habit and efficient mechanisms of dissemination like presence of bristly pods, persistent sutures, seeds with a long dormancy period, etc., has helped it to evolve as a weed that spreads very fast and is difficult to eradicate. Its weedy habit has been further invigorated by polyploidy coupled with out-breeding.

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## ENHANCEMENT OF *IN VITRO* POLLEN GROWTH OF *CYAMOPSIS TETRAGONOLOBA*

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THE requirements of pollen to germinate in an artificial medium vary with species. Pollen of some species germinate even in distilled water, while that of others requires either simple or mineral supplemented sugar medium. Even in standard medium containing sucrose, calcium and boron, pollen of a few species show very poor germination and under such conditions the medium is supplemented with various growth substances to improve pollen germination. The present study is to find out whether addition of a few chemical substance to the pollen germination medium can cause enhancement in pollen germination and tube growth of *Cyamopsis tetragonoloba* and also to understand how far the *in vitro* pollen growth could be manipulated which might throw some light on the control of pollen growth *in vivo*. In addition to the effect of added growth promoting substances like indole acetic acid (IAA), gibberellic acid (GA) and cytokinin on *in vitro* pollen growth, addition of compounds like EDTA and ascorbic acid, which are reported to be related to IAA action has also been studied.

Fresh pollen of *C. tetragonoloba* were cultured by 'hanging drop' method using the basal medium, consisting of sucrose (25%), calcium nitrate (0.03%) and boric acid (0.01%), as the control. Although 93.5% of *C. tetragonoloba* pollen are fertile, only 40.5% germinate in sucrose solution (25%). When sucrose was supplemented with optimal concentrations of calcium chloride (0.03%) and boric acid (0.01%), an increase in the percentage of germination (55%) and tube length (1008 $\mu$m) was observed. Experiments were also carried out supplementing various concentrations of IAA, GA, Cytokinin, EDTA, and ascorbic acid independently and also in combination with one another. In the present communication data relating to optimal concentrations—pertaining to pollen germination and tube growth—are presented (table 1). Measurements were taken after 4 hr of incubation and each experiment consisted of a minimum of five replicate sowings. Percentage of germination was calculated by taking an average of 50 microscopic fields and pollen tube length by an average of 10 maximally grown pollen tubes. Pollen fertility was calculated using Alexander's stain[1]

All the growth substances tested showed promotive effect at optimal concentrations when added individually to the basal medium but a combination of all of these resulted in inhibition (table 1). IAA enhanced the percentage of germination by 30% over the control at 0.0001% and increased the tube length (1489 $\mu$m) signficantly by 48% at 0.00004%. Higher concentrations of IAA induced the formation of paired pollen tubes in a few pollen grains. On the other hand, a marginal increase in pollen germination by 11% was observed at lower concentrations of GA (0.00005%), whereas a higher concentration of GA (0.0002%) enhanced the pollen tube length (1577 $\mu$m) by 56%. The effect of cytokinin is relatively small on pollen germination but pollen tube length is promoted by 32%. Addition of EDTA did not affect germination whereas pollen tube length was promoted significantly (55%). A marginal enhancement of pollen germination and tube length was observed with the addition of ascorbic acid. When all the growth substances were added in a mixture to the basal medium, as a test for synergistic effect, inhibition of pollen tube length was observed (table 1).

TABLE 1

*Effect of chemicals on in vitro pollen growth of Cyamopsis tetragonoloba*

| Substances | Optimal concentration | | Pollen germination (%) | Pollen tube length ($\mu$m) |
|---|---|---|---|---|
| Control (BM) | Sucrose | 25% | | |
| | Calcium nitrate | 0.03% | 55 | 1008 |
| | Boric acid | 0.01% | | |
| BM + IAA | 0.0001% | | 71 | 672 |
| | 0.00004% | | 56 | 1489 |
| BM + GA | 0.00005% | | 61 | 1028 |
| | 0.0002% | | 57 | 1577 |
| BM + Cytokinin | 0.0005% | | 58 | 1330 |
| BM + EDTA | 0.005% | | 59 | 1559 |
| BM + Ascorbic acid | 0.01% | | 63 | 1140 |
| | 0.02% | | 70 | 917 |
| Mixture I | BM + IAA | 0.00004% | | |
| | GA | 0.0002% | | |
| | Cyt. | 0.0005% | 54 | 883 |
| | EDTA | 0.005% | | |
| | AA | 0.01% | | |
| Mixture II | BM + IAA | 0.0001% | | |
| | GA | 0.00005% | | |
| | Cyt. | 0.0005% | 57 | 648 |
| | EDTA | 0.005% | | |
| | AA | 0.02% | | |

