

meters, simple correlations, partial correlations and regressions between protein content, yield and seed weight were computed.

Significant differences existed between exotic and indigenous cultivars for protein content (table 1). A comparison of the overall mean seed protein of the two groups of cultivars indicated that the exotic types had significantly less protein in seeds than the indigenous cultivars. As shown by the range and the genotypic and phenotypic coefficient of variation, there was substantial amount of genetic variation for improving protein content by selection.

**Table 1** Analysis of variance and variability parameters for seed protein in fababeen.

A. Analysis of variance			
Item	DF	Mean squares	
Cultivars	92	15.3*	
Exotic	83	12.6*	
Indigenous	8	41.3*	
Exotic vs. Indigenous	1	30.5*	
Error	184	1.2	

  

B. Variability parameters			
Parameter	Exotic cultivars	Indigenous cultivars	All cultivars
Mean ( $\pm$ S.E.)	18.1 $\pm$ 0.61	19.5 $\pm$ 0.61	18.3 $\pm$ 0.61
Range	12.3 - 25.6	12.5 - 25.9	12.3 - 25.9
Phenotypic coefficient of variation (%)	11.3	19.50	12.5
Genotypic coefficient of variation (%)	10.8	18.75	11.8

\* Significant at  $P < 0.01$

The first task of legume breeders must be to increase yield and then to improve protein content. In practice, legume breeders have generally reported negative correlations between yield and seed protein<sup>3</sup> and positive correlations between yield and seed weight<sup>4</sup>. In the present investigation, however, the simple and partial correlations, and partial regression coefficients between seed protein, yield and seed weight were insignificant. The results thus suggested that seed yield and seed weight had no role in influencing the protein content of seeds and these traits were independently heritable. Hence, selection for increasing protein content in seeds may not cause any undesirable effect upon seed yield and seed weight. Clearly, in fababeen there is considerable scope for improvement of both

protein and yield by selection because these two characters showed no negative relationship.

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1. Sharma, N. K. and Singh, C. B., *Indian Farming*, 1984, 6, 7.
2. Berthelem, P., *FABIS Newslett.*, 1980, 2, 9.
3. Brim, C. A., *Soybean. Improvement, production and uses*, (ed) B. B Caldwell, Am. Soc. Agron. Inc. Publisher, Madison, USA, 1973, p. 155
4. Yassin, T. E., *J. Agric. Sci. (Camb.)*, 1973, 81, 445

## RESISTANCE OF RICE CALLUS TISSUES TO SODIUM CHLORIDE AND POLYETHYLENE GLYCOL

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CALLUS tissues of higher plants have been used to select numerous variant cell lines<sup>1-2</sup>. The variant cells are usually selected from a population of cells by imposing a particular stress or selection pressure on the population. It may be possible to select mutations for increased salt tolerance and drought resistance at the cellular level using tissue culture. Resistance to certain types of stresses like drought and salt has potentially large agricultural value. Nabors *et al*<sup>3</sup> reported the selection of a sodium chloride tolerant line of tobacco cells. Cells of tomato capable of an enhanced ability to grow in the presence of water stress were obtained by exposure of cultured cells to a medium containing polyethylene glycol (PEG) by Bressan *et al*<sup>4</sup>. The addition of polyethylene glycol to the nutrient medium of cultured plant cells stimulates water stress by acting as a non-penetrating osmotic agent which lowers the water potential of the medium in which the cells are growing. Thus, cells resistant to water stress might be selected from populations by using PEG as the stress agent. We report here the isolation of plant cells resistant to PEG and sodium chloride and the subsequent regeneration of whole plants from such cells.

