Research Article

In-Vitro Management of Phytohormones for Micropropagation of Sugarcane

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Abstract | Micropropagation is a phenomenal technique to serve rapid multiplication of vegetatively propagated plants through *in vitro* culture. This study was conducted to establish the optimal concentrations of plant growth regulators and sucrose for micropropagation of sugarcane. Sugarcane is cultivated through cane sets and therefore, multiplication of any new genotype through traditional practices requires interminable period of time. Eight sugarcane genotypes *viz.* NIA-2004, SPF-234, NIA-2012, BL4, AEC92-1208, Thatta-10, Gulabi-95 and NIA-1026-P7 were subjected to tissue culture using four different combinations of shoot induction media and three disparate recipes of rooting media. It was seen that both of the factors i.e. genotype as well as growth medium played significant role in inducing propagation of the sugarcane. Shoots were observed to initiate earliest in NIA-2004, NIA-2012 and NIA-1026-P7 in MS media supplemented with 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP and 20 g l^{-1} sucrose. Regarding number of shoots, shoot length and number leaves of the plantlets, BL4 was seen to produce excellent results in MS media containing 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP and 20 g l^{-1} sucrose. Mean number of shoots, leaves and shoot length, were all recorded to be highest for this composition of media (1.42, 1.54 and 3.90, respectively). The results indicated critical role of BAP and Kin in shoot development and elongation. On rooting, profuse root formation was observed in MS media supplemented with 3.00 mg l^{-1} IBA + 4.00 mg l^{-1} NAA and 30g l^{-1} sucrose. All of the varieties showed root development under this composition except NIA-1026-P7. The results of this experiment also showed that IBA and NAA are vital towards root formation. Moreover, it was seen to be evident that optimal media concentrations for micropropagation varied from genotype to genotype. Establishment of mature micropropagation protocols for new elite sugarcane cultivars can help in rapid multiplication of disease-free potential material to occupy large area in short time span, giving the phenomenon paramount economic importance.

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Introduction

Sugarcane (*Saccharum officinarum* L.) is world's largest crop with respect to total production (FAOSTAT, 2017). Sugarcane is grown in more than a hundred countries (Premachandran et al., 2011). It is one of the most important cash crops of Pakistan and is significantly important for sugar and

sugar related industries of the country (Khan et al., 2017a, 2019c). It provides raw material for the sugar sector-the second largest agroindustrial segment after textiles (Khan et al., 2017b; Ahmad et al., 2019; Raza et al., 2019). Sugarcane production accounts for 3.6 percent in agricultural value addition and 0.7 percent in overall GDP of Pakistan (Iqbal and Iqbal, 2014).

The sugarcane production has increased in recent years in Pakistan as good economic returns encouraged the growers to bring more area under cane cultivation (Ministry of Finance, 2018). The average yield of sugarcane crop in the country is about 48.9 t ha^{-1} as compared to significantly high average of the world that is approximately 65.5 t ha^{-1} (Seema et al., 2011). Brazil is the largest producer of sugarcane in the world, whereas the next five major producers in descending order are India, China, Thailand, Pakistan and Mexico (Khan and Khan, 2019; Khan et al., 2019b). Among 105 countries of the world growing sugarcane, Pakistan rank 5th in total production. Pakistan is also the largest per capita consumer of sugar in South Asia (FAOSTAT, 2017).

Sugarcane does not produce flowers in agroclimatic conditions of Pakistan except a few locations like coastal areas of Sindh (Thatta), Murree (in Punjab), and Dargai (in Khyber Pakhtunkhwa) (Khan et al., 2017c; Seema et al., 2017). Therefore, its cultivation as well as breeding is intricate and suffers from several limitations (Khan et al., 2018b). The cultivation is done through cane sets which also transfer the diseases present in parent plants impacting the overall yields and increase the expenses for control measures (Khan et al., 2018a). *In vitro* micropropagation, in this regard, provides an opportunity for rapid multiplication of sugarcane and production of healthier, disease free plant material, true to parent type (Lee, 1987; Khan et al., 2019a). Also, once an elite sugarcane genotype is developed, its multiplication is a challenge since it takes several years to multiply the material in field conditions to occupy a significant cropping area. Rapid multiplication of new genotypes is significantly important as otherwise, insects pests and microbial communities accumulate genetic changes to invade the newly developed cane genotypes harming the overall economic value of the newly developed varieties (McDonald and Linde, 2003; McDowell and Woffenden, 2003; Palloix et al., 2009; Pangga et al., 2011).

