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Evaluation and identification of early sugarcane varieties under *in vitro* condition

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Abstract
The research was carried out in the Sugarcane Hi-tech Laboratory, SRI, DRPCAU, Pusa, Samastipur, Bihar. The goal of evaluating the regenerative response of six mid-late sugarcane clones under *in vitro* conditions with three different types of growth media *i.e.* root induction and root elongation on media ½ MS basal + NAA (5.0 $mgl^{-1)}$ + 5% sucrose (M_1), MS basal + NAA (5.0 mgl^{-1}) + 5% sucrose (M_2) respectively while acclimatization of *in vitro* developed plantlets and callus formation were recorded on medium MS basal + 2,4-D (3.0 mgL^{-1}) (M_3). A genotypic difference for all tissue culture responses were found in the six selected cultivars. CoP16437 has the best response among these six cultivars, while CoP9301 has the lowest response.

Keywords: Types of growth, selected cultivars, lowest response

Introduction
Sugarcane is a tall, perennial grass that belongs to the Poaceae family. It is native to Southeast Asia, but is now widely cultivated in tropical and subtropical regions around the world, particularly in countries such as Brazil, India, China, Thailand and Mexico. Sugarcane is primarily grown for the production of sugar, which is extracted from the plant's juice. The juice is obtained by crushing the sugarcane stalks and then processing the resulting juice through a series of boiling, filtering and evaporating steps. The final product is crystallized sugar that can be used in a wide range of food and beverage products.

Sugarcane is a vital crop for many countries, providing income for farmers and supporting local economies. However, the sugarcane industry also faces challenges such as diseases, pests, and environmental concerns related to land use and water management. Researchers are working on developing new varieties of sugarcane that are more resistant to pests and diseases, as well as more efficient and sustainable production practices to ensure the continued success of the industry.

The cultivation of sugarcane involves several stages. It begins with the selection of suitable varieties or hybrids based on factors such as yield, disease resistance and sugar content. The crop is usually propagated through stem cuttings or by planting the top portions of mature stalks. After planting, the crop requires adequate irrigation, fertilization and weed control to ensure optimal growth.

Apart from sugar production, sugarcane also has various other applications. It is used for the production of bioethanol, a renewable fuel alternative to gasoline and in the manufacturing of other by-products such as bagasse (fibrous residue used for bioenergy and pulp production), wax, rum and alcoholic beverages.

Sugarcane cultivation plays a significant role in the agricultural economies of many countries, providing income and employment opportunities for millions of people. However, the industry also faces challenges such as pests, diseases, environmental sustainability, and fluctuations in global sugar prices. Efforts are being made to develop more efficient and sustainable cultivation practices, including the use of advanced technologies and the diversification of sugarcane by-products.

Micropropagation, also known as tissue culture, is a powerful technique used for the rapid multiplication and propagation of plants under controlled laboratory conditions. It has emerged as a valuable tool for the propagation of sugarcane. Its ability to produce disease-free and genetically uniform plantlets offers numerous benefits to the sugarcane industry, including enhanced productivity and the preservation and dissemination of desirable traits. Further research and advancements in Micropropagation techniques are expected to contribute to the

sustainable production and improvement of sugarcane crops worldwide.

Materials and Methods

The present research was conducted at Sugarcane Research Institute at Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India. The material included in the experiment consisted of six early maturing clones of sugarcane *viz*. CoP9301, CoP11437, CoP11438, CoP16437, CoP18437 and BO153 employed as the source of explants. Sugarcane plots produce standing crops of healthy, disease-free sugarcane tops.

Explants containing shoot apex were taken from each genotype, sterilized by a standard procedure (Siddiqui *et al.*, 1994) and cultured on modified MS medium (Murashige & Skoog, 1962) supplemented with different concentrations of growth regulators. The supplement to be incorporated such as sucrose 30g/l into the basal medium were added before final adjustment of the volume prepared by double distilled water and further plant growth regulators were added and the pH of the medium was adjust at 5.8 ± 0.5 using either 0.1 NaOH or 1N HCl. Data on Root induction and root elongation on medium ½ MS basal + NAA (5.0 $mgl^{-1)}$+ 5% sucrose (M_1), MS basal +NAA (5.0 mgl^{-1}) + 5% sucrose (M_2) respectively while acclimatization of *in vitro* developed plantlets and callus formation were recorded on medium MS basal + 2,4-D (3.0 mgL^{-1}) (M_3).

