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Evaluation of genotypic and hormone mediated callus induction and regeneration in sugarcane (*Saccharum officinarum* L)

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Abstract
Eight elite sugarcane cultivars i.e., HSF-242, HSF-240, CP-43, CPF-237, CPF-238, SPF-245, CPF-213 and SPF-234 were evaluated in response to different hormones for callus induction and regeneration. Leaf sheaths used as explant. Various levels of 2,4 D, NAA and IBA were exploited to induce callus and regenerate sugarcane callus of selected genotypes. Likewise, Cytokinin and Auxin were provided in disparate quantities to initiate shoot and root development from regenerated callus. The optimum level of callus formation was induced in variety HSF-242 was 96% and 91%. It was observed that 2,4 D and IBA should be maximum at 3.0mg/l and 2.5mg/l respectively. The hormone NAA has shown zero effect on callus formation. IBA was not largely effective for callus induction. Embryogenic callus percentage was seen to be maximum in sugarcane genotype HSF-240. Similarly, maximum level of regeneration was also observed in HSF-242 stands at 91%. Shoot regeneration in all selected sugarcane varieties proved to be maximum at 1.0mg/l for both BAP and kinetin hormones. Rooting of the sugarcane plantlets was also established on half strength MS medium. Percentage of rooted shoots was optimal at 3.0mg/l for all auxin hormones.

Keywords: hormonal influence, embryogenesis in sugarcane, genetic effects, cultivars, tissue culturing

Introduction

Sugarcane (*Saccharum officinarum*) is considered to be a major source of worldwide sugar production (McCormick *et al*., 2008) [24]. Nearly 90% of total sugar availability depends upon this crop at global level (Ramírez *et al*., 2012) [32]. Unfortunately, this crop has been facing severe threats pertaining to abiotic and biotic stresses. Abiotic stresses included high salt, water shortage issues and cold stress causes substantial losses in overall yield of this crop (Sanahuja *et al*., 2014) [34]. Likewise, biotic stresses such as insect pest and viral attacks also damaged this crop to a great extent. Situation demands development of such sugarcane cultivars exhibit better callus induction and high regeneration capacity (Nam *et al*., 2014) [27]. Such characteristics will also help in transformation through micro-projectile bombardment in undifferentiated mass of cells (callus). Genetically Modified callus can also help in producing resistance against abiotic and biotic threats to this crop (Kumlay and Ercisli, 2015) [20].

In-vitro isolation of efficient strains from callus culture is a necessary instrument which leads to several sugarcane improvement programs either through breeding or biotechnological mechanisms (Birsin and Ozgen, 2004) [6]. Every cultivar's ability to produce callus depends largely on its genetics. It means the capacity of every genotype to produce callus and regenerate shoots to induce full fledge plant is obvious. Genotypes are selected in breeding program only if they promote promising aptitude towards in-vitro culture so that embryogenic callus can be evaluated. Plantlets can be regenerated abundantly (Rao and Jabeen, 2013) [33]. Callus culture developed in-vitro largely depends upon many factors like growth conditions, hormonal level (auxin, Cytokinin, kinetin, ABA and Gibberellin) and dark conditions. The explant type, media constitution and culture conditions also contribute heavily towards its induction and regeneration (Naz *et al*., 2017) [29]. The genotypes of the donor plants used as explant in callus culture is the most crucial factor in inducing callus formation and reflects the efficacy of in-vitro culture. It was observed that in wheat (*Triticum aestivum*) crop, genotype of explants having the same age group and identical combinations of hormones impart strong impact on assembly and regeneration ability of callus (Jackson, 2009) [17]. Similar outcomes manifested and reported in *Oryza sativa* (Aftab *et al*., 1996) [1].

