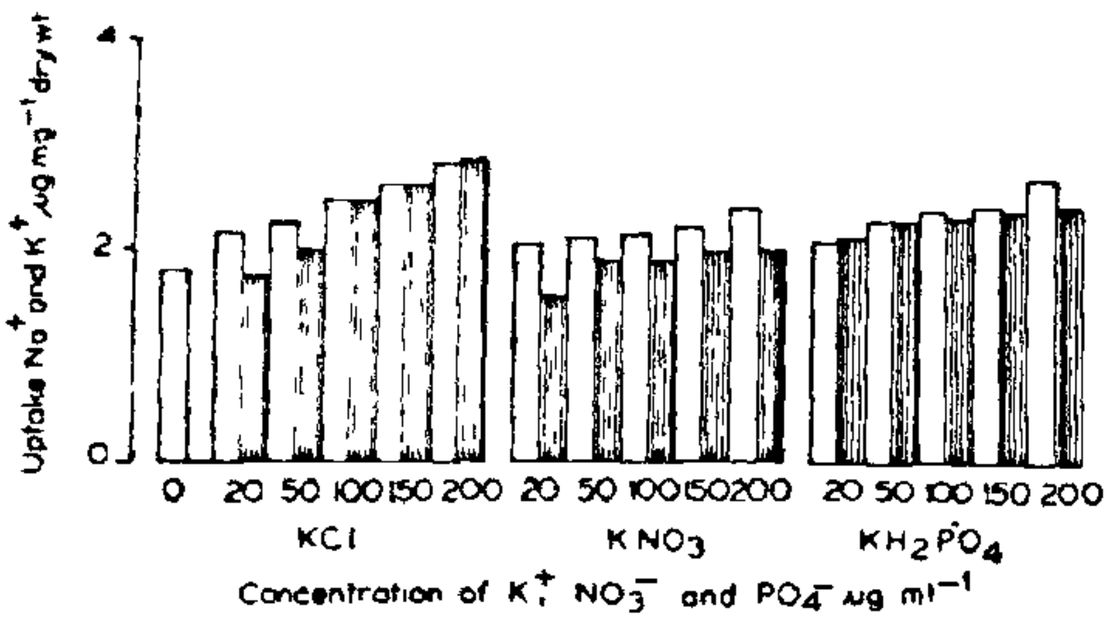

Figure 1. Uptake of Na^+ by *W. prolifica* in presence of fixed concentration of NaCl (23.4 mg NaCl/ml) and graded concentrations of K (20, 50, 100, 150, 200 μg/ml) as KCl, KNO_3 and KH_2PO_4.

Nitrogenase activity measured as acetylene reduction activity was higher in the presence of NaCl-stress. Supplementation of the growth medium with K^+ as salts of chloride, nitrate and phosphates showed that K^+ as KCl was either inhibitory or neutral while NO_3^- and PO_4^- were stimulatory. These results indicate that nutrients like nitrogen and phosphorus are limited under salt stress. Many cyanobacterial strains have been found to grow and tolerate high levels of salinity. These include halophilic forms such as *Microcoleus chthonoplastes* (20–25% NaCl)[9], *Spirulina subsala* (> 3 M NaCl)[10], *Calothrix scopulorum* (5% NaCl)[11], *Anabaena torulosa*[12] and euryhaline forms which could grow in freshwater and in varying degrees of salinity[13] and increased nitrogen fixation by salt adapted *Nostoc muscorum* has also been reported[14].

Na^+ uptake in the presence of fixed level of NaCl and varying concentrations of KCl, KNO_3 and KH_2PO_4 was a concentration-dependent phenomenon up to 200 μg/ml KCl and not in the presence of KNO_3 and KH_2PO_4 (figure 1). *W. prolifica* maintained a low internal Na^+ concentration (2.05 to 2.8 μg/ml dry weight of alga) and this uptake seems to be related partially with K^+. *Anabaena torulosa* showed an increased nitrogen demand under salt-stress and a similar response was also observed with *W. prolifica* where external supply of combined nitrogen could partially improve the level of chlorophyll (table 1). These results show that partial recovery in chlorophyll content is possible by supplying nitrogen as KNO_3 and Na^+ uptake is influenced by the presence of K^+ in the salty environment.