The explants were kept in the dark for 15, 30, 45 and 60 days of intervals were subjected to check the root induction and root elongation on (M_1 & M_2 media), acclimatization of *in vitro* developed plantlets and callus formation on (M_3 media). The inoculated culture tubes and bottles were transferred to the tissue culture chamber having controlled environment conditions such as temperature 25º ± 2ºC and relative humidity (RH) 50% to 80%. The continuous light of about 2 kilo lux was maintained through tube lights. The tissue culture responses were assessed with respect to their frequency as percentage of cultures showing that response and their magnitude either in numbers or in groups namely, low, moderate, good and excellent. The % rate of survival of explants calculated out of total tubes cultured. The average number of root induction and root elongation of each cultivar was calculated by random selection of five culture bottles out of total established bottles, whereas one culture bottle was selected out of the total established cultures to measure average shoot elongation as well as root elonagtion.

Root induction

To assess the average number of roots in the proliferated shoots of all the selected six cultivars of sugarcane another experiment was conducted. Medium M_1 (half MS basal with 5.0 mgl^{-1} NAA) and M_2 (full MS basal with 5.0 mgl^{-1} NAA) were selected for rhizogenesis. The additional 5% sucrose was added in both root inducing media for better response.

After 30 days of inoculation on medium M_1 (½ MS basal + 5.0 mgl^{-1} NAA), the proliferated shoots of all the selected six cultivars showed the root induction. The average number of roots was observed to be the highest in the cultivar, CoP16437 (15.8), followed by CoP18437 (14.6), BO153 (12.4), CoP11437 (12.2), CoP11438 (11.2), whereas the lowest eas observed in the cultivar CoP9301 (10.4) (Table 1 and Fig 1).

However, on medium M_2 (MS basal + 5.0 mgl^{-1} NAA) the cultivar, CoP16437 (20.4) showed the highest number of root induction followed by CoP18437 (19.4), CoP11438 (18.4), BO153 (18.2) and CoP11437 (17.6) while the lowest was observed in the cultivar CoP9301 (16.8) (Table 1 and Fig 1). Thus, the pooled average number of roots induction on both medium M_1 (½ MS basal + 5.0 mgl^{-1}NAA) and M_2 (MS basal + 5.0 mgl^{-1} NAA) was found highest in the selected cultivar, CoP16437 followed by CoP18437, BO153, CoP11437 and CoP11438 whereas the lowest was found in CoP9301.