As sugarcane crop has a highly complex genome with complex ploidy level very little information is known about its genotypic ability to induce callus. Genetic transformation in *Saccharum officinarum* L. against all stresses can only be done through callus by particle bombardment (Silveira *et al*., 2013) [36]. Therefor it is important to discuss the role of many genotypes in induction ability of callus ultimately bringing a valuable genetically modified sugarcane crop (Sanahuja *et al*., 2014) [34]. Another study analyzed three different sugarcane cultivars in their ability to form callus and regenerate plants (Al-Hussaini *et al*., 2015) [2]. He exploited mature in-vitro caryopses as explants and reported the effects of genotype on callus formation (Al-Hussaini *et al*., 2015) [2]. He also calculated the data concerning the importance of genotype and revealed that data about sugarcane callus was scarce and it is necessary to do work in this regard. Previous studies largely based on three cultivars reported that calli induction ability in *Saccharum spp*. is dependent largely upon genotype used as an explant (Heringer *et al*., 2015) [13].

Sugarcane crop has a lot of micro-propagation techniques to produce a new generation (Gulbarga and Rao, 2013) [11]. Tissue culturing is performed in sugarcane to produce undifferentiated mass of cells to introduce new characteristics in Saccharum spp. Tissue culture techniques in plants occurred to be a big instrument in improving crops (Arjun and Srinath, 2015) [3]. The callus was differentiated into shoots or roots. Leaf sheaths acted as explants on MS medium to synthesize callus inoculated on modified MS media enriched with 2, 4-D as hormones (Kumlay and Ercisli, 2015) [20]. This work presents the effects of many concentrations of auxin, Indole-3-butyric acid (IBA) and 1-Naphthalene acetic acid (NAA), Cytokinin included 6-Benzyleaminopurine (BAP) and Kinetin (Kn) and 2, 4 Dicholorophenoxyacetic acid (2,4 D) regulators that were added to testify callus initiation and shoots emergence in *Saccharum officinarum*. Moreover, it also deduced the impact of various genotypes towards callus synthesis and embryogenic calli induction.

Materials and Methods

Plant Materials

Commercial eight varieties (genotypes) of *Saccharum officinarum* varieties collected from Ayub Agriculture Research Institute (AARI) Faisalabad, Pakistan for experimental purposes. These cultivars included: HSF-242, HSF-240, SPF-234, CPF-213, CPF-237, SPF-238, SPF-245, CP-43. Before sowing, stalks of above mentioned cultivars were disinfected with 70% ethanol and subjected to fungicide treatment to prevent fungal diseases. Sugarcane nodal stalks were sown in pots in green house. Irrigation of the pots was done with tap water on daily basis. Following sprouting and germination of seedlings, plants were subjected to grow under the similar conditions for approximately 24 weeks.

Explants for Callus Induction

Young scaling leaves from sugarcane plants having age between 3-6 months were collected from eight selected cultivars mentioned above. In sugarcane plant, leaf sheaths are used as explants for callus induction procedure. The outer layers of leaves and apical shoot parts were removed gently. Leaf sheath was cut into 12 cm long piece. The inner most whorl of leaves were cut into small leaf cylinders having 1cm^2 diameter. Disinfection was done with the help of 0.01% $HgCl_2$ for 5-7 minutes. These small pieces of cylinders were rinsed by sterile distilled water at least four times under asceptic conditions. Sterilized basal leaf cylinders were air dried on filter paper. These cylindrical leaf explants were injected on Murashige & Skoog (MS) (Murashige and Skoog, 1962) [26] callus media provided with sucrose 30g/l, MS basal salt 4.43g/l, Casein hydrolyzate 1g/l, myoinositol 0.20g/l and different conc. of 2,4,Dichlorophenoxy acetic acid was used to induce callus. The conc. of 2, 4 D was used differently at [0.5, 1.0, 1.5, 2.0, 2.5, 3.0] mg/l respectively. The pH of callus media was maintained at 5.8 with the help of 1N NaOH solution. Agar was used at 8gl$^-$, media was autoclaved for 20 min at120°C under 200psi pressure. Three explants were planted on one callus media plate and plates were kept at dark with temperature at 25±1°C. Fresh media was provided to these inoculated explants in every week. The Percentage of callus formation was observed and calculated after 4-5 weeks.

