Sugarcane of Rapid Multiplication by Callogenesis

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Abstract. The results showed that shoot apical meristem of sugarcane has the ability to stimulate and induction to callus in vitro by plant tissue culture. The results showed 2,4-D regulator 1.5 mg/L was more potent in the induction of the callus and callus subsequent growth. The effected interactions of auxin and cytokinin regulators were not significant with respect to the formation of callus. The best regeneration response was achieved by using growth regulators. In Co8371, best induction of the shoot response was found on MS medium with concentration of 1.0 mg/L BAP compared to Co85004. MS medium with the using of 0.5 mg/L BAP and 0.25 mg/L Kinetin regulator exhibited best organogenesis responding.

Keywords. Callus, 2, 4-D, BAP, Organogenesis.

1. Introduction
Sugarcane belonging to a family knowing as poacea family that is considered as one of the most industrial important cash crops used as major source of sucrose and ethanol in the world [1]. Brazil is considered as one of the largest sugarcane producers [2]. Sugarcane is a long standing crop, is widely grown in tropical and subtropical regions of the world as an important and industrial cash crop in ninety countries. Sugarcane is cultivated over large areas in five continents of world. Sugarcane yielded over the world with about 80% of raw materials used in the producing of sugar in the world [3]. The industry of sugar primarily depends on the sugarcane crop that is important source for the production of sugar and generates a profitable return, as well as the possibility of loading some field crops. The industry of sugar also provides an additional return to the farmer and provides food commodities. The sugar industry uses as well as sugar production for other purposes such as paper Plant tissue culture technology which provides the best way to grow sugarcane in high quality and free of disease and in large quantities in a short period of time comparing to traditional breeding methods [4]. However, the limited availability of sugarcane seeds in a timely manner takes time to obtain [5]. Moreover, some seeds begin to deteriorate due to exposure of biotic and abiotic stresses. Plant tissue culture technology had provided alternative ways for the improvement of crop. Sugarcane is one of the crops that produces the largest amount of biomass in the area unit cultivated [6]. Commercial sugarcane varieties obtained through conventional propagation programs took 10 to 15 years, so plant tissue culture has provided many ways to grow sugarcane with large scales over a short time period [7]. Growth regulators are the most important compounds used in the cultivation of plants by plant tissues culture technique such as auxins, cytokinins and gibrelins regulators [8]. They provide good growth to tissue or organ transplanted. The concentration
of these compounds vary according to the tissues and the target of the experiment [9]. The target of the research for growth regulators in the induction of callus and shoot formation in two varieties of sugarcane.

2. Materials and Methods
Meristem apical Shoot collected from leaves in plant were directly used for the induction of the callus. Explants were obtained from plants grown in laboratory. The vegetable parts were cleaned and washed with the running pure water to get rid of the lingering dust after removing the surrounding leaves with the continuous sterilization of the old alcohol with 70% concentration when removing the leaves. The plant parts of the antioxidant solution, which is made up of citric acid and ascorbic acid, are concentrated at 100 and 150 mg/L [10]. The explants after that immersed in sodium hypochlorite NaClO 6.50 % aqueous solution for 20 min and were rinsed thoroughly [11]. The sterilized parts were washed with sterilized distilled water three times to remove the traces of sterile material and the washing was carried out inside the airflow table. MS medium were supplemented using twelve concentrations of [auxin, cytokinins regulators] using 3% sucrose and activated with charcoal 1.0 g/L. The pH of the medium was accurately adjusted to 5.7, media was sterilized and autoclaved at 121 °C for 15 minutes [12]. Data were recorded to the frequency induction of callus and percentage of callus formation. Different concentrations of plant growth regulators were used to standardize the medium for the regeneration frequency of callus. The ability of callus regeneration was found by transferring it to MS free medium and after that to supplemented media with different concentrations of auxin and cytokinins. All the prepared cultures were studied using light intensity with 104 lux with temperature about 25 ± 2°C and photo period about 16 hrs with 8 hrs dark period at every 24 hrs cycle with 7 replicates per variety and concentration and took the traits after four weeks of planting. All trials were performed using CRD and global trials. The results were analyzed using the Genestate program. The LSD test was compared with the 5% [13].