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1. Flowers, T. J., Troke, P. F. and Yeo, A. R., *Annu. Rev. Plant Physiol.*, 1977, **28,** 89.
2. Szalay, A. A. and MacDonald, R. E., In: *Genetic engineering of osmoregulation*, (eds) D. W. Rains, R. C. Valentine and A. Hollaender, Plenum Press, New York, 1980, p. 321.
3. Roychoudhury, P., Kaushik, B. D. and Venkataraman, G. S., *Curr. Sci.*, 1985, **54,** 1181.
4. Jha, M. N., Venkataraman, G. S. and Kaushik, B. D., *Mircen J.*, 1987, **3,** 307.
5. Stanier, R. Y., Kunisawa, R., Mandal, M. and Cohen-Bazire, G., *Bacteriol. Rev.*, 1971, **35,** 171.
6. MaCkiney, G., *J. Biol. Chem.*, 1941, **140,** 315.
7. Kaushik, B. D. and Venkataraman, G. S., *Curr. Sci.*, 1983, **52,** 321.
8. Barber, J., *Biochem. Biophys. Acta* (*Amsterdam*), 1968, **150,** 618.
9. Van Ballen, C., *Botanica*, 1962, **4,** 129.
10. Fogg, G. E., *The biology of blue-green algae*, (eds) N. G. Carr and B. A. Whitton, University California Press, Los Angeles, 1973, p. 368.
11. Tel-Or, E., *Appl. Environ. Microbiol.*, 1980, **40,** 689.
12. Thomas, J. and Apte, S. K., *J. Biosci.*, 1984, **6,** 771.
13. Richardson, D. C., Reed, R. H. and Stewart, W. D. P., *FEMS, Microbiol. Lett.*, 1983, **18,** 99.
14. Blumwald, E. and Tel-Or, E., *Arch. Microbiol.*, 1982, **132,** 163.

SOMATIC HYBRIDIZATION ATTEMPTS BETWEEN *SORGHUM BICOLOR* (L.) MOENCH AND *ORYZA SATIVA* L.

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PROTOPLAST fusion is widely used for transferring nuclear and cytoplasmic traits between sexually incompatible species. Somatic hybridization in the gramineae has proved difficult but varying degrees of success could be achieved ranging from the identification of heterokaryons in fusion between sorghum and maize[1] to that of successful plant regeneration from somatic hybrids of rice and

barnyard grass[2]. Since several methods have been developed for plant regeneration from rice protoplasts[3–8], and since apomictic genotypes are available in the grain sorghum[9], the present investigation attempts to determine the feasibility of protoplast fusion between rice and sorghum.

