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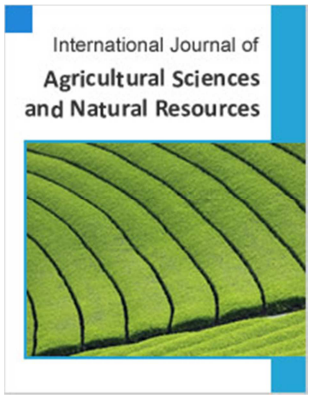

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The Effects of α -Naphthalene Acetic Acid (NAA) on *In Vitro* Rooting of Sugarcane (*Saccharum officinarum* L.) Genotypes

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Abstract
Profuse rooting of *in vitro* grown shoots is an essential step for the establishment of the plantlets in the field, similarly hardening is a vital process prior to transplantation of rooted shoots to the soil. Hence, the experiment was conducted to optimize a protocol for profuse *in vitro* rooting of two sugarcane genotypes (B4906 and Pr1013). It was carried out in completely randomized design (CRD) with 2x5 factorial treatment arrangements of genotypes and NAA (2.0, 3.0, 4.0, 5.0 and 6.0 mgl^{-1}) in combination. Separated shoots were cultured on ½ MS media supplemented with NAA. The results showed that the interaction effects of NAA and genotypes significantly influenced *in vitro* rooting. Half MS medium with 2 mgl^{-1} NAA resulted in 91.67% rooted shoots with 12.58±0.23 roots and 2.54±0.04 cm root length in B4906 whereas 4 mgl^{-1} resulted in 66.67% rooted shoots with 7.83±0.70 roots and 2.60±0.05 cm root length in Pr1013. Rooted plantlets acclimatized in greenhouse and 96.1% of plantlets survived successfully using soil, compost and sand (1:1:1) as potting mixture.

1. Introduction

The success of *in vitro* propagation relies on efficient rooting in regenerated shoot and their subsequent acclimatization. Once the sufficient numbers of shoots have been generated, portion of explants that contain one or more shoots could be transferred to a medium that contains higher concentration of auxin, resulting in root formation. The initiation of roots is easily achieved in some species by reducing the cytokinin level [1] or on MS medium with or without the addition of extra root promoting auxins [2]. In sugarcane, auxins especially IBA from 0.5-3 mgl^{-1} [3, 4] and NAA from 0.5-7 mgl^{-1} [1, 5, 6] alone or in combination are the most common used auxins for rooting.

Most of researchers reported *in vitro* regenerated roots of sugarcane shoots on MS basal medium fortified with auxins [5, 7, 8]. Khan *et al*. [4] reported vigorous root development on MS medium containing 6% table sucrose + 1 mgl^{-1} IBA among the

combinations used. However, the MS medium without growth regulators promoted rooting in more than 90% of two cultivars after 30 days of culture [2, 9]. In addition to the presence and absence of growth regulators, rooting was greatly dependent on the strength of MS medium in various plant species. Half MS media supplemented with elevated sucrose were reported more responsive than full MS medium for rooting of sugarcane [10-12].

Rooting was highly influenced by the different types and concentrations of auxin used. Even if there also results reported on the IBA and IAA, NAA was the most efficient auxin for root initiation of sugarcane *in vitro* propagation [3 4]. NAA was better than IBA either alone or in combination with other hormones for rooting of sugarcane [11]. In addition, NAA at 5 mgl^{-1} was good for rooting as reported [5, 13-15], but more than 5 mgl^{-1} NAA inhibits rooting [16]. In contrary, many researchers obtained best rooting at lower concentration of NAA from 0.5-3 mgl^{-1} [1, 17, 18]. This is due to different genotypes had different physiological requirement of plant growth regulators for *in vitro* shoot regeneration and thus different types of plant growth regulator has been given different physiological response [19]. Therefore, this study was developed to optimize the optimum concentration of NAA for profuse rooting of two sugarcane genotypes specifically B4906 and Pr1013.

2. Material and Methods

Shoots that have above 3 cm length were separated and cultured on hormone free MS medium to avoid carryover effect. After two weeks, the healthy looking and conditioned shoots were transferred to the experimental media, and the leaves that became yellow at the bottom of the shoot were removed before placing them on the medium. MS [20] media augmented by different levels of NAA (2.0, 3.0, 4.0, 5.0 and 6.0 mgl^{-1}) with elevated amount of table sugar (60 gl^{-1}) were used. The completely randomized design (CRD) in 2x5 (two genotypes and five levels of NAA) factorial arrangement with 12 shoots per treatment were used. Data on the number of roots, length of root and number of rooted plantlets were recorded after 30 days of culture. Then thirty plantlets from each genotypes were transferred to plastic pots containing hardening medium composed of soil, compost and sand (1:1:1), covered by perforated white plastics to maintain the moisture for plantlets. Thereafter, plantlets were kept under box in the greenhouse for one week. Then they were exposed to direct sunlight in the acclimatization room. The plantlets were watered twice a day, and 0.2% potassium phosphorus (KH_2PO4) was given for a day. Finally, numbers of dead and survived plantlets were counted after a month.