Micro-Shoot Regeneration

Tissues or explants which were administered to form callus were shifted to MS media having variable conc. of cytokinin (BAP and Kinetin), imperative for shoot development. This variable conc. of cytokinin was used in the range of [0.5, 1.0, 1.5, 2.0 and 2.5mg/l] for each of this growth regulator.

Rooting of *In-Vitro* Grown Micro-Shoots

Root inducing fortification in MS media is inevitable for roots development in micro shoots. In-vitro cultivated micro-shoots were relocated to 50% enriched MS media provided with altered assemblage of auxins. Indole-3-Butyric Acid (IBA), Naphthalene Acetic Acid (NAA) and Indole-3-Acetic Acid (IAA) are different types of Auxin hormones used for root development in micro-shoots. These three Auxin hormones were used in different concentrations of [1.0, 2.0, 3.0, 4.0 and 5.0 mg/l]. Data was recorded in the form of n. of rooted shoots, percentage of rooted shoots and length of roots exhibited by various micro shoots.

Estimation of Embryogenic Callus

Embryogenic calli can be separated from non-embryogenic calli on the basis of apparent features of calli (Benderradji *et al*., 2011) [5]. Embryogenic calli exhibit various phenotypes than that of non-embryogenic calli. Embryogenic callus is compact in form, glossy in appearance and white to creamy in color. Non embryogenic calli are fragile in form exhibits wet look. Such calli are translucent and brownish in color. For eight different genotypes used in this study, percentage of embryogenic calli was recorded after four weeks. The percentage for embryogenic calli is determined by calculating total no. of calli produce embryos divided by total number of calli obtained multiplied by one hundred.

Acclimatization of Regnerated Sugarcane Plants

The plantlets obtained after successful regeneration were aseptically trans-located into similar regeneration medium after every 2 weeks. After 5 weeks regenerated plantlets were young enough to be shifted to soil pots. Plantlets containing minimally 5 roots were shifted into pots with autoclaved high humid soil (>90%). Before translocation, porous soil was autoclaved under 121°C for 15-20 minutes under 200 psi pressures. Autoclaved soil in pots must be kept under high humidity (> 90%). Pots must be covered with plastic bags to prevent transpiration. These plants were kept in a growth chamber with temperature set at 25±1°C with 16 hours light period.

Results and Discussion

Callus Formation

Fortified MS media having embedded explant leaf sheaths started producing callus nearly one and half week later. Induction of callus can be observed visually. As already described earlier, various clustering of 2, 4 D along with other recipients were added to supplement MS media for callus induction. Besides it, various doses in IBA and NAA also added to MS media to check their response towards dedifferentiation of leaf explants. Results of these nutrients can be seen clearly and are described in Table 1st. It exhibits that NAA has no role in inducing callus induction of sugarcane. Varying conc. of Naphthalene Acetic Acid (NAA) was used but it shows zero results in terms of callus induction. Similarly, very low impact of Indole-3-Butyric Acid (IBA) was observed in sugarcane callus induction

experiment. IBA shows maximum 11% callus induction when it was used at 2.5mgL^{-1}. While callus synthesis was seemed to be highly dependent on conc. of 2, 4 Dicholoro-phenxyacetic Acid (2,4D) used in MS media as supplementation. Minimum level of callus induction (11%) was observed in petri plates having 2, 4 D at0.5mgl^{-1}. Maximum effects of 2, 4 D was seen in plates fortified with 2, 4 D at 3mgl^{-1} observed at 89% in different cultivars of sugarcane. While 28%, 39% and 67% callus induction was seen when 2, 4 D conc. was at 1.5, 2.0 and 2.5mg/l respectively. On the other hand, 72% callus synthesis percentage was determined by using 2, 4 D at3.5mg/l. These results clearly indicated that 2, 4 D has direct role in callus formation (Sengar and Sengar, 2012) [35]. As the amount of 2,4 D was enhanced it boosted callus induction but up to a certain threshold level which stands at 3mg/l, above this level, it started declining gradually. Results of the callus induction by a large number of sugarcane genotypes were confirmed by the work done by (Ather *et al*., 2009) [4], (Benderradji *et al*., 2011) [5], (Gulbarga and Rao, 2013) [11] and (Distabanjong *et al*., 2015) [7].

