Research Article

Optimization of Sugarcane Varieties for In vitro Regeneration and Multiplication through Tissue Culture

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ABSTRACT

Sugarcane is an important crop for sugar and bioenergy production and belongs to the family Poaceae. Current sugarcane varieties have a highly complex and large genome, with 100-130 chromosomes. The demand for sugar, fiber, bagasse and molasses is increasing in Pakistan due to the rising population. Varieties of sugarcane (*Saccharum officinarums* L.) that are produced by the breeding method take approximately 8-10 years, with the number of pests and diseases causing a decrease in cane yield. Improving the productivity of sugarcane varieties is a major challenge. Tissue culture is a way for quick multiplication of desired varieties and to develop disease-free healthy plants. Therefore, using high-yielding sugarcane varieties can help us in increasing production. This research aimed to optimize embryogenic callus for subsequent regeneration in sugarcane varieties, specifically CPF-251, HSF-240, and CP-77-400 to device micropropagation. Callus induction was initiated in four weeks culture of explant developed from spindle leaves by employing various concentrations of plant growth regulators (PGRs) such as Kinetin (0.2 mgL^{-1}, 0.3 mgL^{-1}, 0.4 mgL^{-1}) and 2, 4-D (1.5 mgL^{-1}, 2.5 mgL^{-1}, 3.5 mgL^{-1}) on Murashige and Skoog (MS) medium. Micro shoots emerged after three weeks of callus culture of media supplemented with differing concentrations of BAP (0.3 mgL^{-1}, 0.4 mgL^{-1}, and 0.5 mgL^{-1}) on MS medium. After three weeks, roots developed, when the shooting plants were placed in the rooting medium containing varying concentrations of PGRs such as IBA (0.3 mgL^{-1} 0.4 mgL^{-1}, 0.5 mgL^{-1}) and NAA (3.5 mgL^{-1}, 4.5 mgL^{-1}, 5.5 mgL^{-1}) on MS medium. The most effective shoot growth of variety HSF-240 was obtained at MS + BAP 0.5 mgL^{-1} + IBA 0.5 mgL^{-1} + NAA 5.5 mgL^{-1} with 4.9 cm shoot length, 2.7 cm length of root and 8 numbers of roots. Similarly, for variety CP-77-400, optimal shoot growth was observed at MS + BAP 0.4 mgL^{-1} + IBA 0.4 mgL^{-1} + NAA 4.5 mgL^{-1}, with a shoot length of 4 cm, root length of 2 cm, and 7 numbers of roots. For variety CPF-251, the most conducive conditions for shoot growth were at MS + BAP 0.3 mgL^{-1} + IBA 0.3 mgL^{-1} + NAA 3.5 mgL^{-1}, resulting in a shoot length of 3 cm, root length of 1.5 cm, and 5 numbers of roots formation. Plants developed through this study proved useful for producing high-yielding, high-sugar recovery cane of existing commercial varieties.

Keywords: Micropropagation, Embryogenic callus, Meristem, Sugarcane, In-vitro, Plant hormones

Article History

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INTRODUCTION

Sugarcane is a kind of perennial grass native to South Asia. The genus name comes from the Greek word 'Sakcharon,' which denotes sugar, particularly sucrose. The juice of sugarcane is commonly known as a raw ingredient used in the production of sugar. Although refined sugar is the major output of sugarcane juice, additional important products such as molasses, and jaggery are produced in an unrefined form throughout the process [1]. Sugarcane is sometimes referred to as a chewing cane. Sugarcane flourishes well enough in tropical climates. Sugarcane

crops require humus-rich soil with only a pH range of 7.5 - 8.5 and as well as a hot and humid climate [2].

Sugarcane belongs to the Poaceae family [3]. Sugarcane is a major perennial crop in tropical and subtropical regions, which produces 80% of global sugar production [4]. Sugarcane is thought to have originated in the region surrounding New Guinea, which includes parts of Southeast Asia and the Pacific. It is thought to have been grown as a garden crop by the indigenous people of these region about thousands of years ago, then it spread throughout the world via trade and exploration, eventually becoming a major crop in many tropical and subtropical regions [5]. There are a number of superior sugarcane cultivars that are inter-species of different varieties of the genus Saccharum: *S. officinarum, S. barberi, S. sinense*, and one wild species *S. spontaneum* [6]. In modern sugarcane cultivars, approximately 80% of the chromosomes come from Saccharum officinarum, 10% from *S. spontaneum*, and the remaining 10% from other species like *Saccharum barberi* or *S. sinense*. This genetic composition was achieved through selective breeding to create varieties with desirable characteristics such as high sucrose content, disease resistance, and adaptability to various environmental conditions [7].