Micropropagation through *in vitro* techniques has been widely used in horticulture and other agriculture fields to realize the mass propagation of crop plants (George and Sherrington, 1984; Dodds, 1991; George, 1993; Das et al., 1996). It is an excellent approach for multiplication of promising elite genotypes of sugarcane in short time span. Therefore, this technique can serve provision of a new cane genotype to large number of farmers. It has also become an attractive and powerful tool for genetic manipulation, and conservation of plant genetic resources and valuable germplasm (Lal and Singh, 1994). Moreover, micropropagation also helps in developing disease free and quality plant material for commercial purposes (Lal and Krishna, 1994; Lorenzo et al., 2001).

This study was conducted to develop optimal protocols of micropropagation for eight different genotypes of sugarcane. The genotypes included already released elite varieties as well as new candidate lines. Various concentrations of growth hormones and sucrose were utilized in the tissue culture media to investigate their effects on sugarcane shoot and root development. This study also explored the role of BAP and Kin in shoot formation; and that of IBA and NAA in root formation. Hence, the article provides an insight into the role of these growth hormones in micropropagation of sugarcane, and also reports optimal protocols for micropropagation of some of the most important sugarcane varieties of the country.

Materials and Methods

This research was conducted at Nuclear Institute of Agriculture, Tandojam using complete randomized design (CRD) with three replications. Fresh plant materials (healthy young meristems) were collected from six months old field grown plants by removing the leaf sheath. These young meristems were cut into thin pieces of 1.0 to 1.5 cm length. The explants were washed thoroughly under running tap water for five minutes and transferred to laminar air flow cabinet. The young meristem explants were treated with 70 % alcohol for one minute, followed by 10% sodium hypochlorite treatment. Finally, the young meristem cuttings were washed thoroughly three times using sterile distilled water before inoculation into the sterilized nutrient agar media. All of the above operations were performed under aseptic conditions in laminar airflow cabinet.

Culture medium preparation

The young meristem cutting explants were inoculated in sterilized MS medium (Murashige and Skoog, 1962) supplemented with different concentrations of plant growth regulators. The culture media contained macro nutrients, micro nutrients, sugar and gelrite along with vitamins (Thiamine and Myo-inositol). The pH of the media was adjusted to 5.8, followed by heating in microwave for ten minutes until it became transparent. The media was poured into the bottles and was autoclaved (121^{0}C) at 15psi after plugging.

Micropropagation

Micropropagation response of eight sugarcane varieties *viz*. NIA-2004, SPF-234, NIA-2012, BL4, AEC92-1208, Thatta-10, Gulabi-95 and NIA-1026-P7 was analyzed under varying concentrations of growth regulators and sucrose. Following four different concentrations of the said role players were used for shoot induction.

1. MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose
2. MS + 1.50 mg l^{-1} IAA + 1.50 mg l^{-1} Kin + 1.50 mg l^{-1} BAP + 25 g l^{-1} sucrose
3. MS + 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP + 20 g l^{-1} sucrose
4. MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} Kin + 2.00 mg l^{-1} IBA + 20 g l^{-1}

Whereas, for root induction, three different concentrations of growth regulators and sucrose were employed as below.

1. ½MS + 2.50 mg l^{-1} IBA + 3.00 mg l^{-1} NAA + 20 g l^{-1} sucrose
2. ½MS + 3.00 mg l^{-1} IBA + 4.00 mg l^{-1} NAA + 30 g l^{-1} sucrose
3. and ½MS + 4.00 mg l^{-1} IBA + 5.00 mg l^{-1} NAA+ 40 g mg l^{-1}

To evaluate the response of genotypes against mentioned concentrations used in the media, the recorded observations included days taken to shoot initiation, number of shoots (per bottle), shoot length, number of leaves (per bottle), and number of roots (per bottle). The parameters were recorded after sub-culturing two times.

