

## CALLUS INDUCTION AND PLANTLET FORMATION FROM KALLAR GRASS (*LEPTOCHLOA FUSCA*)

NAFEES ALTAF AND KAUSER A. MALIK

*Nuclear Institute for Agriculture & Biology,  
Faisalabad, Pakistan.*

### Abstract

In studies on callus induction from seeds and seedling explants 32% seeds produced calli when BA ( $10^{-7}$  M) + 2, 4-D ( $10^{-5}$  M) + NAA ( $10^{-6}$  M) were used. Mode of regeneration was embryogenesis in a combination of BA ( $10^{-7}$  M) + NAA ( $10^{-6}$  M) + 2, 4-D ( $10^{-6}$  M) + IAA ( $10^{-6}$  M). Numerous small embryoids developed within the callus mass which were easily separated from each other and produced plantlets when cultured on BA ( $10^{-7}$  M).

### Introduction

Plants are exposed to many nutritional factors. A major mineral stress of plants involves the saline environments. Under increasing salt stress the response of proliferating cells callus has been compared to that of whole plants in different species (Warren & Gould, 1982). The mechanism of salt tolerance was more of a cellular and less of whole plant nature. A high correlation between the salt tolerance of other plants and their corresponding callus has been found. Plants which exhibited mechanisms for tolerance based on whole plant structure in some instances did not show tolerance at the callus level (Stavarek & Rains 1983).

Kallar grass (*Leptochloa fusca*) is being widely used for utilization of salt affected lands because of its salt tolerant qualities (Malik, 1978; Sandhu *et al.*, 1981). No information is available regarding the conditions for tissue culture of Kallar grass and its salt tolerance. Somaclonal variation *in vitro* cultures provides a method to improve the response of the crop to salinity stress. This paper describes the conditions for callus induction, its maintenance and plant regeneration from callus.

### Materials and Methods

Kallar grass seeds washed with surf detergent, surface sterilized with (40%) sodium hypochlorite and rinsed thoroughly with sterile distilled water were germinated on moist filter paper to produce sterile seedlings. Leaves and nodes from 5-6 cm tall seedlings were put on the callusing medium containing 3% sucrose. BA, K, IBA, IAA, NAA, 2, 4-D, IPA, GA, ABA and 2, 3, 5-TIBA were used as growth regulators. Other additives were malt extract and cas-amino acids. Salt composition, vitamins and amino acids were the same as

for the medium B5 (Gamborg *et al.*, 1968) and MS (Murashige & Skoog, 1962). pH of the medium was 5.6 before autoclaving. Cultures were kept at  $25 \pm 2^\circ\text{C}$  at low intensity light.

### Results and Discussion

Visibly healthy seeds kept on the callusing media showed that only 49 out of 150 seeds responded to callusing conditions. Since Kallar grass is propagated mostly by vegetative means the heterogenous genotypes of seeds may be the factor in variable callusing and other cultural responses. General genetic background is very important in cell proliferation and differentiation in Gramineae (Hanna *et al.*, 1984). Salt composition of B5 and MS media gave similar callus formation. Calli were produced on media containing cytokinin and auxins. Benzyl adenine seems to be more effective as compared to kinetin. Benzyl adenine had a tendency of giving compact calli which in case of seed derived calli produced embryogenic structures (Fig. 1). Among the auxins tried 2, 4-D was most effective for callusing and differentiation followed by NAA. A lower concentration of 2, 4-D ( $10^{-8}$ ) in the medium tended to grow shoot initials rather than callusing, while at higher concentrations reverse was the case. NAA stimulated both callusing and shoot growth but this auxin was not as effective as 2, 4-D for cell proliferation when compared at ( $10^{-5}$  to  $10^{-6}$  M) levels. IAA had a favourable effect on regeneration. IPA and IBA did not produce any positive response in Kallar grass cultures. Malt extract was not needed as the cultures without it showed better growth. At higher concentrations of auxins ( $10^{-4}$  M to  $10^{-3}$  M), calli quickly turned dark brown, indicating a degeneration of callus tissue. Severe growth inhibition occurred on media containing GA and ABA each at 0.5 and 1 mg/l. However 2, 3, 5-TIBA (2 mg/l) produced calli with small green nodule like structures on the callus. These structures on regeneration medium never developed into plants. On transferring to the callusing medium they produced calli.

