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# EFFECTS OF VARIOUS IRRIGATION TREATMENTS ON ACCLIMATIZATION OF *IN VITRO* REGENERATED SUGARCANE (*SACCHARUM OFFICINARUM* L.*)* PLANTS

**Beena Naqvi[1], Yasmeen Tariq[1], Ali M. Dahri[1], Tabassum Bibi[1] and Raiha Qadri[2]**

[1]*Plant Tissue Culture Lab, Pakistan Council of Scientific & Industrial Research (PCSIR) Laboratories Complex Karachi, Pakistan*
[2]*Department of Botany, University of Karachi, Karachi-75270, Pakistan.*
e-mail: nbeena25@hotmail.com

## ABSTRACT

This study aimed at the development of protocol for effective acclimatization of sugarcane plantlets (cv. SPC-79) regenerated through tissue culture. After successful *in vitro* multiplication and rooting, sugarcane plantlets were transferred to the green house under partially controlled conditions for acclimatization. To achieve maximum survival efficiency of *in vitro* regenerated plants, these were transferred in soil medium supplied with various types of nutrient solutions i.e. ½ MS salts, 10 % coconut water, 1 % DAP + 1 % urea in 1:1 (w/w) and 5 % farm yard manure. It was found that among all the nutrient solutions tested during acclimatization, application of ½ MS salts solution proved to be the most effective treatment closely followed by 10 % coconut water, for achieving significant survival efficiency by improving the growth characteristics of *in vitro* grown sugarcane plants.

**Key-words:** Plant tissue culture technique, regeneration of sugarcane, survival, growth.

## INTRODUCTION

Sugarcane (*Saccharum officinarum* L.*)* is an important crop for many tropical and sub tropical countries (Tiel *et al*., 2006) and a main source of sugar production in the world. In Pakistan sugarcane is the second most important cash crop (Naz, 2003). Pakistan is one of the major sugarcane producing countries with respect to area however it's per hectare production is far below its actual capacity. One of the major underlying reasons is the unavailability of superior quality and disease free planting material. Production of sugarcane varieties with improved characteristics through conventional means is time consuming and laborious job and it takes years to introduce a new variety (Cheema and Hussain, 2004). Therefore, emphasis has been placed upon the use of modern biotechnological methods to accelerate the production of high quality sugarcane varieties (Bibi *et al.,* 2010; Khan *et al*., 2009).

Many reports have been published on *In vitro* multiplication of sugarcane via direct and indirect regeneration methods (Baksha *et al*., 2002; Khan & Kathri, 2006; Ghulam *et al*., 2010). Considerable efforts have been made for the optimization of i*n vitro* conditions for different stages of sugarcane micropropagation however studies on acclimatization stage of sugarcane remain inadequate. After *in vitro* shoot multiplication and rooting sugarcane plantlets are passed through acclimatization phase in semi controlled *ex vitro* conditions leading to the gradual adaptation to the natural environment. The high mortality ratio of large proportion of plantlets during this phase is the major bottleneck in successful micropropagation (Hazarika, 2003).

The effective acclimatization is the key factor for success of *in vitro* propagation system. The plants produced through tissue culture technique although green in color, do not prepare sufficient food for their survival in natural conditions (Chandra *et al.,* 2010). Therefore, at this stage plantlets require application of sufficient nutrient supplements to attain ability to synthesize food naturally and develop cuticular wax covering on the surface of plant (Chandra *et al.,* 2010). The selection of precise mixture of nutrient solutions is decisive for acclimatization of plantlets for the maximum survival (Lavanya *et al*., 2009). In view of these difficulties and of the absence of data regarding acclimatization of the genus *Saccharum*, the present work had the objective of evaluating the behavior of *in vitro* regenerated sugarcane (cv. SPC-79) plantlets, on supplementation of various mixtures of nutrient solutions during acclimatization process.

## MATERIALS AND METHODS

The tissue cultured plantlets of sugarcane (cv. SPC-79) of uniform height (7 cm) each with 5-7 healthy roots (4-5 cm long, 0.8 -1 mm thick) roots were selected for acclimatization. The adhering agar from roots of plantlets was washed away in luke warm water. To protect the plantlets from fungal damage they were dipped in 1 % Belyton solution for 5 minutes. Then the plantlets were transplanted in plastic pots (10 cm) filled with washed and autoclaved sandy loam soil. The pots were covered with transparent polyethylene bags to retain high humidity and

sufficient light for photosynthesis. The plastic covers were gradually removed after one week to expose to the green house conditions with 60-75 % humidity.