Statistical analysis

The experimental data were subjected to factorial design of analysis of variance (ANOVA) under linear models of statistics to observe differences among the studied traits of sugarcane genotypes. Student Edition of Statistix (SWX), version 8.1 was used for this purpose. Least significant difference (LSD) test was also applied to determine the level of significance among various combination means (Gomez et al., 1984).

Results and Discussion

Days to shoot initiation

The statistical analysis of variance for days to shoot initiation indicated that varieties, media concentrations, and their interaction, all produced highly significant outcomes at 5 % probability level (Figure 1; data are presented in Table 1; analysis of variance for the studied parameters is presented in Supplementary Material: Table 1). The results of varieties showed that early (mean) days to shoot initiation were recorded in NIA-2004, AEC92-1208 and BL4 (8.41, 8.42, and 8.75 days to shoot initiation, respectively), followed by Gulabi-95 (10.00 days). On the other hand, late days to shoot initiation were observed for NIA-1026-P7 and Thatta-10 (10.75 and 11.50 days).

The results concerning different concentrations of the plant growth regulators showed early days to shoot initiation (mean) under the media containing MS + 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP + 20 g l^{-1} sucrose (7.29 days), while late days to shoot initiation (mean) were recorded under MS media supplemented with 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose (12.79 days). Comparing a combinatorial effect of both factors (genotypes + media concentrations), it was observed that BL4 initiated the shoot development in earliest time span of 5.33 days when cultured in MS media having 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP and 20 g l^{-1} sucrose. NIA-2004, NIA-2012 and NIA-1026-P7 were also seen to show early shoot development in various media concentrations used. However, in absence of Kin and BAP, same genotype e.g. NIA-1026-P7 showed slowest shoot initiation (15.67 days under MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose) which indicated that BAP and Kin were playing paramount role in shoot initiation. The response of NIA-2004, SPF-234, BL4, AEC92-1208, and Gulabi-95 was also similar which all demonstrated slow initiation of shoot in said media.

