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Influence of Apical Meristem and Chemotherapy on Production of Virus Free Sugarcane Plants

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Abstract

The combination of chemotherapy and the meristem culture increases the virus free production of sugarcane plants, even when the meristem is infected. In vitro culture technique has introduced a new dimension to plant multiplication for the production of well-organized, genetically unwavering clonal germplasm. The present investigation was to study the influence of meristem tip culture and the action of chemotherapy in the elimination of virus. The intention of this work is to determine the effect of various levels of chemical treatment on in vitro shoot multiplication and also assessing the elimination of virus. In this experiment three different varietiesof sugarcane were taken such as Co85004, Co91010, Co86032. These three varieties were tissue cultured using apical meristem as the explant in combination with antiviral agents of different concentrations such as 2.5mg, 5.0mg, 7.5mg, 10.0mg, 12.5mg, 15.0mg. At higher concentrations phytotoxicity was observed. About 95% virus elimination was attained with the addition of ribavirin at 10mg/l in MS medium along with meristem culture. In this experiment good response for shoot initiation was observed in variety Co 85004 compared to other varietiesstudied. The total number of days for culture establishment in Co 85004 was around 14 days. In all the three varieties the elongation was found to be more or less same in 28 – 29 days. In the case of multiplication, the number of days for shoot multiplication was only19 days in Co 86032 whereas in Co 85004 and Co 91010 it was 21 days. So, combination of antiviral chemotherapy and meristem tip culture was found to be more effective in sugarcane mosaic virus elimination.

Keywords: Micropropagation, Ribavirin, sugarcane, Meristem,Tissue Culture,Viruses,Chemotherapy.

Introduction

In Indiasugarcane (*Saccharumofficinarum*L.*)* is one of the noteworthy cash crops grown ubiquitously. Nearly 70 percent of the world sugar productions were from sugarcane so, sugarcane ranks first as a major source of commercial sugar accounting. The world agriculture ranks sugarcane as the top commercial crop. The world's 70 % sugar (sucrose) is from sugarcane and rest from sugarbeet. In India sugarcane is cultivated in about 4.3 million hectares of land with 290 million tonnes of annual cane production. The plants which are affected with the virus are withpoor quality and the yield also got reduced to a significant level[1-4]. It has been promptly reported that by replacing the virus infected stock with healthy stock (virus free) has reported to have a higher yield[5].

There are three methods which are currently used in the elimination of viruses: thermotherapy, meristem culture and chemotherapy. For more than a century heat has been used in the elimination of plant pathogens[6]. About 70% viruses inactivated in plants by the application of heat treatmentwereonly during the late 1960's[7]. It is not well understood that what is the effect of heat on viruses. However, it is assumed to be helpful in inhibiting replication of the virus and by blocking transcription at molecular level and theproduction of various proteins by virus is stopped[8].

Sugarcane tissue culture initiation studies gave a better knowledge about the sugarcane virus elimination under *invitro* conditions about the medium and various chemical constituents[9]. Later intensive work about sugarcane development by using the above technique was amended byLiu[10] byinculcating the callus induction and following regeneration by the application of immature inflorescence, apical meristem, young leaves and pith parenchyma. Elimination of viruses by using apical meristem has become more popular as the time passed[11].

Usage of antiviral agents has also been reported to be effective in the elimination of various viruses[12]. These antiviral compounds can be sprayed directly on the crop or else added along with the medium during the preparation of mediumwhich is taken upon by the plants during the *invitro* growth and they inhibit virus replication[13]. The contemporaryexploration is to study the influence of meristem tip culture and the action of chemotherapy in the elimination of virus. The aim is to determine the effect of various

concentrations of chemical treatments on *in vitro* shoot multiplication and also assessing the elimination of virus.

Material and Methods

Apical meristem was chosen as the explants because the cells are undifferentiated and the meristematic cells are actively dividing and the most important reason is that there is no exposure of virus in the apical meristem and the production of virus free sugarcane is possible. Co85004, Co 91010, Co 86032 are the varieties chosen for the experiment. Young shoots of sugarcane were collected from different varieties of the sugarcane plants from the field of Sugarcane Breeding Institute. The leaves were cautiously trimmed off using scalpel and the shoot is surface sterilized prior to taking into the laminar air flow. The contiguous leaf sheaths around the tops of sugarcane were carefully removed one by one until the inner white sheaths are discernible under a Zoom stereo dissection microscope. The length of the tops should be 10 cm length in such a way the top is cut down, by cutting off at the two ends and fixing the growing parts of the shoots. All the shoot tops were washed in the flask containing Tween20. The washing process was carried out for five minutes to remove the waxy material sticking over the leaf sheaths, followed by rinsing with distilled water four to five times to remove the detergent. After washing the shoots were sterilized thoroughly using ethyl alcohol. The sterilized shoots were dissected using the dissection tool and the excised apical meristem was transferred onto the medium with charcoal at different chemotherapy treatments (table-2). The chemotherapeutant used to eradicate viruses from sugarcane meristems tissues was ribavirin. The concentrations of ribavirin used for experimentation were between 2.5 - 15 mg/l. A control was maintained without the antiviral agent to compare the result. The presence of the virus in the plants regenerated from the meristems was assayed and proved using RT PCR

