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An efficient Micropropagation Protocol for Nutritionally Rich Varieties of Sweet Potato (*Ipomoea batatas* L.)

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Abstract

An efficient and reproducible plant regeneration protocol for nutritionally rich varieties of sweet potato (*Ipomoea batatas* L.), Gouri and Bhu Sona was developed. The effect of different hormone combinations and types of explants on shoot regeneration was evaluated to optimize the regeneration protocol. Among the explants and hormone combinations tested, only nodal explants with axillary bud of both the varieties produced shoots and the maximum per cent shoot induction in Gouri (100%) was observed in MS medium supplemented with 1.0 mg l^{-1} BAP and 1.0 mg l^{-1} BAP along with 1.0 mg l^{-1} GA_3. Similarly, maximum per cent shoot induction (96.67%) in Bhu Sona was observed in the medium supplemented with 0.5 mg l^{-1} BAP along with 1.0 mg l^{-1} GA_3 and 1.0 mg l^{-1} BAP. Leaf, petiole and root explants produced compact callus, which did not regenerate into shoots in shoot induction medium. Regenerated shoots were transferred to different rooting media and maximum rooting in both the varieties were observed in MS medium supplemented with 0.5 mg l^{-1} IBA. An efficient, reproducible plant regeneration protocol developed for sweet potato varieties, Gouri and Bhu Sona can be used for mass production of planting materials of these varieties and/or any other plant biotechnological approaches.

Key words: Nutritionally rich sweet potato, micro-propagation, regeneration, tissue culture

Introduction

Sweet potato (*Ipomoea batatas*) plays a major role as a staple food crop, especially in developing countries. It is a dicotyledonous tuberous crop, belonging to Convolvulaceae family. Sweet potato is being cultivated as a valuable source of human food, industrial raw material as well as animal feed (Jarret and Florkowski, 1990) in over 50 countries. Sweet potato ranks fifth most important food crops (FAOSTAT, 2017) and important for food security in tropical, subtropical and temperate regions of the world not only for its high dry matter per unit area per unit time but also as the cheapest source of minerals, vitamins and antioxidants. Owing to its vast utilization in domestic and industrial use, these crops were biofortified to combat malnutrition (Mukherjee et al., 2008). Especially, orange-fleshed and purple-fleshed sweet potato cultivars are being targeted for use in biofortification to improve the accessibility of diversified nutrition (Laurie et al., 2015; Burri, 2011). Despite having a huge reservoir of nutrients, sweet potato faces biological drawbacks in propagation. This crop is propagated by vine cuttings or tuber, which limits the production of healthy planting materials in sufficient quantity when it is required. As well as, maintenance of genetic resources of sweet potato in field gene bank makes the crop more vulnerable to biotic and abiotic stresses (Mukherjee,1999). Environmental factors and diseases prevent the sweet potato from reaching its maximum yield potential. sweet potato weevil and viral diseases have been identified as the main cause of low productivity (Alula et al., 2018). Continuous cultivation of same varieties despite root yield degeneration and critical shortage of healthy planting materials of superior

varieties are the major cause of cultivar decline (Gibson et al., 1998). Micropropagation and *in vitro* germplasm maintenance can help to a great extent to overcome these limitations (Mukherjee, 1999).

To secure healthy stock, both conventional and biotechnological breeding programs need to be applied. Genetically uniform and healthy plantlets may be raised through plant tissue culture techniques. Plant tissue culture techniques have been employed in large number of important agriculture crops. The plant tissue culture applications become more widely used for many crops in both developed and developing countries. Futhermore, it enables the production of a large number of genetically uniform plantlets in short period of time in limited space as well as short and medium–term maintenance of germplasm under controlled conditions in small spaces with reduced labour requirements and to modify the germplasm itself (Rabbani et al., 2001; Ravi and Indira, 1999; Hashem et al., 1990).