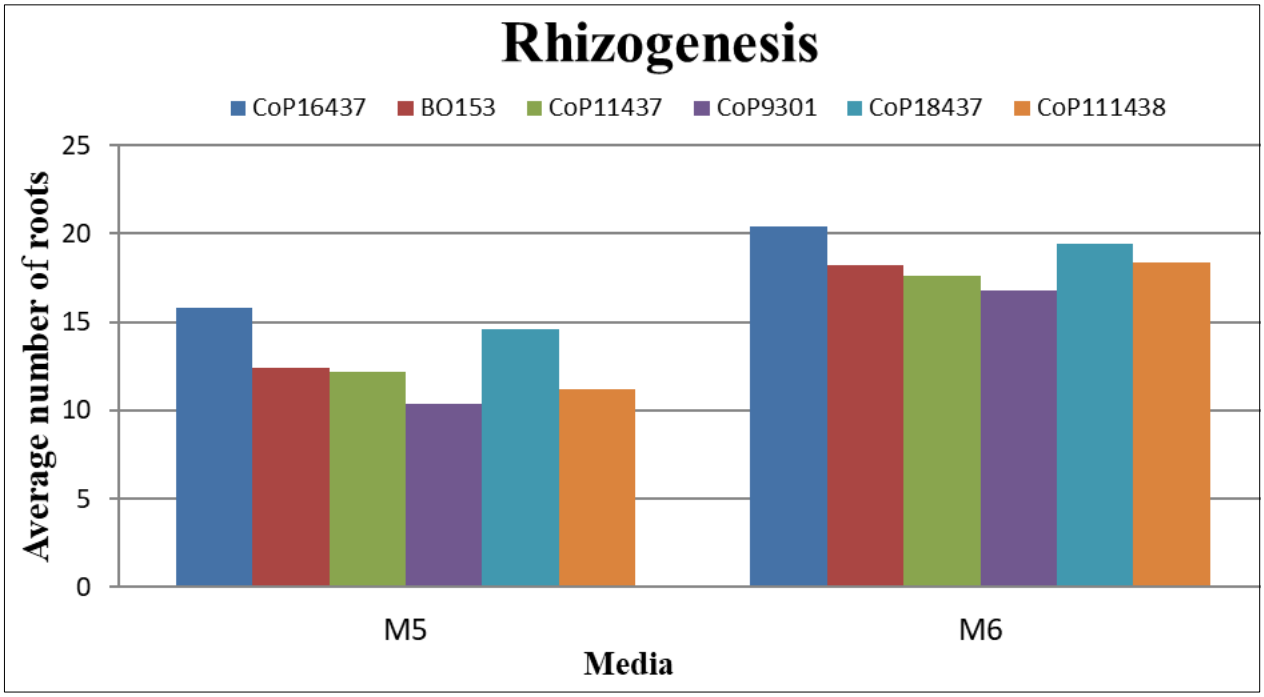

Fig 1: Sugarcane cultivars showing percent rate of Rhizogenesis

Table 1: Regeneration potential of root induction in selected cultivars of sugarcane on M_1 and M_2

Sl. No.	Name of Cultivar	Average number of root induction after 30 days of inoculation	
		M_1 (½ MS basal + 5.0 NAA mgl^{-1})	M_2 (MS basal + 5.0 NAA mgl^{-1})
1.	CoP16437	15.8	20.4
2.	BO153	12.4	18.2
3.	CoP11437	12.2	17.6
4.	CoP9301	10.4	16.8
5.	CoP18437	14.6	19.4
6.	CoP11438	11.2	18.4

Root elongation

The average elongation of differentiated roots from *in vitro* developed shoots of all the selected six cultivars was observed on M_1 (½ MS basal + 5.0 mgl^{-1} NAA) and M_2 (MS basal + 5.0 mgl^{-1} NAA) at 30 days of interval. On medium M_1 (½ MS basal + 5.0 mgl^{-1} NAA), the average root elongation was observed to be the highest in the cultivar, CoP16437 (9.5 cm), followed by CoP18437 (9.3 cm), BO153 (9.2 cm), CoP111437 (8.5 cm) and CoP11438 (8.3 cm), while the lowest was observed in the cultivar, CoP9301 (7.3 cm) (Table 2). However, on medium M_2 (MS basal + 5.0 mgl^{-1} NAA) the cultivar CoP16437 (10.5 cm) had the highest root elongation, followed by BO153 (10.4 cm), CoP18437 (10.1 cm), CoP11437 (9.8 cm) and CoP11438 (9.5 cm), whereas the lowest was found in the cultivar, CoP9301 (8.5 cm) (Table 2). Thus, the pooled average root elongation on both media M_1 (½ MS basal + 5.0 mgl^{-1} NAA) and M_2 (MS basal + 5.0 mgl^{-1} NAA) was found the highest in the cultivar, CoP16437 followed by BO153, CoP18437, CoP11437 and CoP111438 whereas the lowest was found in CoP9301. (Fig: 3)

Table 2: Regeneration potential of root elongation in selected cultivars of sugarcane on M_1 and M_2 media

Sl. No.	Name of Cultivar	Average roots elongation (cm) after 30 days of inoculation	
		M_1(½ MS basal + 5.0 mgl^{-1} NAA)	M_2 (MS basal + 5.0 mgl^{-1}NAA)
1.	CoP16437	9.5	10.5
2.	BO153	9.2	10.4
3.	CoP11437	8.5	9.8
4.	CoP9301	7.3	8.5
5.	CoP18437	9.3	10.1
6.	CoP11438	8.3	9.5