Sugarcane is a perennial plant that grows in clumps, featuring several unbranched stems. These stems can reach height of up to 5 cm and vary in color from green to pinkish purple. Nodes are typically located at the bases of alternate leaves, signifying their joint structure. Variations in diameter among joints are significantly influenced by growth conditions [2]. A network of rhizomes forms under the soil which sends up secondary shoots near the parent plant. The stems vary in color, being green, pinkish, or purple. They are joined, nodes being present at the bases of the alternate leaves. The environmental factors affect the pigments of the stalk observed at the internodes. For instance, exposure to sunlight may completely alter the color of the stalk [8]. Typically, the leaves joined to the node. The leaf bases of sugarcane are joined to the stem. The leaf has a thick midrib that is green from the below side and white from the above side. This change suggests that the growth point no longer produces inflorescences [9].

It is believed that consuming sugarcane juice daily promotes clear and efficient urine flow, facilitating the effective functioning of the kidneys. For enhanced benefits, it is often combined with lime and ginger, which is particularly beneficial for alleviating stomach pain [10]. Sugarcane juice possesses diuretic properties that facilitate the elimination of toxins and illnesses from the body. It is effective in treating urinary ailments and kidney stones, ensuring proper kidney function. Additionally, sugarcane juice serves as a digestive remedy for digestive issues. Its potassium content helps to regulate pH levels in the intestines [11].

Plant tissue culture is becoming an efficient method for the fast growth of sugarcane cultivars. Throughout the year, thousands of plants can be produced [12]. For generating a significant amount of plant material of genotypes with less time 1-2 years it may show to be an effective to the standard multiplication system. The removal of viruses via apical meristem culture is a common technique in plant biotechnology, including the production of virus-free sugarcane plants. Apical meristem culture is the process of cultivating a small piece of tissue from a plant's growing tip (meristem) in a sterile nutrient medium. This technique allows for plant propagation while eliminating viral infections because meristem tissue is typically virus-free [13].

Viruses have been effectively removed from sugarcane plants using meristem culture. The plants are healthy if samples are obtained from disease-free plant material [14]. Meristem culture has shown to be a successful method for removing viruses from plants, making it possible to provide propagation material that is free of disease. It is needed to increase sugarcane yield in order to meet the demand for sugar [15].

The MS medium is a common growth medium for plant tissue culture. Basal Medium includes organic additions including agar, carbohydrates, vitamins, and growth regulators together with macronutrients with high amounts of nitrate [16]. Dichlorophenoxyacetic acid is the most popular auxin utilized in the callus induction of sugarcane. The physiological action of auxins varies, as does the amount to which they transfer in tissue. In this method of sugarcane, Indole butyric acid (IBA) and Benzyl aminopurine (BAP) can both be used to promote callus development and plant growth [17]. Sugarcane callus development is influenced by the type of explant used as well as the plant's genetic characteristics. Furthermore, shoot regeneration has been successful using both basic Murashige and Skoog (MS) medium and media supplemented with cytokinins such as BAP and kinetin (Kn). These hormones promote shoot growth from callus tissue [18]. Naphthalene acetic acid (NAA) has important for root induction. Induced rooting was done in clumps. Auxins, a type of plant hormone, are essential for the development of sugarcane roots. These combinations effectively initiated the rooting process. When sugarcane shoots were placed on MS medium containing 7% sugar and 5 milligrams per liter of NAA, roots began to develop. The researchers found that NAA was good for rooting [19]. In plant cells, tissues, and organ cultures, light is important key factor that promotes growth of plants. The (PGRs) and regulation of hormone levels can both

be affected by light. Acclimatization is the process of gradually removing plantlets from totally controlled environments, so they are ready to live in their natural environment [20]. Our research is a big support in sugarcane tissue culture to improve cane yield by optimizing different media concentrations for different varieties.

MATERIALS AND METHODS

The explants of three genotypes CPF-251, HSF-240 and CP 77-400 of sugarcane for callus induction were collected from 8 months old field-grown sugarcane plants. The samples of these sugarcane varieties were collected from Fatima Sugar Research and Development Centre (Fatima Sugar Mills) Sanawan, Kot Addu. Following the collecting of explants, meristems and spindle leaves were washed under tap water for about 10 to 15 minutes before being cleaned with autoclave distilled water and cut into little pieces. All subsequent procedures were performed in a sterile environment inside a laminar air flow hood. For around 5 to 10 minutes, explants were disinfected with mercuric chloride (0.1%). Explants were then placed in laminar air flow on filter paper to dry. The protocol optimized for callus induction of three varieties of sugarcane on MS medium with different concentration of Kinetin (Kn) ranged from 0.2- 0.4 mg/L and 2-4-D (1.5-3.5 mg/L) were used. Callus regeneration was done by using different concentrations of benzyl amino purine (BAP) ranging from 0.2- 0.5 mg/L. The rooting was optimized by using different concentrations of indole butyric acid (IBA) 0.1-0.5 mg/L and naphthalene acetic acid (NAA) ranged from 1.5-5.5 mg/L. Three replications of each treatment and one control have been used for callus regeneration. Data was observed based on characteristics such as how many days it takes for callus regeneration, number of shoots and number of roots. Completely randomized design (CRD) was used to study the interaction of genotypes on different hormonal treatments in sugarcane, calculated data observed under variance analysis (ANOVA) [21].