The present study can lead to a greater understanding of the mass propagation of nutritionally rich sweet potato varieties. Proper selection of explants used for regeneration and different plant growth regulators and combination for *in vitro* shoot multiplication and *in vitro* rooting. Several researchers studied regeneration of sweet potato from nodal explants, meristem, stem, root, petiole explants and protoplast (Ravi and Indira, 1999). Encapsulation and regeneration of nodal explants of sweet potato (Mukherjee, 2002) and cassava (Vivek et al., 2016) were also reported. Plant hormones have a profound effect on the morphology of the tissue developed from the explants as they can enhance not only the growth of some cultured slow growing tissues but also determine the study of development pathways. *In vitro* plant regeneration from cells, tissues and organ cultures are the fundamental process of biotechnology application in plant propagation, plant breeding and genetic improvement. Several factors including genotypes, explants used for regeneratin, nature and doses of different growth regulators are found to determine the regeneration of sweet potato (Shaibu et al., 2016).

Hence, the present study investigated the effect of the different concentrations and the combinations of growth regulators application for optimal regeneration of nutritionally rich varieties of sweet potato.

Materials and Methods

The experiment was conducted at ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI), Thiruvananthapuram. Nutritionally rich varieties of sweet potato (*Ipomoea batatas* L.) developed and released by ICAR-CTCRI, Gouri and Bhu Sona were selected for this study. These two varieties have orange flesh, which is rich in β-carotene. The variety, Gouri has 4.5 - 5.5 mg/100g β-carotene and 16.5% starch. Similarly, Bhu Sona has very high β-carotene of 14.0 mg/100g as compared to 2.0 - 3.0 mg/100g in popular varieties and 20% starch.

Explants like leaf, petiole, node and root were taken for regeneration experiments, obtained from 20-30 days old *in vitro* grown cultures. Each explant was cut into 0.5-0.7 cm long pieces and then cultured to the surface of the medium. Each petri plate was inoculated with 7-9 explants. The petri plate was then sealed with parafilm and cultures were incubated in a culture room at $25 \pm 2^{o}C$ temperature under a photoperiod of 16 hours light and 8-hour dark. The MS basal medium (Murashige and skoog, 1962) were supplemented with different concentrations and combinations of plant growth regulators to study the regeneration response of sweet potato explants (Table 1). To establish an optimum condition of regeneration media, twelve combinations (T1 to T12) of plant hormones such as BAP, TDZ, GA_3 and IBA were formulated (Table 1).

Table 1. Media compositions used for *in vitro* shoot formation and rooting

Media compositions for *in vitro* shoot formation	
T1	MS Basal
T2	MS + BAP (0.5 mgl^{-1})
T3	MS + BAP (0.5 mgl^{-1}) + GA_3 (1.0 mgl^{-1})
T4	MS + BAP (1.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1})
T5	MS + BAP (1.0 mgl^{-1})
T6	MS + TDZ (0.5 mgl^{-1})
T7	MS + TDZ (0.5 mgl^{-1}) + GA_3 (1.0 mgl^{-1})
T8	MS + TDZ (1.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1})
T9	MS + TDZ (1.0 mgl^{-1})
Media compositions for *in vitro* rooting	
T10	MS Basal
T11	MS + IBA (0.5 mgl^{-1})
T12	MS + IBA (1.0 mgl^{-1})

The well elongated individual shoots generated and obtained from shooting medium were separated and transferred to MS medium supplemented with auxins (T10, T11 & T12), IBA of different concentrations (0.0, 0.5, 1.0 mgl^{-1}) for root induction (Table 1). Observations like days taken for shoot induction, number of shoots per explants, shoot length, height of the plant, number of shoots, length of root, number of roots and number of leaves were recorded.

The mean and standard errors were worked out from triplicate data obtained from the experiment. The per cent data and numerical data were transformed using angular and square-root transformation and analyzed following Completely Randomized Design (CRD).