3. Results
Normally callus could be practically obtained from growing explants of any part of the original plant; the callus formation was shoot apical meristem.

Auxins with different concentrations were used to induce callus, maximum induction were achieved at 1.5 mg/L (M3) of 2,4-D (Table 1). Auxins exhibit the highest activity as a growth regulator in the induction and multiplication of callus [14]. Different growth regulators like auxins (2,4-D) reported to have the highest induction and multiplication of callus in the field of crops. 2,4-D was reported to have higher vitality in most of fields of crops [15].

2,4-D 1.5 mg/L gave 98% the formation of callus in Co8371, while 85% formation of callus were resulted with the same concentration of 2,4-D Co85004 figure 1, the formation of callus almost depends upon the used concentration of 2,4-D table 1. This may be due to the role of auxin in stimulating the formation of plant parts in the food circles, which is achieved by encouraging cells to divide, elongate and grow [16]. Auxin-cytokinins growth regulators eight combinations were also prepared and tested (M5 to M8) showed in table 1.

2,4-D-BAP combinations didn't gave significant formation of callus in Co8371 variety, while a higher formation of callus was practically obtained in Co85004 variety table 1.

2,4-D-Kinetin exhibited higher formation of callus with Co85004 variety opposite to Co8371 which gave poor formation of callus table 1.

The multiplication and highest proliferation resulted by growth regulators with sub-culturing was increased in third sub-culturing table 2. The data reveal that the varieties Co8371 and Co85004 with the selected medium regarding callus induction and multiplication was (M3) 2,4-D at the 1.5 mg/L.

Table 1. Biochemical Details Identification of isolates bacterial species by Vitek II system. report of Aeromones hydrophila /caviae with probability (98%)
Table 2. Effect of sub-cultures on callus growth

| Varieties | Main culture | 1st sub-culture | 2nd sub-culture | 3rd sub-culture | 4th sub-culture | 5th sub-culture | 6th sub-culture | 7th sub-culture | L.S.D 0.05 |
|-----------|--------------|-----------------|-----------------|-----------------|----------------|----------------|----------------|----------------|------------|
| Co8371    | 1.33         | 1.58            | 1.73            | 1.94            | 1.63           | 1.32           | 1.24           | 1.11           | 0.044      |
| Co85004   | 1.28         | 1.45            | 1.70            | 1.91            | 1.52           | 1.31           | 1.22           | 1.00           | 0.048      |

Two kinds of callus morphogenic and non-morphogenic was achieved from third sub-culturing after an inoculation of period of eight weeks of further sub-culturing either on plant growth regulators free or supplemented MS medium for efficient organ induction. The rate of regeneration the same when the morphogenic callus of the varieties above was transferred to growth regulators MS free (D1), which was 62% in Co8371 and 61% in Co85004 table 3. For further accelerate of the rate of regeneration MS medium was supplemented with different combinations of BAP and kinetin concentrations. In the case of Co8371 variety, the maximum shoot differentiation was achieved in sixth sub-culturing with D2 Medium (MS in 1.0 mg/L of BAP) table 3. At in Co8371 was 78% while in Co85004 it was 65%. In case of Co8371 was 76% while in Co85004 it was 63%.