Table-2
The MS (Murashige and Skoog) were prepared with different concentrations of Ribavarinas

Treatments	Concentrations (mg/l)
T_0(Control)	-
T_1	2.5
T_2	5.0
T_3	7.5
T_4	10.0
T_5	12.5
T_6	15.0

Results and Discussion

Shoot initiation, elongation and multiplication: The shoot tips started growing from the merstemwitin 3 to 4 days and it attained two to three leaf within 3 to 4 weeks and it is transferred to multiplication medium and after 2 to 3 weeks, young plantlets are observed (Figure-1 for Co 85004) for illustration.

Table-3
Effect of Chemotherapeutant in virus eliminationof sugarcane plants

S.No	Treatments	Co 85004	Co 91010	Co 86032
		Presence/ Absence		
		SCSMV		SCMV
1.	T_0	+		+
2.	T_1	+		+
3.	T_2	+		+
4.	T_3	+		-
5.	T_4	-		-
6.	T_5	-		-
7.	T_6	-		-

Effect of chemotherapeutant on shoot multiplication: Chemotherapeutant namely ribavirin amended in the meristem culture medium at various concentrations (2.5mg, 5.0mg, 7.5mg, 10.0mg, 12.5mg, 15.0mg/l). Infected shoots of varieties such as Co 85004, Co 91010, Co 86032 were subjected for virus elimination by meristem tip culture and assessing the virus elimination. In this present study the MS medium with six different concentrations of ribavirin was used to assess the effect on SCSMV and SCMV elimination and shoot multiplication. Sugarcane varieties Co 85004, Co 91010, Co 86032 were subjected to virus elimination. In this study it was found out that shoot multiplication and growth were decreasing from decreasing from lower concentration to higher concentration of ribavirin (figure-1) below.