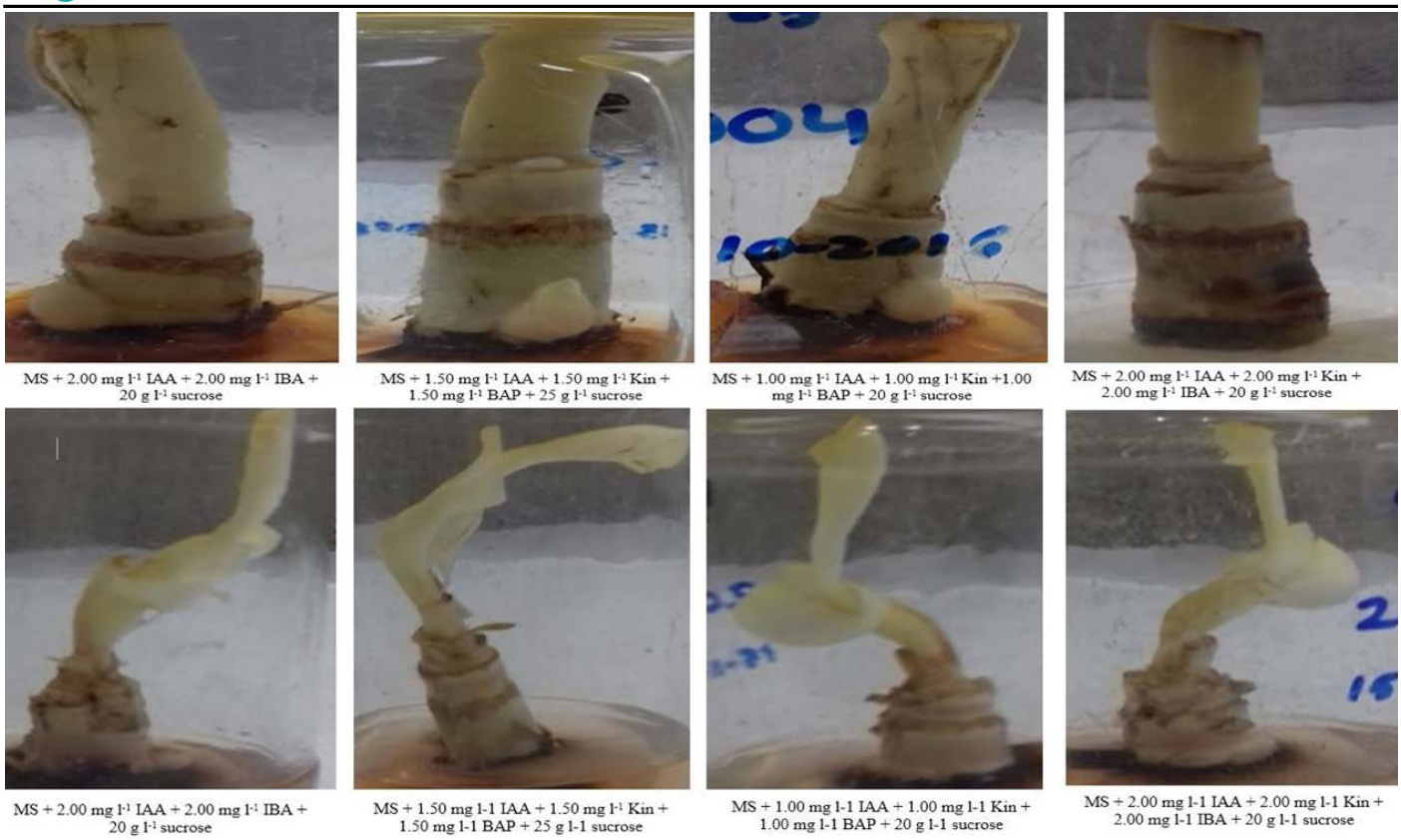

Figure 1: *Shoot initiation under different concentrations of plant growth hormones in variety NIA-2004 and BL4.*

Table 1: *Effect of different concentrations of plant growth regulators and sucrose percentage on days to shoot initiation (days).*

Varieties	Concentrations				Mean
	MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose	MS + 1.50 mg l^{-1} IAA + 1.50 mg l^{-1} Kin + 1.50 mg l^{-1} BAP + 25 g l^{-1} sucrose	MS + 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP + 20 g l^{-1} sucrose	MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} Kin + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose	
NIA-2004	12.67 b-e	8.33 h-k	6.33 kl	6.33 kl	8.41 d
SPF-234	14.33 a-c	11.66 c-f	7.00 j-l	8.67 g-k	10.42 ab
NIA-2012	13.66 a-d	15.00 ab	6.33 kl	7.33 j-l	10.58 ab
BL4	11.67 c-f	8.33 h-k	5.33 l	9.67 f-j	8.75 cd
AEC92-1208	10.33 e-i	8.00 i-l	7.00 j-l	8.33 h-k	8.42 d
Thatta-10	11.33 d-g	14.33 a-c	11.33 d-g	9.00 f-k	11.50 a
Gulabi-95	12.67 b-e	11.00 d-h	8.66 g-k	7.66 i-l	10.00 bc
NIA-1026-P7	15.67 a	14.67 ab	6.33 kl	6.33 kl	10.75 ab
Mean	12.79 a	11.41 b	7.29 c	7.91 c	

Varieties SE (0.6741), LSD (5%) (1.3476); Concentrations SE (0.4767), LSD (5%) (0.9529); V x C SE (1.3483), LSD (5%) (2.6952); Means followed by common letters are not significantly different at p < 0.05.

Ali et al. (2008) have earlier reported similar results mentioning that optimum shoot formation can only be obtained in MS medium containing BAP and Kin. They observed excellent shoot formation when Kin and BAP, both were added to the tissue culture media for sugarcane micropropagation. Our results also agreed to the report of Tarafdar et al. (2014) who demonstrated the shoot initiation from shoot tips containing axillary meristem within 7-10 days after culturing on MS medium supplemented with 1.0 mg l^{-1} BAP. The results were also supported by a similar report of Rahman et al. (2018) who argued that the days to shoot initiation were dictated by concentrations of BAP in MS medium.

BAP is a synthetic cytokinin which plays vital role in plant development, growth and elongation of shoot meristems (Glocke et al., 2006; Victório et al., 2012). Reilly and Washer (1977) reported that BAP is responsible for inducing shoot

organogenesis during tissue culture, whereas Lam (1975) also suggested BAP to be a critical factor for inducing shoot formation - the outcome we observed in this study. Kin, on the other hand, is a cytokinin involved in cell division (Abu-Romman et al., 2015). Hence, for shoot initiation, elongation and development, it is also an essential component as observed (Müller and Leyser, 2011; Premkumar et al., 2011). Gopitha et al. (2010) also presented similar effects of Kin on shoot initiation.