RESULTS

Days to Callus Induction.

A significant variation was recorded for callus induction in three genotypes at ($p \leq 0.01$, Table 1). Results of the said parameters depicted that there was a significant effect of genotypes while highly significant results of treatments and interaction between genotypes × treatments were observed.

Table 1. Level of significance for days to callus induction.

Source	DF	SS	MS	F	P
Genotype	2	10.4	5.21	0.44	0.3466*
Treatment	9	31017.0	3446.33	290.54	0.0000**
Genotype x Treatment	18	923.6	51.31	4.33	0.0000**

*= Significant **=highly significant at $p \leq 0.01$, DF (Degree of freedom), SS (Sum of square), MS (Mean square), F (F-value), P (P-value)

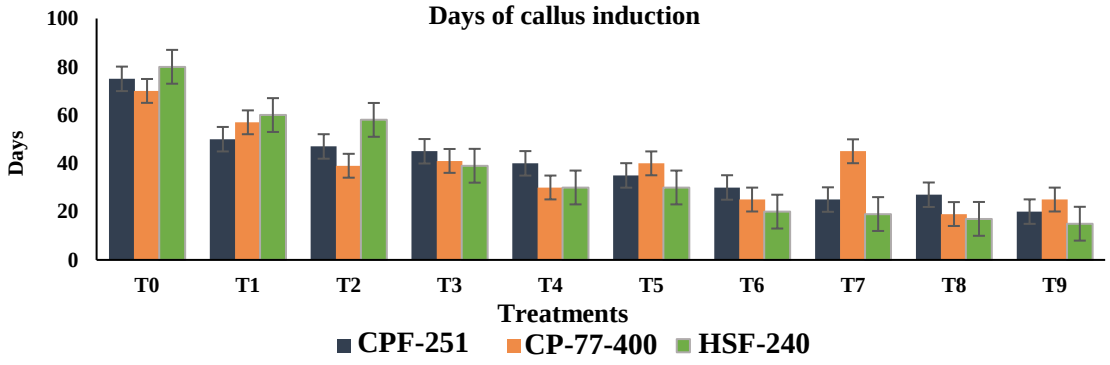

Figure 1. Performance of varieties for days to callus induction

Sugarcane varieties for days to callus induction. The variety HSF-240 has taken a maximum (80) days in the control treatment and a minimum (15) days at treatment 9 for callus induction in sugarcane.

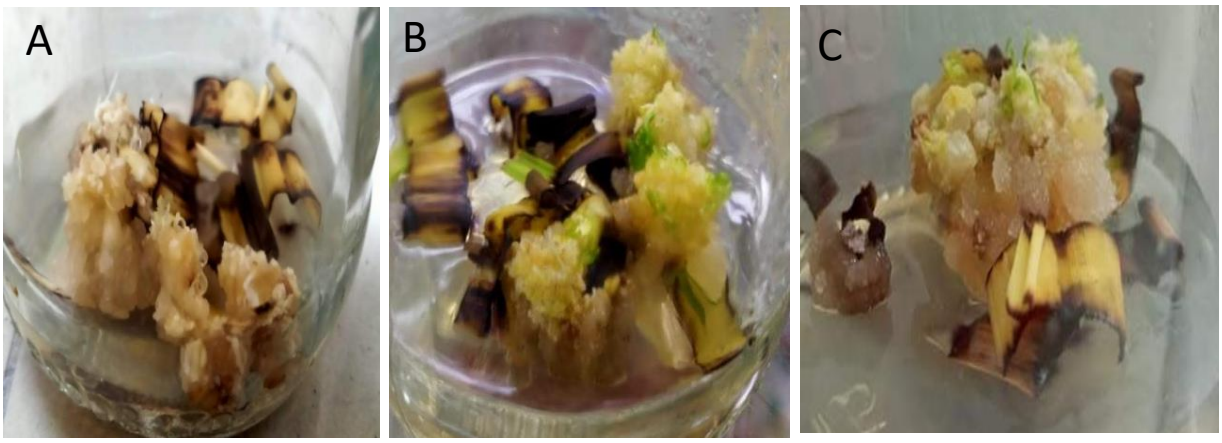

Figure. 1.1 (A). CPF-251 at treatment 9 has taken (20) days for callus induction. (B). CP-77-400 at treatment 8 has taken (19) days. (C). HSF-240 at treatment 9 has taken (15) days for callus induction.