The best combination for calls induction was BA ( $10^{-7}$  M) + 2, 4-D ( $10^{-5}$  M) + NAA ( $10^{-6}$  M) whereas Casamino acid 0.1-0.5 g/l had no effect. Mode of regeneration was embryogenesis and it occurred with BA ( $10^{-7}$  M) + NAA ( $10^{-6}$ ) + 2, 4-D ( $10^{-6}$  M) + IAA ( $10^{-6}$  M). Individual embryos with distinct shoot and root primordia were grown on BA ( $10^{-7}$  M) alone to get plantlets. These plantlets were similar to the control seedlings. When these embryos were separated and developed on the same medium, they produced more callus (Fig. 2 & 3). In this study some morphogenetic responses such as small globular calli with a simple root growth were difficult to explain (Fig. 4). They might have originated by the callusing of the shoot pole of an embryoid or due to formation of callus in a detached root or by formation of single root in a callus aggregate. The differences in the capacity of regeneration among the callus pieces derived from the same seed is difficult to explain. Calluses isolated from the primary cultures on 2, 4-D medium were reproducible on the same medium but a prolonged incubation of the callus cultures resulted in aerial root differentiation.

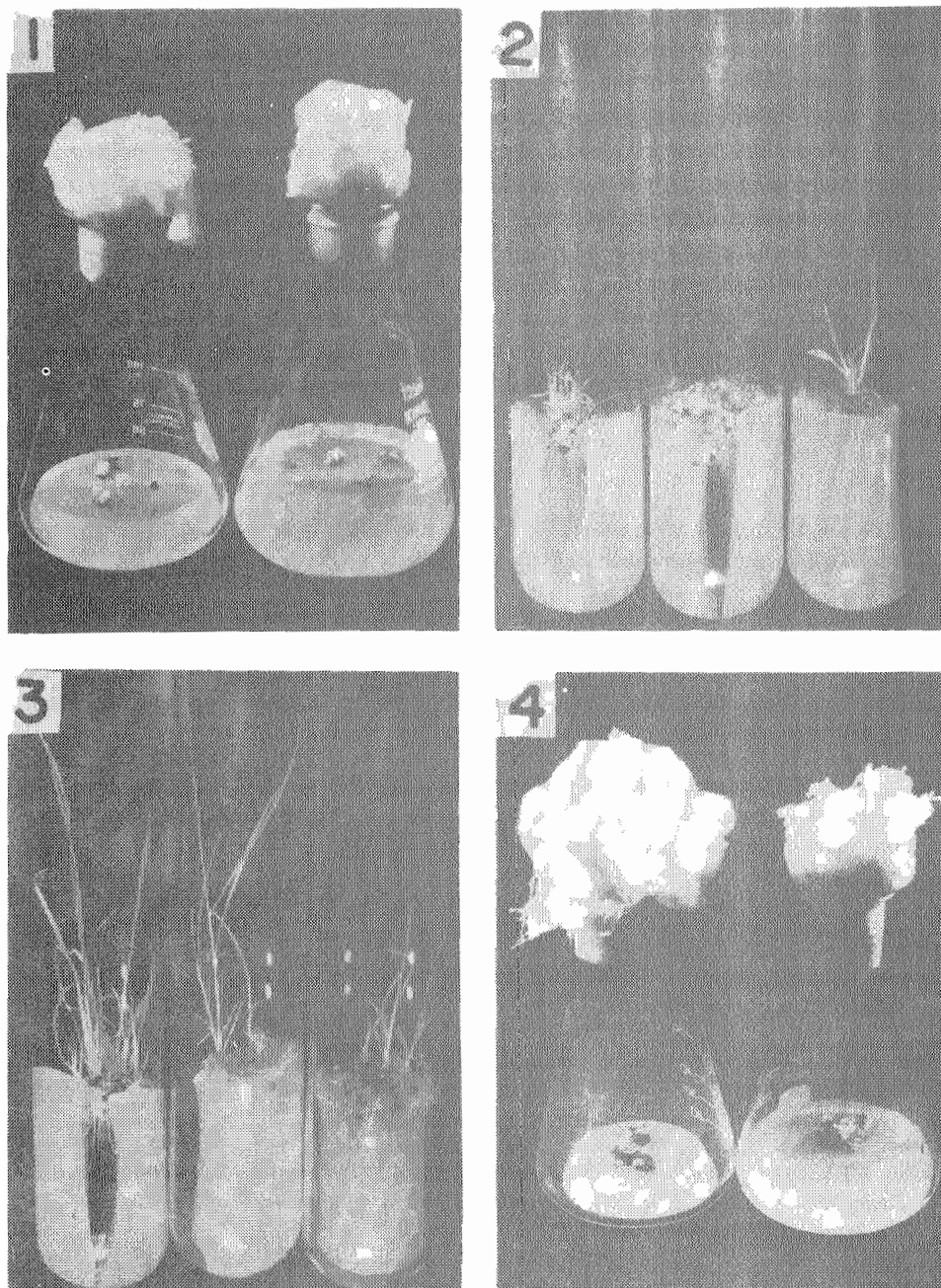


Fig. 1. Small globular structures in compact calli. Fig. 2 & 3. Embryogenesis with callusing. Shoot and root development. Fig. 4. Rooting in callus.

From the explant derived calli, no plantlets were found. Nodes were better explants for callus induction as compared to leaf explant. Under the conditions tested, roots but not shoots were formed from the callus. In this study, callus derived from leaf and node explants were not embryogenic and shoots were also not formed perhaps because of the high endogenous levels of auxins. These calli were comparatively fast growing than the embryogenic calli derived from seeds. The embryogenic callus was compact and composed of small globular structures scattered on the surface. The embryoids usually differentiated from compact and the friable callus seemed to be non-embryogenic