Number of shoots

The statistical analysis of variance for number of shoots (per tissue culture bottle) showed that the influences of varieties and media concentrations were highly significant in producing differences at 5% probability level; however, their combined interaction was non-significant (Figure 2, Table 2, Supplementary Table 1). Maximum number of shoots per bottle, on mean basis, were achieved in BL4 and NIA-2004 (1.58 and 1.42 shoots, respectively), followed by AEC92-1208 (1.33 shoots). On the other hand, minimum number of shoots were recorded in Gulabi-95. The mean results regarding different concentrations of growth regulators used in the media indicated that highest number of shoots (per bottle) were developed in media composing of MS + 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP + 20 g l^{-1} sucrose (1.42 shoots), followed by plantlets grown under media containing MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} Kin + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose (1.21 shoots). Contrarily, minimum number of shoots (per bottle) were recorded under the MS media having 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose (0.63 shoots).

The combinatorial effect of both factors *viz*. varieties + varying compositions of media produced maximum number of shoots (per bottle) in varieties BL4 and NIA-2004 under MS media supplemented with 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP + 20 g l^{-1} sucrose, both of which recorded 2.33 shoots (per bottle). Minimum number of shoots (0.33 shoots per bottle) were observed in varieties SPF-234 as well as Gulabi-95 under the MS medium containing 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA and 20 g l^{-1} sucrose. Again, from these observations, a critical role of BAP and Kin was evident in shoot development.

The number of shoots formed directly depend on hormones influencing the cell division; therefore, Kin was seen to play critical role regarding this parameter as well (Müller and Leyser, 2011; Premkumar et al., 2011). Moreover, crucial impact of BAP concentrations was also seen. Similar results have been reported by Ali et al. (2008) who asserted that a combination of BAP and Kin was required for good shoot formation. They also observed variable response in shoot development with respect to varieties and mentioned good shooting in CP 77400 in media compositions having BAP. Likewise, Baksha et al. (2002) observed multiple shoots when the plantlets were cultured on MS media supplemented with BAP in the range of 0.5-2.0 mg l^{-1}. Similarly, Khan et al. (2006) reported rapid multiplication and shoot development when sugarcane genotypes were cultured on MS medium containing 1.0 mg l^{-1} BAP and IAA along with low concentration of Kin (0.1 mg l^{-1}). Gopitha et al. (2010) suggested that the best regeneration of sugarcane shoot was attained when it was cultured on MS medium supplemented with 1.0 mg l^{-1} BAP and 0.5 mg l^{-1} IBA during micropropagation. Their results also demonstrated an important role of Kin in shoot development. Results of this study are also supported by Mamun et al. (2004) who proposed that BAP is one of the major role players for good shoot proliferation.

Shoot length

The statistical analysis of variance for shoot length indicated that varieties and composition of media contributed significantly towards producing differences, whereas interaction of both of these factors resulted in non-significant differences at 5 % probability level (Table 3). Mean data regarding varieties showed that BL4 and AEC92-1208 produced longest shoots of 4.18 and 3.40 cm respectively, whereas shortest plantlets were produced by NIA-1026-P7 (1.52 cm). Regarding mean data for growth hormones, longest average length of shoots was observed in MS media having 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP and 20 g l^{-1} sucrose. The said media produced average shoot length of 3.90 cm while highest shoot length of 7.07 cm was also observed in this media for the variety BL4. Lowest mean shoots lengths (1.42 cm) were observed in media composition of MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose. All of the varieties were observed to produce extremely short shoots in this media; the minimum observations were recorded for SPF-234 (0.53 cm). These results agreed to earlier report of Singh et al. (2006) who reported successful micropropagation protocol of sugarcane using media

Figure 2: *Number of shoots developed under different concentrations of plant growth hormones in variety NIA-2004 and BL4.*

Table 2: *Effect of different concentrations of plant growth regulators and sucrose percentage on number of shoots (per bottle).*

