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.

One of the authors (GS) thanks UGC, New Delhi, for financial assistance.

17 October 1988; Revised 20 January 1989

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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

Table 1 *Effects of temperature and a substituted phthalimide AC 94,377 on acquisition of thermotolerance in mung bean seedlings*

Treatment	Hypocotyl length (mm)	Primary root length (mm)	Whole seedling length (mm)
28°C (5 h)	95^a	75^a	170^a
28°C (2 h), 45°C (3 h)	40^d	36^e	76^f
40°C (2 h), 45°C (3 h)	63^c	66^b	129^d
40°C + Phthalimide (2 h), 45°C (3 h)	66^c	64^b	130^d
40°C + Phthalimide (2 h), 45°C + phthalimide (3 h)	83^b	68^b	151^b
28°C + Phthalimide (2 h), 45°C (3 h)	65^c	50^d	115^e
28°C + Phthalimide (2 h), 45°C + phthalimide (3 h)	80^b	60^c	140^c

Values are means of 20 readings. Means within the same column with the same letter in the superscript are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

applied during the 40°C pretreatment, phthalimide did not significantly enhance the thermotolerance response; however, hypocotyl growth was further enhanced when its application was continued during the heat-shock period. When the phthalimide treatment was given at 28°C, statistically similar increases were observed in respect of hypocotyl growth, but increase in root growth was less. The application of phthalimide to seedlings grown entirely at 28°C had a rather small growth-promoting effect.

Thus, a prior treatment of seedlings at an elevated temperature within a permissive range (40°C, 2 h) imparted protection to seedlings against heat-shock stress. Similar observations have been made by other workers[1,2]. The presence of phthalimide during the 40°C pretreatment and the heat-shock period enhanced the protective response, as has been observed[1] for GA_3. Hence AC 94,377 is capable of mimicking the heat-shock response induced by GA_3. In the present study, the phthalimide protection against heat-shock was provided by pretreatment not only at 40°C but also at the normal temperature of 28°C. In addition, both hypocotyl and root appear to be the site of the phthalimide effect, whereas in the case of GA_3 it is mainly the hypocotyl[1].

Application of phthalimide warrants further investigation to understand the mechanisms through which it triggers a protective response.

Thanks are due to Dr A. D. Pavlista of the American Cynamid Co. for a gift of AC 94,377.

24 October 1988; Revised 30 December 1988

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COLORIMETRIC METHOD FOR THE ESTIMATION OF *p*-COUMARIC ACID FROM THE BARK OF *OROXYLUM INDICUM* VENT

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p-COUMARIC acid has been estimated colorimetrically using ammonium vanadate–perchloric acid reagent[1], by reverse-phase high-pressure liquid chromatography[2], spectrophotometrically using 4-hydroxybenzaldehyde[3], and by high-pressure TLC with UV and fluorescence[4]. *p*-Coumaric acid from sunflower seeds was determined by titration with $KMnO_4$(ref. 5). This method was rapid and simple, and gave good results.

p-Coumaric acid was extracted from the bark of *Oroxylum indicum* (Bignoniaceae) according to Subramaniyan and Nair[6]. It was further purified by preparative TLC using silica gel GF 254 as adsorbent and benzene:dioxane:acetic acid (90:25:4)[7] as solvent. The prominent spot obtained at R_f 0.45 was scraped out and the compound so obtained was recrystallized from methanol. The compound was confirmed as *p*-coumaric acid from its m.p., R_f and IR spectrum[8].

A more sensitive colorimetric method was established by reacting *p*-coumaric acid with molybdophosphoric acid in alkaline medium. A reference solution was prepared by dissolving 10 mg of *p*-coumaric acid in 100 ml of ethyl alcohol. To prepare