The variety HSF-240 has taken maximum (80) days at treatment T_0 containing MS medium 4.43 gL^{-1} for callus induction. In treatment T_1 containing MS + 2-4-D (1.5 mgL^{-1}) has taken (60) days. In treatment, T_2 containing MS + 2, 4-D (2.5 mgL^{-1}) has taken (58) days. In treatment, T_3 containing MS + 2, 4-D (3.5 mgL^{-1}) has taken (40) days. Treatment T_4 containing MS + Kinetin (0.2 mgL^{-1}) has taken (60) days. In treatment, T_4 containing MS + 2, 4-D (2.5 mgL^{-1}) has taken (58) days. Treatment T_5 containing MS + Kinetin (0.3 mgL^{-1}) has taken (25) days. In treatment, T_6 containing MS + Kinetin (0.4 mgL^{-1}) has taken (20) days for callus induction. In treatment, T_7 containing MS + Kinetin (0.2 mgL^{-1}) + 2, 4-D (1.5 mgL^{-1}) has taken (19) days for callus induction. In treatment, T_8 containing MS + Kinetin (0.3 mgL^{-1}) + 2, 4-D (2.5 mgL^{-1}) has taken (18) days for callus induction. In treatment T_9 containing MS + Kinetin (0.4 mgL^{-1}) + 2, 4-D (3.5 mgL^{-1}) has taken a minimum of (15) days for callus induction. The creamy white fresh callus was formed under medium 9 for a variety HSF-240 containing 3.5 mgL^{-1} 2, 4-D.

Days to Shoot Formation

A significant variation was recorded for days to shoot formation in three genotypes at ($p \leq 0.01$, Table 2). Results of the said parameters depicted a significant effect of genotypes while highly significant results of treatments and interaction between genotypes × treatments were observed. HSF-240 has taken a maximum of days at treatment T_0 containing MS medium 4.43 gL^{-1} for shoot formation. Treatment T_1 containing MS + BAP (0.1 mgL^{-1}) has taken (30) days. In treatment, T_2 containing MS + BAP (0.2 mgL^{-1}) has taken (38) days. Treatment T_3 containing MS + (0.3 mgL^{-1}) has taken (31) days. Treatment T_4 containing MS + BAP (0.4 mgL^{-1}) has taken (25) days.

Table 2. Level of significance for days to shoot formation.

Source	DF	SS	MS	F	P
Genotype	2	35.8	17.91	6.37	00.45
Treatment	5	16240.8	3248.15	1156.19	0.0000*
Genotype Treatment	10	25.1	2.51	0.89	0.5497

*=Significant difference **= highly significant at $p \leq 0.01$, DF (Degree of freedom), SS (Sum of square), MS (Mean square), F (F-value), P (P-value).

Treatment T_5 containing MS + BAP (0.5 mgL^{-1}) has taken a minimum (12) days. The variety CP-77-400 has taken a maximum (75) days at treatment T_0 containing MS medium 4.43 gL^{-1} for shoot formation. Treatment T_1 containing MS + BAP (0.1 mgL^{-1}) has taken (20) days. In treatment, T_2 containing MS + BAP (0.2 mgL^{-1}) has taken (25) days. Treatment T_3 containingMS + BAP (0.3 mgL^{-1}) has taken (40) days. Treatment T_4 containing MS + BAP (0.4 mgL^{-1}) has taken a minimum of 16 days.

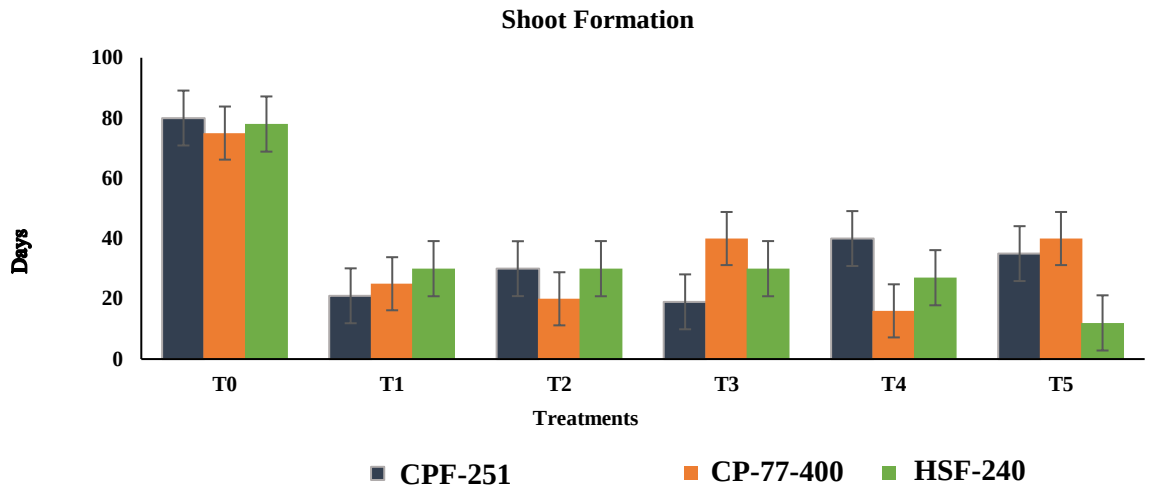

Figure 2. Performance of varieties for days to shoot formation.

