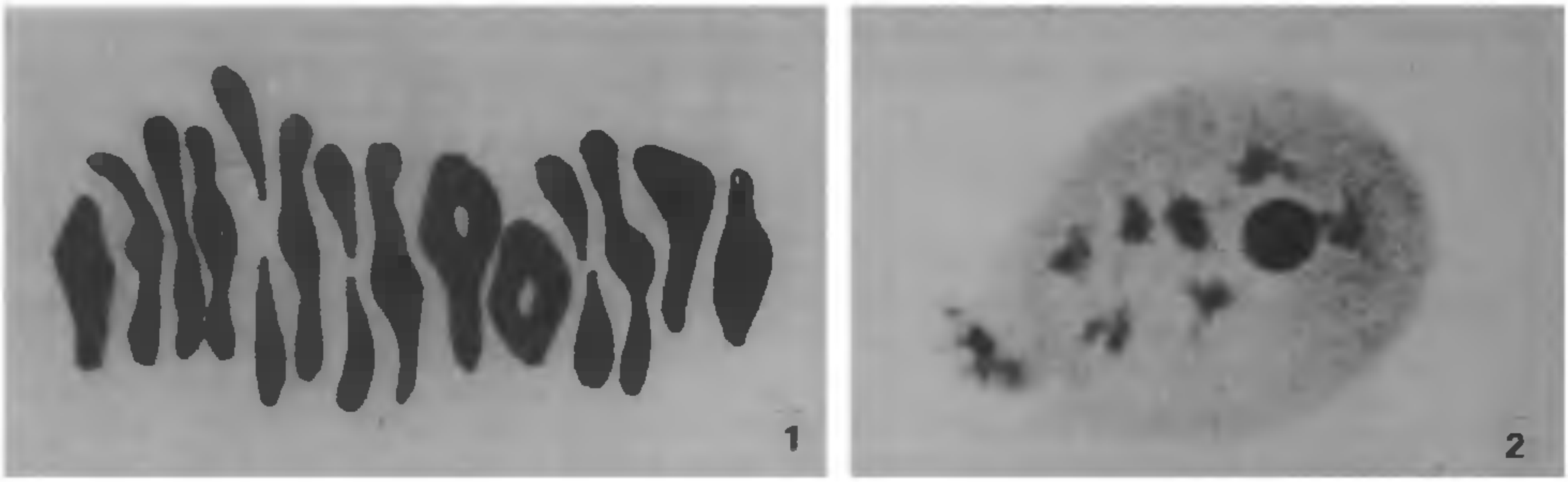

Figures 1 and 2. Chromosomes of *S. grandiflora*. **1**, PMC showing 14 bivalents at metaphase I (×2640). **2**, PMC showing 7 bivalents at diakinesis (×1260).

altitude. For the present studies, material was collected from different locations. Young floral buds were fixed in Carnoy's fluid for 24 h and then transferred to 90% ethyl alcohol. Meiotic preparations were made usually by squashing the developing anthers in 1% acetocarmine. Vouchers are preserved in the Herbarium, Department of Bio-Sciences, HPU, Shimla.

Cytological investigations revealed that meiosis was perfectly normal in all the individuals studied. A chromosome number of 14 bivalents at diakinesis and metaphase-I (figure 1) was obtained for the material collected from Shimla (2350 m), Theog (2350 m) and Shilaru (2500 m), while 7 bivalents were recorded at diakinesis (figure 2) and metaphase-I for the material collected from Baghi (2800 m). Normal bivalent formation with equal segregation of chromosomes has been observed in both the cytotypes (diploid and tetraploid). The average pollen fertility for the diploid is 94% and for the tetraploid 97%. There is no significant difference in the morphology and size of the pollen grains between the cytotypes.

The present count of $n=14$ agrees with the previous report[2,3] of $2n=28$. The diploid cytotype with $n=7$ has been reported for the first time from India. However, its existence in Nepal has already been reported[4].

The diploid plant is short compared to the tetraploid plants. By and large, the diploid cytotype can be distinguished in the filed by its large but thin and light-coloured leaves compared to the tetraploid cytotype.

Of the three species studied so far, all are tetraploid with $n=14$. The intraspecific diploid with $n=7$ has been recorded for *S. grandiflora* only. So the genus seems to be monobasic with $x=7$ as the base number.

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PLANT REGENERATION FROM POLYETHYLENEGLYCOL ADAPTED CALLUS OF RICE

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THE use of *in vitro* techniques akin to the selection of variants in bacterial and fungal cultures has been proposed and employed in very few higher plants[1-3]. Plant cell culture methods allow selection of variants, such as those resistant to water stress[4,5], which would have practical application in agriculture.

