less similar and did not show very significant differences either in susceptibe or in resistant host and even weakly virulent isolates Ss-2 showed fairly good degree of activity which was higher than that of highy virulent isolate Ss–3. In the susceptible host, all the 4 isolates showed considerably good degree of activity from the 5th day and only slight increase in activity was observed during further observations which suggest that this enzyme might be playing a secondary role in concert with other enzymes at various stages of pathogenesis. In case of resistant cultivar enzyme activity was lower than that of susceptible ones and the maximum activity was observed on 10th day after which a decrease in activity was recorded (Table I). Mahadevan and Chandramohan[4] have also reported a lower protease activity in resistant plants as compared to the susceptible ones in a wilt disease of cotton caused by *Fusarium oxygsporum*. Khare and Bompeix[5] have shown a high protease activity of *S. sclerotiorum in vitro* and *in vivo* and have found it to be related with the pathogenicity. These authors have also reported that this fungus can reduce the pH of the medium of the infected tissue to the value most favourable for the activity of their own proteases. In the case of the present disease also, there was a fall in the pH of the infected tissue with the advancement of the disease which might be favouring the activity of protease. From the findings of the present study it appears that in this disease, the protease possibly causes the tissue maceration by degrading the structural wall protein and thus facilitating the spread and development of the pathogen.

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BARLEY X-RYE HYBRIDS (*HORDECALE*) THROUGH EMBRYO CULTURE

INTERNATIONAL efforts are being made not only to improve the existing cultivars of various cereals, but to synthesize new ones[1] which are high-yielding, disease-resistant, and rich in nutritional quality. In this connection, various *in vitro* techniques[2,3] would facilitate the cereal improvement programmes involving wide hybridization.

The present investigation is an attempt to hybridize barley (*Hordeum vulgare*) × rye (*Secale cereale*), with a view to exploring the possibility of incorporating the relatively high contents of protein and lysine into the grain, and resistance of rye to yellow rust into barley. In nature, such a cross is incompatible because of an early abortion of the endosperm and the embryo. However, by spraying the spikes with growth-regulators, combined with the culturing of young embryos, this incompatibility can be overcome[4–6].

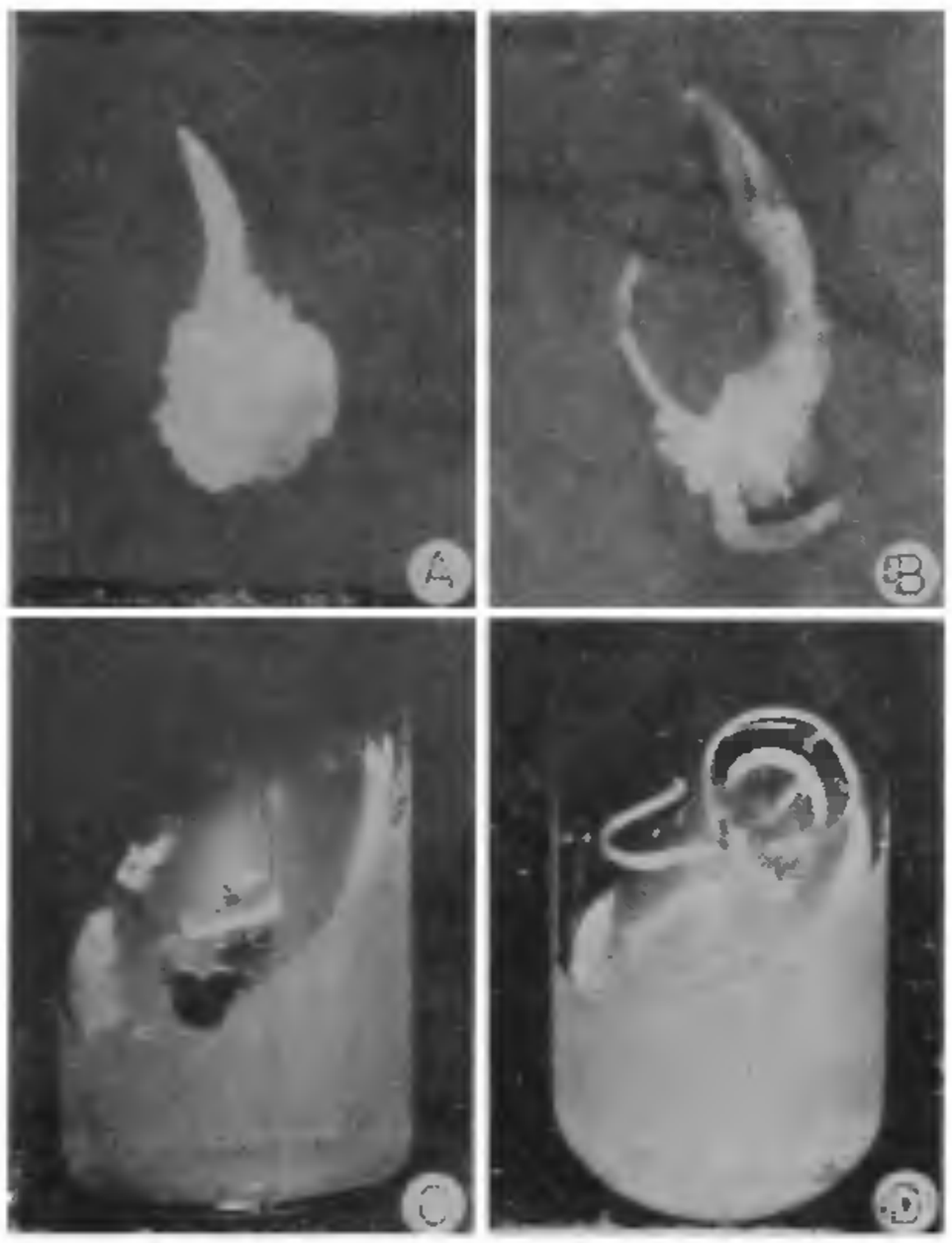
Six lines of field-grown barley were fertilized with pollen from nine strains of rye and about 5,000 pollinations involving 250 spikes were carried out during February–April 1978 and 1979. The pollinated spikes were treated with the solution of a mixture of various concentrations (25–100 mg/l) and combinations of gibberellic acid (GA) and kinetin (0·1–1 mg/l) by using two methods : (i) by pouring the solution on the spike twice a day, and (ii) by wrapping up the spike with a wad of wet cotton. In some cases, occasionally one or two florets developed further. Seven to nine days after pollination, young embryos were dissected out from such florets and cultured on various media. All manipulations were carried out aseptically in a Laminar Flow Chamber (Klenzaids, Bombay).

