Pak. J. Bot., 36(3): 549-556, 2004.

IN VITRO CONSERVATION OF SUGARCANE (*SACCHARUM OFFICINARUM* L.) GERMPLASM

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Abstract

In order to conserve the sugarcane germplasm *in vitro*, studies were undertaken at the Plant Genetic Resources Institute, National Agricultural Research Centre, Islamabad. The cultures were successfully established for var. Katha on MS medium containing BA. Establishment of 8 other sugarcane varieties on the same medium composition suggested its suitability for conservation of sugarcane germplasm. *In vitro* conservation of var. Katha by osmotic stress showed that cultures with 2% mannitol were healthy up to 165 days (showing 75% survival rate), while the cultures with 1 and 3% mannitol showed 100% survival up to 105 days of storage. The technique has great potential for the conservation of plant genetic resources and more studies are required to further extend the subculture period by combining the osmotic stress with incubation conditions management.

Introduction

Tissue culture techniques are of great interest for the collection, multiplication and storage of plant germplasm (Engelmann, 1991). Tissue culture systems allow propagating plant material with high multiplication rates in an aseptic environment. Virus-free plant can be obtained through meristem culture in combination with thermotherapy thus ensuring the production of disease-free stocks and simplifying quarantine procedures for the international exchange of germplasm. The miniaturization of explants allows reducing space requirements and consequently labouring cost for the maintenance of germplasm collections.

Tai & Miller (2002) have stressed on the characterization of the World Collection of Sugarcane for effective preservation and use of genetic resources. Sugarcane include genotypes both sterile and orthodox seeds. Orthodox seeds are generally highly heterozygous and are of limited interest for conservation (Roberts, 1973). These species are thus mainly maintained as clones. Conservation in the field present major drawbacks like disease, disaster limiting its efficacy and threatening the safety of plant genetic resources conserved in this way (Withers & Engels, 1990). Tai *et al*., (1994) also reported that the clones of wild sugarcane (*Saccharum spontaneum*) in the World Collection of Sugarcane and related grasses, which are being maintained vegetatively, are very costly and subject to the perils of man and nature. Shaw (1990) stated that the present and near term applications of *in vitro* techniques in sugarcane involve both meristem and callus based micropropagation, development of disease-free clones and genetic resources conservation.

The main concerns in genetic resources conservation are the genetic stability and survival. Genetic stability is maintained when the cultures are derived from meristematic cells. Kazim & Shahid (2001) worked with micropropagation of 8 sugarcane clones using meristem tip culture method and found that 4-mm size of meristem tips was the most

suitable for establishment of culture. Their results indicated that the micropropagated plants were phenotypically similar to the mother plants. Whereas, when variability is required to be induced, cultures are grown from explant sources other than meristem and callogenesis is initiated at first. Thus Shahid *et al*., (2001) have developed tissue culture technique using leaf explant and MS (Murashige & Skoog) growth medium with varying levels of 2,4-D. They developed clonal variants of 7 sugarcane genotype. Similarly, Sorory & Hosien (2000) compared the explants from young leaf tissue and apical meristem of sugarcane for callus initiation. Their analysis showed that the leaf explant compared with apical meristem explant took less time to initiate callus. Thus the concern about genetic variability can be overcome by the proper methodology. Gonzalez *et al*., (1999) found no differences for 6 agronomic traits between plants regenerated from control and cryopreserved apices of two sugarcane varieties.

Paulet & Glaszmann (1994) stated that the biotechnological advances have benefited sugarcane extension and exchange. An *in-vitro* sugarcane collection (almost 400 varieties or clones, 10 specimens of each) has been set up as a supplement to the International Quarantine Service organized by CIRAD, Montpellier, France. This "vitrotheque" does not take much space and enables rapid distribution of a broad range of healthy plants.

Sugarcane is a major cash and industrial crop of Pakistan. Its germplasm is being maintained in our field genebank where danger of loss of valuable germplasm due to perils of man and nature exist. Considering all the advantages of the *in vitro* conservation, research studies were initiated with sugarcane at the *In vitro* Preservation Laboratory of Plant Genetic Resources Institute, National Agricultural Research Centre, Islamabad, 45500, Pakistan which is reported herein.

