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SALINITY TOLERANCE AT CELLULAR LEVEL OF SOME HIGH YIELDING LOCAL CULTIVARS OF *ORYZA SATIVA* (RICE)

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ABSTRACT

Five cultivars of rice (Oryza sativa) were tested for salinity tolerance at cellular level. Seed callus of the cultivars (BW 351, AT 354, BG 94-1, BG 403, AT 402) was developed on Murashige and Skoog medium (1962) with Casein hydrolysate (1.0 g/L), 2,4-D (2.0 mg/L) and BAP (1.5 mg/L) on semi-solid medium. Cell cultures from the callus were developed on IRRI basic composition containing 2.0 mg/L BAP. They were exposed to salinity levels of 2,4,6,8 and 10 ds/m. Total number of single cells, percent of plasmolyzed cells and the percent viability were measured every week. There was considerable variation in the cell tolerance to salinity levels. In general, AT 354 and AT 402 showed a lower percentage of plasmolysis than other cultivars at all salinity levels: at 10 ds/m salinity, viable cells were shown only in these two cultivars while, all the cells were plasmolyzed in others. It was also observed that tolerance shown in the field by the cultivars was reflected at the cellular level. Another significant observation was that every cultivar showed the presence of at least a few tolerant cells at salinity levels higher than the recorded level in the field.

INTRODUCTION

Rice (*Oryza sativa*) is the staple food in Sri Lanka and, about 1.8 million farmers are engaged in rice cultivation throughout the country. The annual per capita consumption of rice is around 100 kg/Y and attempts are still being made to achieve self sufficiency in rice production mainly by extended irrigation facilities, developing desirable cultivars and providing infra-structural support for fertilizer and seed distribution (Gunesena et al. 1977). However in the recent past, the rice yield has stagnated and efforts are being made to overcome the difficulties (Abeysiriwardena, 1996). Therefore, improvement of rice cultivars is given prominence in Sri Lanka. Over the past few decades several high yielding cultivars have been developed at Rice research and Development Centers in Sri Lanka. These high yielding cultivars have been grown continuously and have contributed considerably to improve the national production.

It is known that the rice yield has stagnated for the last few years and one reason is the deterioration of rice soils. Also, with continuous irrigation, frequent drought, seepage, and due to many other reasons, salinity of rice soils has been affected (Thenabadu, 1972) and in fact has increased, and there is a great possibility of further increase. One of main drawbacks of many high yielding cultivars is their poor tolerance to salinity. Improvement of salt tolerance in high yielding cultivars would undoubtedly increase and stabilize rice yields especially in salt affected areas. Therefore, improvement for salinity tolerance has become an important rice research programme in many countries.

Conventional breeding programmes, requiring longer periods of time, are commonly being used in many of these studies. However the role of tissue culture technologies especially in shortening this period is now realized. Use of tissue culture technologies in rice improvement is variable; one use is based on the selection of tolerant cell lines after an exposure of cells to salt

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stress and regeneration of tolerant cells into plants and, finally field testing of regenerated plants. Tissue culture technologies have been used for rice breeding programmes at IRRI in Philippines and in many other international laboratories (IRRI News Letter, 1979; Yoshida et al. 1983; Zapata, 1991). A difficulty encountered had been the variation of salinity tolerance with the development of the plant.

The main objective of the study was to determine a possible relationship between the salinity tolerance at cellular level and in the field, the final objective being improvement of high yielding cultivars for salinity tolerance.

MATERIALS AND METHODS

The cultivars tested were BW 351, AT 354, BG 94-1, BG 403 and AT 402: high yielding and commonly cultivated in Sri Lanka (AT, BW & BG cultivars have been developed at Ambalantota, Bombuwela & Batalagoda Rice Research and Development Centers respectively). Dehusked rice grains were used as explants for callus development. Grains of the cultivars were obtained from Bombuwela and Ambalantota Rice Research and Development Centers (RRDC), Sri Lanka. They are either 3 ½ or 3 month cultivars and field tested salinity tolerance was highly variable, 'AT' cultivars with higher tolerance than 'BW' (Rice records from Department of Agriculture, Sri Lanka - seed certification center, Gannoruwa).