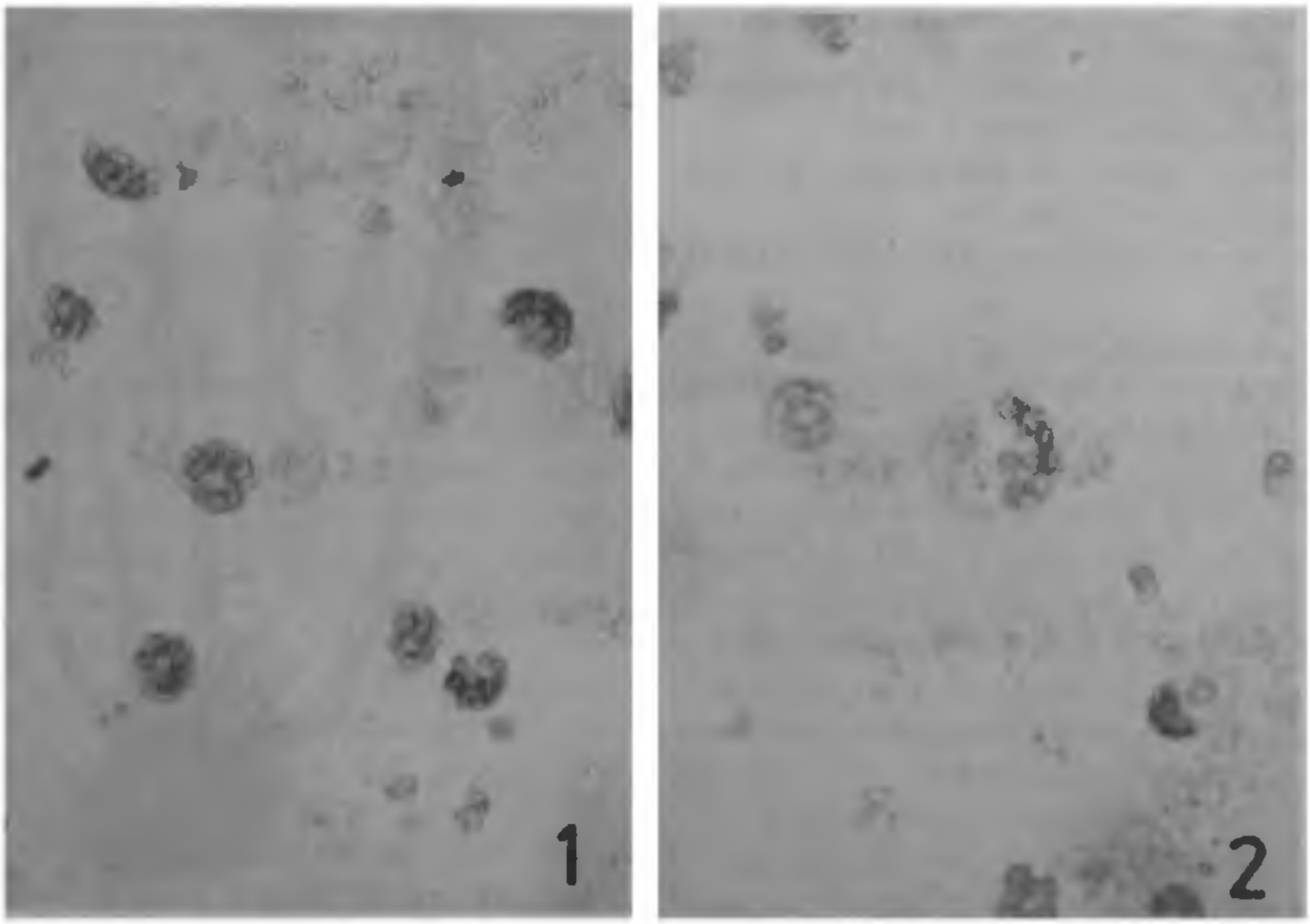
The source of protoplasts in sorghum was the apomictic line R473. Seedlings were raised in the greenhouse and 3-week-old seedlings were used to isolate the mesophyll protoplasts. Leaves were surface-sterilized in 8% 'Domestos' solution for 30 min and washed 6 times with sterile water. The leaves were chopped into small linear strips under aseptic conditions. The tissue was plasmolyzed[10] in CPW 13M for 1 h and then digested in the enzyme mixture 155 13M for 6 h. The enzyme mixture (20 ml) was used for incubating 1 g of the tissue. After incubation, the enzyme mixture together with the leaf tissue was filtered through 30 μm mesh. The protoplasts were then pelleted in a centrifuge at 100 *g* and purified by floatation on sucrose[11].

Protoplasts of rice were simultaneously isolated[7] from an embryogenic cell suspension culture LB-1 of the variety T309. Rice protoplasts were stained with fluorescein diacetate (FDA) by adding 3–4 drops of FDA solution (5 mg/ml in acetone) to the enzyme mixture during incubation. Before fusion, protoplasts of both rice and sorghum were washed twice in electrofusion solution (11% mannitol and 0.5 mM $CaCl_2$, $2H_2O$) and mixed in 1:1 ratio at a density of 10^4/ml. The mixture transferred to 5 × 5 repliplates (Sterilin Ltd. UK); was fused using the electrofusion equipment based on Watts and King[12].