Materials and Methods

Three experiments were carried out in sequence after culture establishment of sugarcane variety Katha. **A**: Effect of incubation period (*in vitro* storage) on culture survival. **B**: Varietal response of 8 other sugarcane cultivars compared to Katha cultures (control) on same media and culture conditions as in experiment A. **C**: Effect of slow growth conditions (using osmotic stress) for extending the storage period without effecting the culture survival.

Culture establishment

The materials (Ex-plant source) used in the studies were lateral buds of field grown sugarcane var. Katha. The, bud clumps covered with muslin cloth were kept under running tap water for 10 minutes then treated with 30% Clorox for 20-30 minutes and rinsed with sterile water three times in laminar flow cabinet. Size of buds was reduced to minimum possible with naked eyes before culturing one bud in each culture tube. MS medium with BA (benzyl adenine) 1.125 mg/l, 30 g sugar was used. pH was adjusted to 5.8. and 15 ml media was dispensed per culture tube and autoclaved. The cultures were incubated in the dark at 17^{o}C for about 1 week and than transferred to 16 h light at 25^{o}C. Transfers to fresh media (sub-culturing) was done after every 5-6 weeks.

A. Effect of incubation period (*in vitro* storage) on culture survival: Established cultures of var. Katha were sub cultured on MS medium containing BA 1.0 mg/l with 10

replicates of each treatment. Data on shoot length (mm) was taken at 15, 45, 75, 105 and 195 days of culture, number of shoots were taken at 15, 45 and 75 days and number of leaves were recorded at 15 and 45th day only, over a storage period of 6 months. The data were processed to % increase over the initial growth for height, number of shoots and leaves, averaged and compared for inference. The details of treatments are as under:

T1 25^{o}C incubation temperature under light 16h, (Control).
T2 17^{o}C incubation temperature under light 16h.
T3 17^{o}C incubation temperature under dark.
T4 10^{o}C incubation temperature under light 8h.
T5 10^{o}C incubation temperature under dark.

B. Response of 8 other sugarcane cultivars compared to Katha cultures (control) on same medium and culture conditions as in experiment A: This protocol was applied to 8 other varieties to determine their response and compared with Katha cultures (control). Medium as mentioned above was used with similar culture conditions i.e. 16 h light at 25^{o}C. Data on culture biomass was recorded after 3 months and compared.

C. Effect of slow growth conditions (osmotic stress) for extending the storage period without effecting the culture survival: Established cultures of var. Katha were sub cultured on MS medium containing BA 1.0 mg/l, and 4 different concentrations of osmotica added to the medium i.e. 0, 1, 2, 3, and 4% W/V mannitol. There were 10 replicates of each treatment. The osmotic stress was evaluated for its influence in retarding the culture growth in terms of improved survival over the period of six month. Data on survival of cultures was mainly targeted.

Results and Discussions

A. Effect of incubation period

Shoot growth: Over six months culture growth period, the % increase in shoot length showed that sugarcane cultures in control grew healthy and vigorously up to 45 days after subculture (DAS). Later growth was normal up to 105 DAS after which it sharply declined and none of the cultures survived (Fig. 1). All other incubation conditions showed reduced growth as compared to control.

At low temperature regime (10^{o}C), under both light and dark conditions, cultures showed poor performance as the shoots degenerated after 45 DAS and cultures did not survive as in case of control (Fig. 1). In moderate temperature regime (17^{o}C) the cultures grew healthy with reduced growth in comparison to control while, under illumination conditions the cultures died before the control (75DAS). Cultures under dark at 17^{o}C survived a period at par to that of control. This experiment suggests that culture growth could be reduced at 17^{o}C under dark condition, but storage period could not be increased to maintain healthy cultures. This aspect needs further experimentation to prolong the subculture period. It also suggests that low temperature of 10^{o}C is not suitable for sugarcane *in vitro* storage.