Dehusked grains were surface sterilized in 70% (v/v) ethanol for 1 min, followed by 20 min in 30% (v/v) clorox solution (NaOCl 5.25%), with frequent agitation. Grains were then washed with sterile distilled water. Callus initiation and development was on Murashige and Skoog (MS) medium (1962) supplemented with Casein hydrolysate (1.0 g/L), 2,4-D (2.0 mg/L), BAP (1.5 mg/L) 3% sugar and solidified with 0.8% agar (BDH) (Hirimburegama & Kulasekera, 1994).

Cell cultures for salinity studies were developed on IRRI basic mineral composition (Yoshida et al. 1983) with 2.0 mg/L BAP in the culture medium. The salinity of the media was adjusted to 2,4,6,8 and 10 ds/m levels using 1 M NaCl. The control did not contain NaCl. A volume of 15 ml was dispensed into 125 ml Erlenmeyer flasks. The pH was maintained at 5.8 ± 0.1 with 0.1 N NaOH. A volume of 10 ml was dispensed into tubes (2.5 cm diameter, Pyrex). Culture media were autoclaved at 103.4 kPa and 121 ^{0}C for 20 min. Dehusked, surface sterilized seeds were inoculated on the culture media in an upright position, where the embryo was placed on the culture medium (Hirimburegama & Basnayake, 1995). Cultured were incubated at 27 ^{0}C for 24 h photoperiod provided with white cool fluorescent light. RH was 70-80%. The fresh weight of callus was recorded every week for 6 weeks. Each treatment had 30 replicates.

Flasks containing liquid culture media were inoculated with 4 pieces of calli (0.5 x 0.5 mm^2, at 6 weeks after callus initiation). Flasks with calli were placed on an orbital shaker at 60-70 rpm. Samples from the cultivars were taken every week. One set was stained with Lugol's solution, and were observed through a light microscope. Another set was stained with FDA (Fluorescein Diacetate) and observed through a fluorescent microscope (Nikon). The total number of round shaped cells and cell clusters were counted in a field of x400 of light microscope. The percentage of plasmolysis was recorded. The percentage of viable cells was counted through a fluorescent microscope (Nikon, Japan). Measurements were taken for 30 days.

RESULTS AND DISCUSSION

Callus was developed from embryos of all the cultivars tested, but callus initiation and the rate of growth were highly variable. In some cultivars, callus initiation was observed one week after inoculation while in others it took 3-6 weeks. However, in all cultivars the callus growth (taken as fresh weight) increased exponentially up to 6 weeks after which the growth rate was reduced, thus giving a typical sigmoid type of growth (Figure 1). This shows that the growth of cells is normal and follows the typical sigmoid type, which is generalized for the growth of single cells, tissues, organs and organisms.

