In vitro Multiplication and Conservation of *Saccharum* Germplasm

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Sugarcane germplasm have been maintained in the world germplasm repository at Sugarcane Breeding Institute Research Centre in the field gene bank. Field gene bank is always at the risk of biotic and abiotic factors and complimentary strategies need to be worked out to support the field gene bank. *In vitro* techniques offer different methods of conservation, and slow growth preservation is being successfully employed in many vegetative propagated crops. In this study, attempt was made to standardize optimal *in vitro* multiplication of different species of *Saccharum* and also studied to delay the sub-culturing period by retarding the growth of the *in vitro*-maintained material. It was observed that MS medium supplemented with 1 mg/l Kn and 6 mg/l BAP was more effective in inducing multiple shoots from the shoot apex in most of the species studied and 2% mannitol was found effective in controlling the growth.

Key Words: *Saccharum*, Sugarcane, *In vitro* multiplication, Micropropagation, *In vitro* conservation, Field gene bank

Introduction

Saccharum germplasm includes five species, *viz.*, *S. officinarum*, *S. spontaneum*, *S. barberi*, *S. sinense* and *S. robustum*. *S. officinarum* is the thick cane, high sugared tropical sugarcane which is also known as the 'noble' cane. *S. barberi* and *S. sinense* are the north Indian and Chinese forms, respectively, which were under cultivation in the sub-tropical areas before the advent of man made hybrid varieties in the early part of twentieth century. At present, these varieties are not commercially cultivated for sugar and are mainly confined to collections in research stations only. The related genera of *Saccharum* such as *Erianthus, Miscanthus, Narenga, Sclerostachya, Sorghum, Imperata,* etc. and the commercial hybrids developed in India and foreign countries were also the part of sugarcane germplasm which are also maintained in field gene bank at Kannur. The germplasm assembly at SBI Research Centre, Kannur now consists of 3368 accessions. These include *S. officinarum* (759), *S. barberi* (42), *S. sinense* (30), *S. robustum* (145), *S. spontaneum* (79), Indian hybrids (1027), Foreign hybrids (611), IND collections (393), IA clones (130) and allied genera (152). The germplasm accessions are clonally propagated every year and are maintained in the field. The field gene bank is exposed to various diseases and pests and other natural calamities like drought, flood, etc. Genetic resources being the safe deposit for the present and future crop improvement programmes, it is imperative to maintain the germplasm through whatever mode of conservation available to safeguard it from any kind of loss during conservation. The viability of the *in vitro* technique in micropropagation and for crop improvement have been studied by Sreenivasan and Sreenivasan, 1984 and 1992. Potential of *in vitro* meristem culture technique in sugarcane storage of germplasm was also reported by Sreenivasan and Srinivasan, 1985. Hence to adopt a complementary strategy to support field gene bank *in vitro* multiplication and slow growth conservation studies were conducted in the sugarcane germplasm.

Materials and Methods

Two clones each from all five species were studied for *in vitro* multiplication in MS media supplemented with Kinetin 1mg/l and BAP at 0, 2, 4, 6, 8 mg/l. The shoot apex were taken from the field and surface sterilized by wiping with 95% ethyl alocohl. The outer leaf sheaths were removed and the shoot apex of 1-2 cm length with two whorls of leaf sheath were inoculated in the liquid media in three culture tubes each in three replication. Explants were collected from two clones each from five species *viz.*, *S. officinarum* (White transparent and NC 25), *S. barberi* (Lalri, Matnashaj), *S. sinense* (Uba reunion, Uba seedlling), *S. robustum* (NG77-213, NG 77-90) and *S. spontaneum* (IND 81-318, PLAG 84-8). The cultures were transferred to same media after one week to avoid the excess phenolics exudated to the medium. After three weeks of inoculation the response were observed and sub cultured in the same media for three weeks. The number shoots induced were recorded. For rooting, fully matured 60 shoots were inoculated in MS solid medium supplemented with 1 mg/l IBA; and for hardening, 50 rooted plantlets were transplanted to plastic cups with 1:1 sand and soil and kept in high humidity condition under shade for two weeks. For prolonging the sub culturing period by growth retardation mannitol were supplemented to the basal MS medium at 0%, 1%, 2% and 4% level. Ten fully grown plantlets with roots were

inoculated in the media in three replications on a filter paper bridge in liquid media and another set in media solidified with 0.75% Agar. Initial shoot length and fresh weight were recorded. The fresh shoot weight and shoot length were measure at one month intervals after culling out two tubes per replication. The observations were recorded till the shoots started drying.