The graph shows that the variety HSF-240 has taken maximum of (80) days in the control treatment and a minimum of (12) days at treatment 5 for shoot formation in sugarcane.

Figure 2.1 (A) *In-vitro* micro shoots formation of variety HSF-240 (B) *In -vitro* micro shoots formation of variety CP-77-400 (C) *In-vitro* micro shoots formation of variety CPF-251

Shoot Length (cm)

A significant variation was recorded for shoot length in three genotypes of sugarcane at $p \leq 0.01$, Table 3). A highly significant effect on shoot length was observed for genotype and treatment. Results of the said parameter (shoot length) also showed that highly significant effects were also present between (genotypes × treatments) interaction.

Table 3. Level of significance for shoot length

Source	DF	SS	MS	F	P
Genotype	2	0.8844	0.4422	0.56	00.4339*
Treatment	5	58.0172	11.6034	14.62	0.0000**
Genotype Treatment	10	7.7267	0.7727	0.97	0.4835*

*=Significant, ** highly significant at p≤0.01, DF (Degree of freedom), SS (Sum of Square), MS (Mean Square), F (F-value), P (P-value)

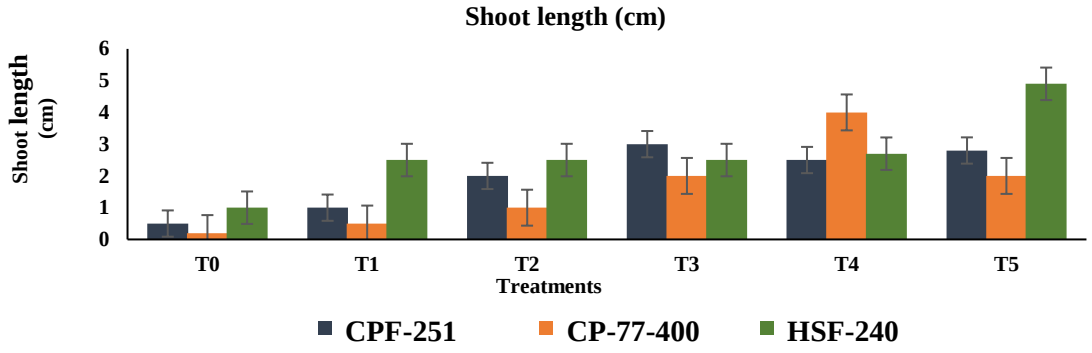

Figure 3. Performance of sugarcane varieties for shoot length (cm)

The figure shows HSF-240 has a maximum shoot length (4.9) at treatment 5 as compared to the other two varieties (CPF-251, CP-77-400).

The graph shows that the variety CPF-251 has (0.5 cm) shoot length in the control treatment and has a maximum (3cm) shoot length at medium 3. CP-77-400 has (0.2 cm) shoot length in the control treatment and has (4 cm) at treatment 4. HSF-240 has (1 cm) shoot length in the control treatment and has a maximum (4.9 cm) at treatment 5.

The variety HSF-240 has more shoot length (4.9 cm) as compared to the varieties CP-77-400 and CPF-251. It was observed that under medium 3 containing MS + BAP (0.3 mgL^{-1}) of a variety, CPF-251 has (3 cm) shoot length. CP-77-400 has (4 cm) shoot length and under medium 4 containing MS + BAP (0.4 mgL^{-1}).

Days to Root Formation

A significant variation was recorded for days to root formation in three genotypes at (p≤0.01, Table 4). Results of the said parameters depicted that there was a significant effect of genotypes while highly significant results of treatments and interaction between genotypes × treatments were observed.

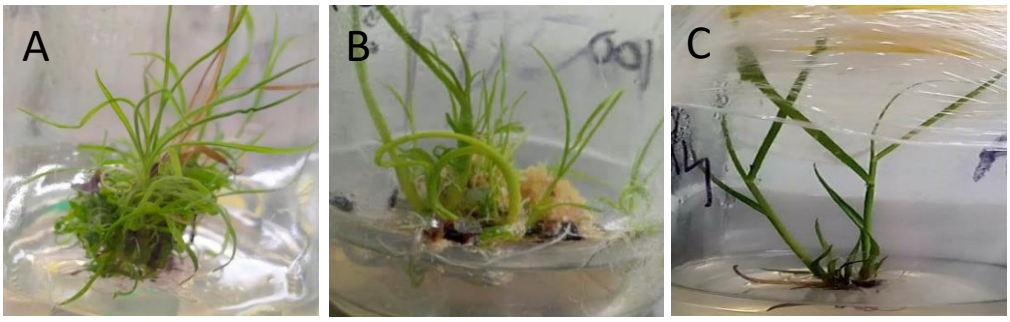

Figure 3.1 (A) *In- vitro* micro shoots formation of variety CPF-251 at treatment 3 (B) *In- vitro* micro shoots formation of variety CP-77-400 at treatment 4 (C) *In- vitro* micro shoots formation of variety HSF-240 at treatment 3.