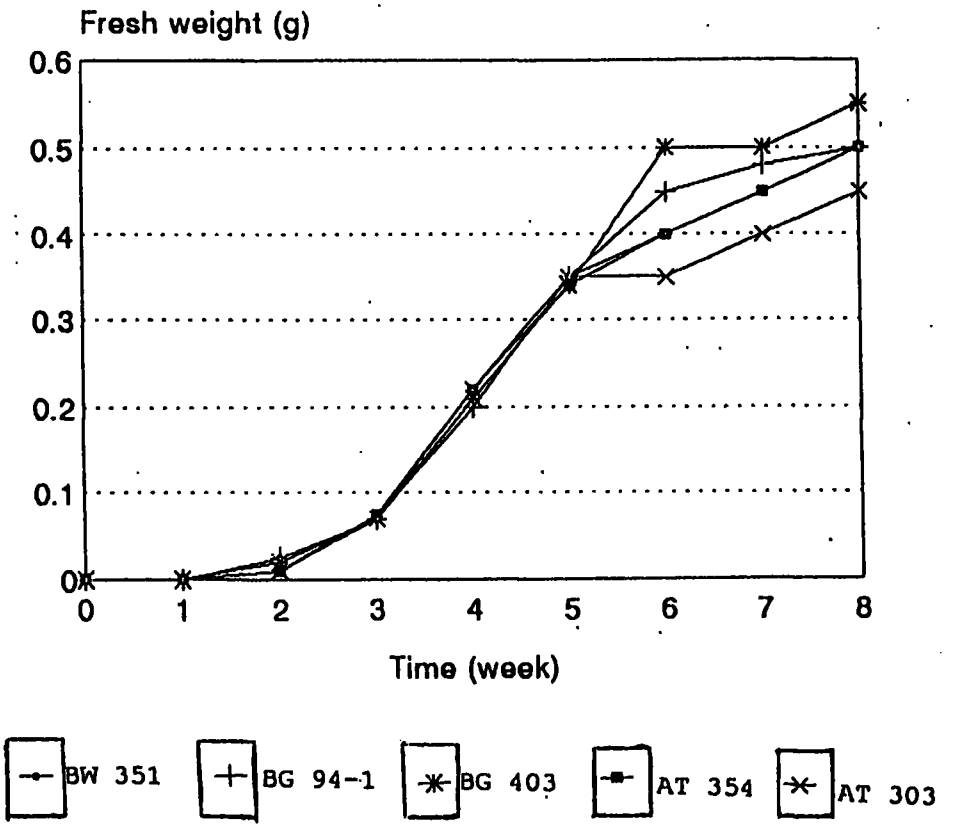


Figure 1. Callus growth (fresh weight) of rice varieties

In all the cultivars, calli predominantly contained parenchyma cells (Figure 2). They were separated by groups of cells which appeared to have meristematic characters (dense cytoplasm, distinct nucleus, very few vacuoles etc.) (Williams and Maheswaran, 1986).

Upon transfer of callus to liquid media, single cells and mini clusters were produced by all cultivars. However, the total number of cells was different, especially at different salinity levels. The percentage plasmolysis and viable cell count also varied in cultivars at salinity levels (Figure 3). In general, AT 354 and AT 402 showed a lower percentage of plasmolysis than other cultivars at all salinity levels. In fact, at 10 ds/m level, live cells were shown only in the above two cultivars (Figure 3 b), suggesting that at cellular level, the two cultivars show a better salinity tolerance than the others. According to reports, at field level, the same two cultivars show a better tolerance than the others (Rice records, Department of Agriculture, Sri Lanka- seed certification center, Gannoruwa). This indicates that tolerance shown at field level is also reflected at cellular level. However studies with more cultivars are needed to generalize this fact.

In a few studies, a change in the proline content has been measured as it is reported to be related with salt tolerance. However, it is also reported that free proline content may also be increased in salt-sensitive varieties and thus the proline content may not always be a suitable marker in examining salt resistance in rice (Gonzales and Labrada, 1995). Therefore, this parameter was not taken in the present study.

Another significant observation was that every cultivar showed the presence of tolerant cells at salinity levels higher than the recorded level in the field (2-4 ds/m). This indicates that in a plant there are cells having higher salinity tolerance than that of the complete plant. This effect is better seen in cell cultures where individual or mini cluster of cells exist. This also suggest the potential of establishing cell lines with the higher tolerant cells (at 8 ds/m), for subsequent plant regeneration.

Since salinity tolerance at field level is reflected at cellular level in the cultivars tested, it could be postulated that plants regenerated from tolerant cell lines may also express the same tolerance at field level. The main difficulty in such studies, as reported in other cultivars of rice, had been the variation of salinity tolerance with the development of the plant. It is also reported that tolerance at cellular level may not be reflected at field level throughout, by the whole plant. This is reported not only for rice but also for several other crops (Jennings et al, 1976; IRRI News Letter, 1979; Zapata, 1991; Cano et al, 1996). However, results of the present study reveal the possibility of screening the rice cultivars at cellular level for the salinity tolerance in the field. Confirmation of this fact with more local varieties, is under study.