The data was subjected to SAS program (SAS, 2008 version 9.2) and then treatment means were separated using Duncan Multiple Range Test (DMRT) at 5% probability.

3. Result and Discussion

Analysis of variance indicated that the interaction effect of genotype and NAA was very highly significant ($p< 0.001$) for percentage of rooted shoots, number of roots per shoot and average root length of the two sugarcane genotypes tested. Fine roots began to be induced from the basal portion of the shoots after 15 days in both genotypes on ½ MS media fortified by 60 gl^{-1} with and without (control) of NAA, but the roots in the control were not well elongated. This is due to the presence of elevated table sugar, which has increased the cell metabolism and stress to induce rooting, and high source of energy is required to induce cells that are different from the source cell. Singh *et al*. [2] obtained 100% root induction from plant growth regulator free ½ MS media fortified by elevated 60 gl^{-1} sugar.

B4906 gave the highest (91.67%) rooted shoots on ½ MS medium with 2 mgl^{-1} NAA and the lowest 33.33% on ½ MS medium with 6 mgl^{-1} NAA. Whereas Pr1013 produced a maximum of 75% at ½ MS with 2 mgl^{-1} and 3 mgl^{-1} of NAA, and minimum of 41.67% rooted shoots at 5mgl^{-1} NAA (Table 1). This result indicates that each genotype responded differently due to their endogenous auxin amount. Each genotype requires different concentrations based on the amount of their endogenous auxin concentration [2]. By increasing the concentration of NAA from 2mgl^{-1} to 6mgl^{-1}, percentage of rooted shoots decreased continuously from 91.67% to 33.33% in B4906, and discontinuously decreased from 75% to 41.67% in Pr1013. In Pr1013, root induction percentage increased from 41.67% at 5mgl^{-1} to 66.67% at 6mgl^{-1} NAA, but the roots were not well grown, and they were more stunted at 6 mg/l than 5 mgl^{-1}. Generally, this indicates that low concentration of NAA promotes more root induction and elongation than higher concentration that inhibited rooting in both genotypes. In contrary, many researchers reported that higher level of NAA was better for root induction [5, 15].

B4906 gave the highest (12.58±0.23) roots/shoot with 2.54±0.04 cm average root length on ½ MS medium with 2 mgl^{-1} NAA (Table 1 and Fig. 1). On the same media composition, only 5.67±0.27 roots/shoot with 1.49±0.12 cm average root length were observed for Pr1013. In Pr1013, a maximum of 8.17±0.31 and 7.83±0.70 roots/shoot with 1.49±0.06 and 2.60±0.05 cm average root length were obtained on ½ MS supplemented with 3 and 4 mgl^{-1} NAA respectively (Table 1 and Fig. 1). On the same media, B4906 produced only 9.50±0.15 and 5.75±0.32 roots/shoot with 2.08±0.10 and 1.87±0.06 cm average root length respectively. This indicates that rooting was highly influenced by the concentrations used. Hence, appropriate amounts of auxin in the rooting medium are crucial for root induction. Treatments with 3 mgl^{-1} and 4 mgl^{-1} NAA were not statistically significant in terms of root number, but 4 mgl^{-1} was optimum to get better average root length in Pr1013.

The average number of roots produced per shoot ranged from 2.67 to 12.58. The highest and the lowest roots per

shoot were recorded on B4906 at 2 mgl^{-1} and 6 mgl^{-1} NAA. This indicates that it needs further lower (< 2 mgl^{-1}) levels of NAA to get more number of roots, as higher concentrations inhibit rooting in B4906. Nawaz *et al*. [1] obtained maximum number of roots on MS plus 0.5 mgl^{-1} NAA. The root length also ranged from 0.54 cm for B4906 at 6mgl^{-1} NAA to 2.6 cm for Pr1013 at 4 mgl^{-1}. In general, the effect of NAA was different in two genotypes in terms of percentage, number and length of roots, B4906 showing better results in lower concentrations of NAA than Pr1013.

In this study, the result of B4906 are in line with [7, 16] who obtained the highest 8.8 and 8 number of roots on ½ MS+ 2 mgl^{-1} NAA respectively. Whereas result from Pr1013 is corroborated with [18, 21], who obtained the highest roots number on ½ MS+3 mgl^{-1} NAA. Rooted plantlets acclimatized in the greenhouse and 96.1% of plantlets survived and acclimatized successfully on hardening medium composed of soil, compost and sand (1:1:1) (Figure 2). This is agree with the results of [22] who found 96% successfully survived plantlets using farmyard manure with garden soil (1:4) as a potting mixture. In contradictory, [11] reported the highest survival of 75% and 50% plantlets using vermicompost: soil: sand (1:1:1), and press mud: soil: sand (1:1:1) respectively.