Table 4. Level of significance for days to root formation

Source	DF	SS	MS	F	P
Genotype	2	84.3	42.13	8.63	0.0009*
Treatment	5	15797.0	3159.40	646.82	0.0000**
Genotype x Treatment	10	17.7	1.77	0.36	0.2165*

*= Significant, **= highly significant at p≤0.01, DF (Degree of freedom), SS (Sum of square), MS (Mean square), F (F-value) P (P-value)

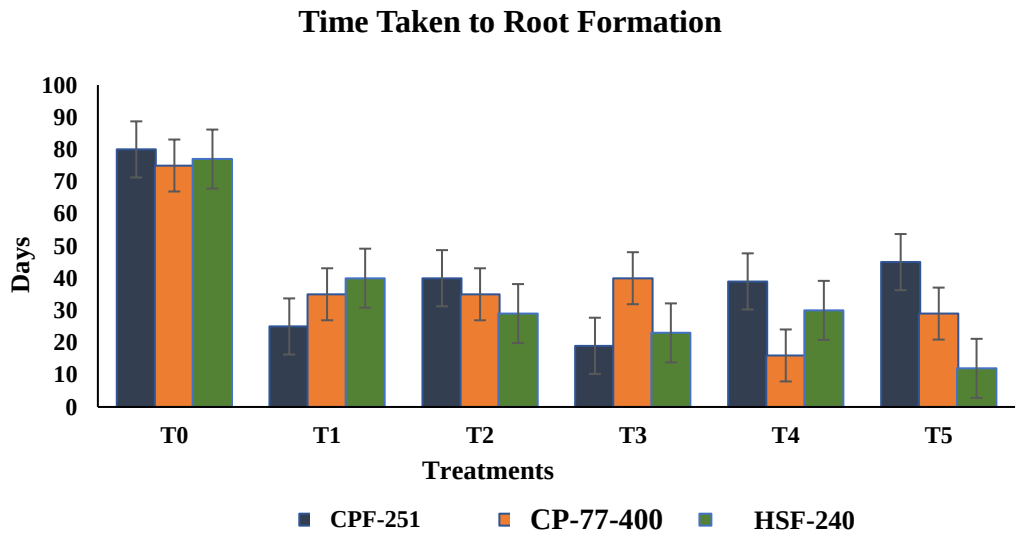

Figure. 4. Performance of varieties for days to roots formation

The graph shows that the variety CPF-251 has taken a maximum (80) days in control treatment and minimum (19) days at treatment 3 for root formation. The variety CP-77-400 has taken (75) days in control treatment and has taken minimum (16) days at treatment 4 for root formation. The variety HSF-240 has taken maximum (77) days in control treatment and has taken minimum (12) days at treatment 5 for root formation in sugarcane.

Figure 4.1 Root formation of variety HSF-240

The variety HSF-240 has taken maximum (77) days at treatment T_0 containing MS medium 4.43 gL^{-1} for root formation. In treatment T_1 containing MS + IBA (0.1 mgL^{-1}) + NAA (1.5 mgL^{-1}) has taken (40) days.

In treatment T_2 containing MS + IBA (0.2 mgL^{-1}) + NAA (2.5 mgL^{-1}) has taken (29) days. In treatment T_3 containing MS + MS + IBA (0.3 mgL^{-1}) + NAA (3.5 mgL^{-1}) has taken (23) days. In treatment T_4 containing IBA (0.3 mgL^{-1}) + NAA (3.5 mgL^{-1}) has taken (30) days. In treatment T_5 containing MS IBA (0.4 mgL^{-1}) + NAA (4.5 mgL^{-1}) has taken minimum (12) days for root formation.

Root Length (cm)

A significant variation was recorded for root length in three genotypes of sugarcane at p≤0.01, Table 5). A highly significant effect on root length was observed for genotype and treatment. Results of the said parameter (root length) also showed that highly significant effects were also present between (genotypes × treatments) interaction.