Table 1: Effects of different conc. of 2, 4 D, NAA and IBA on induction of callus are percentage are described

Growth Regulators	Concentration Mg/L	No. of explants used	No. of explants induce callus	Callus Induction (%)
NAA	0.5	18	0	0
	1.0	18	0	0
	1.5	18	0	0
	2.0	18	0	0
	2.5	18	0	0
	3.0	18	0	0
	3.5	18	0	0
2,4 D	0.5	18	2	11
	1.5	18	5	28
	2.0	18	7	39
	2.5	18	12	67
	3.0	18	16	89
	3.5	18	13	72
IBA	0.5	18	0	0
	1.0	18	0	0
	1.5	18	0	0
	2.0	18	1	5
	2.5	18	2	11
	3.0	18	0	0
	3.5	18	0	0

Genotypic Effects on Callus Induction

Callus emergence capacity of eight sugarcane (*Saccharum officinarum*) cultivars (genotypes) was studied in this article. Callus emergence percentage varied from 61-96% pertaining to different genotypes used as explants. Saccharum cultivars HSF-242 showed maximum (96%) while CP-43 exhibited minimum level (61%) of callus formation. Sugarcane variety SPF-245 stands at 93 % and HSF-240 has 79% capacity to trigger callus synthesis. Genotype SPF-234, CPF-213, CPF-237 and SPF-238 manifested 72, 64, 78 and 82% callus induction respectively (Figure 1st). It indicates that these eight genotypes are more than good in dedifferentiation of sugarcane leaf tissues. Significant variations ($p < 0.05$) observed between genotypes HSF-242.SPF-245which have the greatest callus generation percentages (96 and 93%, respectively) while CP-43 has the lowest (61%).

These results are the clear manifestations of the fact that callus induction ability depends upon genotypes. These findings are in agreement with those reported in sugarcane crop (Gandonou *et al*., 2005) [8]. These findings are in complete agreement with the work governed by (Ikeuchi *et al*., 2013) [15] Ikeuchi *et al* 2013 [15] and Sabaz Khan *et al* 2009 (Khan *et al*., 2009) [4]. Similarly, Khamrit *et al* 2012 [18] evaluated different genotypes in response to callus induction prior to chitinase transformation and form different callus. His results clearly respond and confirmed findings drawn from this study (Khamrit *et al*., 2012) [18].

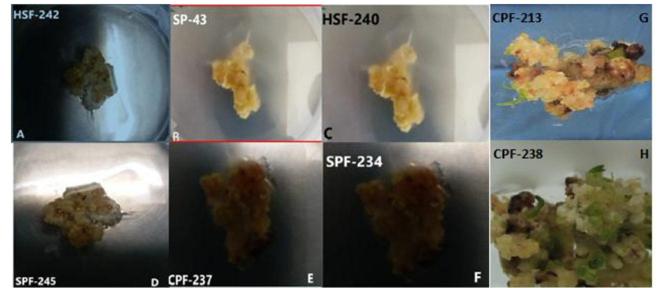

Fig 1: *In-vitro* Callus Induction from Eight Sugarcane Genotypes by the Variable Application of 2, 4 D.