After one week of transplantation, the plantlets were irrigated with various nutrients solutions (Table 1) in appropriate quantity. These nutrient solutions were supplied to plants at the interval of one week in the duration of 21 days. During this period, plantlets were irrigated with tap water daily. After five weeks, the plantlets were transferred to open nursery for final stage of acclimatization and data recorded for number of tillers, height of tillers and number of leaves per plant (Table 2). The success of acclimatization was determined by calculating survival percentage.

Table 1. Various Nutrient Solutions used for irrigation of sugarcane plantlets during acclimatization for 3 weeks with one week interval, with daily tap water supply as a control.

| **Nutrient solution code** | **Type of Nutrient Solution** | **Application Quantity(ml/pot)** | **No. of Replicates** |
|---|---|---|---|
| NS-1 | Tap Water | 10 | 10 |
| NS-2 | 5 % Farm yard manure | 10 | 10 |
| NS-3 | 1 % DAP and Urea (1:1) | 10 | 10 |
| NS-4 | 10 % Coconut Water | 10 | 10 |
| NS-5 | ½ strength Murashige and Skoog Salts | 10 | 10 |

**RESULTS AND DISCUSSION**

Sugarcane requires a fertile and well-drained soil for its growth (Yousuf *et al*., 2002). The availability of nutrients plays an important role in the development and growth of a normal cane crop (Naz *et al*., 2009). Height and number of tillers and number of leaves are directly affected by the availability of fertilizers (Lavanya *et al*., 2009). Nitrogen is essential for plant growth, phosphorus for roots development, and potassium for promoting cell activity and growth, increasing resistance to infection and lodging, and improving sucrose content. *In vitro* plantlets, growing on artificially supplemented media have the low capacity to synthesize nutrients in sufficient amount to meet growth requirements at the initial stage (Hazarika, 2003). When these plantlets are transplanted from *in vitro* to *ex vitro* conditions they have to switch to fully autotrophic mode of life. However photosynthetic competence takes some time to be instituted in them due to the marked differences in internal anatomy and physiology from the field grown plants (Chandra *et al*., 2010). To support the *in vitro* regenerated sugarcane plant at this sensitive stage the application of certain nutrient mixtures not only speeded up the acclimatization process but also significantly reduced the mortality rate (Figure 1) by improving all the growth characteristics of plants (Table 2).

Application of various nutrient solutions revealed significant differences in plant survival, height, number of tillers and leaves per plant during acclimatization of sugarcane plantlets (Table 2). The highest survival percentage (98%) was noted with application of ½ strength Murashige and Skoog (1962) salts solution (Figure 1). This treatment resulted in induction of multiple shoots with maximum height and leaves on plants after five weeks of *ex vitro* transfer. These results are in agreement with Vanegas *et al*., (2002) and Kothari and Chandra (1984), who observed similar results in plant acclimatization under natural conditions, showing that this is a good system for acclimatization of *in vitro* regenerated plants. However, Mirada-Ham *et al*., (2006) emphasized the use of Hoagland solution (Hoagland & Arnon 1950) during acclimatization.

The application of coconut water during acclimatization also showed promising results (Table 2). It was the second most effective treatment with 80 % survival of plantlets (Figure 1). It was previously reported (George, 1993) that the addition of coconut water to the culture media resulted in the plants with a greater nutritional and carbohydrates contents. Our results showed that all the growth parameters were highly influenced by the addition of coconut water and growth was much better in the soil supplemented with coconut water as compared the soil without coconut water. These results are in accordance with the results obtained by Nasib *et al*. (2008). Coconut water itself is a complex organic supplement and also possess plant growth promoters which not only help in rapid growth of plants but provide strength to resist physiological shocks at transition of plants from *in vitro* to *ex vitro* conditions (Silva *et al*., 2003; Ather *et al*., 2009).