Varieties	Concentrations				Mean
	MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose	MS + 1.50 mg l^{-1} IAA + 1.50 mg l^{-1} Kin + 1.50 mg l^{-1} BAP + 25 g l^{-1} sucrose	MS + 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP + 20 g l^{-1} sucrose	MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} Kin + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose	
NIA-2004	0.67 cd	1.00 b-d	2.33 a	1.67 a-c	1.42 ab
SPF-234	0.33 d	1.33 a-d	1.33 a-d	1.00 b-d	1.00 a-d
NIA-2012	0.67 cd	0.67 cd	1.00 b-d	1.33 a-d	0.92 b-d
BL4	1.00 b-d	1.33 a-d	2.33 a	1.66 a-c	1.58 a
AEC92-1208	0.66 cd	1.33 a-d	2.00 ab	1.33 a-d	1.33 a-c
Thatta-10	0.66 cd	0.66 cd	1.00 b-d	0.67 cd	0.75 cd
Gulabi-95	0.33 d	1.00 b-d	0.67 cd	0.67 cd	0.67 d
NIA-1026-P7	0.66 cd	0.66 cd	0.66 cd	1.33 a-d	0.83 b-d
Mean	0.63 b	1.00 ab	1.42 a	1.21 a	

Varieties SE (0.3050); LSD (5%); (0.6097); Concentrations SE (0.2157); LSD (5%) (0.4311); V x C SE (0.6100); LSD (5%) (1.2194); Means followed by common letters are not significantly different at p < 0.05.

supplemented with various combinations of 1.0 mg l^{-1} – 6.0 mg l^{-1} BAP + 1.0 mg l^{-1} – 6.0 mg l^{-1} Kin. Moreover, shoot elongation have been suggested to be superior on MS media supplemented with 2.0 mg l^{-1} Kin + 2.0 mg l^{-1} IBA + 2.0 mg l^{-1} IAA by Khan et al. (2006). In an agreement to our results, they also reported excellent shoot elongation in BL4 variety of sugarcane. Parallel observations have been reported by Warakagoda et al. (2007) as well.

Development of leaves

Numbers of leaves were also analyzed in order to investigate the growth of plantlets of different varieties in various media compositions. The statistical analysis of variance for number of leaves (per bottle) revealed that the effect of media concentrations was highly significant, while the consequences of varieties and combinatorial interactions of varieties x concentrations were non-significant at 5 %

Table 3: *Effect of different concentrations of plant growth regulators and sucrose percentage on shoot length (cm).*

Varieties	Concentrations				Mean
	MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose	MS + 1.50 mg l^{-1} IAA + 1.50 mg l^{-1} Kin + 1.50 mg l^{-1} BAP + 25 g l^{-1} sucrose	MS + 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP + 20 g l^{-1} sucrose	MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} Kin + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose	
NIA-2004	2.03 e-k	1.83 e-k	6.17 ab	2.46 d-k	3.12 bc
SPF-234	0.53 k	1.23 i-k	4.33 b-d	1.83 e-k	1.98 d
NIA-2012	1.40 h-k	1.27 i-k	1.07 jk	2.70 d-j	1.60 d
BL4	2.53 d-k	3.73 c-e	7.07 a	3.40 c-h	4.18 a
AEC92-1208	1.47 g-k	3.47 c-g	5.13 a-c	3.57 c-f	3.40 ab
Thatta-10	1.26 i-k	1.60 f-k	3.20 c-i	1.00 jk	1.76 d
Gulabi-95	1.17 i-k	2.57 d-k	2.43 d-k	2.40 d-k	2.14 cd
NIA-1026-P7	1.00 jk	1.16 i-k	1.80 e-k	2.13 e-k	1.52 d
Mean	1.42 c	2.11 bc	3.90 a	2.43 b	

Varieties SE (0.5094); LSD (5%) (1.0183); Concentrations SE (0.3602); LSD (5%) (0.7200); V x C SE (1.0188); LSD (5%) (2.0365); Means followed by common letters are not significantly different at p < 0.05.