Results and Discussion

In the present study, the regeneration potential of two nutritionally rich varieties of sweet potato was studied in different hormonal combinations. Different cytokinins at varying concentrations induced callus in all the explants and showed significant variation in rate of callus formation, shoot induction and root initiation among the varieties. The highest frequency of callus induction in all the explants of two varieties was observed in medium containing 0.5 mgl^{-1} or 1.0 mgl^{-1} TDZ and in combination with 1.0 mgl^{-1} GA_3. In this medium, leaf, petiole and nodal explants of variety Gouri induced 100% callus and recorded 93.33% callus from root explants on medium containing 0.5 mg/ml TDZ (Fig. 1). Similarly, 100% callus induction was observed in leaf and nodal explants of variety Bhu Sona in all the hormonal combinations and 100% callus induction was observed in petiole and root explants on the medium supplemented with different levels of TDZ alone and TDZ with GA_3 (Fig. 2). These callus were compact and highly recalcitrant that remained unresponsive to shoot regeneration. Earlier studies on the *in vitro* propagation of sweet potato have also reported low regeneration frequencies for various explants like stem and leaf explants (Moran et al., 1998; Sivparsad and Gubba, 2012).

Since shoot regenerated only from nodal explants of both the varieties, showed significant changes in their response towards each hormonal medium. The medium supplemented with varying concentrations and combinations of BAP induced callus with in 3.33 days of culture, whereas medium with TDZ recorded maximum of 5.33 days to induce callus from nodal explants of varieties Gouri and Bhu Sona. In contrast, nodal explants of Bhu Sona took minimum of 4.33 days for callus induction in the medium with 0.5, 1.0 mgl^{-1} TDZ alone as well as in combination with 1.0 mgl^{-1} GA_3 and maximum of 5.0 days observed for callus induction in the medium containing varying concentrations and combinations of BAP and 1.0 mg/ml TDZ (Table 2 and 3).

The minimum days required (4.67 days) for shoot initiation was recorded in the variety Gouri from nodal explants cultured in MS medium supplemented with 0.5 mgl^{-1} BAP and produced 1.09 shoots (Table 2). Similarly, the variety Bhu Sona produced shoots after 5.33 days of inoculation on the medium supplemented with 0.5 mgl^{-1}

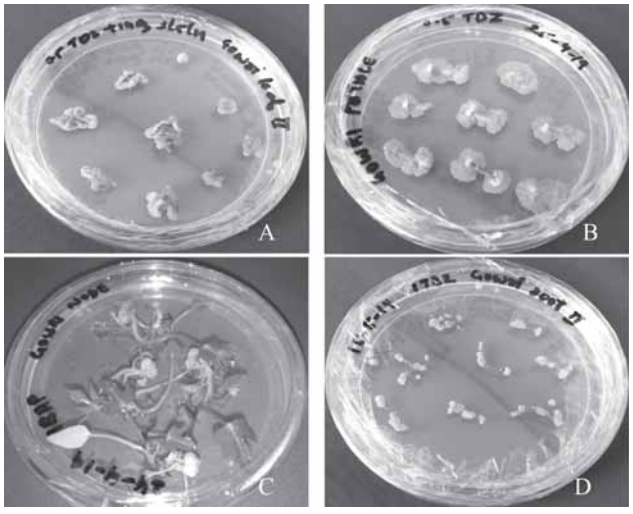

Fig. 1. Regeneration of cultured explants of variety Gouri; Leaf (A), Petiole (B) Node (C) and Root (D)

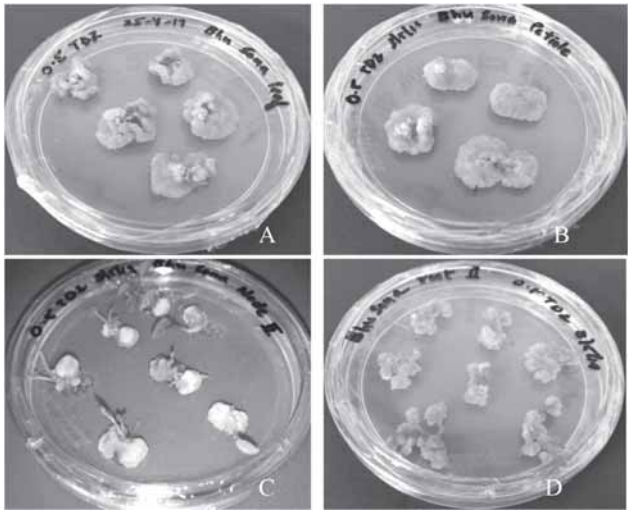

Fig. 2. Regeneration of cultured explants of variety Bhu Sona; Leaf (A), Petiole (B) Node (C) and Root (D)