The highest organogenic response was achieved with D10 medium (MS in 0.5 mg/L BAP and 0.25 mg/L Kinetin) table 3. The differences between varieties are due to genetic differences among them and to differences in the content of the internal hormones and their role in different growth processes [17], the reason for the superiority of BAP in the number of branches may be due to the stimulation of BAP in stimulating cells to divide, differentiate and increase growth, cytokinins act to break capillary sovereignty and create attractions in side buds that stimulate the rapid transfer of nutrients, Cell growth and growth of buds [18] these findings were confirmed by the researchers [19, 20, 21, 22]. Maximum organogenic response from morphogenic callus was noticed after ten week of inoculation in fifth subculture.
Table 3. Organogenesis from morphogenic callus

| Treatment No. | Media          | Concentration mg/L | Varieties |  |  |
|---------------|----------------|--------------------|-----------|---|---|
|               |                |                    | Co8371    | Co85004 |
| D1            | MS Free        |                     | 62        | 61 |
| D2            | MS + BAP       | 1.0                 | 78        | 65 |
| D3            | MS + BAP       | 2.0                 | 55        | 43 |
| D4            | MS + BAP       | 3.0                 | 44        | 42 |
| D5            | MS + BAP       | 4.0                 | 35        | 33 |
| D6            | MS + Kin       | 1.0                 | 43        | 41 |
| D7            | MS + Kin       | 2.0                 | 45        | 43 |
| D8            | MS + Kin       | 3.0                 | 26        | 22 |
| D9            | MS + Kin       | 4.0                 | 13        | 11 |
| D10           | MS + BAP + Kin | 0.5 + 0.25          | 76        | 63 |
| D11           | MS + BAP + Kin | 1.0 + 0.5           | 75        | 55 |
| D12           | MS + BAP + Kin | 1.5 + 0.75          | 69        | 35 |
| D13           | MS + BAP + Kin | 2.0 + 1.0           | 46        | 23 |
| D14           | MS + 2,4-D     | 1.0                 | 45        | 33 |
| D15           | MS + 2,4-D     | 1.5                 | 32        | 34 |
| D16           | MS + 2,4-D     | 2.0                 | 56        | 45 |
| D17           | MS + 2,4-D     | 2.5                 | 32        | 34 |
|               | L.S.D 0.05     |                    | 0.045     | 0.036 |

Generally, non-morphogenic callus resulted in low organogenesis percentage. The using of D2 showed 42% and 40% for both varieties at maximum organogenic response table 4. For the two studied varieties Co8371 showed best organogenic response compared to Co85004, the differences between varieties are due to genetic differences among them and to differences in the content of the internal hormones and their role in different growth processes [23].

Table 4. Organogenesis from non-morphogenic callus

| Treatment No. | Media          | Concentration mg/L | Varieties |  |  |
|---------------|----------------|--------------------|-----------|---|---|
|               |                |                    | Co8371    | Co85004 |
| D1            | MS Basal       |                     | 35        | 32 |
| D2            | MS + BAP       | 1.0                 | 42        | 40 |
| D3            | MS + BAP       | 2.0                 | 26        | 24 |
| D4            | MS + BAP       | 3.0                 | 20        | 11 |
| D5            | MS + BAP       | 4.0                 | 23        | 20 |
| D6            | MS + BAP + Kin | 0.5 + 0.25          | 46        | 42 |
| D7            | MS + BAP + Kin | 1.0 + 0.5           | 43        | 40 |
| D8            | MS + BAP + Kin | 1.5 + 0.75          | 35        | 32 |
| D9            | MS + BAP + Kin | 2.0 + 1.0           | 11        | 10 |
|               | L.S.D 0.05     |                    | 0.031     | 0.028 |

The resulting cultivars were transferred to the center of rooting with 1.5 mg/L of IBA for four weeks [24]. The syrup and the peptos were sterilized in the sterilization apparatus and mixed with 1:1. The plants were then planted in bots [25] figure 2.
Figure 1. Response of two varieties of sugarcane to callus induction at 1.5 of 2.4-D after four weeks of planting Var.1: Co85004, Var.2: Co8371.

Figure 2. Sugarcane plants for Co8371 produced from agriculture in MS with IBA 1.5 mg/L and treatment BAP 1.0 mg/L that succeeded in localization and living in normal conditions after four weeks of planting.

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