12 hr of exposure in both tetraploid and hexaploid wheats. On the other hand, under stress conditions the two genotypes behaved differently. Tetraploid wheat accumulated $^{44}$Ca in its root and shoot significantly as compared with the control after 5 minutes of exposure and this continued to rise till 6th hour. After 12 hr the radioactivity continued to decline and at the end of 24 hr the accumulation of $^{44}$Ca in the shoot was found to be only 50-70% of that of the control plant. However, in root, the drop in radioactivity accumulation was noted in the range of 15-25% only. In marked contrast to this in hexaploid, the accumulation of $^{44}$Ca in root and shoot did not alter significantly below that of control plants till the termination of stress exposure. These results indicate that root membrane integrity and permeability are very much distorted in tetraploid under salinity stress whereas in hexaploid the effect was noted to be minimal. Waisel also reported least disturbance in root permeability in salinity resistant halophytes. From the results of this experiment, it is evident, that in hexaploid wheat cv Kharcha the maintenance of root permeability is one of the physiological basis for salt resistance.

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**PATCHOULY PLANTS DIFFERENTIATED IN VITRO FROM STEM TIP AND CALLUS CULTURES**

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The Patchouli plant (*Pogostemon cablin* Benth. syn. *P. pancho il* Pellet var. *sweiti* Hook (Family: Labiatae) is cultivated for the Patchouli oil of commerce. The oil is almost a perfume by itself and it is indispensible in cosmetics, soaps and incense. The Patchouli plant is invariably affected by mosaic disease and the vegetative propagation of the plant promotes the spread of the virus disease. Since apical meristem culture offers an efficient and reliable method for eliminating systemic viral infections$^{2-5}$ it will be advantageous to obtain disease-free plants from infected parent stock by culturing the shoot-tips. This communication describes the *in vitro* method of virus elimination and propagation of healthy Patchouli plants/clores from shoot-tips and callus cultures.

Stem cuttings of Patchouli plants showing virus infection with three nodes were planted in pots and the upper cut ends sealed with paraffin wax. The pots were maintained in a green house and the cuttings sprouted within 6-8 days. Shoot-tips measuring approximately 0.5-1 mm in length were aerobically dissected and cultured in test tubes (7.5 x 2.5 cm) containing 20 ml of the following sterile media solidified with 0.7% Difco Bacto agar; Murashige and Skoog (MS)$^a$, B5$^b$, Eriksson (ER)$^c$ and Halperin.$^d$ The media were supplemented with 2,4-D (1 mg/l), 6-benzyladenine (0.5 mg/l) and indole 3-acetic acid.

**Fig. 1.** Four week old culture showing multiple shoot development of *Pogostemon cablin* in Halperin medium.
(1 mg/l). The pH was adjusted to 5.8 before autoclaving. The tubes containing the shoot-tips were incubated in a growth chamber with 16 hr photoperiod at a light intensity of 30,000 lux (provided by cool white fluorescent lamps) 26°C and 70% relative humidity.

The growth response and morphogenesis were found to vary in different culture media. In MS, B5 and ER media, the growth response was more or less similar with low incidence of callus formation at cut ends of explants (10-15%). In Halperin medium the callus formation was high with multiple shoot production within 20-25 days (60-65%). However, the root development was poor (Fig. 1). The callus tissues developed on other media also when transferred to Halperin medium differentiated into shoots. On further subculturing in Halperin medium with high concentration of 6-benzyladenine (1-2 mg/l) or 6-furfurylamino uracil (2-5 mg/l), there was a high proliferation of shoots (Fig. 2). In about 35-40 days the total number of multiple shoots from a single shoot-tip was as high as 60-80. Shootings about 3 cm long and carrying three pairs of leaves were removed from 6-8 week old cultures and placed aseptically on filter paper bridges\(^{10}\) dipped in White’s\(^{6}\) liquid medium containing a-naphthaleneacetic acid (2 mg/l) for rooting. The plants developed profuse roots in about 10-15 days. The whole plants were transferred to pots containing sterilized soil mix and kept under diffused sunlight in the laboratory at 28°C for a week. The plantlets rapidly developed into healthy plants. The plants obtained by in vitro culture appeared morphologically true to type at maturity with square stems bearing opposite, ovate leaves with serrate margins and acuminate tips (Fig. 3). The plants were healthy and no virus symptoms were noticed in any of the plants. ‘Plant tests’ made for *Pogostemon* virus\(^{11}\) with extracts of the leaves of the in vitro developed plants from shoot-tips and callus gave a negative reaction on *Nicotiana glutinosa* L., showing the elimination of virus in these plants.

The totipotency of plant cells is well established in many species\(^{12}\) and the present report shows that cells of *Pogostemon cablin* Benth. have high regenerative capacity. The study offers a method for obtaining ‘virus tested’ plants directly from shoot-tips and callus cultures of virus infected parent plants. The results also might open up the possibility of rapid propagation of specific genotypes with high essential oil content.

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**Fig. 2.** Proliferation of shoots of *P. cablin* in Halperin medium with high concentration of cytokinin three weeks after subculture.

**Fig. 3.** Plant regenerated *in vitro* growing in pot (3 months old).
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CHROMOSOME NUMBERS IN THE GENUS PIPER

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The genus Piper includes several economically important species like P. nigrum, P. betle, P. longum, etc. Despite this, cytological information available on the genus is confined to reports of chromosome numbers in P. betle, P. nigrum, P. longum, P. geniculatum, and the chromosomal numbers and morphology of a few cultivated and wild varieties of P. nigrum, P. longum, P. betle and an undetermined species.

From an island wide collection of 69 accessions of germplasm of Piper maintained at the Minor Export Crops Research Station, Matale, 30 collections were studied for their chromosome number using root tip squashes in acetocarmine. The results are reported here.

The data given in Table I presents interesting discussion. Chromosome numbers 2n = 26, 39 and 65 (Figs. 1-3) are reported for the first time in the genus Piper. The 2n chromosome numbers of P. nigrum (52) has been confirmed. The chromosome numbers of P. zeylanicum, P. argyrophyllum, P. attenuatum, P. thwaitsei, P. chuuya, P. sylvestre and P. triburon are the first reports for these species. The 2n numbers of 26 and 52 presently observed for P. longum and P. betle, respectively, are at variance with the earlier reports of 2n = 52 and 78 for these two species.

Based on the earlier reports of the lowest haploid number of 26 for this genus, it had been hypothesised that the 52 chromosome of P. nigrum are diploids, P. betle (2n = 78) triploid and the unidentified species of Piper with 2n = 104 tetraploid. The 2n = 26, 39, 52, 65 and 78 reported in the present study clearly demonstrates that the basic number of the genus Piper is x = 13. In the light of this, 52 chromosome of P. nigrum is tetraploid. However, considering the 2n number 24, 43, 64 and 96 (Table I) reported earlier, the possibility of another basic number x = 12 existing in the genus through dysploid changes cannot be ruled out. The chromosome numbers of 26 and 52 for P. longum, 39 and 65 for P. thwaitsei, 52 and 78 for P. betle, and 52, 78 and 104 for P. nigrum are so far appear to suggest the presence of polyploid races or cytotypes at the intraspecific level.

Figs. 1-3. Mitosis in three species of Piper, Fig. 1. P. argyrophyllum, 2n = 26; Fig. 2. P. zeylanicum, 2n = 39; Fig. 3. P. thwaitsei, 2n = 65, × 3,000.