In vitro screening for salinity resistance in cotton (*G. hirsutum*)

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ABSTRACT

Cotton (Gossypium spp.) is an important commercial crop of our country. It is grown in an area of 7.0 m.ha with production of 16m bales of cotton lint. The productivity of the country is 300 kg lint/ha and it is very low compared to the world productivity (550 kg lint/ha; Anonymous, 1999). As much as 65 percent of the total cotton growing area is under rainfed conditions, where the productivity is very low ranging from 35-40 kg to 150 kg/ha and this is one of the reasons why our national productivity is low. The unassured and erratic distribution of rainfall is resulting in moisture stress during critical crop growth stages and consequently yields are low. There is 20 to 30 percent losses in cotton production due to abiotic stress. Breeding for drought resistance is challenging, right from screening stages. Drought resistance is a combination of effect of several characters. Development of effective selection indices for drought resistance on morpho-physiological basis is essential. Screening germplasm to develop such effective selection indices is very tedious, time consuming and labor intensive. The regeneration system via somatic embryogenesis/organogenesis and meristem culture in in vitro *is a landmark in the history of life sciences. They are useful to induce genetic variability, genetic transformation and* in vitro *screening/selection. A large number of germplasm lines can be screened with minimum effort in terms of space, labor and time. In the present study, 35 germplasm lines have been screened for drought resistance using NaCl, $MgSO_4$, $NaHCO_3$ and $CaCl_2$ as* in vitro *drought inducers. The observations at different stages of plant growth on different concentrations of above salts at the levels of EC at 2 and 12 ds/m have been studied. Jayadhar (G.* herbaceum*) genotype has shown tolerance at 2 ds/m but is susceptible at 12 ds/m. By keeping these two concentration ranges (2-12 ds/m) it is possible to screen other genotypes. Such studies can be used to establish easy and effective selection indices for field selection.*

Introduction

Plants vary widely in their ability to withstand saline environments. The optimal salt concentration for growth of halophytes is about 0.5 M NaCl (Flowers *et. al.,* 1977), which is roughly equivalent to salt concentrations in seawater. But most crop plants are glycophytes (salt sensitive plants), such as *Medicago sativa,* which is killed by growth on 0.1 M NaCl (Smith and Mc Comb, 1981). Because of an increase in the salinization of agricultural lands, in the world, it is of utmost importance to identify genotypes that can withstand salinity. In India, over utilization of canal water during irrigation by the farmers have also been resulting in salinization of commond areas. Plant breeding for tolerance to salt stress has been difficult, as salt tolerance has been considered to be a quantitative trait, and also because of variety of physiological parameters contribute to tolerance (Norlyn and Epstein, 1984; Dvorak *et al.,* 1985; Dvorak *et al.,* 1988). At cellular level, there are increased growth and maintenance costs as a result of increased environmental salinity (Handa *et al.,* 1983; Stavarek and Rains, 1984; Rhodes *et al.,* 1986).

Varietal halo-tolerance was first studied as a possible source of plant breeding material for salt tolerance genes in barley (Epstein and Narlyn, 1977; Epstein *et al.,* 1980; Harkman *et al.,* 1989). Some other plant species that show varietal differences in salt tolerance are tomato (Tal *et al.,* 1978), rice (Flowers and Yeo, 1981; Akbar *et al.,* 1986) and alfalfa (Smith and Mc Comb, 1981; Allen *et al.,* 1986; Kapulnik *et al.,* 1989).

Salinity resistant genetic resources in cotton were also identified by several workers (IAEA, 1992; Singh, 1993; Singh *et al.,* 1996; Rajamani, 1994; Rajeshwari, 1995; Zafri and Ahmad, 1994; Khan *et al.,* 1995; Uma *et al.,* 1995; Lin Jin Ding *et al.,* 1995, Hebbara *et al.,* 1996).

Among several activities the development of salt tolerance in crop plants and the identification of salt (salinity) tolerant genetic resources through screening is most important. Screening for salt tolerance under natural conditions is difficult because of non-availability of uniformly salt affected fields, time consuming, and labor intensive. So the conventional breeding methods including screening methods have been slow in improving salt tolerance. The alternative approach of utilizing plant cell culture and regeneration of salt tolerant plants from potential cell mutants has received increased attention. In cotton regeneration directly via cell cultures is very limited (Katageri *et al.,* 1998; Suresh Kumar *et al.,* 2001; Khadi *et al.*, 1990). But shoot apical meristems can directly be used to regenerate cotton (Katageri *et al.,* 1998; Gould *et al.,* 1991). Besides obtaining somaclonal mutants through *in vitro* culture, it can also be used to screen existing natural genetic resource, as it requires less space, less labor intensive and short duration. So the present investigation was carried out to standardize the protocol for *in vitro* screening for salt tolerance in cotton using shoot apical meristem culture.

Experimental procedure

The experiment was conducted at the tissue culture laboratory, Agricultural Research Station Dharwad Farm, Dharwad (India) during 2001-03.

Genotypes

Cotton varieties/hybrids belonging to all four cultivated *Gossypium* species have been grown in India. In order to know the sensitiveness of all four species to an *in vitro* screening system, one variety belonging to each species were chosen as they are popular directly as variety or parents of hybrids. Genotypes chosen for the study were as follows.