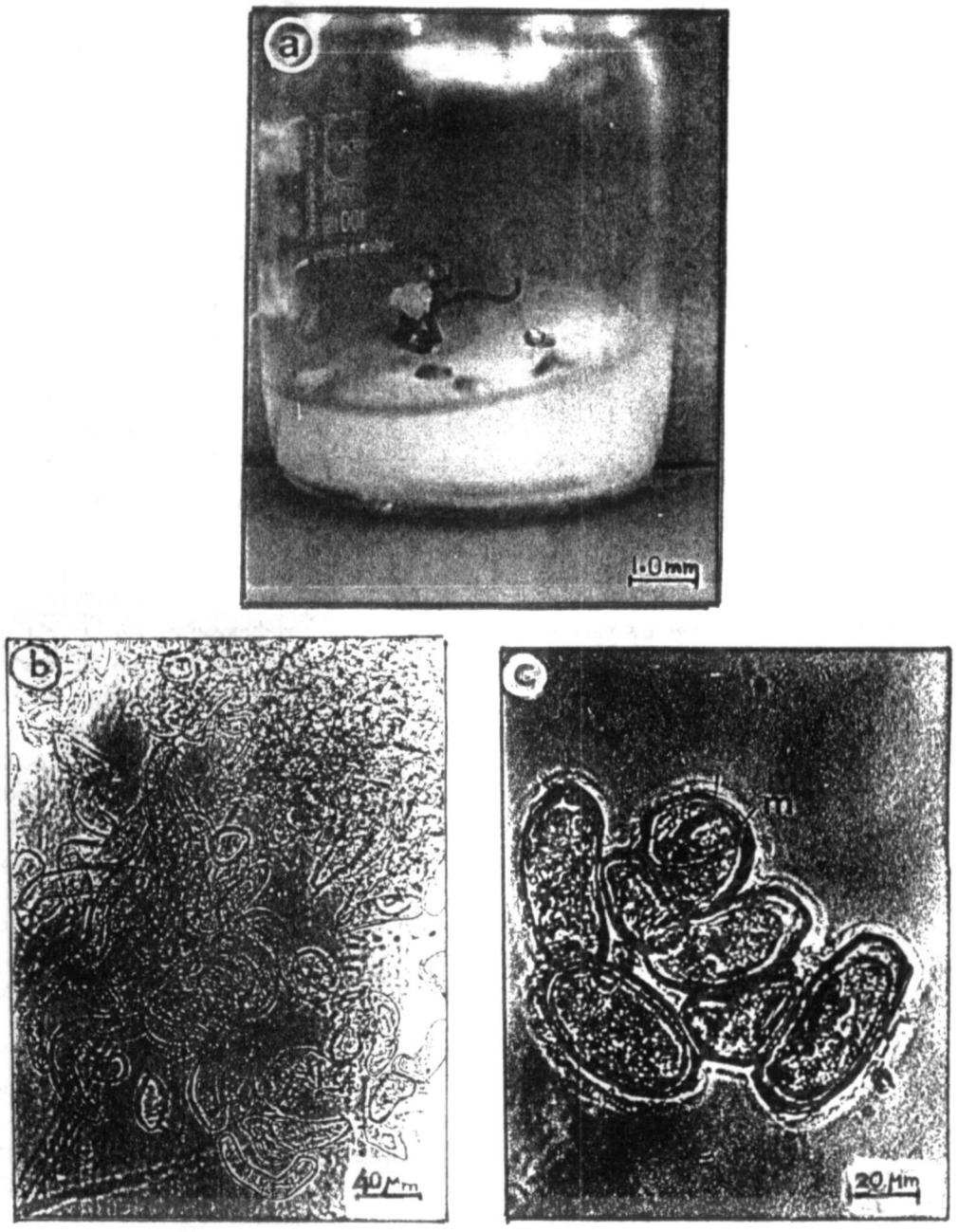
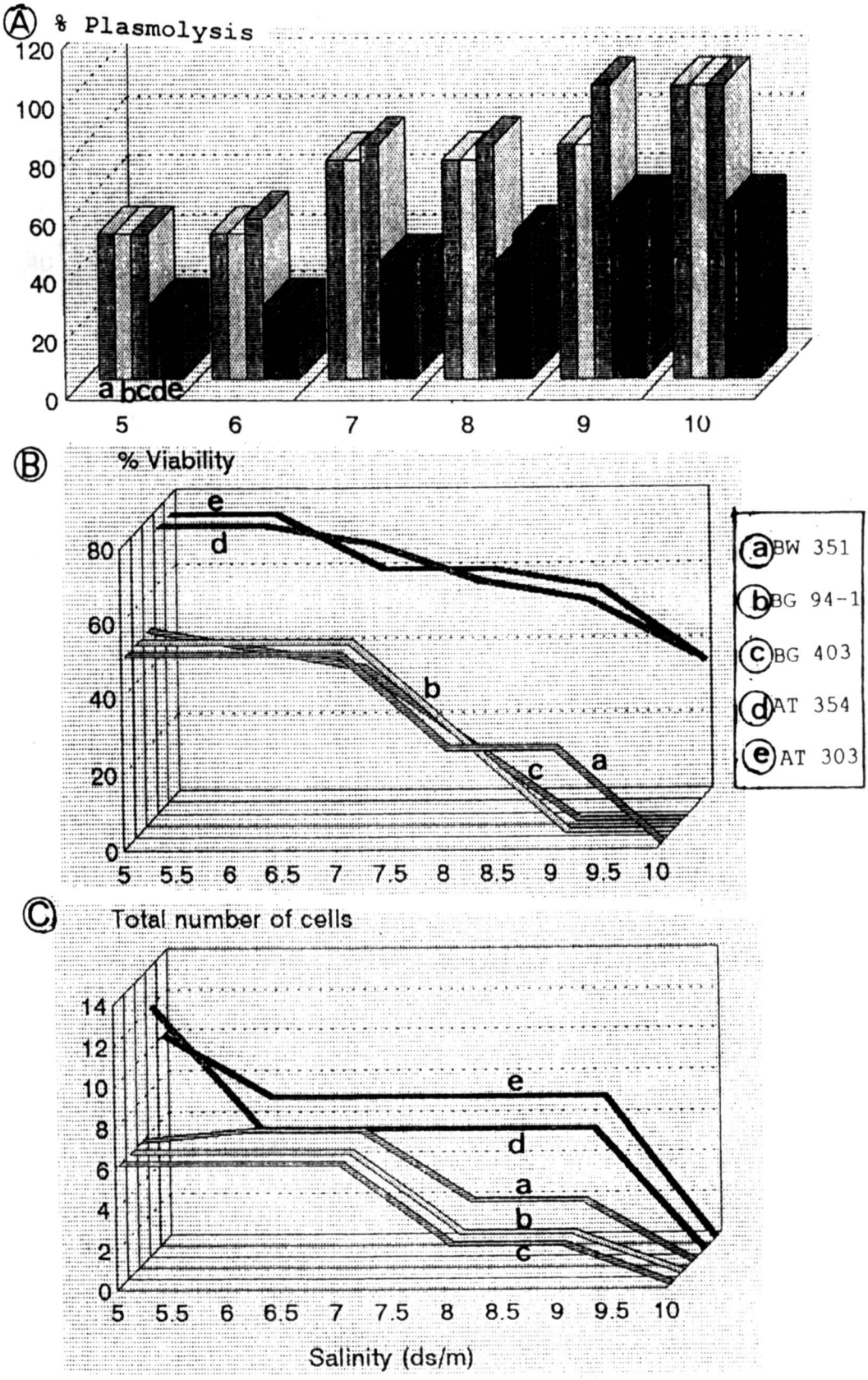


Figure 2. The callus and cells in culture of rice
a: callus of AT 354, b & c cells in culture (p: parenchyma, m: meristematic)



A: % Plasmolysis **B: % Viability** **C: Total number of cells**

Figure 3. Tolerance of cells in culture at salinity levels

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