collected from *Phytophthora*-infested *Colocasia* fields in Hyderabad. Sufficient sterile water was added to keep the soil moist. The plates were incubated for one week at 15° C ± 2° C in a cold room. After the incubation the root bits were removed and washed thoroughly with sterile water for several times and blotted dry. Then the root bits were plated on oat meal agar medium aseptically. After 4 days white fluffy growth of *P. palmiovora* was observed and the fungus was transferred to oat meal agar slants.

The present root baiting technique has given promising and more reproducible results than any other baits tried and would be useful in survival studies of *P. palmivora* in soils.

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PRODUCTION OF PROTEASE BY *SCLEROTINIA SCLEROTIORUM* CAUSE OF WHITE ROT OF *BRASSICA JUNCEA*

WHITE ROT caused by *Sclerotinia sclerotiorum* is a very destructive disease and causes heavy economic losses to *Brassica juncea* crop in north-eastern India[1]. During the course of the physiopathological studies, certain aspects of the pathogenesis were investigated and the present paper deals with the production of enzyme protease in susceptible and resistant cultivars at different stages of pathogenesis.

Experimental

Four isolates belonging to 4 different groups, *viz.*, "Most highly", "Highly", "Moderately" and "Weakly" virulent isolates and cultivars of *B. juncea* showing maximum and minimum disease reactions against these pathogens. Preflowering plants of both the varieties were inoculated by agar disc method of Rai and Dhawan[2]. Infected plant parts were harvested after 5, 10 and 15 days of inoculation. Samples were homogenized with 0·1 M phosphate buffer (pH 7·0) in a ratio of 1 : 5 (w/v), strained and centrifuged at 6000 rpm for 15 minutes, supernatants were used as crude enzyme preparation.

Enzyme assay : Proteolytic activity was determined by the modified method of Davis and Smith[3]. Reaction mixture consisted of 2 volumes of 1% casein in 0·1 M phosphate buffer (pH 7), 1 volume of the same buffer and 1 volume of the enzyme preparation was incubated at 30° C. Aliquots of 1·0 ml of reaction mixture were withdrawn after 2 hrs and immediately treated with 2 ml of ninhydrin reagent which stopped the reaction. Tubes were kept in boiling water bath for 20 minutes to develop the colour and were cooled to room temperature. Final volume was made up to 50 ml with 50% *n*-propenol and the colour intensity measured at 570 mμ. The enzyme activity was expressed as nanomoles of α-amino nitrogen released at 30° C per ml of reaction mixture in the specified time of reaction.

Results and Discussion

During this study all the isolates of *S. sclerotiorum* were found to produce significant amount of protease in both the host varieities (Table I). It was more or

TABLE I

Protease activity (nanomoles of aminonitrogen released/ml of reaction mixture/2 hr) in the tissue extract of susceptible and resistant* B. juncea *plants after 5, 10 and 15 days after inoculation with 4 different isolates of* S. sclerotiorum

Isolates of *S. sclerotiorum*	Days after inoculation	Susceptible host tissue	Resistant host tissue
Ss-1 (MHV)	5	1201·97	1156·62
	10	1220·81	1173·62
	15	1245·91	1154·88
Ss-2 (WE)	5	1151·73	1082·73
	10	1161·11	1117·25
	15	1201·97	1054·88
Ss-3 (HV)	5	1085·86	1011·20
	10	1114·10	1098·41
	15	1139·21	950·91
Ss-4 (MV)	5	1180·01	1121·95
	10	1189·43	1132·93
	15	1220·71	1062·22

MHV, Most highly virulent; MV, moderately virulent; HV, highly virulent; WE, weakly virulent.

* Data presented in the table are average of 3 independent assays.

Note : Control sets showed no activity.

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