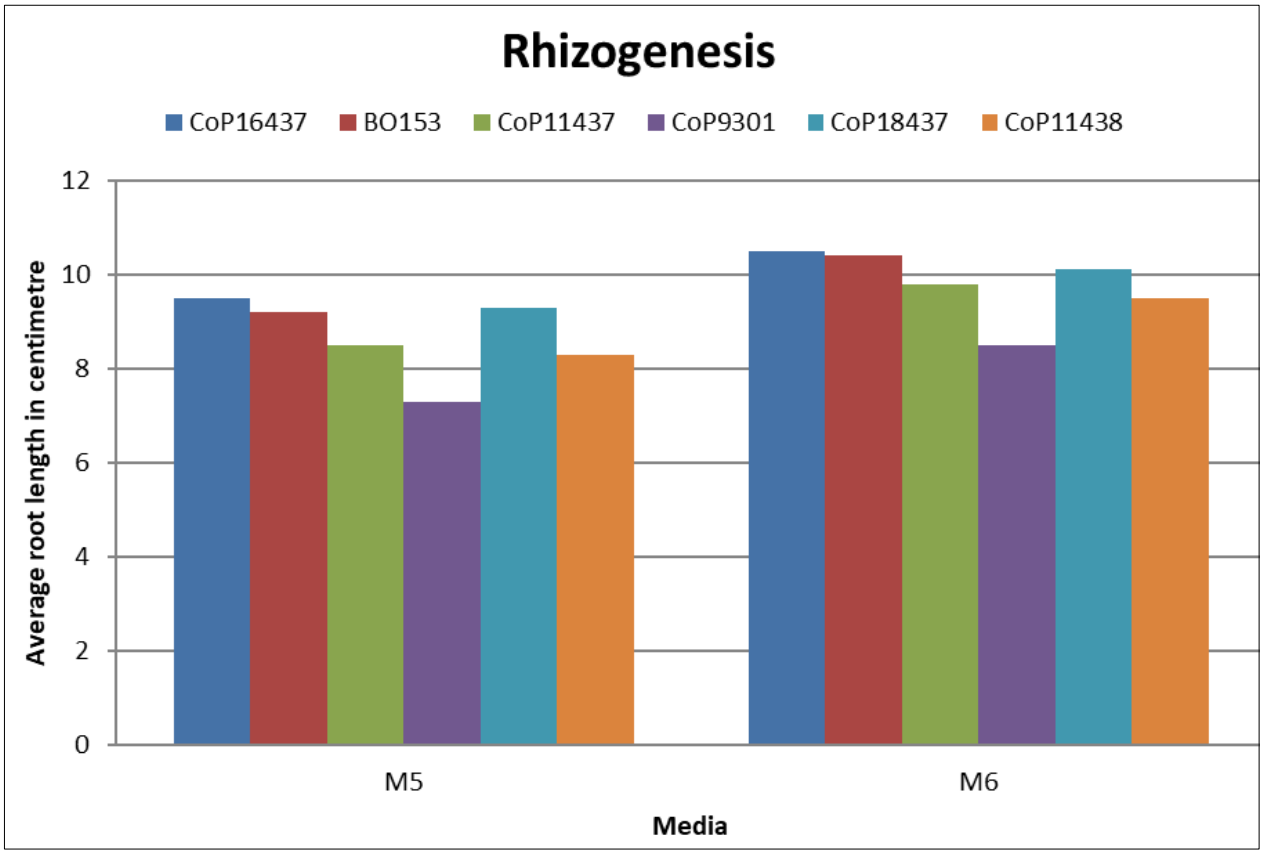

Fig 2: Sugarcane cultivars showing percent rate of Rhizogenesis

Fig 3: Sugarcane cultivars showing root elongation: (A) in the cultivar CoP9301 on 30days, (B) in the cultivar CoP16437 on 30 days

Acclimatization

In the present study rooted plantlets of selected six cultivars were hardened on potting mixtures containing autoclaved sand, soil, white sand and farm yard manure (FYM) under shade and high humidity condition. However, some of the plantlets decayed due to softening of tissues in excess moisture.

The highest acclimatization was observed in the cultivar, CoP16437 (95.5%) followed by CoP11437 (90.5%), BO153 (89.0%), CoP18437 (88.0%) and CoP11438 (86.0%) while the lowest was observed in the cultivar CoP9301 (76.5%) (Fig 4 A, B, C, D, E & F and Fig 5).