Table 2. Effects of different concentrations of growth regulators on regeneration of nodal explants of sweet potato variety Gouri

Treatment	Days to callus formation	Days to shoot formation	Number of shoots per explant	Days to rooting per explant	Number of roots per explant
Control	3.33 (2.49 ± 0.00)	5.00 (2.44 ± 0.00)	1.00 (1.41 ± 0.00)	17.67 (4.31 ± 0.17)	1.33 (1.47 ± 0.29)
TDZ (0.5 mgl^{-1})	5.00 (2.42 ± 0.23)	8.67 (3.10 ± 0.14)	1.40 (1.55 ± 0.03)	6.33 (2.67 ± 0.34)	2.67 (1.91 ± 0.08)
TDZ (0.5 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	5.33 (2.50 ± 0.17)	8.67 (3.10 ± 0.14)	1.13 (1.45 ± 0.05)	6.33 (2.67 ± 0.34)	1.67 (1.56 ± 0.37)
TDZ (1.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	5.00 (2.42 ± 0.23)	8.67 (3.10 ± 0.14)	0.81 (1.34 ± 0.04)	6.33 (2.67 ± 0.34)	2.00 (1.71 ± 0.16)
TDZ (1.0 mgl^{-1})	5.00 (2.42 ± 0.23)	14.33 (3.89 ± 0.37)	1.11 (1.44 ± 0.07)	8.00 (2.90 ± 0.54)	1.00 (1.38 ± 0.21)
BAP (0.5 mgl^{-1})	3.33 2.06 ± 0.20)	4.67 (2.33 ± 0.32)	1.09 (1.44 ± 0.09)	5.67 (2.49 ± 0.45)	4.00 (2.21 ± 0.23)
BAP (0.5 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	3.33 (2.06 ± 0.20)	5.67 (2.57 ± 0.12)	0.93 (1.38 ± 0.04)	3.33 (2.06 ± 0.20)	1.00 (1.38 ± 0.21)
BAP (1.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	3.33 (2.06 ± 0.20)	6.00 (2.65 ± 0.10)	1.16 (1.46 ± 0.11)	5.00 (2.49 ± 0.00)	1.67 (1.57 ± 0.29)
BAP (1.0 mgl^{-1})	3.33 (2.06 ± 0.20)	5.67 (2.57 ± 0.12)	1.07 (1.43 ± 0.02)	4.00 (2.21 ± 0.23)	3.67 (2.08 ± 0.41)
C.D. (0.05)	NS	0.589	NS	0.994	NS

TDZ alone as well as in combination with 1.0 mgl^{-1} GA_3 and produced 0.73 and 0.78 shoots respectively (Table 3). Whereas, the maximum days required for shoot initiation was observed in the variety Bhu Sona from the nodal explants (Table 3) cultured in medium with 0.5 mgl^{-1} BAP (8.0 days) and 1.0 mgl^{-1} TDZ along with 1.0 mgl^{-1} GA_3 (8.33 days). Similarly, the variety Gouri produced shoots after 14.33 days of culture in the medium with 1.0 mg/ml TDZ (Table 2). Appropriate hormonal concentration for shoot regeneration of variety Gouri was found to be 0.5 mgl^{-1} TDZ resulted in highest shoots (1.40) and roots (2.67) per nodal explants. The variety Bhu Sona produced maximum shoots (1.03) and roots (1.00) on the medium supplemented with 1.0 mgl^{-1} BAP (Table 2 and 3).