because it was only proliferating and not regenerating. Friable callus which was attached to the embryogenic callus was removed as when subcultured at monthly intervals, it retained its embryogenic capacity for over 4 months. Friable callus never regenerated and was faster in proliferation than the compact callus. However, non-embryogenic nature of friable callus is not universally true (Evans *et al.*, 1983).

#### References

- Evans, D.A., W.R. Sharp, P.V. Ammirato and Y. Yamada. 1983. *Hand book of plant cell culture*. Vol. 1 *Techniques for propagation and breeding*. Collier Macmillan Publishers, London.
- Gamborg, O.L., R.A. Miller and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Expt. Cell Res.*, 50: 148-151.
- Hanna, W.W., C. Lu and I.K. Vasil. 1984. Uniformity of plants regenerated from somatic embryos of *Panicum maximum* Jacq. (Guinea grass). *Theor. Appl. Genet.*, 67: 155-159.
- Malik, K.A. 1978. Biological Methods of Reclamation of salt-affected soils. In: *Technology for Increasing Food Production*, (Ed.) J.C. Holmes. Proc. 2nd FAO/IAEA Seminar on Field Food Crops in Africa and Near East, Lahore, Pakistan, Sept. 18-Oct.5, 1977. pp 105-108.
- Malik, K.A., Z. Aslam and S.H.M. Naqvi. 1986. *Kallar grass a plant for saline land*. NIAB, Faisalabad, Pakistan.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15: 473-497.
- Sandhu, G.R., Z. Aslam, M. Salim, A. Sattar, R.H. Qureshi, N. Ahmad and R.G. Wyn Jones. 1981. The effect of salinity on the yield and composition of *Diplachne fusca* (Kallar grass). *Plant, Cell and Environment*, 4: 177-181.
- Stavarek, S.J. and D.W. Rains. 1983. Cell culture techniques: Section and physiological studies of salt tolerance, p. 321-334. In: *Salinity tolerance and plants: strategies for crop improvement*. (Ed.) R.C. Staples. J. Wiley, New York.
- Warren, R.S. and A.R. Gould. 1982. Salt tolerance expressed as a cellular trait in suspension cultures developed from the halophytic grass *Distichlis spicata* (L.) Greene. *Pflanzen Physiol.*, 107: 347-356.

(Received for publication 21 December 1985)