Results and Discussion

Shoot elongation was recorded in all the clones in the treatment T1 where only one or two side shoots were observed (Table 1). All clones except *S. spontaneum* clones showed maximum shoot induction in media supplemented with Kn 1mg/l + BAP 6 mg/l. *S. spontaneum* showed maximum shoot production in media supplemented with 1 mg/l Kn + 2 mg/l BAP. The maximum multiplication rate of 1:11 was observed in NC 25. In BAP, concentration above 6 mg/l did not have any advantages and in *S. spontaneum* browning of explants was noticed. The result indicated that BAP at 6 mg/l was better for induction of multiple shoots from the shoot apex for most of the clones and for *S. spontaneum* lower concentration was desirable. The multiplication rate of 1:10 was desirable in view of maintenance of germplasm where faster multiplication rate is discouraged to reduce the chance of somaclonal variation. Hundred per cent rooting was observed in MS medium supplemented with 1 mg/l IBA with in three weeks. The hardened plants under shade and high humid condition were transplanted after two weeks to pots and 90% of the plants could be established.

One of the difficulty that arises during maintenance of germplsm clones under *in vitro* conservation is that it continues to grow in the culture medium and difficult to accommodate in the culture vessel and need trimming and constant replacement of culture media (Sreenivasan and Srinivasan, 1985). Slow growth preservation by using osmotic regulators (mannitol) and growth retarding substances like ABA in culture medium have been employed successfully in other crops (Ashmore, 1997; Chandran *et al*., 2001). In this study, the treatment with 2% mannitol in liquid medium was found effective in retarding the growth in good health condition up to 120 days in culture. After 120 days the medium get completely exhausted and required to be replenished or sub culturing had to be done. Compared to solid medium the liquid medium was found better for *in vitro* maintenance. This may be due to the exudated phenols get dispersed in the liquid medium whereas in solid medium it accumulate on the surface where the explant is in contact with the medium resulting death of cells at the cut end and affect the proper absorption of the nutrients. The increase in fresh weight was more in liquid medium as the absorption rate of the nutrient is faster in liquid medium compared to solid medium. A slow and linear increase was observed in medium supplemented with 1% and 2% mannitol (Table 2). In solid medium supplemented with 2% mannitol, the increase in fresh weight was not observed after 90 days in culture; this may be due to the drying of shoots. In medium supplemented with 4% mannitol, the shoots were completely dried within 90 days.

Table 1. Shoot induction in 5 different species of *Saccharum*

Genotype	No of shoot tip cultured	Average no of shoots induced/explant				
		T1	T2	T3	T1	T5
		1 mg/l Kn	1 mg/l Kn+ BAP 2 mg/l	1 mg/l Kn+ BAP 4 mg/l	1 mg/l Kn+ BAP 6 mg/l	1 mg/l Kn+ BAP 8 mg/l
S. officinarum						
White transparent	45	1.3	2.0	2.8	9.2	5.9
NC 25	45	1.0	2.1	2.7	11.0	4.0
S. robustum						
NG 77-213	45	2.4	3.0	3.2	9.1	5.3
NG 77-90	45	2.0	2.7	3.3	8.0	3.7
S. barberi						
Lalri	45	1.7	3.3	3.1	10.7	3.1
Matnashaj	45	1.0	2.6	2.8	6.0	3.5
S. sinense						
Uba reunion	45	1.6	2.5	3.3	6.3	5.0
Uba seedling	45	1.7	3.7	2.2	8.2	4.7
S. spontaneum						
IND 81-318	45	2.0	6.0	2.1	2.3	2.0
PLAG 84-8	45	1.9	8.3	2.0	2.7	1.7

Table 2. Fresh weight increase in different treatment of mannitol in culture medium

		Initial fresh weight of shoot (mg)	Fresh wt after 30 days (mg)	Fresh wt after 60 days (mg)	Fresh wt after 90 days (mg)	Fresh wt after 120 days (mg)
T1	Liquid medium + 0% Mannitol	104.0	338	902	702	1200
T2	Solid medium + 0% Mannitol	104.6	228	493	353	508
T3	Liquid medium + 1% Mannitol	104.8	155	724	550	800
T4	Solid medium + 1% Mannitol	104.6	240	910	721	942
T5	Liquid medium + 2% Mannitol	90.0	200	215	525	610
T6	Solid medium + 2% Mannitol	105.0	150	425	425	0
T7	Liquid medium + 4% Mannitol	90.0	125	348	100	0
T8	Solid medium + 4% Mannitol	105.0	120	350	0	0