The present communication deals with the successful isolation of rice calli adapted to 10, 20, 30 and 50 g of polyethyleneglycol (PEG, mol. wt 6000) and subsequent regeneration of plants. PEG induces water stress in the medium by acting as a non-penetrating osmotic agent that lowers the water potential of the medium. Callus cultures of *Oryza sativa* L. cvs. Tellahamsa and Sureka (susceptible to water stress) were initiated from mature embryos on Linsmaier and Skoog's[6] (LS) medium containing 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 2% sucrose. All the cultures were incubated under continuous, cool white fluorescent light (2000 lux) at 26±2°C. Callus cultures were maintained on the same medium for six subcultures of 25-day duration (control). Callus tissues of both cultivars were then transferred to LS medium supplemented with 2 mg/l 2,4-D, 2% sucrose and different concentrations of PEG (10, 20, 30 and 50 g/l).

Healthy callus masses were isolated and sub-cultured on the respective media for six passages of 25 days each. Callus cultures of cv. Sureka growing on LS medium without PEG (control) and on 20 g/l of PEG are shown in figures 1A and B. Calli growing on different concentrations of PEG appeared healthy and yellowish compared to control (without PEG) calli, which were slightly brown. Regenerating ability of all the callus cultures was tested (figure 2) on LS basal medium containing 1 mg/l indole-3-acetic acid, 4 mg/l kinetin, 10 g/l PEG and 2% sucrose. Callus cultures of both cultivars developed green spots during the first 20 days of culture on the

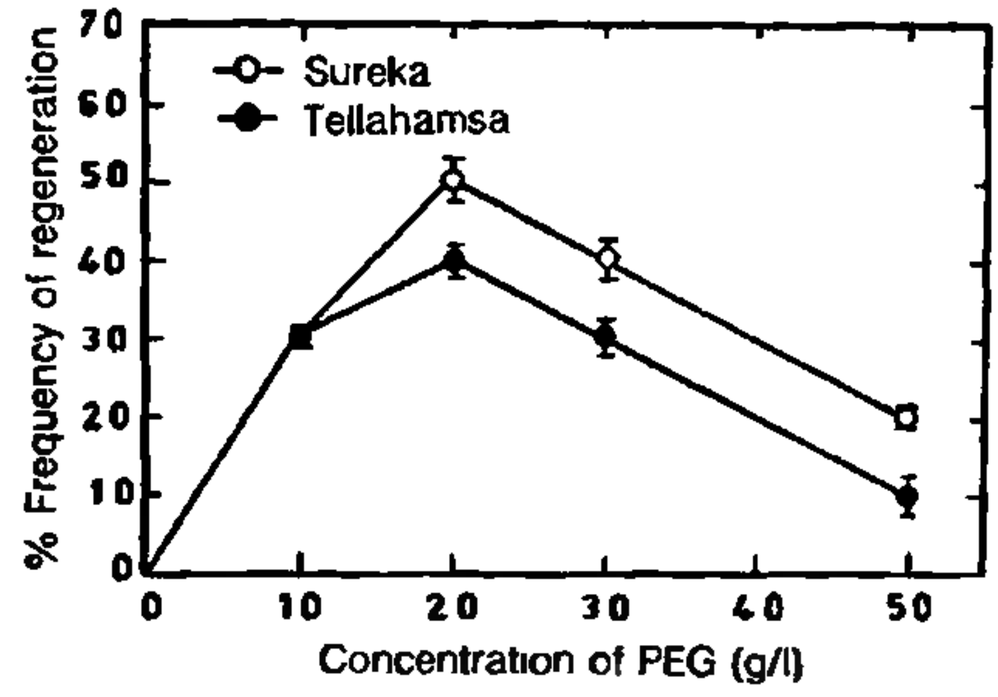

Figure 2. Regenerating ability of rice cultivar Sureka (○) and Tellahamsa (●) callus cultures adapted to different concentrations of PEG. Data are average of 10 replicates ±SE.

LS basal medium. A second passage on the same medium but without PEG was necessary for successful whole-plant regeneration from these tissues.

Callus cultures of both cultivars maintained on medium without PEG (control) however failed to give any organogenetic response after 12 passages (300 days). Plantlet regeneration from a 20 g/l PEG-adapted callus of cv. Sureka is shown in figure 3.

