CHARCOAL ROT OF GARDEN BALSAM—A NEW DISEASE

DURING September–October, 1976, some plants of garden balsam (*Impatiens balsamina* L.) growing in ornamental flower beds were showing severe stem rot symptoms (Fig. 1) particularly involving swollen joints and resulting in toppling of plants and showing numerous, shiny black sclerotia on the smoky or discoloured surfaces.

FIG. 1

Isolations from surface-sterilized portions of the stem on potato dextrose agar medium yielded a pure culture of *Rhizoctonia bataticola* (Taub.) Butler with dark greyish brown and slightly fluffy mycelium. The fungus mycelium from pure culture was inoculated in the basal portion of healthy stem of the host *in situ* by cork borer method using cotton plugs or paraffin wax, as this method has been found to be suitable especially for succulent tissues[1]. Within 4–5 days, the typical symptoms of charcoal rot in the initial stage appeared on stem at the soil line and the infection advanced upward, severely affecting the nodal joints. Reisolations from diseased portions confirmed the identity of the original pathogen. The toppled and otherwise affected plants were left over as such and at the end of 6–8 weeks, pycnidia were noticed on the dried up surfaces of the stem. The pycnidia were sub-carbonaceous, globose, ostiolate, 74·0–11·0 μm; pycnidiospores ovate-elongate, hyline, aseptate, and variable in size, but not curved, 14·8–22·2 × 3·8–7·4 μm (the characteristic 3 : 1 ratio). Sclerotia in culture black, smooth, globose to slightly irregular in shape and size, 44·0–167·0 μm. The fungus was identified as *Macrophomina phaseolina* (Tassi.) Goid. The pure culture was maintained by transfer of single sclerotia on PDA and their germination was easily obtained. Efforts to induce pycnidia on artificial medium by employing the usual methods[2] were unsuccessful.

In India, *Rhizoctonia solani* Kuehn. has been reported to infect collar and roots of balsam in Assam[3]. *Rhizoctonia bataticola* (Taub.) Butler (=*Sclerotium bataticola*) has been reported to be pathogenic on garden balsam in Pakistan[4], and only sclerotial stage has been reported to occur; their attempts to find or induce the pycnidial stage were unsuccessful. The pycnidial strain reported here is a new record on this host not only in India, but elsewhere also[5]. Moreover, sclerotia of the Pakistan strain are smallar (48·0–80·0 μm) and rather regular in shape and size compared to those of the Indian strain (44·0–167·0 μm). On the basis of sclerotia, pycnidia and pycnidiospore size, the Indian strain is akin to those found on leguminous crops, such as soybean, peanut and gram[6] as also jute[7], whereas those of the strains on sunflower[8], dahlia[2,9] and several cucurbits[10] are much bigger, although a wide variability governed by the host, medium or temperature is known to exist in this fungus[11].

A large number of fungi causing leaf spot diseases and obligate pathogens causing both powdery and downy mildew are known to infect balsam in India mostly exhibiting symptoms on leaves and blossoms, but those affecting stems or roots are only a few. Among these, *M. phaseolina* causing charcoal rot quickly destroys the plant under continued high temperatures (above 30° C) and post-rainy season warm sunshine coupled with acute or sudden stress of low soil moisture as found by several workers[2]. Such conditions appeared to exist at the time when mortality in the beds was fairly high at Delhi. The culture has been deposited in I.T.C.C., New Delhi under Accession No. 2577.

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HISTOCHEMICAL LOCALIZATION OF ENZYME ACID PHOSPHATASE IN THE ANTHERS OF MALE-FERTILE, CYTOPLASMIC, GENIC AND INDUCED MALE-STERILE PLANTS

HISTOCHEMICAL localization of the enzyme acid phosphatase during different developmental stages in the anthers of male-fertile (MF), Cytoplasmic (CMS), Genic (GMS) and induced male-sterile (IMS) plants of *Allium cepa, Capsicum annuum, Cucumis melo, Cucurbita maxima, Daura alba, Solanum melongena* and *Triticum aestivum* was undertaken.

Flower buds of the above mentioned plants were fixed in 80% alcohol at 25° C for 12 hours. These were quickly dehydrated, cleared and embedded by usual procedure. Serial microtome sections of 6–15 μ thick were cut and Haupt's adhesive was used for affixing the sections to the slides. Fresh sections of the anthers of some of the above mentioned plants were also cut with a sledge microtome using dry ice. For the localization of enzyme acid phosphatase procedure of Jensen[1] was used as summarised in Table I.

These sections were rinsed in water, placed in 95% ethanol and then in 100% ethanol and finally transferred to pure xylene and mounted in balsam.

Table II shows the distribution pattern of enzyme acid phosphatase in the anthers of MF, CMS, GMS and IMS plants at different developmental stages. Anther development had been divided into six stages. The sporangial cells were initially evident, and the last stage ended near anthesis, after the tapetal protoplasts in MF anthers have degenerated and the anthers were ready to dehisce. The intensity of enzyme reaction has been arbitrarily divided into five parts : low (+), moderate (++), high (+++), higher (++++) and highest (+++++).

A. Sporogenous tissue stage : At this stage (Fig. 1), in the anthers of MF plants, the enzyme reaction was high in the cells of epidermis, tapetum, pro-cambial strand and pollen mother cells (PMCs); moderate in other wall layers and connective parenchyma of the

TABLE I

Method used for the localization of enzyme acid phosphatase

Incubation of sections (fresh or fixed)	pH	Temperature	Time	Dilute yellow ammonium sulphide	Control
Substrate + sodium glycerophosphate (0·6 gm lead nitrate in 500 ml of 0·5 M acetate buffer at pH 4·5, added 50 ml of 0·1 M sod. glycero-phosphate)	5	37° C	4–6 hours	15 min. (1–2 ml of yellow amm. sulphide in a coplin jar of water)	1. Sections placed in the substrate minus sod. glycerophosphate. 2. Section placed directly in yellow ammo sulphide. 3. Heated sections were carried throughout.

TABLE II

Evaluation of enzyme acid phosphatase in the anthers of MF and MS (CMS, GMS and IMS) plants at different stages of development. Enzyme activity has been arbitrarily evaluated into five parts : Low (+), Moderate (++), High (+++), Higher (++++) and Highest (+++++)

Stage of Development	Strain	Epidermis	Endothecium	Middle layers	Tapetum	Sporogenous tissue	PMCs	Microspore tetrad	Microspore	Pollen grains	Connective parenchyma	Vascular tissue
A.	MF	+++	++	++	+++	+++	..	..	..	..	+++	+++
	MS	+	+	+	+	+	..	..	..	..	+	++
B.	MF	++++	++	++	++++	..	++++	..	..	..	+++	++++
	MS	++	+	+	+	..	+	..	..	..	+	++
	MF	++++	+++	+++	+++++	..	..	+++++	..	..	++++	+++++
C.	MS	++	+	+	++	..	..	++	..	..	+	++
D.	MF	++++	++++	+	++++	..	..	..	++++	..	+++	++++
	MS	++	+	+	++	..	..	..	++	..	+	++
E.	MF	+++	++	..	..	..	..	..	..	+++++	++	++++
	MS	+	+	+	+	..	..	..	..	++	+	++
F.	MF	+++	+	..	..	..	..	..	..	+++++	++	++++
	MS	+	+	+	+	..	..	..	..	++	+	++