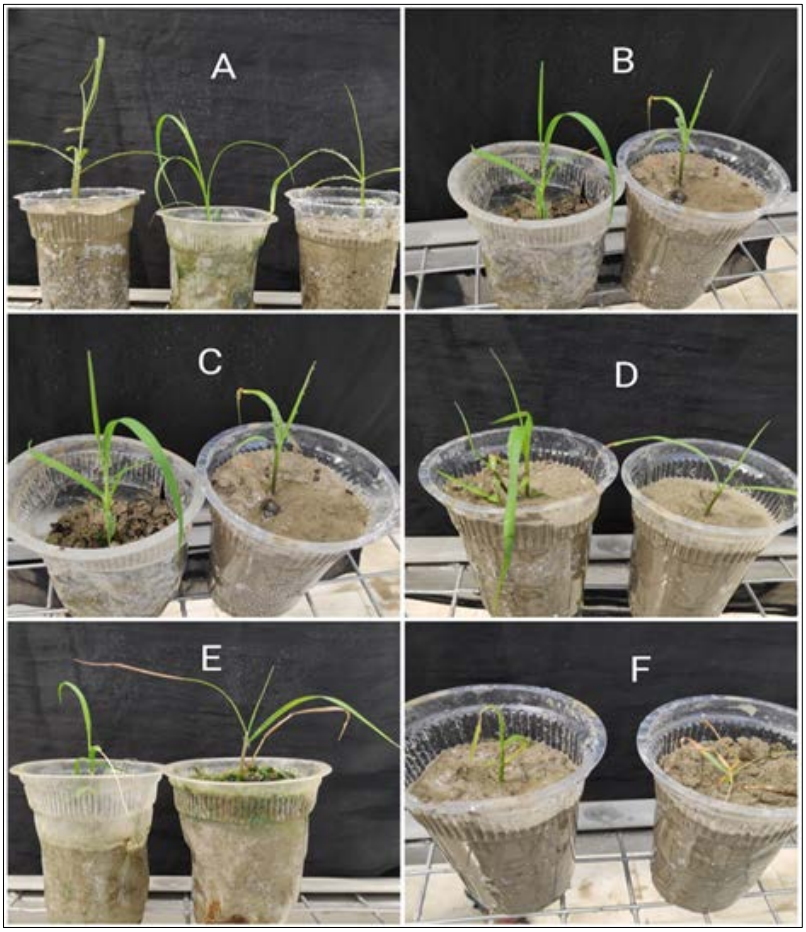

Fig 4: Hardening of *in vitro* developed shoot apex of six selected cultivars of sugarcane (A) CoP16437 (B) BO153 (C) CoP18437 (D) CoP11437 (E) CoP18437 (F) CoP9301

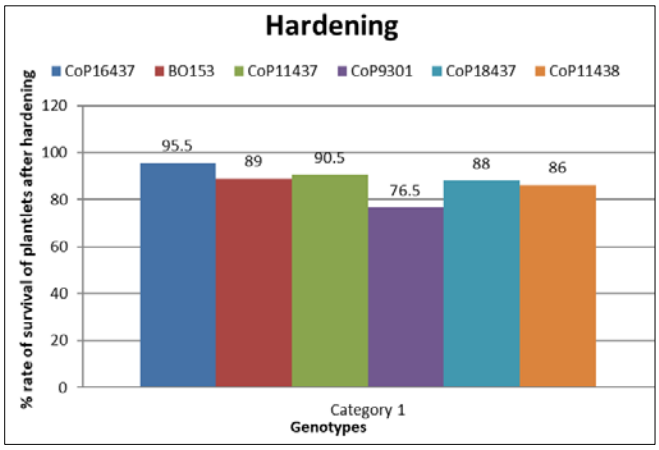

Fig 5: Sugarcane cultivars showing percent rate of survival of *in vitro* developed plantlets after

Callogenesis

Cultured scaly leaves of the six selected cultivars showed callogenesis on medium M_3 with 3.0 mgl^{-1}, 2, 4-D. The callus was initiated after 11 to 23 days of inoculation. The callogenesis was earliest in cultivar, CoP16437 (11-15 days), followed by BO153 (13-15 days), CoP18437 (15- 17 days), CoP11438 (16-18 days), CoP11437 (19-21 days), while the most delayed in the cultivar, CoP9301 (20-23 days). The highest frequency of callus formation was found in the cultivar, CoP6437 (94.45%), followed by BO153 (88.23%), CoP18437 (82.35%), CoP18437 (81.25%) and CoP11437 (73.33%), while the lowest in the cultivar CoP9301 (58.33%) (Table 3).

The growth of callus was poor to excellent. The excellent growth of callus was observed in the cultivar, CoP16437, good in cultivar BO153, CoP11437 and CoP18437, moderate in CoP11438, whereas poorest in the cultivar, CoP9301 (Table 3). The colour of callus was observed was greenish cream in the cultivar, CoP16437 (Fig 6 A), brownish cream in BO153 (Fig 6 B), whitish cream in CoP11437 (Fig 6 C), brownish in CoP9301 (Fig 6 D), and creamish yellow in both cultivars CoP18437 (Fig 6 E) and CoP11438 (Fig 6 F). The nature of callus was friable with some compact region in the cultivar CoP16437, CoP11437 and CoP11438 compact in the cultivar BO153 and CoP9301 whereas the friable callus was observed in the cultivar CoP18437 (Table 3).

