and cultivated in green house

It was observed the BAP and NAA combination showed effective result, but organogenesis was observed in case of eye bud explants when inoculated in the MS media containing various concentrations of cytokinins. The two different cytokinin BAP and kinetin was used for regeneration of shoot in case of eye bud. The ideal formation of shoot was observed when eye bud explants were inoculated in MS media containing BAP (4.0mg/l) and IBA (0.5mg/l). The height % of explant with shoot induction was 85% in duration in 13 to 15 days (Fig.10). For further multiplication of shoot in case of explant eyebud of sugarcane variety 018, the explants were subculture in different concentrations of auxin and cytokinin. Shoot induction was observed when explants were subcultured in the MS media containing BAP (o.1 mg/l), Kn (0.5 mg/l) and NAA (0.25mg/l). The percentage of explants produced shoots was 86% and the number of shoots per explant was 11 and the average length of the usual shoot was 4.4cm.

Regenerated shoots were transferred to rooting medium, the plantlets developed tiny roots which later on were the cause of formation of root system of the plants. Best rooting was observed ½ MS medium supplemented with

NAA (3mg/l). The highest number of roots/ micro shoots was 11 and the average length of roots 2.8cm found in the above case (Fig 6).Root can be easily induced on cultured shoots by their transfer to another medium with or without NAA, where optimal growth was observed with ½ MS medium[10] In comparison to NAA, MS medium along with IBA at a concentration of 3.5mg/l[remove brackets] gave highest number of roots / shoots that is 10 and average length of root was found 2.2cm Rooted plants were taken from the media washed with distilled water then put in poly bags containing agro peat mixed soil, sand and compost (1:1:1) for pre-hardening. After acclimatization plants were transfer to green house with 85% of survival rate. This is considered as the higher survival rate.

CONCLUSION

This protocol achieved the successful establishment of *in-vitro* propagation procedure for variety 18 which is established and the success rate after post hardening was found 85% which is very much satisfactory and can be employed for the mass scale propogation.

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