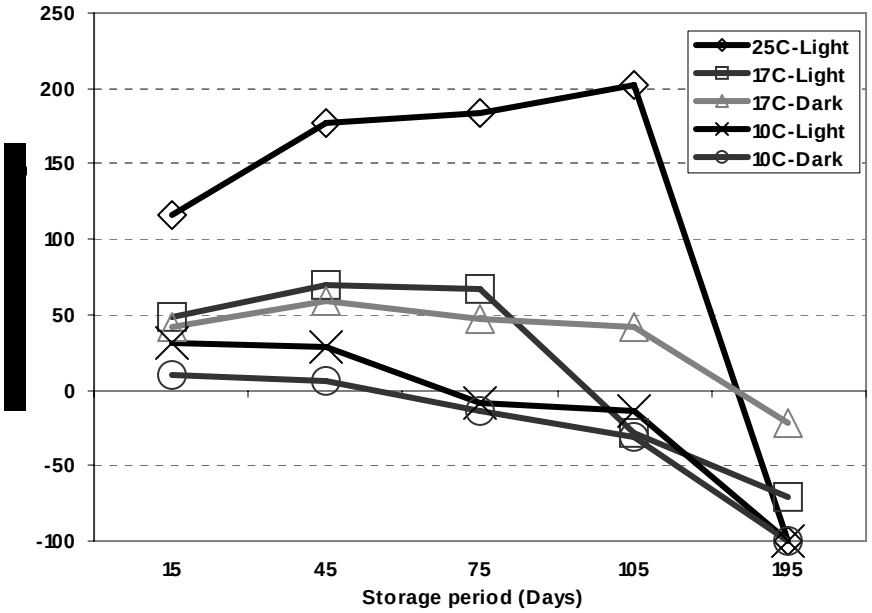

Fig. 1. Percent increrase in shoot length (mm) of sugarcane var. Khata cultures over initial growth as influenced by photoperiod and temperature variation over a period of six months

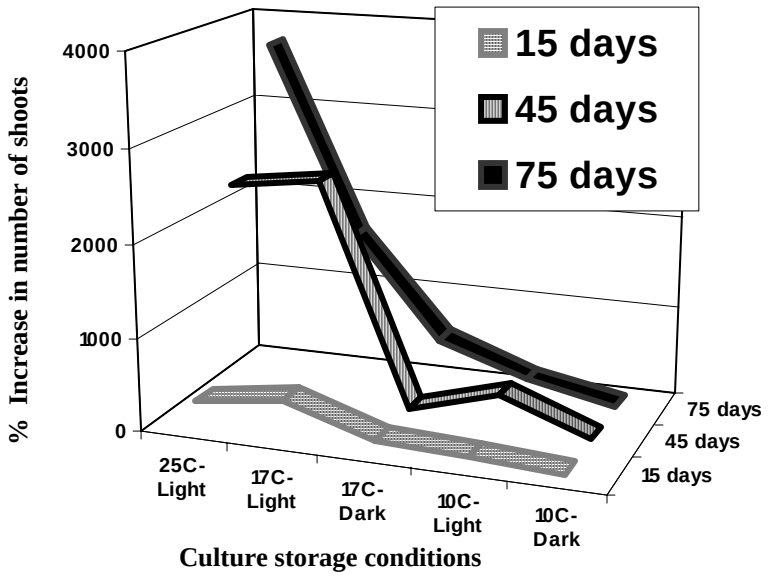

Fig.2: Percentage increase in number of shoots of sugarcane var. Khata cultures as influenced by storage conditioned over a period of six months.