Table 4. *Effect of different concentrations of plant growth regulators and sucrose percentage on number of leaves (per bottle).*

Varieties	Concentrations				Mean
	MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose	MS + 1.50 mg l^{-1} IAA + 1.50 mg l^{-1} Kin + 1.50 mg l^{-1} BAP + 25 g l^{-1} sucrose	MS + 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP + 20 g l^{-1} sucrose	MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} Kin + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose	
NIA-2004	0.67 cd	1.67 a-c	2.00 ab	1.00 b-d	1.33 ab
SPF-234	0.33 d	1.33 a-d	1.67 a-c	0.67 cd	1.00 ab
NIA-2012	0.67 cd	0.67 cd	0.67 cd	1.00 b-d	0.75 b
BL4	1.33 a-d	1.00 b-d	2.33 a	1.00 b-d	1.42 a
AEC92-1208	0.67 cd	1.33 a-d	1.67 a-c	1.00 b-d	1.17 ab
Thatta-10	0.67 cd	1.00 b-d	1.33 a-d	0.67 cd	0.92 ab
Gulabi-95	0.33 d	2.00 ab	1.67 a-c	0.67 cd	1.16 ab
NIA-1026-P7	1.00 b-d	0.67 cd	1.00 b-d	1.00 b-d	0.91 ab
Mean	0.71 c	1.21 ab	1.54 a	0.88 bc	

Varieties SE (0.31.05); LSD (5%) (0.6206); Concentrations SE (0.2195); LSD (5%) (0.4388); V x C SE (0.6209); LSD (5%) (1.2412); Means followed by common letters are not significantly different at p < 0.05.

probability level (Table 4, Supplementary Table 1). On mean basis for genotypes, highest number of leaves (per bottle) were produced by BL4 (1.42 leaves) followed by NIA-2004 (1.33 leaves). Conversely, minimum number of leaves (per bottle) were noticed for NIA-2012 (0.75 leaves). Mean outcomes of the media compositions produced significant variation; highest number of leaves (1.54 leaves per bottle) were seen in MS media + 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP + 20 g l^{-1} sucrose. While lowest mean values for number of leaves were observed in media comprising of MS + 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 20 g l^{-1} sucrose.

Analyzing individual performance of varieties under different concentrations of growth hormones, highest number of leaves were observed in BL4 cultured in MS media containing 1.00 mg l^{-1} IAA + 1.00 mg l^{-1} Kin + 1.00 mg l^{-1} BAP and 20 g l^{-1} sucrose, while lowest number of leaves were recorded in SPF-234 under MS media supplemented with 2.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA and 20 g l^{-1} sucrose. In a similar report, Tolera et al. (2014) have also proposed statistically significant changes in development of leaves when concentrations of growth hormones were changed in the media; however, on contrary, they observed highly significant changes for genotypes as well, which was not the case here.

From results of this study, it can be speculated that BAP as well as Kin have extremely important role to play in sugarcane growth and development.

Table 5: *Effect of different concentrations of plant growth regulators and sucrose percentage on number of roots (per bottle).*

Varieties	Concentrations		
	½MS + 2.50 mg l^{-1} IBA + 3.00 mg l^{-1} NAA + 20 g l^{-1} sucrose	½MS + 3.00 mg l^{-1} IBA + 4.00 mg l^{-1} NAA + 30 g l^{-1} sucrose	½MS + 4.00 mg l^{-1} IBA + 5.00 mg l^{-1} NAA+ 40 g l^{-1} sucrose
NIA-2004	+	++	-
SPF-234	-	+	++
NIA-2012	++	+	+
BL4	+++	+++	++
AEC92-1208	+	+++	-
Thatta-10	-	+	-
Gulabi-95	-	+	+
NIA-1026-P7	-	-	+

- No rooting; + weak rooting; ++ good rooting; +++ Profuse rooting.

Similar results were reported by Pawar et al. (2002) who analyzed the effect of growth regulators on *in vitro* multiplication of sugarcane cultivars including Co86032, Co-740 and Co-8014. They found significant effects of different concentrations of Kin and BAP for development of leaves on main shoot. They also observed highest number of leaves in media containing 1.0 mg l^{-1} Kin +1.0 mg l^{-1} BAP, while shoot elongation was seen to be maximum in media supplemented with 1.5 mg l^{-1} Kin + 1.0 mg l^{-1} BAP.

