branes, scattering of chromosomes and deterioration of stain are avoided by the presence of lactic acid in the LPO staining fluid as observed by Dyer[4]. This method is particularly suitable for a correct determination of the haploid number of chromosomes in cases of plants where the meiotic number is variable through meiotic abnormalities[1]. It is by far the most convenient method of studying pollen mitosis and determining the gametic number of chromosomes inside the pollen which fail to produce pollen tube.

Thanks are due to Dr C. A. Ninan and the Kerala University for facilities.

9 October 1984

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IMPROVED PRODUCTION OF CALCIUM GLUCONATE BY MUTANTS OF *PENICILLIUM FUNICULOSUM*

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A PROJECT was undertaken to obtain the mutant strains of *Penicillium funiculosum* with a better yield of calcium gluconate than the parent strain isolated[1]. Solution of $NaNO_2$ (2 M) in acetate buffer (pH 4.5) was used as a mutagen.

The parent strain of *P. funiculosum* used in the investigation was selected as the best out of 15 isolates of *Penicillium*. It produced 26.2 g of calcium gluconate from 35 g of glucose per 100 ml of medium. The fermentation medium used was the modified medium[2] and had (g/l); glucose 35, glutamic acid 0.50, KH_2PO_4 0.19, $MgSO_4 \cdot 7H_2O$ 0.05 (pH 6.5). Calcium carbonate (1 g for every 4 g of glucose) sterilized separately, was added to the sterilized medium. To avoid precipitation of calcium gluconate, due to higher concentration of glucose, boric acid at 25 mM level was added to the medium[3–5]. Fifty ml of the medium were taken in 500 ml Erlenmeyer flasks, inoculated with 1 ml of aqueous suspension of spores (10^7/ml) and incubated on a rotary shaker. Calcium gluconate was measured by estimating calcium in the broth by the EDTA titration method[6]. The identity of gluconic acid in the culture filtrate (after removing calcium by ion exchange resin treatment) was confirmed by paper chromatography as well as by co-chromatography using an authentic sample of gluconic acid as standard. Ethanolic solution (0.04%) of bromophenol blue was used as a spraying reagent for the development of spot on the chromatogram.

For the development of high yielding mutants the parent strain of *P. funiculosum* (93 g) was treated with 2 M solution of $NaNO_2$ for 0, 5, 10, 15 and 20 min at 37°C. The method for mutagenesis was essentially that of Calvori and Morpurgo[7] and of Das[8]. Treated spores were washed thoroughly with 0.1 M phosphate buffer (pH 7.2) and finally plated on optimized mineral salt agar medium containing 0.04% alcoholic solution of bromocresol green as an indicator and were incubated for 3–4 days at 30°C. The number of colonies was counted and the acid unitage of the surviving strains was determined to select the high yielding mutants. Acid unitage was calculated by following the formula of Roy and Das[9]

$$\text{acid unitage} = \frac{\text{mycelial zone} + \text{acid zone}}{\text{mycelial zone}}$$

Of the surviving strains, 300 clones having acid unitage greater than the wild type were selected and tested for their ability for calcium gluconate production by growing them in 50 ml liquid medium in 500 ml flasks for 11 days when growth and calcium gluconate were estimated. Among the clones tested the mutant strain N10-287 was found to be the best and produced 29.2 g of calcium gluconate per 100 ml of fermentation broth and this yield amounted to an increase of 11.2% over the parent isolate. From the experimental results it is evident that $NaNO_2$ is an effective mutagen for this fungus and is able to produce mutants with improved production of calcium gluconate.

The authors thank CSIR, New Delhi for financial assistance to S. K. Mandal.

19 September 1984; Revised 14 November 1984

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SEQUENCE OF SENESCENCE IN SORGHUM SEED DURING ACCELERATED AGEING

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AGEING is an inevitable and irreversible process in any living being and seed is no exception. Roberts[1] considers it reasonable to suppose that there could be a group of key cells most of which have to remain functional if the seed is to retain germinability. So long as the cells concerned with the assimilation and transportation of food and the meristematic regions are alive, the seed remains viable and capable of germination. Banerjee[2] reported the mesocotyl region including the root and shoot meristems to be the most vital and 'key tissue' in the seeds of onion and barley.

An experiment was conducted with CSH 5 hybrid sorghum seeds to monitor the sequence of senescence during ageing. Two hundred grams of seeds retained by 9/64″ round hole sieve were kept in the accelerated ageing cabinet maintained at 40 ± 1°C and 95% relative humidity. Samples of seeds were drawn every two days for 16 days and tested for viability by the topographical tetrazolium test. The germinability of aged seeds was simultaneously assessed by the ISTA method[3].