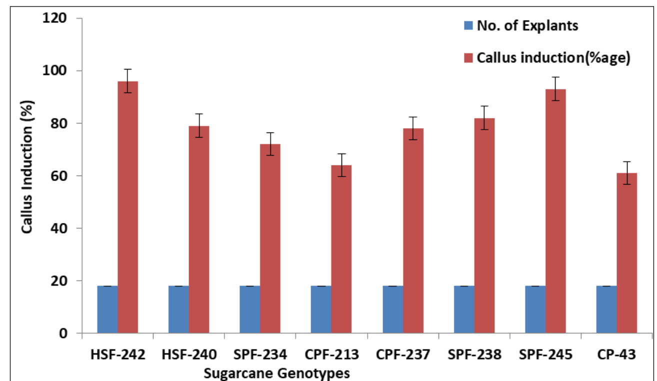

Fig 2: Estimation of Percentage of in-vitro callus induction from eight sugarcane genotypes recorded after 28 days of explants inoculation.

Genotypic Effects on Percentage of Embryogenic Calli

Embryogenic calli are those callus which gives rise embryo from its mass of cells. Embryogenic calli percentage was observed in the range of 50-94%. Sugarcane genotype HSF-240 showed maximum embryogenic calli (94%) while CP-43 manifested the least embryogenic calli stands at 53%. Genotypes HSF-242 and SPF-245 both showed 89% of embryogenic calli while SPF-237, SPF-238, SPF-234 and SPF-213 showed intermediate level of embryogenic calli in the range of 55, 78, 67 and 61% respectively. Figure II revealed the percentage of these results. Some studies also revealed the occurrence of another type of calli which stands intermediate between embryogenic and non- embryogenic calli. It is basically non embryogenic callus covered by a layer of embryogenic calli, mostly reported in sorghum and sugarcane cultivars studies on previous studies by Warchol *et al* (Warchol *et al*., 2015) [39]. In this study, the third type was also included in embryogenic calli because overall it showed embryogenic behavior and grows faster as compared to non embryogenic calli. For embryogenic calli percentage determination, we observed that different genotypes of sugarcane showed different embryogenic calli percentage in response to whole calli. Another valuable

work done by Iqbal *et al* in differentiating embryogenic and non- embryogenic by creating their NMR metabolomic profiles. His work revealed that different sucrose, fructose and other carbohydrates were present more abundantly in embryogenic calli as compared to non-embryogenic calli. That study suggested that there would be a role of these metabolites in causing somatic embryogenesis as opposite in non-embryogenic callus (Mahmud *et al*., 2015) [23]. As the scope of our study was limited to other aspects it did not focus on evaluating role of different metabolites in inducing somatic embryogenesis. Moreover, other works also reported somatic embryogenesis in *Oryza sativa* strongly confirmed our results and evaluations (Pellegrineschi *et al*., 2004) [31]. Likewise, in other plants biologists also reported similar results. Research work also manifested that embryogenic ability of callus was a stable trait which comes from its ancestors (Lal, 2003) [21].

Fig 3: *In-Vitro* development of embryogenic calli from sugarcane genotypes. Greenish color denotes the development of embryo from regenerated calli.

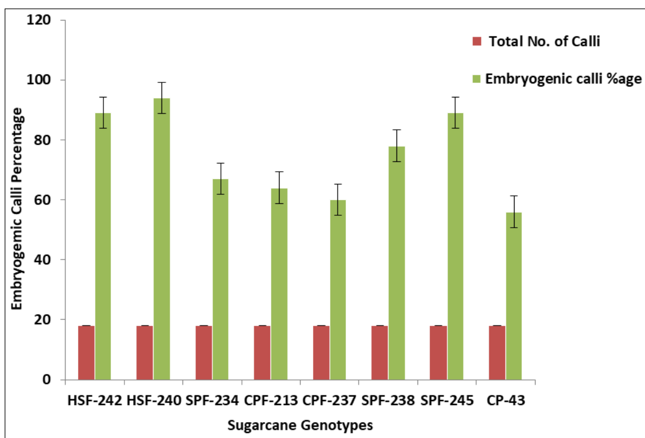

Fig 4: Estimation of percentage of embryogenic calli recorded after 6 weeks of callus induction experiment.