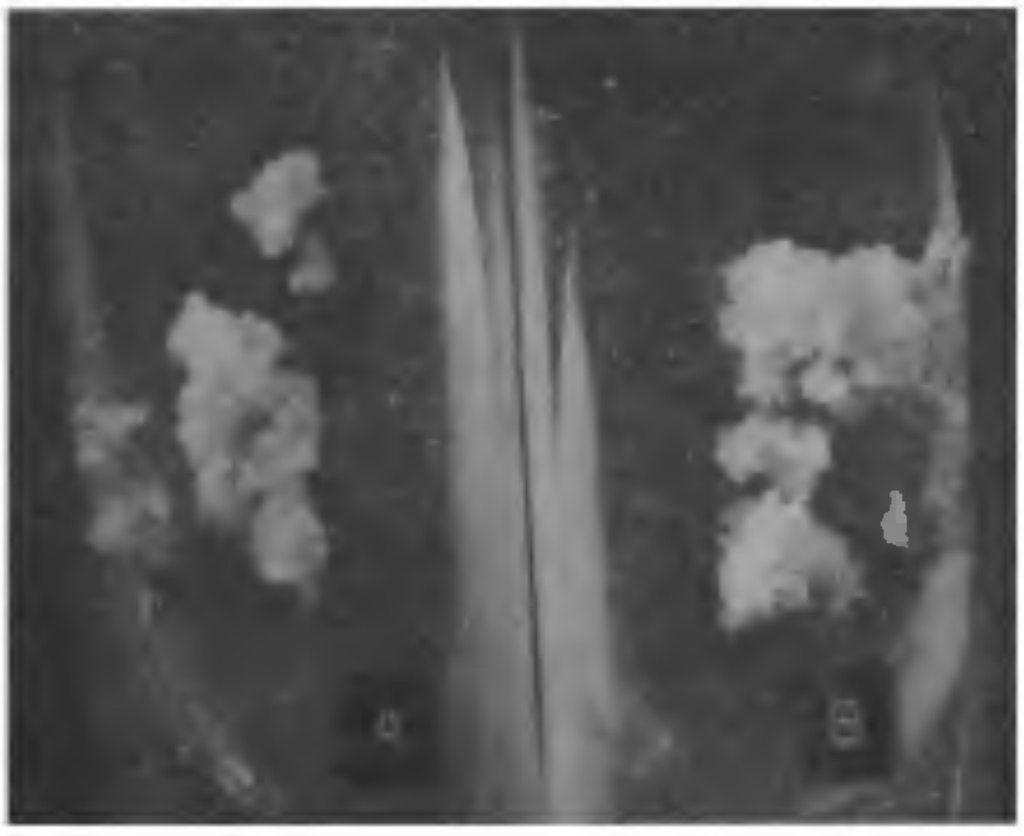

Figure 1A, B. Callus of rice cultivar Sureka on LS medium (A) without PEG (control), and (B) containing 20 g/l PEG.

Figure 3. Regeneration of plantlets from 20 g/l PEG-adapted callus of rice cultivar Sureka.

Regenerating ability of the calli was not very significant (figure 2) in the two cultivars used. The frequency of plant regeneration was lowest in 50 g/l PEG-adapted calli of both cultivars. The plants were transferred successfully to pots for further evaluation. Sievert and Hildebrandt[7] observed variations among tobacco cells for their ability to grow on different carbon sources. Clones that vary in their ability to produce anthocyanin[8] and tropane alkaloids[9], and to grow in the absence of plant growth regulators[10] have been isolated earlier. This shows that within a population of cells, individual cells with different phenotytic characteristics or with altered metabolism also exist. The change in colour of the PEG-grown and calli of rice cultivars may suggest the occurrence of an adaptation process or an epigenetic phenomenon in the cells.

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PROTECTION OF MUNG BEAN SEEDLINGS AGAINST HEAT SHOCK BY A SUBSTITUTED PHTHALIMIDE

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ESSENTIALLY all crops are adversely affected by high temperatures, especially during critical stages of plant development. Where this occurs, it is important that crops develop a certain degree of thermotolerance to cope with the temperature assault. Our knowledge about acquisition of thermotolerance in plants by chemical growth regulators is extremely limited. Exogenously applied gibberellic acid (GA_3) has been shown to enhance thermotolerance of mung bean seedlings[1]. A recently synthesized group of substituted phthalimides has been shown to mimick GA effects, but it is not known whether these compounds could also show a protective response against heat shock. The present study has investigated the effect of a substituted phthalimide AC 94,377 on the heat shock response of etiolated mung bean seedlings.

Etiolated 48-hour-old mung bean seedlings (*Vigna radiata* L. Wilczek. var. ML-131) with an axis length of 2 cm, grown at 28°C, were incubated in 1 mM phosphate buffer, pH 6.0, containing 1% sucrose at appropriate temperatures. In order to study the effect of the substituted phthalimide AC 94,377 (1-(3-chlorophthalimido)-cyclohexanecarboxamide), it was added to phosphate buffer and either applied to seedlings as a pretreatment at 28°C (2 h) or 40°C (2 h), or its application was continued during exposure to the heat-shock temperature of 45°C (3 h). A concentration of 100 μm was found to be the best. After the treatment the seedlings were grown on water in germination paper rolls at 28°C in the dark for 72 h. Length of whole seedlings (root plus hypocotyl), hypocotyls and primary root were then measured.

Compared with the growth of normal seedlings grown entirely at 28°C, the growth of seedlings given a 3-h 45°C treatment was severely inhibited (table 1). Hypocotyl growth was inhibited by 58% and primary root growth by 52%. After a pretreatment at 40°C for 2 h, the seedlings became more thermotolerant. Compared with the heat-shocked seedlings, in pretreated seedlings hypocotyl growth was enhanced by 57% and root growth by 83%. Whe