Table 1. *Mean values of rooting percentage, root number and root length of B4906 and Pr1013 genotypes under influence of NAA.*

Genotype	NAA (mg/l)	% of rooted shoots (Mean±SD)	No. of roots (Mean±SD)	Av. root length (Mean±SD)
	2	91.67^{a}±2.36	12.58^{a}±0.23	2.54^{a}±0.04
	3	75.00^{b}±6.38	9.50^{b}±0.15	2.08^{b}±0.10
B4906	4	58.33^{d}±2.36	5.75^{d}±0.32	1.87^{c}±0.06
	5	50.00^{e}±1.34	4.58^{e}±0.32	1.68^{d}±0.05
	6	33.33^{g}±0.00	2.67^{f}±0.00	0.54^{g}±0.00
	2	75.00^{b}±4.08	5.67^{d}±0.27	1.49^{e}±0.12
	3	75.00^{b}±1.36	8.17^{c}±0.31	1.49^{e}±0.06
Pr1013	4	66.67^{c}±2.72	7.83^{c}±0.70	2.60^{a}±0.05
	5	41.67^{f}±10.41	3.17^{f}±0.53	0.71^{f}±0.09
	6	66.67^{c}±2.72	4.75^{e}±0.65	0.62^{fg}±0.18
CV		7.09	6.7	5.44

Note:*Values given are as mean ± SD. *Means within the same column with different letter (s) are significantly different from each other at $p \leq 0.05$.

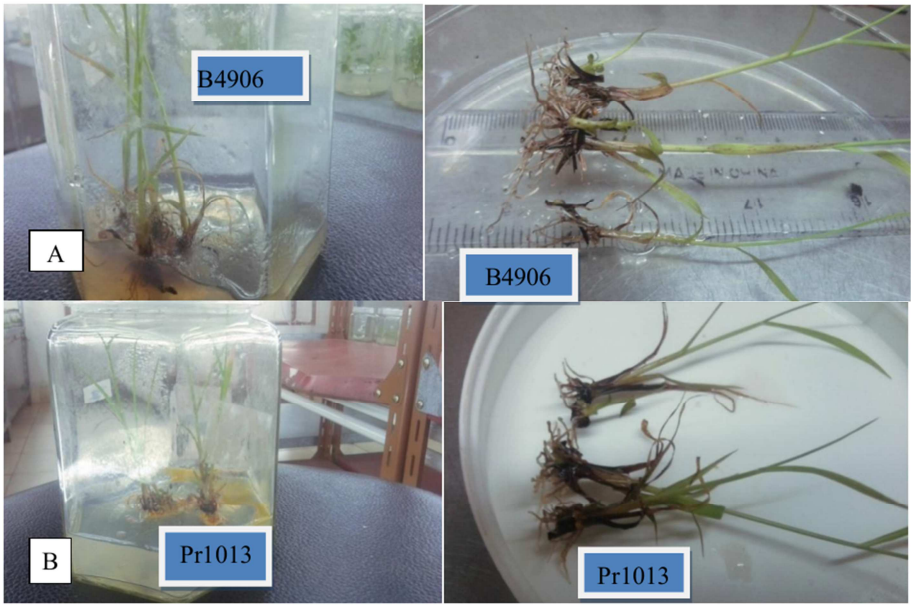

Figure 1. *In vitro rooting of A) B4906 on ½ MS +2 mgl^{-1} NAA and B) Pr1013 on ½ MS + 3 mgl^{-1} NAA.*

Figure 2. *Acclimatized plantlets of B4906 and Pr1013 after 30 days.*

4. Conclusion

In vitro rooting of two sugarcane genotypes, B4906 and Pr1013 has been developed. The analysis of variance indicated that *in vitro* rooting of sugarcane depends on the interaction of genotypes and NAA. On ½ MS medium with 2 mgl^{-1} NAA, 91.67% B4906 shoots induced roots with 12.58±0.23 roots/shoot and 2.54±0.04 cm average root length. Whereas, ½ MS medium with 4 mgl^{-1} NAA produced 66.67% rooted plantlets, 7.83±0.70 roots and 2.60±0.05 cm root length in Pr1013. MS medium with 3 mgl^{-1} also produced similar number of roots like that of 4 mg/l in Pr1013, but its root length were not equally good as they were stunted. Hence, ½ MS medium plus 2mgl^{-1} NAA and 4 mgl^{-1} NAA were found optimum to produce profuse and elongated roots in B4906 and Pr1013 respectively.

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