The protoplasts were aligned in an AC field of 500 kHz volts for 20 s. A 400V DC pulse of 2 μs was found optimal for protoplast fusion. The fusion frequency between sorghum and rice protoplasts was 6% (figures 1 and 2).

The heterokaryons could be identified from chloroplasts using vital staining. They were plated at 2–3 × 10^4/ml in KPR[10] medium solidified with Sea Plaque agarose in 9 cm petri plates. The position of the heterokaryons was marked to study their subsequent development. The heterokaryons formed a wall within 24 h. They completed up to 3 divisions within 10–12 days with no further development of the heterokaryons.

The present study reveals that improved sorghum protoplast culture systems are needed for realizing the potential of somatic fusion. These observations, together with those of Brar *et al*[1], indicate that sorghum can be somatically hybridized with other cereals. Wide hybridization in cereals is useful mainly in the production of haploids by eliminating the genome of one of the parents as well as in the introduction of small genetic elements from one



Figures 1 and 2. **1.** Alignment of rice (light) and sorghum (dark with chloroplasts) protoplasts, and **2.** Heterokaryon of rice and sorghum protoplasts.

species to the other. The advantages of somatic hybridization with sorghum, in particular, are the transfer of apomixis, drought resistance and C_4 synthetic pathway. To achieve the desired objective, successful plant regeneration has to be obtained from the heterokaryons. It should also be noted that the problems of evaluation of the regenerated plants, the introgression of useful genes and the elimination of the undesirable genes are as great as those experienced in conventional breeding.

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1. Brar, D. S., Rambold, S., Constable, F. and Gamborg, O. L., *Z. Planzenphysiol.*, 1980, **92**, 269.
2. Terada, R., Kyozuka, J., Nishibayashi, S. and Shimamoto, K., *Mol. Gen. Genet.*, 1988 (in press).
3. Fujimura, T., Sakurai, M., Akaji, H., Negishi, T. and Hirose, A., *Plant Tissue Cult. Lett.*, 1985, **2**, 74.
4. Coulibaly, M. Y. and Demarly, Y., *Z. Pflanzenzucht.*, 1986, **4**, 85.
5. Yamada, Y., Yang, Z. Q. and Tang, D. T., *Plant Cell Rep.*, 1986, **4**, 85.
6. Toriyama, K., Hinata, K. and Sakai, T., *T. A. G.*, 1986, **73**, 16.
7. Abdullah, R., Cocking, E. C. and Thompson J. A., *Bio/Technology*, 1986, **4**, 1087.
8. Kyojuka, J., Hayashi, Y. and Shimato, K., *Mol. Gen. Genet.*, 1987, **206**, 408.
9. Murty, U. R., *Sorghum News Lett.* (*USA*), 1986, 28.
10. Power, J. B., Chapman, J. V. and Wilson, D., In: *Laboratory of manual plant tissue culture*, Univ. Nottingham, UK, 1984.
11. Gamborg, O. L., Shyluk, J. P. and Shalin, E. A., In: *Plant Tissue Culture: Methods and applications in agriculture*, (ed.) T. A. Thorpe, Academic Press, New York, 1981, p. 15.
12. Watts, J. V. and King, J. M., *Biosci. Rep..*, 1984, **4**, 335.

A NEW SPECIES OF *PHAEORAMULARIA* MUNTANOLA

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DURING periodical surveys of the forest flora for the plant parasitic fungi, an interesting fungal specimen was collected which on detailed taxonomic observation was found to be a new taxon of species rank. It is described below:

Phaeoramularia asiatica A. N. Rai *et* Kamal sp. nov.