Table 3: Regeneration potential of callogenesis in selected cultivars of sugarcane on M_3 medium

Responses	Cultivars of sugarcane cultured on the medium M_3 (MS basal + 3.0 mgL^{-1} 2,4- D)					
	CoP16437	**BO153**	**CoP11437**	**CoP9301**	**CoP18437**	**CoP11438**
% culture Showing callogenesis	94.40	88.23	73.33	58.33	82.35	81.25
Growth of Callus	++++	+++	+++	+	+++	++
Colour of callus	Greenish cream	Brownish cream	Whitish cream	Creamish	Creamish yellow	Creamish Yellow
Nature of Callus	Friable with some Compact region	Compact	Friable with some Compact region	Friable	Friable with some Compact region	Friable with some Compact region

++++ = Excellent, +++ = Good, ++ = Moderate, + = poor.

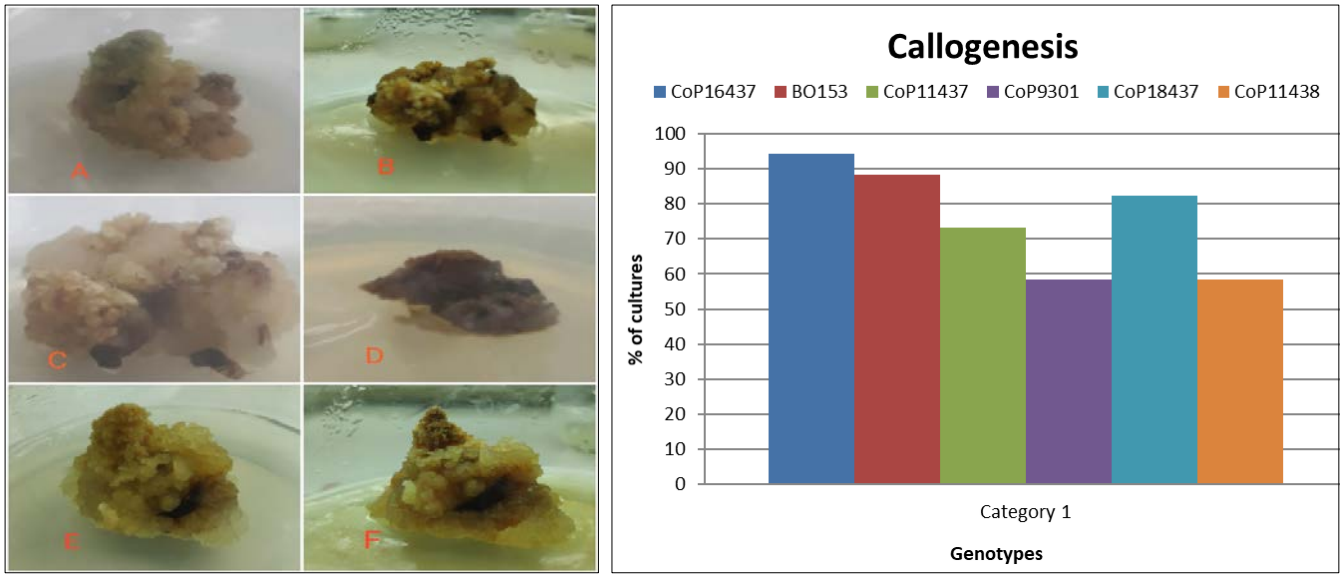

Fig 6: Cultured scaly leaves explants of six selected cutlivars of sugarcane on medium M_3 showing callognesis: (A) greenish cream embryogenic callus in CoP16437 (B) brownish cream in BO153 (C) whitish cream in CoP11437 (D) brownish in CoP9301 (E) creamish yellow in CoP18437 and (F) creamish yellow in CoP11438

Root induction and elongation

Auxin defines niche structure of the stem cells by influencing gene additives actively and. Part of auxin's indirect control can include adjustments in redox, which benefit local, oxidized microenvironments. Forming a QC is necessary for the creation and elaboration of root meristem. Many signals are expected to move through the QC and tissues adjacent to the root meristem. QC absence is synonymous with persistence of origin. It is speculated that roots formed as part of an auxin homeostasis system, considering the several auxin feedback loops (Jiang and Feldman, 2005)