Pollen germination and tube growth, the two distinct phases of pollen growth, were induced by two different concentrations of IAA, GA and ascorbic acid. There are conflicting reports about the causal effect of IAA and GA on pollen germination and tube growth. IAA did not affect pollen germination in *Cassia fistula*[2] whereas in *Tropaeolum*[3] considerable increase in pollen germination was observed. Bandyopadhyay *et al.*[4] observed only inhibition of both the processes in *Catheranthus*. Similarly Virk and Grover[5] observed GA induced inhibition of pollen germination and tube growth in *Petunia*, whereas Chandler[6] observed growth promoting property of GA on the pollen of 27 speices. In *Calotropis*[7] IAA,GA and kinetin promoted tube growth but did not enhance pollinial germination. Our studies showed promotion of both pollen germination and tube growth in *C. tetragonoloba* by the addition of IAA and GA. Cytokinin increased the pollen tube length marginally. The differential action of these substances may be due to different endogenous levels of these substances in the pollen grains. Enhancement of pollen tube length by EDTA may be due to its synergistic effect with endogenous IAA by inhibiting IAA oxidase[8]. Increment of pollen tube growth by ascorbic acid is not significant, although pollen germination is promoted by 27%.

Bose[9] reported in *Pisum* that a combination of GA with kinetin or IAA in the basal medium did not bring about any improvement in pollen growth. Our studies also revealed that all the five chemicals when added together to the basal medium did not affect pollen germination but only inhibited pollen tube growth.

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## PRODUCTION OF MOMILACTONE ASSOCIATED WITH RESISTANCE OF RICE CULTIVARS TO SHEATH ROT DISEASE

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IT is of common occurrence in the field that semi-dwarf rice cvs. are highly susceptible while the tall ones are resistant to sheath rot disease caused by *Acrocylindrium oryzae*[1]. It created an interest and hence it was considered worthwhile to investigate the cause of this differential resistance. Cartwright *et al.*[2] demonstrated that a phytoalexin, known as momilactone (a diterpene lactone) was associated with defence reaction of rice plants and therefore it was decided to ascertain whether momilactone was any way involved in differential resistance of tall and semi-dwarf rice cultivars.

At first biological activities of leaf sheath exudates and diffusates of both Mahsuri (tall and resistant) and Jaya (Semi-dwarf and susceptible) cultivars were tested against fresh spores of *A. oryzae* following the method of Purkayastha and Mukhopadhyay[3]. The results indicate that diffusates from Mahsuri were more antifungal (74% inhibition of germination; 70% inhibition of germ tube length) than that of semi-dwarf one (12% inhibition of germination and 40% inhibition of germ tube length).

No fungitoxicity was, however, detected either in leaf sheath exudates or in spore germination fluid (*i.e.* drops of spore suspension placed on clean glass slides, collected after 48 hr of incubation, combined, centrifuged and the supernatant used as germination fluid) Besides, momilactone A was detected in trace in the diffusates of resistant cv. (Mahsuri—10 week-old) only but not in the exudate.

To obtain greater amount of momilactone A *Acrocylindrium*-infected, dark grown coleoptiles (3 tall and 3 semi-dwarf rice cvs.) and infected leaf sheaths (one tall and one semi-dwarf cvs.) of rice were extracted separately[3]. Momilactone A was isolated after 48 hr of incubation, when no symptom of disease was observed. The infected coleoptiles/leaf sheaths (250 g) of each cultivar were extracted twice with 75% ethanol and filtered. The residue was reextracted overnight at 4° C with 50% ethanol and filtered. The filtrates were combined and evaporated to dryness at 35° C in a rotary evaporator. The residue was dissolved in 100 ml water and extracted thrice with equal volume of diethyl ether. The organic phases were combined, reduced in volume and washed with phosphate buffer (1.4 M, pH 6.3). The ether fraction was evaporated to dryness, residue dissolved in 95% ethanol and applied to a Sephadex LH.20 column. Rest of the procedure was as described[2]. Momilactone A was identified by UV-spectrophotometry, IR-spectrophotometry and thin layer chromatography (TLC) but for quantitative analysis UV-spectrophotometry was used. The results are given in Table 1.

TABLE 1

*Comparison of momilactone 'A' level in different rice cvs.*

| Cultivars | Concentration of momilactone A in $\mu g/g$ (fresh weight) |
|---|---|
| *Tall cvs. (coleoptile)* | |
| Badkalamkati | 21.36 |
| Mahsuri | 16.02 |
| Rupsail | 12.91 |
| Mashuri (*leaf sheath*) | 19.36 |
| *Semi-dwarf cvs. (coleoptile)* | |
| Jaya | 5.59 |
| CR-126 42 1 | 8.13 |
| Ratna | 5.58 |
| Jaya (*leaf sheath*) | 8.64 |