Shoot regeneration in sweet potato was highly influenced by the media formulations containing plant growth regulators. The experiment revealed that low concentrations of TDZ and high concentrations of BAP resulted in highest number of shoots per nodal explant in the variety Gouri. In the variety Bhu Sona, low concentrations of BAP along with GA_3 and high concentrations of BAP alone found better for production of highest number of shoots per nodal explants. The percentage of *in vitro* shoot production was highest (100%) in nodal explant of the variety Gouri cultured in medium supplemented with 1.0 mgl^{-1} BAP and 1.0 mgl^{-1} BAP along with 1.0 mgl^{-1} GA_3 and in MS basal media. The percentage was lowest in nodal explants cultured in different concentrations of TDZ. In the variety Bhu Sona, percentage of *in vitro* shoot production was highest in nodal explants cultured in the MS basal media (100%) and in different concentrations of TDZ along with GA_3 (90-93.3%). Studies by (Kassahun et al., 2018) in three varieties of sweet potato revealed that minimum days were required for shoot initiation in the medium supplemented with 0.5 mgl^{-1} BAP while maximum number of days required for shoot production in hormone-free medium as well as in the medium with 1.0 mgl^{-1} BAP. Another investigator reported that medium supplemented with 0.25 mgl^{-1} BAP resulted in highest percentage of shoot induction (80%) and varied responses at different cytokinins with the highest shoot multiplication rate at 1.0 mgl^{-1} TDZ (El-Afifi et al., 2012).

Table 3. Effects of different concentrations of growth regulators on regeneration of nodal explants of sweet potato variety Bhu Sona

Treatment	Days to callus formation	Days to shoot formation	Number of shoots per explant	Days to rooting per explant	Number of roots per explant
Control	5.00 (2.49 ± 0.00)	6.67 (2.73 ± 0.28)	1.00 (1.41 ± 0.00)	8.33 (3.02 ± 0.28)	4.33 (2.23 ± 0.41)
TDZ (0.5 mgl^{-1})	4.33 (2.30 ± 0.07)	5.33 (2.42 ± 0.46)	0.73 (1.31 ± 0.02)	4.00 (2.22 ± 0.13)	2.33 (1.80 ± 0.19)
TDZ (0.5 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	4.33 (2.30 ± 0.07)	5.33 (2.42 ± 0.46)	0.78 (1.33 ± 0.04)	4.00 (2.22 ± 0.13)	2.00 (1.62 ± 0.43)
TDZ (1.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	4.33 (2.30 ± 0.07)	8.33 (2.91 ± 0.66)	0.55 (1.24 ± 0.04)	3.33 (2.07 ± 0.07)	0.33 (1.13 ± 0.13)
TDZ (1.0 mgl^{-1})	5.00 (2.44 ± 0.11)	7.00 (2.79 ± 0.31)	0.56 (1.24 ± 0.07)	3.67 (2.15 ± 0.07)	0.33 (1.13 ± 0.13)
BAP (0.5 mgl^{-1})	5.00 (2.45 ± 0.00)	8.00 (2.83 ± 0.68)	0.61 (1.27 ± 0.05)	9.33 (3.06 ± 0.70)	6.33 (2.64 ± 0.43)
BAP (0.5 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	5.00 (2.44 ± 0.00)	6.00 (2.57 ± 0.45)	0.97 (1.40 ± 0.03)	6.00 (2.56 ± 0.45)	4.67 (2.28 ± 0.46)
BAP (1.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	5.00 (2.44 ± 0.00)	6.00 (2.57 ± 0.45)	0.79 (1.33 ± 0.04)	6.67 (2.67 ± 0.54)	2.00 (1.66 ± 0.35)
BAP (1.0 mgl^{-1})	5.00 (2.44 ± 0.00)	6.00 (2.57 ± 0.45)	1.03 (1.42 ± 0.04)	9.33 (3.05 ± 0.70)	1.00 (1.41 ± 0.00)
C.D. (0.05)	NS	NS	NS	NS	0.979