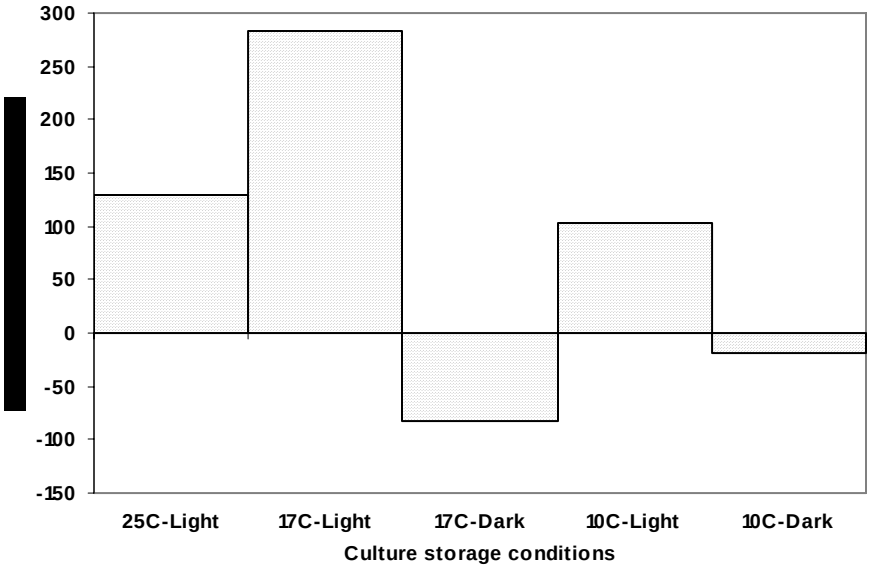

Fig. 3. Percentage increase in number of leaves of sugarcane cultures var. Khata after 15 days as influenced by photoperiod and temperature.

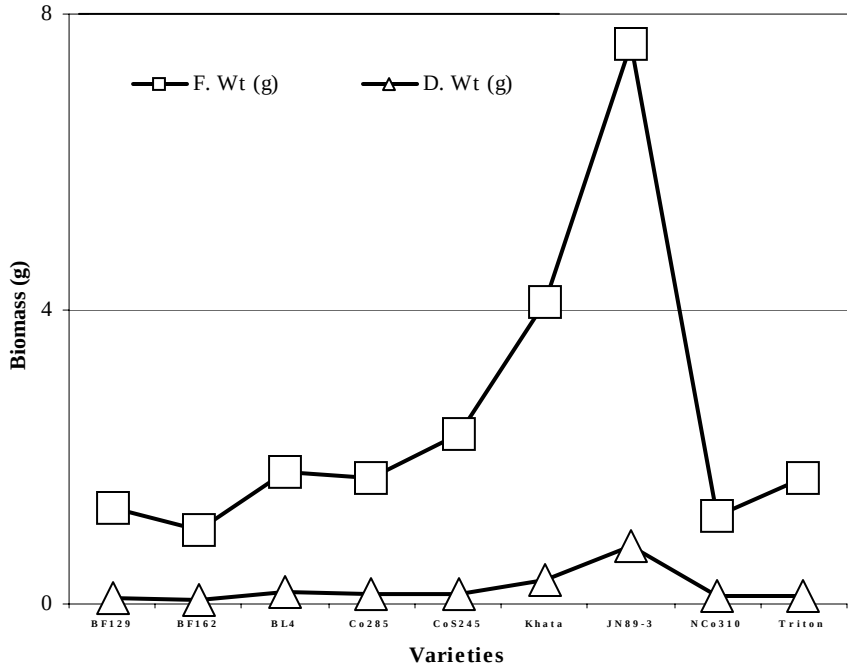

Fig. 4. Varietal response of sugarcane cultures to in vitro preservation for 3 months.

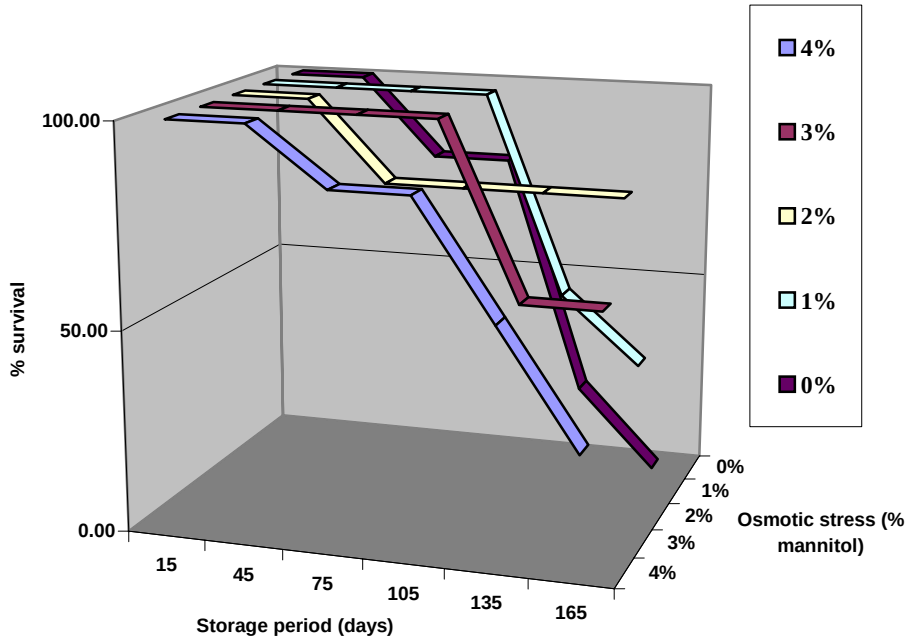

Fig. 5. Percentage survival of sugarcane cultures var. Khata during six month storage as influenced by osmotic stress and incubation conditions of temperature (25oC) under light.