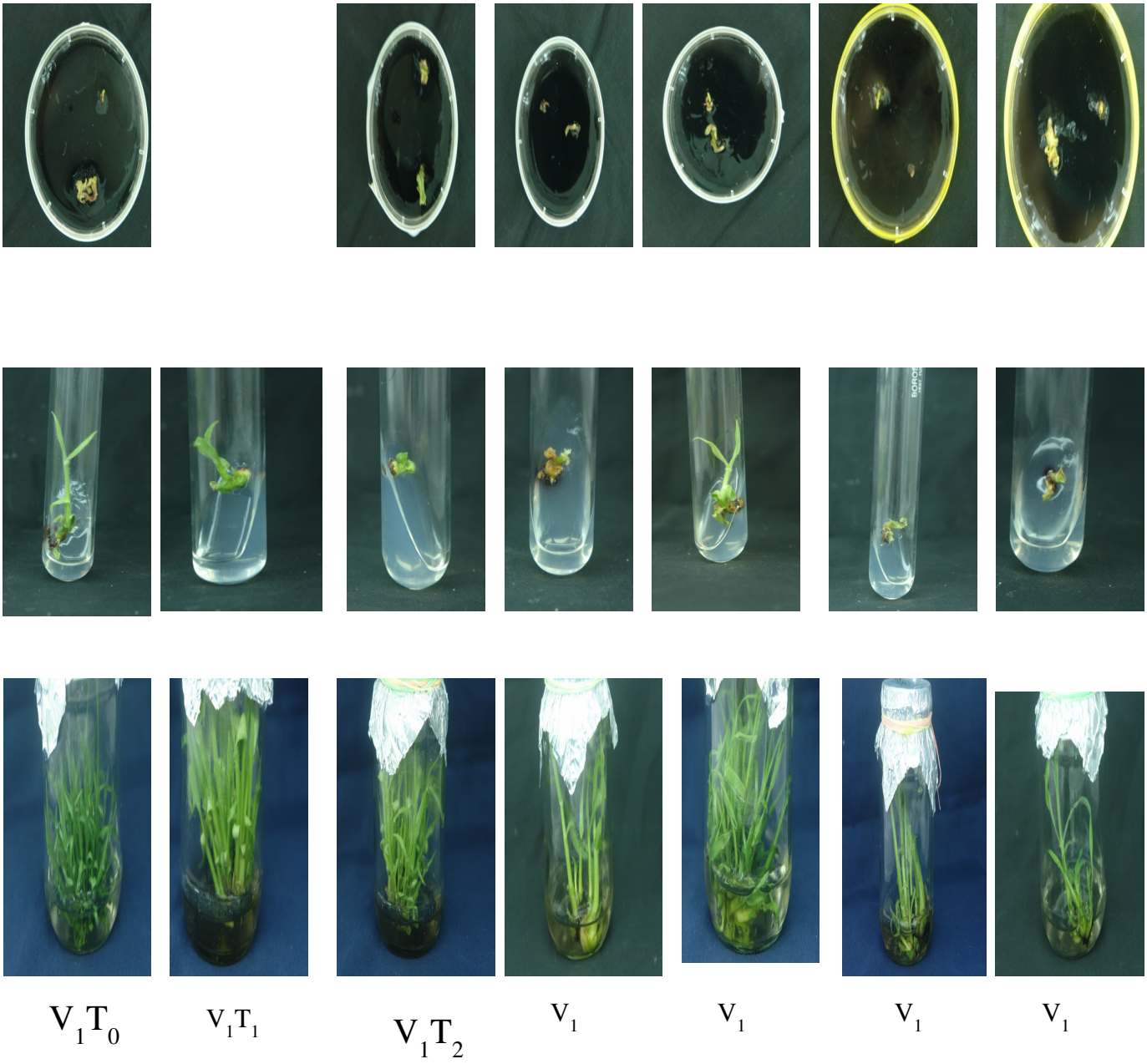

Figure-1
Influence of Ribavrin in Various Stages INCO 85004

Comparison of number of days for initiation, elongation and multiplication: Ribavirin at higher concentration has no critical effect in the initiation, elongation and multiplication stages as illustrated using the following representations (figures2-7).