The regeneration of sweet potato, the correct hormone combination ratio and source of explant are critical factors to consider in obtaining high regeneration efficiency (Sihachakr et al., 1997; Gaba, 2005). In the present study, highest regeneration percentage (93.33%) was recorded in nodal explants of both the sweet potato varieties in all hormone combinations. The regeneration efficiency of nutritionally rich varieties of sweet potato indirectly depends on the presence of Gibberellic acid in the medium. It regulates positively in Bhu Sona whereas negatively in Gouri when it is combined with TDZ (Table 4). Successful regeneration of sweet potato in the media supplemented with BAP, GA_3 and NAA was also reported by Mukherjee (2002) and Onwubiko et al. (2015). Sivparsad and Gubba (2012) obtained highest percentage of shoot regeneration with apical shoot explants (31%) and axillary bud explants (22%) on MS medium supplemented with 0.01 mgl^{-1} NAA and 1 mgl^{-1} BAP. Alula et al. (2018) reported MS medium supplemented with 0.5 mgl^{-1} BAP with 0.5mgl^{-1} kinetin followed by 0.75 mgl^{-1} BAP with 0.5 mgl^{-1} kinetin were best medium for shoot induction and growth of apical meristem of three varieties of sweet potato. Abubakar et al. (2018) observed highest number of shoots (1.30) on MS medium with 0.5 mgl^{-1} BAP and 0.05 mgl^{-1} NAA from axillary buds.

MS media along with various proportions of auxins were tried for rooting of the regenerated shoots of sweet potato varieties, Gouri and Bhu Sona. Roots in regenerated shoots of both the varieties were induced under all the media combinations studied (Fig. 3, 4, 5 and 6). Number

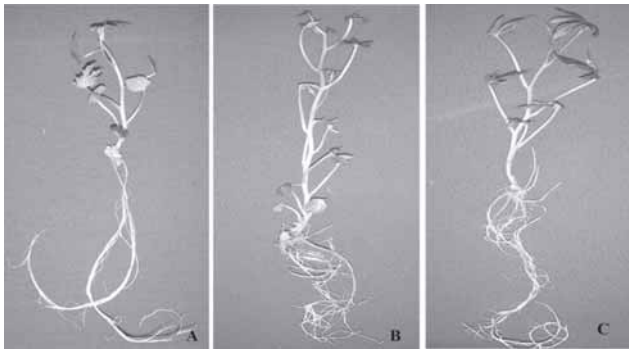

Fig. 3. Root induction of regenerated shoots of Gouri on MS basal medium (A), MS + 0.5 mgl^{-1} IBA (B) and MS + 1 mgl^{-1} IBA (C)

Table 4. Shoot formation of nodal explants cultured in different concentrations and combinations of growth regulators

Treatment	Percentage of shoot formation	
	Gouri	Bhu Sona
Control	100.00(90.00 ± 0.00)	100.00(90.00 ± 0.00)
TDZ (0.5 mgl^{-1})	93.33(81.13 ± 8.87)	93.33(81.13 ± 8.87)
TDZ (0.5 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	70.00(56.77 ± 0.00)	93.33(81.13 ± 8.87)
TDZ (1.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	60.00(50.84 ± 3.40)	90.00(78.93 ± 11.07)
TDZ (1.0 mgl^{-1})	83.33(74.99 ± 15.00)	93.33(81.13 ± 8.87)
BAP (0.5 mgl^{-1})	93.33(81.13 ± 8.87)	86.67(72.77 ± 9.62)
BAP (0.5 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	86.67(68.83 ± 2.71)	96.67(83.85 ± 6.15)
BAP (1.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1})	100.00(90.00 ± 0.00)	80.00(63.90 ± 4.27)
BAP (1.0 mgl^{-1})	100.00(90.00 ± 0.00)	96.67(83.85 ± 6.15)

of roots (11.67), number of nodes (11.0), number of leaves (10.0) and plant height (11.03 cm) were highest in the variety Gouri on MS media supplemented with 0.5 mgl^{-1} IBA. The shoot number per nodal explants was highest (1.33) in both MS basal media as well as MS media with 0.5 mgl^{-1} IBA and average root length was highest (17.00 cm) in MS media supplemented with 1.0 mgl^{-1} IBA (Fig. 3 and 5). Whereas, in the variety Bhu Sona, root number (9.33), leaf number (8.33) and plant height (7.6 cm) increased when cultured in MS media supplemented with 0.5 mgl^{-1} IBA. Average root length (11.57 cm) and node number (9.33) per plant was highest in MS basal media and shoot number was highest (1.67) in both MS basal media as well as MS media with 0.5 mgl^{-1} IBA (Fig. 4 and 6). Negash et al. (2000) reported that the highest number of roots per plant was observed in MS media supplemented with 1.0 mgl^{-1} IBA in combination with 0.5 mgl^{-1} NAA and 0.5 mgl^{-1} IBA in combination with 0.5 mgl^{-1} NAA. Similarly, Alula et al. (2018) obtained optimum root induction and growth from *in vitro* produced shoots on MS medium with 0.5 mgl^{-1} IBA with 0.5 mgl^{-1} NAA.