Table 5. Level of significance for root length

Source	DF	SS	MS	F	P
Genotype	2	2.9287	1.46436	14.38	0.0000**
Treatment	5	33.7381	6.74762	66.28	0.0000**
Genotype Treatment	10	4.4781	0.44781	4.40	0.0005*

*= Significant, **= highly significant at p≤0.01, DF (Degree of freedom), SS (Sum of square), MS (Mean square), F (F-value) P (P-value)

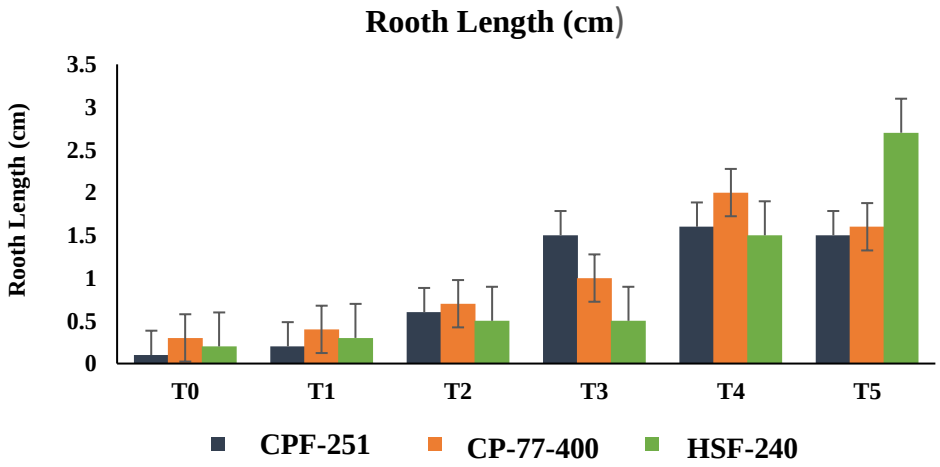

Figure 5. Performance of varieties for root length (cm)

*The figure shows that the variety HSF-240 has a maximum root length (2.7) at treatment 5 as compared to the other two varieties (CPF-251, CP-77-400).

The graph shows that the variety CPF-251 has (0.1 cm) root length in control treatment and has maximum (1.5 cm) root length at medium 3. CP-77-400 has (0.3 cm) root length in control treatment and has (2.00 cm) at treatment 4. HSF-240 has (0.2 cm) root length in control treatment and has maximum (2.7 cm) at treatment 5 root length has been observed.

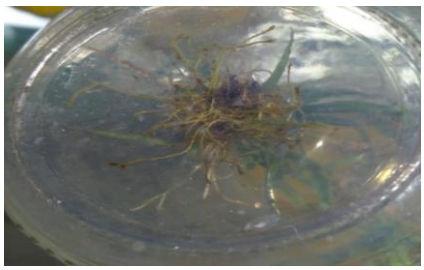

Figure 5.1. Rooting of variety CP-77-400 at treatment 4

The variety CP-77-400 has taken maximum (75) days at treatment T_0 containing MS medium 4.43 gL^{-1} for root formation. In treatment T_1 containing MS + IBA (0.1 mgL^{-1}) + NAA (1.5 mgL^{-1}) has taken (35) days. In treatment, T_2 containing MS + IBA (0.2 mgL^{-1}) + NAA (2.5 mgL^{-1}) has taken (35) days. In treatment,

T_3 containing MS + MS + IBA (0.3 mgL^{-1}) + NAA (3.5 mgL^{-1}) has taken (40) days. In treatment, T_4 containing IBA (0.3 mgL^{-1}) + NAA (3.5 mgL^{-1}) has taken minimum (16) days.

Number of Roots

A significant variation was recorded for the number of roots in three genotypes of sugarcane under different treatments ($p \leq 0.01$, Table 6). A significant effect for the number of roots was observed for genotypes, and a highly significant effect was observed among treatments. The number of roots of all selected genotypes was observed. The number of roots of genotypes (CPF-251, CP-77-400 and HSF-240) were significant.

Table 6. Level of significance for the number of roots

Source	DF	SS	MS	F	P
Genotype	2	2.481	1.2407	5.45	0.0089*
Treatment	5	114.537	22.9074	100.62	0.0000**
Genotype Treatment	10	9.074	0.9074	3.99	0.0012*

*= Significant, **= highly significant at $p \leq 0.01$, DF (Degree of freedom), SS (Sum of square), MS (Mean square), F (F-value) P (P-value)

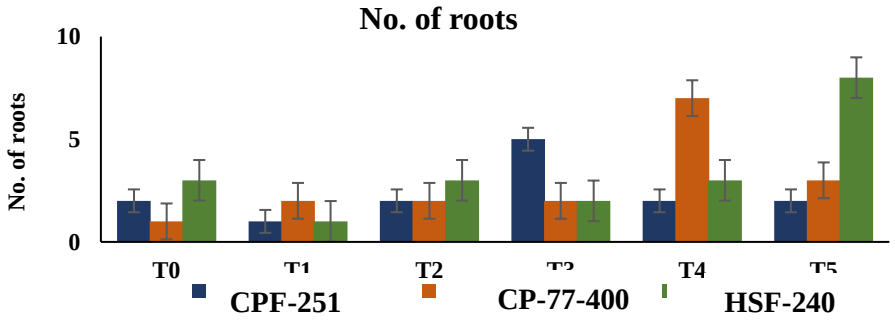

Figure 6. Performance of sugarcane varieties for the number of roots

*The figure shows HSF-240 has a maximum number of roots (6) at treatment 5 as compared to the other two varieties.