Genotypic Effects on Callus Regeneration

Sugarcane produce callus for transformation purposes and producing plants at tissue culture level. Regeneration of callus is a major issue. Regeneration ability of eight different cultivars of sugarcane gives different results. These results are depicted in the form of figure 3rd. Genotype HSF-242 showed maximum regeneration percentage (91%) while SPF-234, CPF-213,CPF-238,SPF-245, CPF-237 and HSF-240 showed regeneration percentage in intermediate range at 84, 76, 67,87,80 and 78% respectively. These cultivars showed regeneration at intermediate level while CP-43 was seen to regenerate at slowest rate than other selected cultivars. Its regeneration percentage was observed to be 62%, lowest than other selected genotypes. These results for all selected sugarcane genotypes are shown in figure 3rd. The regeneration of callus from different genotypes can be exhibited in the figure 3rd. These results are in complete conformity with the results obtained by a scientist also working on sugarcane who conducted experiments on HSF-240 and observed regeneration of different genotypes at different levels (Nasircilar *et al*., 2006) [28]. Patel *et al* 2015 [30] also witnessed regeneration of callus of sugarcane which stands at 85-88%, these results are in agreement with our findings (Patel *et al*., 2015) [30]. A study carried out on initiation and regeneration of callus in *Oryza sativa and Triticum aestivum* also revealed homological results (Benderradji *et al*., 2011) [5]. Another work conducted on Primula spp. and evaluated six different genotypes of this species. He found out their phenotypic expression in terms of callus regeneration and collected data. Their results were slightly different from this study (Guma *et al*., 2015) [12]. They obtained rich regeneration of callus at values and concentrations mentioned in their studies. But overall there is a huge level of consensus exists between their studies and this research work (Gao *et al*., 2011) [9]. This research endorsed clearly the evaluations obtained similarly in Primula spp. and in Triticum (Zare *et al*., 2015) [40]. A group of plant biologists was involved in evaluating embryonic and non-embryonic callus and differentiate between them by creating NMR metabolomics profiles. It helped them in differentiating between embryonic and non-embryonic calluses but also confirmed that callus induction is more successful when 2, 4 D was used at 3.0 mg/ml (Mahmud *et al*., 2015) [23]. The results of that study also support our study.

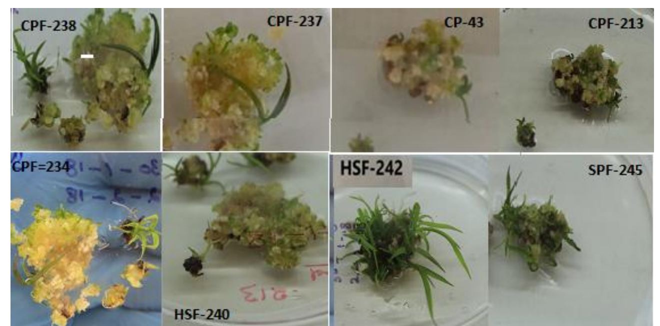

Fig 5: Pictorial Demonstration of In-Vitro Regeneration of plantlets from Calli of different Sugarcane (*Saccharum officinarum* L.) genotypes

Selection of cultivars was always made on the basis of their regeneration ability of callus. If a variety has very weak regeneration capacity it can't survive in in-vitro breeding program. Figure 4th reveals graphical representation of percentages obtained in callus regeneration and it reveals that those were in range between 57 and 92%. Moreover, regeneration media plays a very good role in determining efficiency of regeneration ability. These findings are very much in conformity with outcomes reported in previous studies conducted by other researchers. Casein hydrolysate was used as a natural nutrients complex utilized exclusively for plant regeneration per callus in *Saccharum officinarum*. These findings show that regeneration ability of sugarcane callus was administered by certain gene hence genotype has strong influence on regeneration of callus. Likewise, in rice callus culture was produced and regeneration of different genotypes was estimated for their role by Hoque and Mansfield (Hoque and Mansfield, 2004) [14] and Triticosecale by Birsin and Ozgen (Birsin and Ozgen, 2004) [6].