Development of roots

Profuse root development was observed in half MS media supplemented with 3.00 mg l^{-1} IBA + 4.00 mg l^{-1} NAA and 30g l^{-1} sucrose. All of the varieties showed good rooting under this combination except NIA-1026-P7. Excellent root development was seen in BL4 and AEC92-1208 under said media. However, BL4 also produced profuse rooting in ½MS medium containing 2.50 mg l^{-1} IBA + 3.00 mg l^{-1} NAA and 20 g l^{-1} sucrose. Other two media compositions, as well as remaining varieties, demonstrated marginal root development. Overall, Thatta-10, Gulabi-95 and NIA-1026-P7 exhibited least rooting irrespective of the media concentration they were cultured in.

Behera and Sahoo (2009) investigated root development in sugarcane through *in vitro* culture using young meristem as explants. They reported best response in terms of root induction using ½MS basal media supplemented with 3.00 mg l^{-1} NAA. In our study, the best rooting response was observed in half MS medium supplemented with 4.00 mg l^{-1} NAA. Moreover, our results also agreed to that of Gopitha et al. (2010) who reported profuse rooting in micropropagation of sugarcane varieties on MS medium supplemented with high concentration of NAA. Likewise, Bisht et al. (2011) and Yadav and Ahmad (2013) also published similar reports. However, Singh et al. (2001) suggested profuse root induction on ½MS medium supplemented with higher concentration of NAA (5.0 mg l^{-1}) but lower sucrose contents (6%); an observation which was reported by Baksha et al. (2002) as well. Regarding IBA concentration in rooting medium, Mustafa and Khan (2016) demonstrated excellent root induction in elite sugarcane varieties grown in media having slightly higher concentration of IBA (5.0 mg l^{-1}).

The results of this study agreed to the reports of Gray and Trigiano (2004), Niroula et al. (2005), George et al. (2008) and Soomro et al. (2016) who discussed that success of any tissue culture practice depends on hormonal composition of the culture media. Moreover, the variation in genotypes also showed that the micropropagation was dependent on the parent material and optimal media concentrations varied from one to genotype to other. Other studies confirm similar results in sugarcane as well as other plants (Khatri et al., 2004; Khan et al., 2006; Mustafa and Khan, 2012; Sughra et al., 2014; Ali et al., 2019). The results depicted a strong need to develop individual optimal protocols for sugarcane multiplication based on the genotypes under such consideration.

One of the most important observations from the study was the role BAP and Kin were seen to play in inducing sugarcane shoot development. IAA was also a component of the media which produced good shoot development; however, excellent shooting was

observed only in media having Kin and BAP apart from IAA suggesting that supplementing the media with Kin and BAP played crucial role in sugarcane growth and development. Kin is involved in signaling for cell division whereas specific control on shoot elongation and development is influenced by BAP, which makes it vital for sugarcane organogenesis and proliferation (Müller and Leyser, 2011; Premkumar et al., 2011; Soomro et al., 2016). Therefore, being major role players, concentration of these hormones must be maintained for mass production of sugarcane.

Conclusions and Recommendations

The study concluded that *in vitro* development of plantlets is controlled by genotypic factors as well as culture media composition. Both of these factors play significant role towards sugarcane micropropagation. Hence, success of micropropagation is dependent on parent material and composition of the media. The proper supplementation of growth hormones in the growth media plays crucial part. For shoot development BAP and Kin play vital role and their optimal addition to the media controls this phenomenon. For root development, NAA and IBA are extremely important regulators.

Author's Contribution

AQ executed the experiment. GSN conceived the research idea, and planned the study. MTK helped in tissue culture operations, results interpretation and writing of the manuscript. SY collected the raw observations and experimental data. SKB helped in research and write up as supervisor of the first author. MA, helped in research and write up as co-supervisor of the first author. IAK supervised the project. SA, proof read, critically revised and improved the manuscript. MRN assisted in proofreading, editing and value addition of the manuscript. MAS did statistical analysis of the experimental data.

Supplementary Material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjar/2020/33.1.180.191

Abbreviations

MS, Murashige and Skoog; IAA, Indole Acetic Acid; Indole-3-Butyric-Acid IBA; Kinetin, Kin; 6-Benzyl Aminopurine, BAP; Naphthaleneacetic acid, NAA.

Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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