Table 2. Effects of various nutrient solutions on growth behavior of sugarcane plants in terms of mean height of tillers, number of tillers and number of leaves per plant.

| Nutrient solution code | Type of Nutrient solution | Height of Tillers (cm) | Number of Tillers per plant | Number of Leaves per plant |
|---|---|---|---|---|
| NS-1 | Tap water (control) | 5.2±1.43 | 2.3±0.65 | 2.2±0.61 |
| NS-2 | 5 % Farm yard manure | 2.9±1.19 | 1.5±0.62 | 1.4±o.58 |
| NS-3 | 1 % DAP and Urea (1:1) | 8.4±1.41 | 3.2±0.57 | 3.3±0.56 |
| NS-4 | 10 % Coconut Water | 10.7±0.15 | 4.7±0.15 | 4.2±0.13 |
| NS-5 | ½ strength MS Salts | 3.9±1.31 | 2.1±0.71 | 2.2±0.74 |

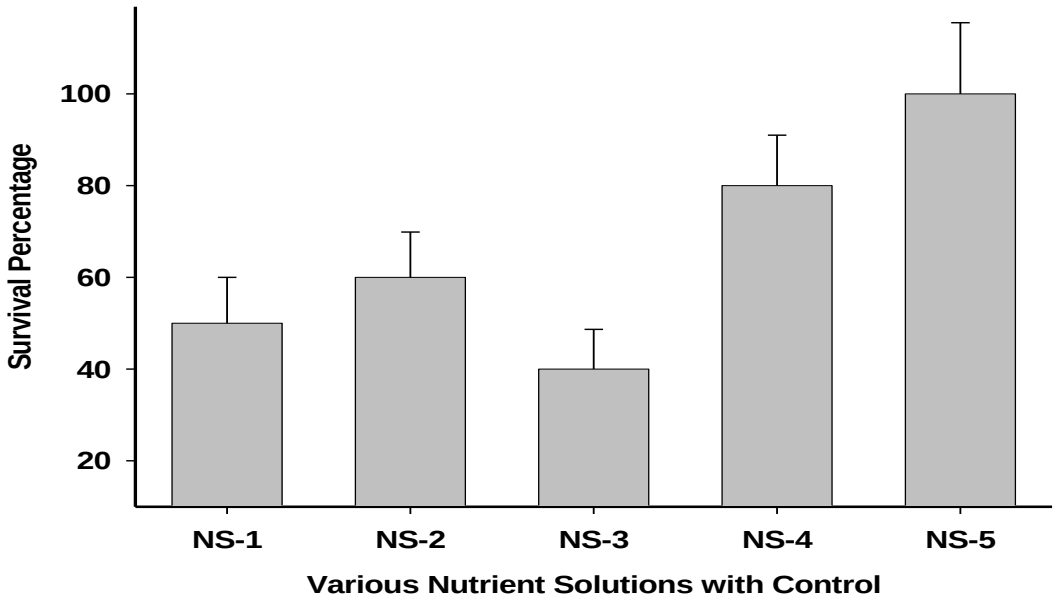


Fig. 1. Effects of various nutrients solution Farm Yard Manure (NS-2), DAP+ Urea (NS-3), Coconut Milk (NS-4), Half MS (NS-5) and tap water (NS-1) on survival efficiency of sugarcane plants.

It was found that by the applications of DAP + Urea the plantlet survival was 40 %, using tap water 50% and by farm yard manure 60 %. Results shows that application of DAP + Urea, farm yard manure and simple tap water were not affective enough to acclimatize tissue culture plants. Our results are in agreement with Hoffmann *et al*., (2001) and Silva *et al*., (2003), who found that use of DAP and urea were not useful for the acclimatization of micro-propagated plantlets and negatively affected the growth of the plantlets. It was observed that farm yard manure is the most common and easily available potting mix used for hardening of tissue culture plants (Ather *et al*., 2009) but we have not found it affective. Naz *et al.*, (2009) obtained best hardening response in mixture of sand: silt: peat (1:1:1) after three weeks of transplantation in the green house. This study depict that half strength MS salt solution as the most effective nutrient application to achieve maximum survival of sugarcane tissue culture plants during acclimatization.

In conclusion, the survival of tissue culture plants during acclimatization is dependent on the availability of appropriate nutrients to the plant in the soil.

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