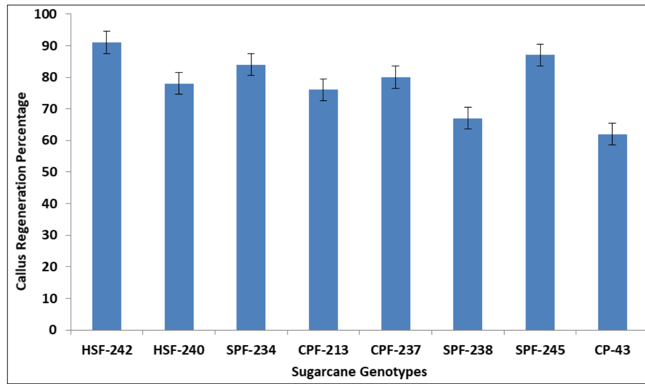

Fig 6: Estimation of Genotypic Effects on Percentage of Callus Regeneration from Eight Cultivars (Genotypes) after two weeks of Calli Induction.

Role of Different Growth Regulators in Shoot Emergence

Cytokinins play an active role in shoot emergence of regenerated callus as per knowledge. Cytokinins growth hormones BAP and Kinetin were involved in triggering micro shoot generation from callus. This research work was conducted to determine the conc. of BAP and Kn which would result maximum micro- shoot induction in selected sugarcane genotypes. The research work manifested that maximum level of shoots were generated when BAP was used at 1.0 mg/l. Calculation of shoots were conducted for explants and according to these results nearly 6 shoots were emerged from 1mg/l of BAP regulator usage. Minimum no. of shoots were observed at0.5mg/l of BAP and it reaches to full level at 1mg/l. Shoot length was also measured at all levels of BAP which revealed maximum (3.4cm) at0.5mg/l while minimum shoot length is observed at1.5,g/l of BAP (TABLE 2nd). Kinetin like other cytokinins showed its effects generously with regards to shoot formation. Study also indicated that Kinetin also performed the best at 1.0mg/l conc. when it produced 40 shoots from explants with 4.8 shoots/explant, moreover, it gives maximum length (3.7cm) at 2.0mg/l of its concentration level. These results were confirmed further by studies conducted by Gopitha *et al* 2010 [10] in his experiments (Gopitha *et al.*, 2010) [10]. In another study conducted by Ithape *et al* 2017 [16] previously, hormonal level influence was observed in model plant Arabidopsis (Ithape *et al.*, 2017) [16]. His research witnessed the role of cytokinins used for shoot development in model plant. Our results also witnessed clearly that cytokinins played decisive role in shoot formation and it also gave insight to the level of these hormones required by sugarcane plant for better shoot emergence. More examples experimented given in the form of study conducted by some researchers (MacKinnon *et al.*, 1986) [22] who endeavored to regenerate shoots from sugarcane embryogenic callus by the use of Thidiazuron and confirmed better shoot development from callus of sweet sorghum. Similarly, impacts of growth regulators were observed in sugarcane callus shoot development by wang *et al* 2017 [38] (Wang *et al.*, 2017) [38]. Our study confirmed the results already obtained in the study described by (Mostafiz and Wagiran, 2018) [25] and (Naz *et al.*, 2017) [29].

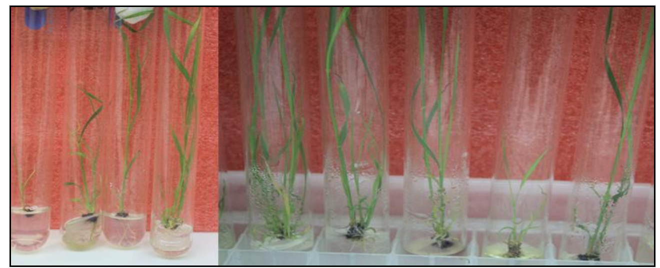

Fig 7: Pictorial demonstration of Shoot formation by the action of various conc. of Cytokinin (BAP and Kn) from Callus of selected Genotypes of Sugarcane (Saccharum Officinarum L.)