Coloniae hypophyllosae, effusae, fusce olivaceae; mycelium hypharum immersum, tenue, angustum, laeve, septatum et ramosum; stromata bene formata, partimimmersa et partim superficialia, erumpentia, bulbosa, pseudoparenchymatosa, moderate vel fusce olivacea, 23–69 μm dimetro; conidiophori caespitosi, in fasciculis magnis, macronematosi, mononematosi, exiliter septati, septa transverse 3 et interdum plura, non ramosi (simplices), sympodiales, erecti vel suberecti, recti vel flexuosi, interdum geniculati, laeves, subhyalini vel pallide oli-

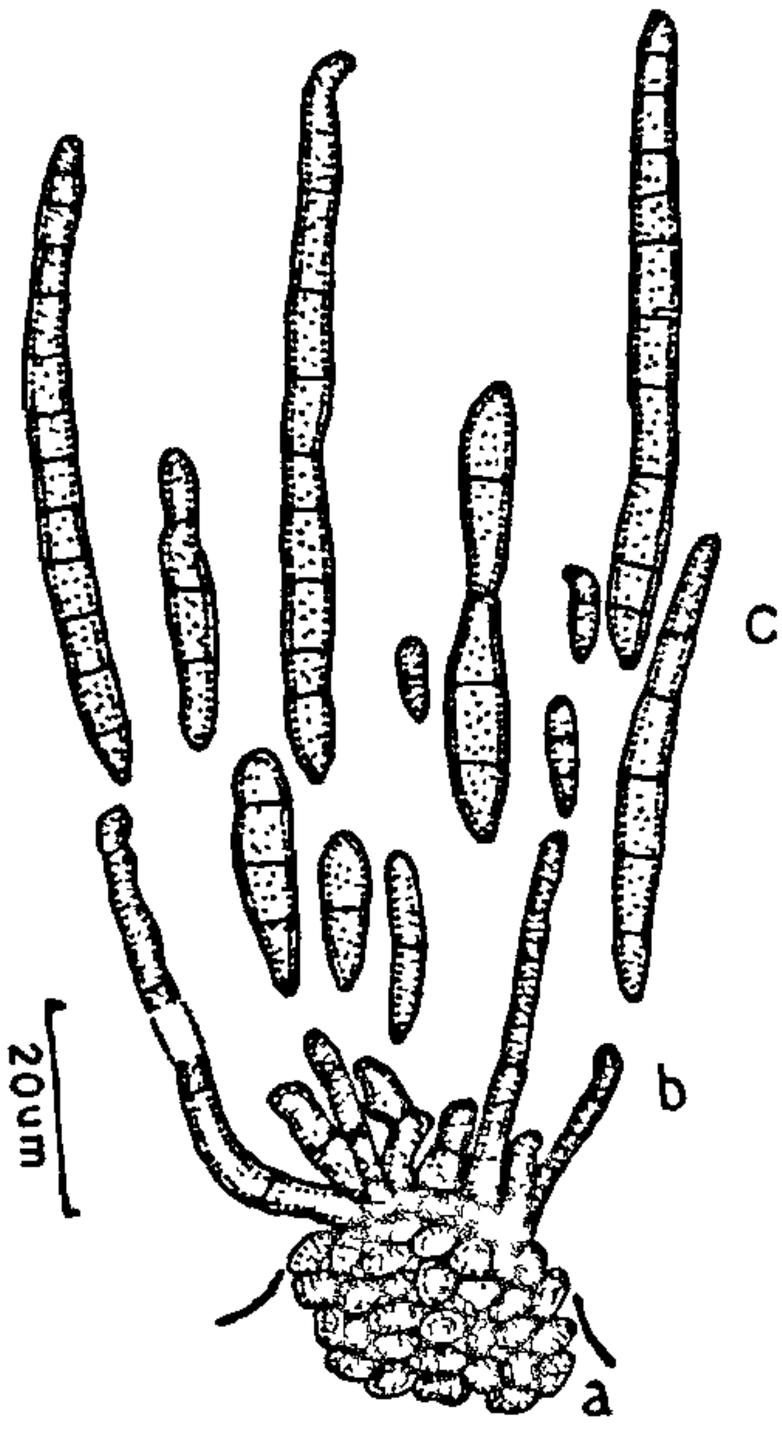

Figure 1. *Phaeoramularia asiatica* A. N. Rai *et* Kamal sp. nov. a–stroma, b–conidiophores, c–conidia.