Shoot elongation was found maximum in medium without any addition of mannitol where it reaches up to 26 cm within 120 days and in treatment the maximum retardation was observed in liquid medium supplemented with 2% mannitol and in solid medium with the same composition and in the medium supplemented with 4% mannitol the shoots were dried with in 120 days of culture (Fig. 1). Hence, it is inferred that 2% mannitol can be supplemented to the liquid culture medium to retard the growth of the shots under *in vitro* condition and can be used in *in vitro* germplasm maintenance without affecting the viability and vigour of the material. The *in vitro* maintenance of germplasm also facilitate the easy exchange of germplasm with minimal quarantine procedures and the supply of the material can also be ensured round the year irrespective of crop season.

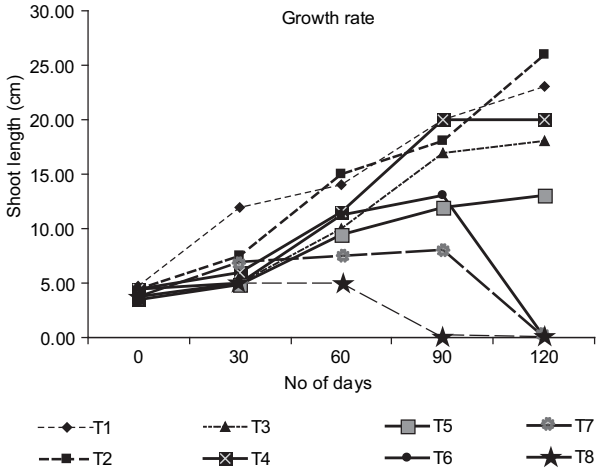

Fig. 1: Growth rate of *in vitro* maintained shoots in different treatment

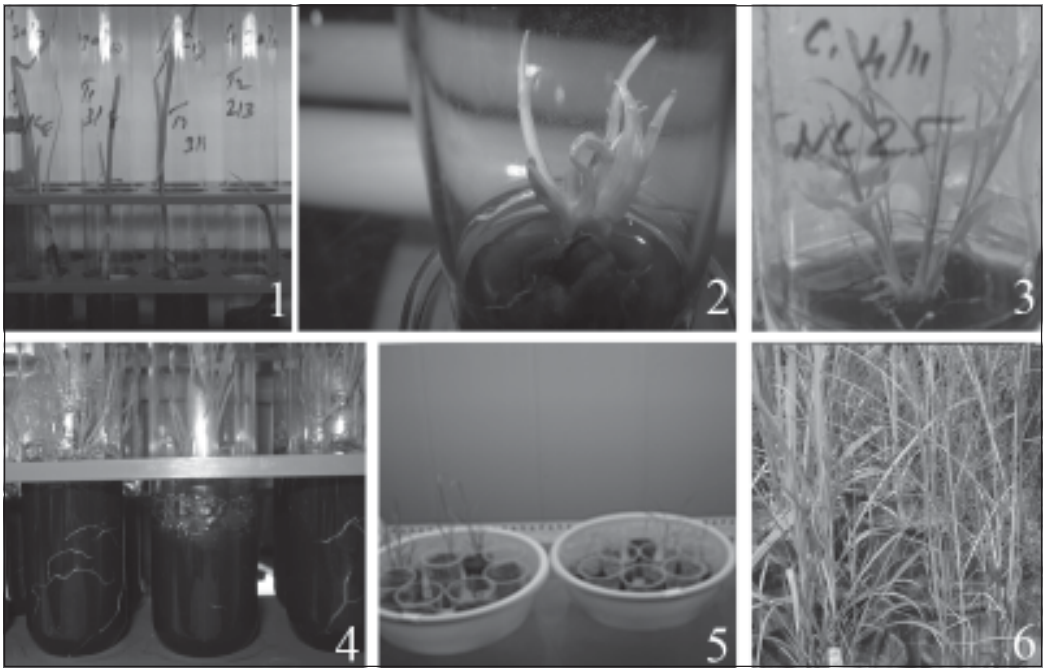

Fig. 2: Various stages of development of shoot in germplasm clones; 1. Elongation of single shoots from explant, 2. Multiple shoot induction, 3. Multiple shoots derived from NC 25, 4. Rooting in solid medium, 5. Hardening in plastic cups, 6. Transplanted plants in pots

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