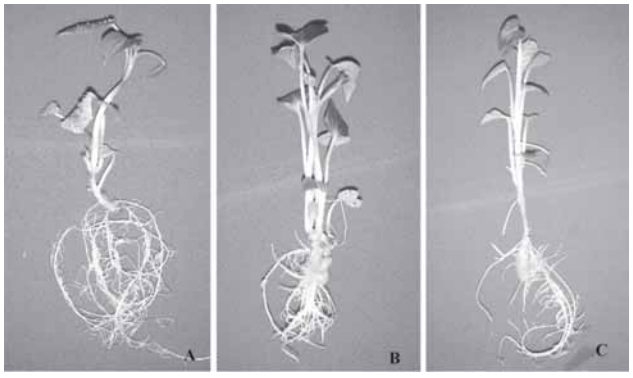

Fig. 4. Root induction of regenerated shoots of Bhu Sona on MS basal medium (A), MS + 0.5 mgl^{-1} IBA (B) and MS + 1 mgl^{-1} IBA (C)

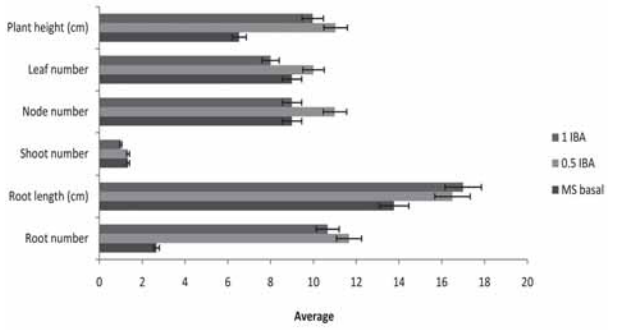

Fig. 5. Effect of different rooting medium on regenerated shoots of Gouri

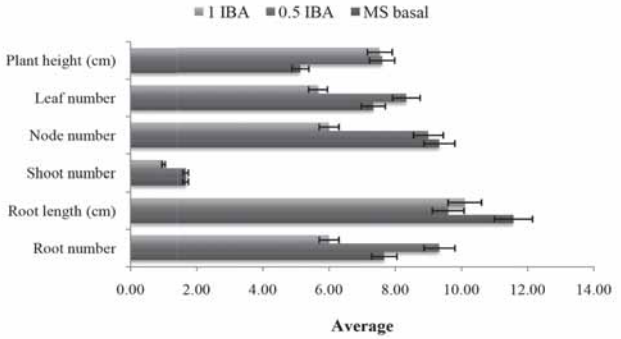

Fig. 6. Effect of different rooting medium on regenerated shoots of Bhu Sona

Conclusion

The present study showed that two nutritionally rich varieties of sweetpotato, Gouri and Bhu Sona, responded differently to the same hormonal combinations, which proves the varietal difference for the regeneration of sweet potato. Among the explants and hormone

combinations tested, only nodal explants of both the varieties induced shoot regeneration. The highest shoot induction (100%) in the variety Gouri was observed in MS medium supplemented with 1.0 mgl^{-1} BAP and 1.0 mgl^{-1} BAP along with 1.0 mgl^{-1} GA_3. Similarly in Bhu Sona, highest shoot induction (96.67%) was observed in the medium supplemented with 0.5 mgl^{-1} BAP along with 1.0 mgl^{-1} GA_3. MS media having 0.5 mgl^{-1} IBA appeared to be beneficial for the highest number of roots in both the varieties. The study revealed that for better root length the optimum level of IBA was 0.5 mgl^{-1}. This study lead to a greater understanding of the mass propagation of sweet potato for *in vitro* shoot multiplication and *in vitro* rooting. Though the results obtained from this research can be used as the guideline for mass propagation of sweet potato.

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