Table 2: Effects of different Cytokinin hormonal levels on percentage of shoot development with given shoot length

Hormone	Conc. mg/l	Shoot percentage	Shoots regenerated per Explant (Avg. no.)	Shoot length (cm)
6-Benzyl amino purine (BAP)	0.5	25	4.2	3.4
	1.0	68	5.9	2.7
	1.5	54	3.8	1.9
	2.0	40	3.5	2.4
	2.5	25	2.1	1.7
Kinetin (Kn)	0.5	12	3.0	2.1
	1.0	40	4.8	2.6
	1.5	32	2.7	3.0
	2.0	24	2.1	3.7
	2.5	18	1.8	2.8

Root Induction from Micro-Shoots by Different Growth Regulators

Role of Auxins in different conc. was observed in roots generated from callus mediated shoots. Different proportions of IBA, NAA and IAA were given to MS media to give data about root developments. It was observed that that percentage of roots was seen to be in range of 49-85% in sugarcane shoots developed from callus. It gives maximum length of 4.2 cm to Roots of shoots when BAP was used at3.0 mg/l. Likewise NAA was seen to give optimum roots (92%) when it was dosed at 3.0mg/l of conc. while maximum root length was also recorded to be 3.9cm at 3.0mg/l of NAA conc. Another auxin hormone IAA also gave maximum roots (52%) used at 3.0mg/l. These results clearly witnessed that auxin hormones have decisive role in rooted shoots of sugarcane callus. It gives maximum results when exploited at 3.0mg/l. In 2008, similar type of study was conducted by (Distabanjong *et al.*, 2015) [7] and evaluated data about the role of auxin in root development of sugarcane callus shoots. His results clearly endorsed the findings we obtained in this study (McCormick *et al.*, 2008) [24]. Other studies conducted on sugarcane callus induction and root development was done previously by Rao (Rao and Jabeen, 2013) [33] and (Solangi *et al.*, 2016) [37] whose research findings with non-significantly difference were according to results shown in this research (Sanahuja *et al.*, 2014) [34].

Fig 8: Pictorial demonstration of Roots developed after obtained after differential application of auxin (IAA, IBA and NAA) hormones in various sugarcane cultivars

Table 3: Role of Auxin hormones at different levels for the induction of rooted shoots with root length

Hormones	Conc. mg/l	Roots (percentage)	Shoots Rooted (Nos.)	Root length (cm)
Indole-3-Butyric Acid (IBA)	1.0	49	7	1.6
	2.0	72	9	2.7
	3.0	85	12	4.2
	4.0	64	10	3.0
	5.0	59	8	3.5
Naphthalene Acetic Acid (NAA)	1.0	56	9	2.0
	2.0	67	11	3.4
	3.0	92	14	3.9
	4.0	75	7	3.1
	5.0	68	8	2.9
Indole 3-Acetic Acid (IAA)	1.0	49	9	1.8
	2.0	34	11	2.9
	3.0	52	13	4.6
	4.0	43	10	3.7
	5.0	35	8	2.8

Conclusion
This study concluded that 2, 4 D give rise to different range of callus induction at different levels of concentrations. At low magnitude the percentage of callus will be reduced while at certain maximum level 2, 4 D induce highest percentage of callus in sugarcane. Hormones like NAA and IBA has no role in callus production. Moreover, callus regeneration to shoots and root emergence depends largely on different auxins and cytokinin hormones applied at variations. There is a need to pursue solid efforts in determining phenomenon to find out genetic basis different sugarcane genotypes induce callus at different ratios. Moreover, it also suggested to work on determining the role of different metabolites involved in inducing somatic embryogenesis in callus.

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