1. *G. hirsutum* var. Abadhita: Released for commercial cultivation in both irrigated and rainfed conditions of South zone covering three states viz., Karnataka, Tamil Nadu and Andra Pradesh as it is boll worm tolerant (Khadi and Katageri, 1991 and Katageri *et al.,* 1997).
2. *G. barbadense* var. SB(YF-425): Male parent of popular inter-specific hybrid, DCH-32 (Katarki, 1981).
3. *G. arboreum* var. A 82-1: Male parent of potential desi hybrid, DDh-2 (Khadi *et al*., 1992)
4. *G. herbaceum* var. Jayadhar: Popularly growing desi cotton under rainfed condition.

Preparation of explants

Shoot apical meristems were isolated from germinating seeds under aseptic conditions.

Preparation of media

MS medium was supplemented with growth regulators like benzyl adenine (2.0 mg/l) and only NaCl was initially added in such a way that stress to the extent of 9.14, 18.28, 27.42, 36.56 and 45.70 had to be developed. Later different salts at different concentrations were used (details are given in Table 1).

Culture of explants

Freshly isolated shoot apical meristems were cultured and maintained at 26 ± 2 °C temperature and 50-60 percent relative humidity. Initially, for one week 1000 lux light intensity was provided and later cultures were maintained at 2000-3000 lux light intensity.

Observations recorded

Observations on number of shoot apical meristems able to produce shoots (two to three leaves) and roots were made for once in 7 days.

Results and Discussion

Initially four genotypes belonging to the four species cultivated were chosen for *in vitro* screening with NaCl. Spontaneous somaclonal cell variants tolerant to NaCl have been selected on media containing NaCl in several plant species (Stavarek and Rains, 1984; Tal, 1984). That is why initially only NaCl was used. Among the four genotypes cultivated on different levels of salt, two genotypes Abadhita and SBYF-425, belonging to tetraploid species, were having less tolerance to salt compared to the genotypes (Jayadhar and A-82-1) belonging to two diploids . Although in both the tetraploid genotypes normal plants survived at 9.14 EC with normal growth, a very high percentage of plants were abnormal at 18.28 EC. Therefore, although more of the plants died at 20 EC, this level was not taken as a critical limit but higher levels were tried and it was found that the percentage of abnormal plants increased as the level of NaCl increased till 36-56 EC. At 45.7 EC, there was no regeneration at all. Although diploid genotypes were having a higher tolerance to salt, abnormal plants were observed in both the herbaceum and arboreum species till 45.7 EC (Table 2). In Figure 1, non-emergence of shoot from shoot apical meristem due to NaCl effect was shown. One two five indicates 9.14 EC to 45.70 EC (as mentioned in Table 2) and 'C' indicates control.

Conclusions

- Shoot apical meristem culture would be used to screen salinity tolerance in cotton.
- In the absence of somatic embryogenesis in Indian cultivars, SAM can be effectively used for *in vitro* screening.
- It is possible to develop less labor intensive, zero land intensive, short duration oriented and more efficient screening for biotic and abiotic stress using salts *in vitro*.

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Table 1. *Salt quantity (g) for different ec levels (for 1 liter solution).*

EC (ds/m)	NaCl	$MgSO_4$	$NaHCO_3$	$CaCl_2$
2	0.3211	0.3000	0.2152	0.1664
4	1.2288	0.6000	0.4304	0.3328
6	1.8432	0.9000	0.6456	4.9920
8	2.4576	1.1867	0.8608	0.6656
12	3.6864	1.8000	1.2912	0.9709

Table 2. *Critical level of salt tolerance of different genotypes belonging to four cultivated species of cotton.*

Genotypes	Salinity level (EC)	Normal plants (%)	Abnormal Plants (%)	Dead (%)
Abadhita (4x)	09.14	87.5	12.5	00.0
	18.28	10.0	90.0	00.0
	27.42	00.0	92.5	07.5
	36.56	00.0	75.0	25.0
	45.70	00.0	00.0	100.0
	Control	100.0	00.0	00.0
SB(YF-425)	09.14	85.0	15.0	00.0
	18.28	05.0	95.0	00.0
	27.42	00.0	90.0	10.0
	36.56	00.0	67.5	32.5
	45.70	00.0	00.0	100.0
	Control	100.0	00.0	00.0
Jayadhar (2x)	09.14	91.5	09.5	00.0
	18.28	31.5	68.5	00.0
	27.42	19.5	80.5	00.0
	36.56	00.0	95.0	05.0
	45.70	00.0	95.5	04.5
	Control	100.0	00.0	00.0
A-82-1	09.14	93.5	06.5	00.0
	18.28	59.5	39.5	01.0
	27.42	18.5	78.5	03.0
	36.56	00.0	97.0	03.0
	45.70	00.0	97.0	03.0
	Control	100.0	00.0	00.0

Table 3. *Number of germplasm lines (4x) which survived at 27 EC.*

10 days	20 days	30 days	40 days	50 days
16	15	5	5	2

Table 4. *Effect of combination of salts on Jayadhar in* in vitro.

Levels of salts in EC (ds/m)	Number of plants established (%)
2	53.33 (46.90)
4	43.33 (41.40)
6	33.33 (35.22)
8	06.66 (24.93)
12	01.11 (03.50)
Control	93.33 (75.31)
Mean	36.11
CV	9.72 %
CD (0.05)	6.385
CD (0.01)	9.084

* Numbers in parenthesis indicate angular transformation values.