Bathing the spikes with a solution of GA (25 mg/l) + kinetin (0·5 mg/l) proved to be best, as suchspikes retained their green colour, whereas the wrapping up with cotton led to fungal infection and rotting. The control florets turned yellow within 3–4 days, whereas the treated ones showed streaks of green coloration, and an occasional development of grains. The frequency of the abortion of the embryos increased with the rise in temperature in April.

The hybrid embryos were cultured on modified Murashige and Skoog's medium (MS)[7]. On MS + 2, 4–D (1 mg/l) the embryo swelled and showed enlargement in general, within a week of culturing, and proliferated to form a mass of callus (Fig. A), with a rudimentary shoot. The callus grew at the expense of the shoot.

On MS + casein hydrolysate (500 mg/l) + IAA (1 mg/l) + kinetin (0·5 mg/l), there was a tendency towards normal regeneration of the plant (Fig. B). Increasing the level of auxin resulted in more of

callusing at the basal end (Fig. C). Plantlets produced were mostly lean and weak (Fig. D). The growth response was genotypically oriented[8]. Of all the different combinations of various lines, the cv. Aohar of barley gave the best response, whereas the source of the pollen did not matter.



FIGS. A–D. *In vitro* growth of *Hordecale* embryos on synthetic media. Fig. A. 3-week-old culture of an excised embryo (9 days after pollination) on MS + 2, 4–D (2 mg/l) : note the profuse callusing. Fig. B. 3-week-old culture on MS + CH + IAA + kinetin showing sparse proliferation and initiation of roots. Figs. C. D. Three and four-week-old hybrid plantlets.

The *Hordecale*, thus produced, will be employed as a bridge for increasing the genetic variability in barley. In addition to these hybrids, some haploids would be expected[9] and they can be incorporated into the barley improvement programme.

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EFFECT OF FAT SOLUBLE VITAMINS ON GROWTH AND SPORULATION OF *PESTALOTIOPSIS*

FUNGAL growth and sporulation is markedly affected by vitamins[1–3]. However, reports on the utilisation of fat-soluble vitamins by fungi are scarce. Therefore, such studies were undertaken on *Pestalotiopsis versicolor* (Speg.) Steyaert, isolated from the leaves of *Gnetum gnemon* L. and *Nepenthes khasiana* Hook., f. and *Pestalotiopsis theae* (Saw.) Steyaert var. *minor* from the leaves of *Callistemon lanceolatus* DC.

Pure isolates were cutlured on vitamin-free Asthana and Hawker's medium. The pH of the medtum was adjusted at 5·5. Heat sensitive vitamins A, K and E were added to the medium after autoclaving. The liquid medium (50 ml.) was taken in 250 ml conical flasks and vitamins (50 ppm each) were added to the flasks. A mixture of 0·5 ml benzene and 0·5 ml of 80% ethanol was used for dissolving the vitamins. Each flask was inoculated with 1 ml of spore suspension at 22° C ± 2° C for ten days and the mycelial mats were filtered through Whatman Filter paper No. 42, washed and dried at 60° C for 48 hours. The average of three replicates were recorded. Sporulation was measured as suggested by Bilgrami and Verma[3].

Maximum mycelial dry weight (210 mg) of *Callistemon* isolate (*P. theae* var. *minor*, IMI No. 226396) was recorded when vitamin D–3 was furnished whereas vitamins A and E were favoured most by both the isolates of *P. versicolor*, *i.e.*, IMI Nos. 226392 (from *Gnetum*) and 226376 (from *Nepenthes*) and the mycelial weights were 330 mg and 400 mg respectively. Growth of *Callistemon*-isolate was retarded a little in the presence of all the vitamns as compared to that of control. However, sporulation on all the meda was generally excellent except in *Callistemon*-isolate under the effect of vitamin E.