Frequency score of the seeds with tissues unstained after tetrazolium colour development along with the percentage germination is presented in table 1. The topographical tetrazolium test conducted on the progressively aged seeds showed increasing deterioration in the form of more unstained tissues. When the sequence was observed, it was the tip of the scutellum which showed the first sign of deterioration closely followed by scutellum bottom and aleurone layer. Coleorhiza, root apex, coleoptile and shoot apex were the next parts of the embryo to exhibit the sign of deterioration. Middle portion of the scutellum and the mesocotyl region were the last to senesce (figure 1).

A close association between the frequency of seeds showing the deterioration of scutellum tip (column No. 6 + 11 of the table 1) and the percentage of seeds

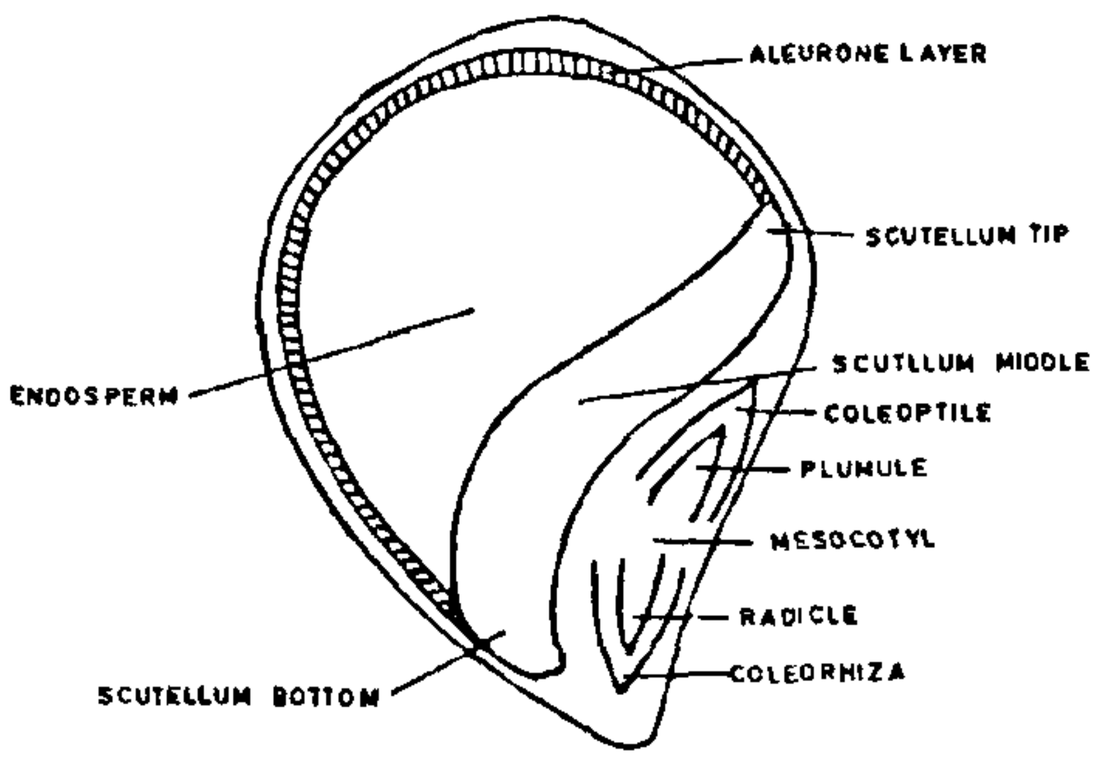

Figure 1. L.S. of Sorghum seed

Table 1 *Frequency score of the seeds aged acceleratedly with tissues unstained after tetrazolium colour development (out of 100 seeds) and percentage germination.*

Ageing (days) 1	Coleoptile 2	Shoot apex 3	Coleo-rhiza 4	Root apex 5	Scutellum tip 6	Scutellum middle 7	Scutellum bottom 8	Meso cotyl 9	Alerone layer 10	Full seed 11	Germination percentage 12
0	4	4	6	2	22	4	34	0	14	4	79
2	11	3	28	9	50	13	64	8	42	3	62
4	12	4	17	7	43	7	50	4	43	7	57
6	10	4	12	4	45	1	40	4	47	8	52
8	14	9	16	4	37	2	39	2	61	25	48
10	12	7	9	4	42	3	29	1	57	39	29
12	7	6	10	4	13	2	7	2	27	65	15
14	5	7	12	5	10	3	9	0	22	78	3
16	5	5	7	5	5	0	5	5	10	90	0