Media M_1 (half MS basal + 5.0 mgl^{-1} NAA) and M_2 (full MS basal + 5.0 mgl^{-1} NAA) were selected for rhizogenesis. The additional 5% sucrose was added in both root inducing media

for better response. The average root induction on both media M_1 (half MS basal + 5.0 mgl^{-1} NAA) and M_2 (MS basal +5.0 mgl^{-1} NAA), the highest rhizogenesis was found in the selected cultivar, CoP16437 followed by CoP18437, BO153, CoP11437 and CoP11438 whereas the lowest was found in CoP9301. The highest concentration of auxin favored the root induction and elongation in cultivars CoP16437, CoP18437, BO153, CoP11437 and CoP11438. In contrast, at higher concentration of auxin, the cultivar CoP9301 favored less root induction and elongation. This inhibition of root initiation and elongation may be attributed to deposition of ethylene on higher concentration of auxins. Because auxins of all types stimulate plant cells to generate ethylene, ethylene retards root elongation particularly when high amounts of synthetic auxins are used (Weiler, 1984).

Acclimatization

A huge number of micro-propagated plants cannot withstand the transition to greenhouse or field ecosystem from *in vitro* conditions. The greenhouse have considerably lower RH, more light levels and specific climate, which are difficult for micro-propagated plants compared *in vitro* conditions. However, the advantages of any micro-propagated device can only be completely realized by effectively moving the plantlets from tissue-culture vessels to the identified ex vitro environmental conditions. Most species grown *in vitro* need a period of acclimatization to ensure adequate numbers of plants thrive and develop vigorously when moved to soil (Hazarika, 2003) [19].

In the current study, rooted plantlets of selected six cultivars were hardened on potting mixture containing autoclaved sand, soil, white sand and farm yard manure (FYM) under shade and high humidity condition. However, some of the plantlets decayed due to softening of tissues in excess moisture. The highest acclimatization was observed in the cultivar, CoP16437 (95.5%) followed by CoP11437 (90.5%), BO153 (89.0%), CoP18437 (88.0%), CoP11438 (86.0%), while the lowest was observed in the cultivar, CoP9301 (76.5%).

Callogenesis

Callus acts as a curing agent under normal circumstances after the plant has been injured, or when any phytopathogenic organism such as *Agrobacterium tumefaciens* causes a tumor. Callus tissue natural formation is a result of a shift in the amount of endogenous growth hormones, typically auxin and cytokinin (Shetty *et al*., 2006). Almost all cultured plant tissues show callogenesis. Callus can be characterized as an amorphous assembly of cellular plants, slightly separated and experiencing rapid proliferation. Nearly all tissues of developed plants exhibit callogenesis. The development of callus from explants under *in vitro* cultures is the reflection of the basic architectural change of the tissue by cell division. Any examples include the disappearance of some types of cells, the creation of new types of cells and the being metabolically more involved cells (Wagle *et al.*, 1987) [20].

Regeneration potential of all the selected cultivars in relation to callogenesis was observed using medium M_7 with 3.0 mgl^{-1} 2, 4-D. The highest frequency of callus formation was found in the cultivar CoP16437 (94.4%), followed by BO153 (88.23%), CoPP18437 (82.5%), CoP11438 (81.25%) and CoP11437 (73.3%), while the lowest in the cultivar CoP9301 (58.33%). Acclimatization was observed best in the cultivar CoP16437 followed by CoP11437, BO153, CoP18437 and CoP11438, whereas the poorest in CoP9301.

Conclusion

As a concluding remark, the best and highly recommended cultivar for the root induction were CoP16437 followed by CoP18437, BO153, CoP11437 and CoP11438 whereas the poorest was CoP9301. And for root elongation CoP16437 followed by BO153, CoP18437, CoP11437 and CoP111438 whereas, the poorest was CoP9301. Thus, theses sugarcane cultivars showed differential response under *in vitro* conditions suggesting development of specific tissue culture protocol for individual genotype and for future breeding programme. The highest acclimatization was observed in the cultivar, CoP16437 followed by CoP11437, BO153, CoP18437 and CoP11438 while the lowest was observed in the cultivar CoP9301.

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