Callus cultures of rice varieties Jaya and Tellahansa were initiated from the mature embryos on Linsmaier and Skoog's<sup>5</sup> (LS) medium containing 2 mg l of 2,4-

dichlorophenoxyacetic acid (2,4-D), 2% sucrose and with or without PEG and sodium chloride. PEG (mw 6000) and sodium chloride were dissolved in the medium prior to adjustment to final volume. In addition to PEG or sodium chloride, sucrose and salts in the medium influence the water potential and their levels do not remain constant as the cells grow. Therefore, the initial osmotic potentials of the medium containing various amounts of PEG and sodium chloride were measured using a Wescor 5100 C Vapor Pressure Osmometer. Suspension cultures of Tellahamsa were initiated from the callus in the same LS liquid medium containing 2 mg/l 2,4-D and 2% sucrose. Growth measurements were made as a function of increase in fresh and dry weights of rice callus tissues.

Embryo derived callus tissues of Jaya and Tellahamsa cultivars adapted to sodium chloride showed reduced growth when compared with similar tissues maintained without sodium chloride (table 1). These results on growth of rice tissues adapted to sodium chloride agrees with the reports of Bernstein<sup>6</sup> that the major effect of sodium chloride on plant growth is a general stunting of all plant parts. Strogonov<sup>7</sup> favoured the idea that negative salt effects are primarily due to specific ion toxicities, whereas Bernstein<sup>6</sup> identified negative osmotic effects are the probable cause of the sodium chloride inhibition of growth in sodium chloride adapted and non-adapted cells. Suspension cultures of Tellahamsa were initiated from the callus using gyratory shaker (80–100 rev/min) at constant temperature room ( $26 \pm 2^\circ\text{C}$ ) and low light intensity (500 lux). Small clumps of

Tellahamsa cells were plated on LS agar media containing 2.5 and 5% PEG. These tissues were light yellow in colour and healthy as opposed to cells never exposed to PEG (non-adapted cells). Non-adapted cells were on the other hand brown in colour and necrotic. The PEG adapted cells showed better growth than the non-adapted cells (table 1). Low levels of stress (2.5 and 5% PEG) resulted in an increase in both dry and fresh weight gain of PEG adapted cells consistently (table 1) for about 250 days. When the PEG was withdrawn from the medium, the characteristic resistance of the adapted cells was lost immediately and necrosis was observed.

The medium used for control cultures in this study had an osmotic potential of 149 milli osmols before the addition of the osmotica. Cell growth was depressed as the osmotic potential was increased beyond 149 milli osmols. Profound compositional changes occur in the cells as the stress conditions increase<sup>8</sup>. Many investigators<sup>9,10</sup> have hypothesized that organic molecules like proline, hydroxyproline, proline betaine and others found in scores of higher plant cells act as osmoprotectants. They propose that these organic molecules accumulate in plant cells during osmotic stress and prevent damage from cellular dehydration. Though these compounds are not measured in the present investigation such possibility could not be ruled out. The data on regenerating ability of salt and PEG adapted cells are given in table 2. From callus cultures of Jaya, adapted to 1% sodium chloride, plantlets were regenerated on LS medium supplemented with 1 mg/l indole-3-acetic acid + 4 mg/l kinetin with 15–16% frequency. On the other hand callus tissues of Tellahamsa adapted to 1% sodium chloride were grown for 120 days and whole plants were differentiated with 20–25% frequency (table 2). Callus tissues of Tellahamsa variety adapted to 2.5 and 5% PEG produced plantlets with 20–30 and 14–15%

**Table 1** Growth of sodium chloride and PEG adapted and non-adapted (control) rice callus\*

Cultivar and treatment	Osmotic potential of medium (milli osmols)	Fresh weight	Dry weight
		mg/culture	mg culture
Jaya control	149	451.8	68.9
Jaya 1% NaCl	448	389.0	59.2
Tellahamsa control	149	603.7	90.8
Tellahamsa 1% NaCl	448	508.2	70.1
Tellahamsa 2.5% PEG	165	692.5	103.4
Tellahamsa 5% PEG	178	649.7	96.1

\* Data were scored at the end of 30 days from 6 replicate cultures

**Table 2** Plantlet regeneration from callus cultures of rice grown on sodium chloride and PEG\*

Cultivar	Conc. of NaCl or PEG	Age of callus (days)	% regeneration of plantlets
Jaya Control		58	20–22
Jaya	1% NaCl	58	15–16
Tellahamsa Control		250	40–45
Tellahamsa	1% NaCl	80	20–25
Tellahamsa	1% NaCl	120	20–25
Tellahamsa	2.5% PEG	250	25–30
Tellahamsa	5% PEG	250	14–15