The graph shows that the variety CPF-251 has (2) the number of roots in the control treatment and has a maximum (5) number of roots at medium 3. CP-77-400 has (1) no. of root in the control treatment and (7) the number of roots at treatment 4. HSF-240 has (3) number of roots in the control treatment and has a maximum (8) number of roots at treatment 5.

Figure 6.1. Rooting of variety CPF-251

DISCUSSION

Sugarcane varieties are quite different and often reproduce vegetatively through stem cutting. The ability to propagate a superior sugarcane genotype faces major challenges due to inadequate multiplication techniques [22]. Thus, current research for *in-vitro* culturing of three cultivars (HSF-240, CP-77-400 and CPF-251) of sugarcane was conducted at MNS-Tissue Culture Laboratory. The main purpose of research is to adopt advanced techniques for micropropagation using shoot tips. Due to the undifferentiated nature of the cells and the fact that meristematic cells actively divide, meristems were chosen as a source of explants. The ability to produce sugarcane plants that are free of viruses is the primary factor. Different scientists reported that micropropagation of sugarcane through meristems and spindle leaves gave satisfactory results [23]. In the current research, Callus initiation was established for genotypes CPF-251, CP-77-400 and HSF-240 of sugarcane by using shoot tip as explants. Callus induction was observed after two weeks of inoculation from the meristems and spindle leaves containing different concentrations of PGRs such as Kinetin (0.2 mgL^{-1},0.3 mgL^{-1},0.4 mgL^{-1}) and 2,4-D (1.5 mgL^{-1}, 2.5mgL^{-1}, 3.5mgL^{-1}) on MS media 4.33gL^{-1}, sucrose 30gL^{-1} and gelzen 3.3gL^{-1}. Initiation of callus was observed within 2-3 weeks from the explants. 3.5 mgL^{-1} of 2, 4-D was shown to provide the most profuse callus induction, with the potential for callus initiation from the explant, even though 2, 4-D was induced at all doses The researchers found similar results in two different varieties of sugarcane, CPF-237 and Co 86032 [24].

According to the present study, it was found that the concentrations and types of growth regulators utilized in the experiment had a significant impact on shoot development. Micro shoots were observed after two weeks of inoculation of callus into media containing different concentrations of plant growth regulators such as BAP on MS medium 4.33gL^{-1}, sucrose 30gL^{-1} and gelzen 4.33gL^{-1}. The maximum length of shoot (4.9 cm) was measured in variety HSF-240 at treatment 5 after 12 days, whereas the (4 cm) shoot length after 16 days was observed in CP-77-400. The same findings were presented by the researchers that the different shoot length was observed in Co 6806 and BL4 varieties of sugarcane [25]. These findings were consistent with Kureel et al., 2006 who also reported potential axillary shoot proliferation of variety CPF-337 optimum multiplication for variety CP-77-400 was obtained at 1.0 mg/l BAP and 0.5 mg/l Kin, with a maximum of 8.5 cm shoot length, 7 number of tillers and 24 number of shoots [26]. In the current study, it was found that the sugarcane genotype HSF-240 has a maximum shoot length that was 4.9 cm at MS + BAP (0.5 mgL^{-1}) as compared to genotype CP-77-400 has a shoot length of 4 cm at MS + BAP (0.4 mgL^{-1}) and CPF-240 has shoot length 3.5 cm at MS + BAP (0.3 mgL^{-1}). The rate of shoot multiplication was much lower in a control experiment using simply MS basal media. The impact of hormones on the culturing of sugarcane genotypes was also reported [27].

In case rooting of protocol, after 2-3 times of subcultures, when the regenerated shoots inoculated into the rooting media. The best results were obtained in the media that was supplemented with MS + IBA (0.5 mgL^{-1}) + NAA (5.5 mgL^{-1}) for a variety HSF-240 which has a root length of 2.7 cm as compared to varieties CP-77-400 has root length 2 cm and CPF-251 has length of root 1.5 cm. These relatively new roots have continued growth. It was important to note that most of the shoots that were inoculated into the medium showed considerable root formation within 15 days. Both NAA and IBA were found to be better responses for profuse rooting.

CONCLUSION

It is concluded from this research that varieties of sugarcane are mostly dependent on the quantities and combinations of growth regulators including 2,4-D, and Kinetin were considered best for callus induction. Callus regeneration is also affected by different concentrations of (BAP) for effective regeneration and the induction of the greatest number of shoots. Hence, Micropropagation is an effective way to deal with problems caused by serious diseases like red rot and rust. In comparison to conventional methods, it might also be utilized to produce a lot of plantlets in a short time under controlled light and climatic conditions.

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