Number of shoots: The % increase in number of shoots of sugarcane cultures var. Katha over a period 75 DAS showed that the culture under control conditions produced the highest number of shoots followed by 17°C light > 17°C dark > 10°C light > 10°C dark (Fig. 2). The results indicate that at every temperature, culture under light produced more shoots compared with dark condition (Fig. 2). The number of shoots declined with the decrease in incubation temperature. It suggests that both dark conditions and low temperature could be exploited to reduce the growth of cultures for *in vitro* storage purposes. Poor growth at 10°C demonstrates that such low temperature is not suitable for *in vitro* storage of sugarcane cultures.

Number of leaves: Dark incubation conditions at 10°C and 17°C showed negative effect on increase in number of leaves after 15 DAS (Fig. 3). Due to profuse growth, leaves count was not possible after 15 DAS. Similar response to low temperature and dark incubation conditions was observed in number of shoots and number of leaves. However, reducing the growth at the cost of survival is not practical for *in vitro* germplasm conservation.

B. Varietal response to *in vitro* storage

Sugarcane var. Katha, showed best response at 25°C under light (control). To evaluate the performance of this medium 9 varieties were cultured under control

conditions (Fig. 4). Cultures were established for all the varieties and were maintained for 3 months. On the basis of culture biomass production, it was observed that the growth of variety JN 89-3 was vigorous and needs modified medium and culture conditions for *in vitro* storage. On the contrary two varieties BF162 and Nco310, produced low biomass and need some modified medium for maintaining healthy cultures during preservation (Fig. 4). It is thus concluded that due to the varietal response of sugarcane cultures generally one protocol (i.e. one medium and incubation condition) is not applicable. In case if one such protocol is followed the cultures have to be grouped according to response and subculture period. However, this medium was found to be suitable for most of the varieties tested for *in vitro* germplasm conservation.

C. Effect of osmotic stress

To extend the culture life (storage period), the effect of osmotic stress was evaluated. Sugarcane var. Katha was subjected to slow growth medium where osmotic stress was induced by adding mannitol (0 to 4% W/V). It was noted that over time the survival rate as compared to control was improved by osmotic stress (Fig. 5). The cultures in control survived for 135 DAS but with low survival rate of about 20%, whereas at 2% mannitol survival percentage was 75% even after 165 DAS. Though the culture survival was not 100% depicting that more cultures than usual (10 cultures/variety) will be required, demanding more space. It was interesting to note that at 1% and 3% mannitol levels, 100% cultures survived for 105 DAS under 25^{o}C and 16 h illumination (Fig. 5). Lal (1993) while working with *in vitro* conservation of the shoot cultures of sugarcane cv. CoS 8436, found that biomass production, multiplication rate and shoot vigour of cultures were highest at 4% sucrose, while shoot chlorophyll concentration was highest at 2% sucrose. The response of cultures change due to nutrient and micro-environment needs detailed investigation in order to develop protocol for a large range of genotypes. Variation in temperature and /or light to this treatment may be evaluated to explore its effect for better *in vitro* storage of sugarcane cultures. Sreenivasan & Sreenivasan (1985) had shown that rooted sugarcane *in vitro* culture plants on White's basal medium were stored for up to 9 months.

There is this great potential for sugarcane cultures to be stored *in vitro* and that most of its varieties can be preserved for a considerable period. However, the current protocol needs improvement; other media also need to be evaluated for *in vitro* conservation of sugarcane or under one protocol regime, grouping for different subculture period may be helpful.

Acknowledgement

The authors are grateful to **JICA** for providing excellent *In vitro*-Genebank facility at Plant Genetic Resources Institute, National Agricultural Research Centre, Islamabad for *in vitro* conservation of plant genetic resources.

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(Received for publication 10 August 2003)