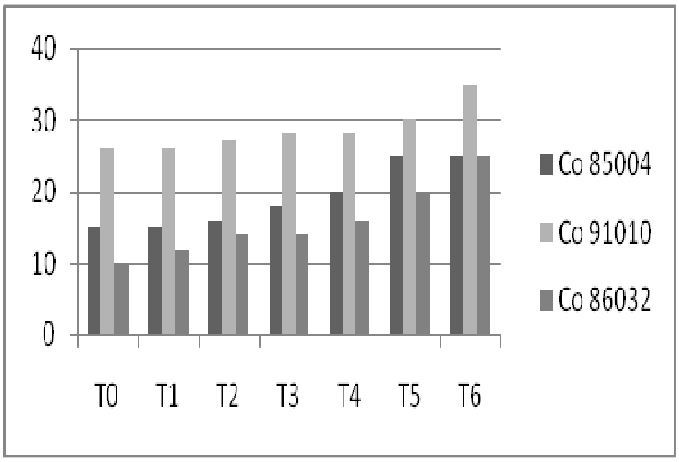

Figure-2

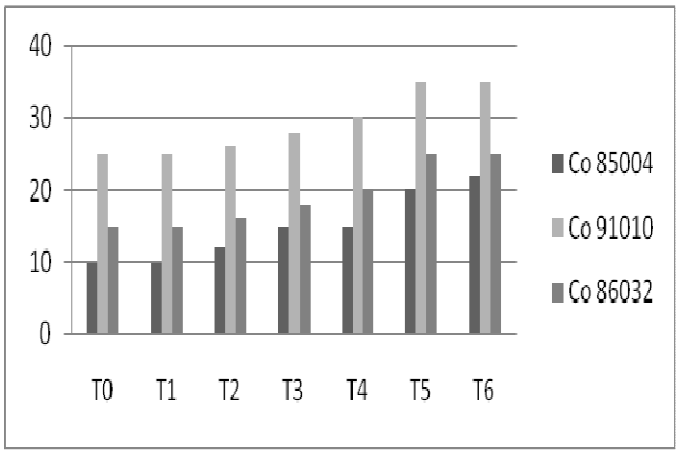

Figure-3

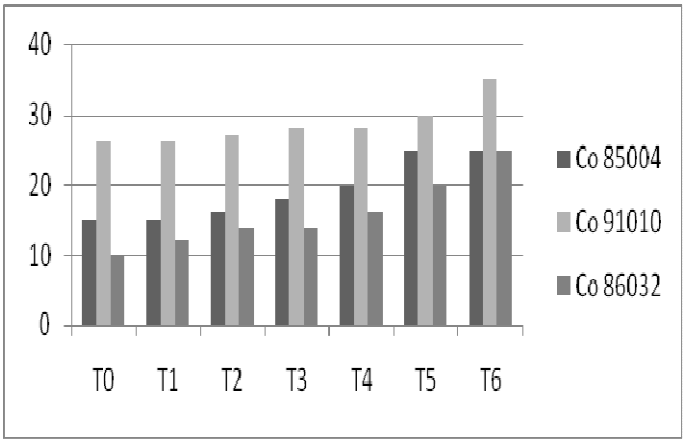

Figure-4

Effect of ribavirin on shoot length, number of shoots and biomass: The effect of ribavirin on shoot length, number of shoots and biomass is illustrated using graphical representation as below (figures-5-7).

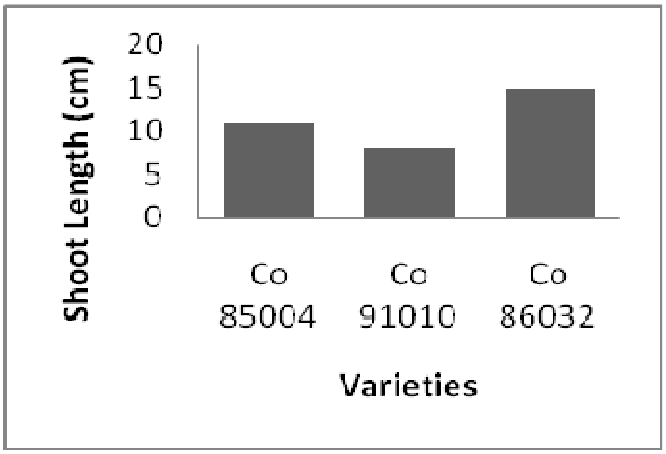

Figure-5

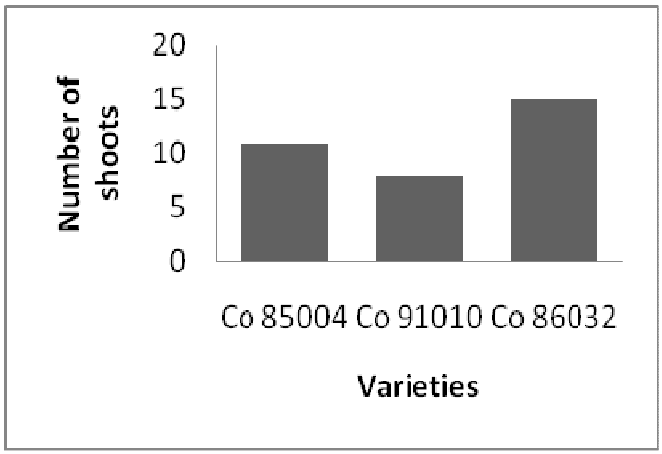

Figure-6

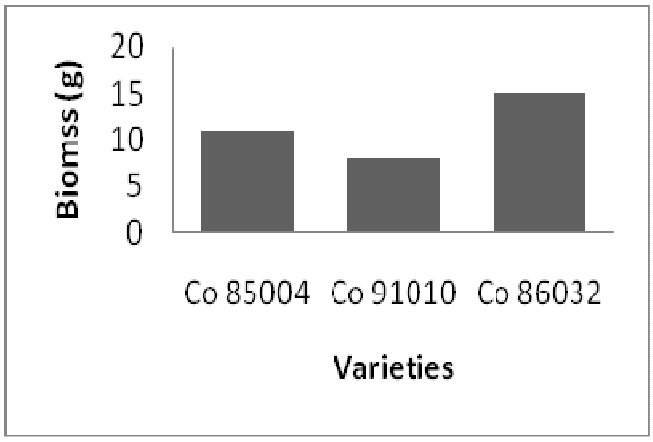

Figure-7

Conclusion

In the present study it is investigated that thechemotherapy at lower concentrations (2.5,5.0,7.5mg/l) has noinfluence on the initiation and growth of the meristem, shoot multiplicationas well as the elongation of shoots. However, there was no elimination of virus. Higher concentrationsresulted in phytotoxicity and found to be negatively affecting the shoot multiplication and growth.

Combined method of antiviral chemotherapy and meristem tip culture was found to be more effective in sugarcane mosaic virus elimination. Amending the MS medium with 10mg/l of ribavirin increased the SCSMV and SCMV elimination from the meristem tip and it did not affect the shoot emergence. Thus shoot multiplication and growth were decreasing fromlower concentration to higher concentrations of the chemotherapeutant.

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