\* Data represent an average of 40–50 replicates

frequency (table 2) respectively in the same regenerating medium compared to the control which differentiated with 40–45% frequency. We cannot, however, tell from our results whether selected cells are the result of a true selection of variant cells within the normal population or the result of adaptation of cells to the PEG imposed water stress. The changes in growth rates on PEG containing medium observed between selected and non-selected cells indicate that physiological characteristics of the cells change once exposed to PEG, suggesting the occurrence of an adaptation process. Nabors *et al*<sup>3</sup> found that tobacco cells selected for resistance to sodium chloride gradually increased their growth rate on medium containing sodium chloride. Regenerated plants which are adapted to sodium chloride and PEG have been transferred to the pots and their performance is being studied now.

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1. Scoweroft, W. R., *Adv. Agron.*, 1977, **29**, 39.
2. Croughan, T., Stavarek, S. and Rains, D. W., *Crop Sci.*, 1978, **18**, 959.
3. Nabors, N. W., Daniels, L., Nadolny, L. and Brown, C., *Plant Sci. Lett.*, 1975, **4**, 155.
4. Bressan, R. A., Hasegawa, P. M. and Handa, A. K., *Plant Sci. Lett.*, 1981, **21**, 23.
5. Linsmaier, F. M. and Skoog, F., *Physiol. Plant*, 1965, **8**, 100.
6. Bernstein, L., *Annu. Rev. Plant Pathol.*, 1975, **13**, 295.
7. Stroganov, B. P., *Israel program for scientific translations*, New York, 1964, p. 16.
8. Marezki, A., Thom, M. and Nickell, L. G., *Hawaiian Planters' Record*, 1972, **58**, 183.
9. Heyser, J. M. and Nabors, M. W., *Plant Physiol*, 1981, **67**, 720.
10. Le Rudulier, D., Strom, A. R., Dandekar, A. M., Smith, L. T. and Valentine, R. C., *Science*, 1984, **224**, 1064.

## A NEW MEDIUM FOR MOUNTING MELIOLACEOUS FUNGI

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MELIOLACEOUS fungi are commonly known as 'Black Mildews' and are often erroneously called 'sooty moulds'<sup>1</sup>. These are epiphyllous fungi possessing superficial, deep brown to dark mycelia, globose perithecia and straight to flexuous mycelial or perithecial setae.

Hansford<sup>2</sup>, in his monograph, used characters like the nature of the colony, arrangement of the hyphopodia, setae, etc to distinguish the genera and species of meliolales. To study such characters in their natural condition, various mounting media like necol<sup>3</sup> collodion-acetone drops<sup>2</sup> and quick-fix<sup>4</sup> have been suggested by different mycologists. The present authors found another equally good mountant for Meliolales, Microthyriales, Dematiaceous Hyphomycetes and other epiphyllous dematiaceous fungi, the details of which are discussed here.

Clean and bright-white thermocol (a material used for packing fragile or delicate articles) was cut into small slices (2–3 mm in diameter) and 2.5 g of these slices were added to 10 ml of isobutyl methyl ketone. The thermocol readily dissolved producing vigorous effervescence. The solution was stirred and kept open for a while to eliminate air bubbles. The transparent solution was stored in an airtight bottle.

A thin layer of this solution was applied on selected fungus colonies and was allowed to dry up for about 20–30 min. A thin hyaline 'flip' was then formed with the colonies firmly embedded in it. These flips were removed with a razor. A drop of D.P.X. was put on a clean slide and the flip spread on it. A little of D.P.X. was again added on the flip and a clean cover-glass was placed over it. A gentle pressure on the cover-glass brought out the excess D.P.X. which can be removed after drying.

This solution can be used for taking stomatal impressions of the leaves even without detaching the leaves from the plants.

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1. Stevens, P. L., *Philippine Agric.*, 1931, **19**, 549.