ISOLATION OF AND ASSAY FOR CERULENIN PRODUCED BY RICE SHEATH-ROT PATHOGEN *SAROCLADIUM ORYZAE* (SAW) GAMS

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SHEATH-ROT disease of rice caused by *Sarocladium oryzae* (Saw) Gams has become a severe constraint for rice production in Asian countries. Yield losses and damage caused by the disease can be as high as 53%[1,2]. The fungus, *S. oryzae* is known to produce two metabolites, helvolic acid[3] and cerulenin[4] in culture. The latter compound is a well-known antibiotic produced by *Cephalosporium caerulens* and has been thoroughly characterized[5]. The structure of cerulenin (figure 1) suggests that it is a 2,3-epoxy-4-oxo–7, 10-dodecadienoyl amide[6]. In the absence of any published work, we examined which of the metabolites is produced by Indian isolates of *S. oryzae*.

An isolate of *S. oryzae* which caused severe symptoms of sheath-rot on naturally infected rice, cv.IR.36, was used in this study. Metabolites from a fungus grown in a seed medium for 4 d were isolated using the procedures developed by Awaya *et al*[7]. The culture filtrate was filtered to remove the mycelium, extracted with 3 separate volumes of chloroform and the choloroform phase of the extract was concentrated by rotary evaporation. This crude extract was chromatographed on a silica gel column and was eluted with choloroform-ethyl acetate (50:1.5, V/V, 600 ml). Fractions (5 ml each) were collected and were monitored for biological activity using the *Candida albicans* (strain KF-1) inhibition assay[8] (figure 2).

Biologically active fractions were pooled and concentrated by rotary evaporation. These fractions yielded 49 mg (dry wt) of toxin/antibiotic. This material was further purified by repeated column chromatography. On TLC plates (silica gel, 0.2 mm, developed with benzene : acetone, 1:1) the toxin/antibiotic moved as a single brownish yellow spot (detected by iodine vapour). In repeated cochromatography runs where an authentic sample of cerulenin was used, both cerulenin and the purified biologically active metabolite of *S. oryzae* had an Rf value of 0.49. The *S. oryzae* metabolite was tentatively identified as cerulenin based on its toxicity to *C. albicans* (which is not inhibited by helvolic acid, the steroid antibiotic)[8] and chromatographic properties. Cerulenin was also identified in rice tissues showing sheath-rot symptoms (Sakthivel, unpublished data).

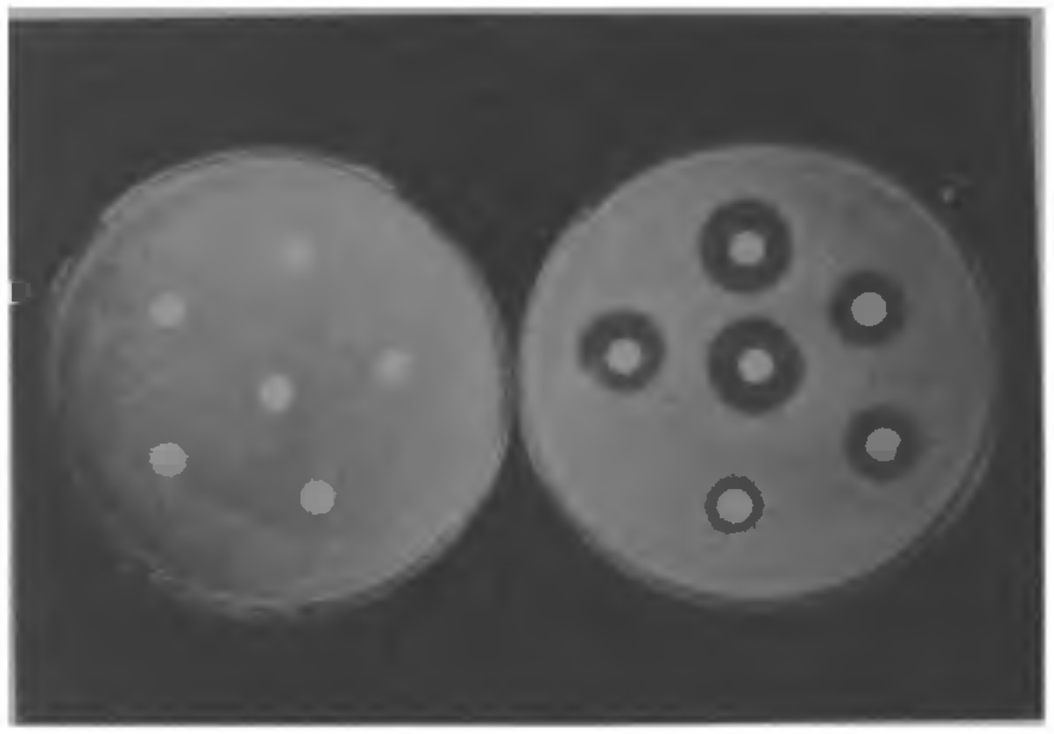

Figure 2. Bioassay for cerulenin by paper-disk method. The biologically active fractions of *Sarocladium oryzae* (2 μl) (on the right) induce the growth-free inhibition zones of *Candida albicans* (KF-1 strain).

The present results reveal that isolates of *S. oryzae* produce cerulenin in culture. Further work is in progress to assess the role of cerulenin in the development of sheath-rot symptoms on rice.

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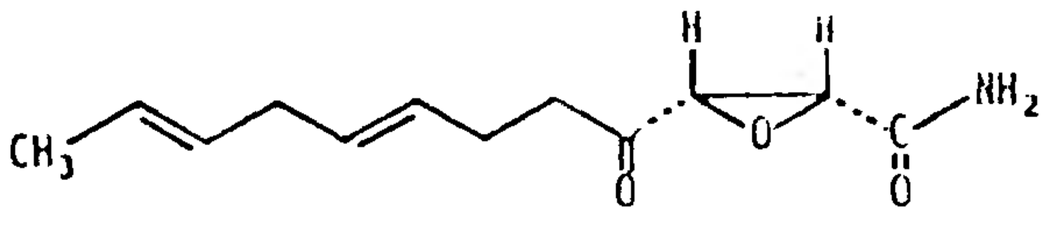

Figure 1. Structure of cerulenin.

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EFFECTS OF BLUE LIGHT AND PHYTOHORMONES ON ENHANCEMENT OF NITRATE REDUCTASE ACTIVITY IN EXCISED OKRA EMBRYOS

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NITRATE REDUCTASE is a key enzyme involved in the nitrate assimilation of plants and its activity is controlled by a number of environmental and endogenous factors[1]. The photocontrol of nitrate reductase activity operating via phytochrome has been demonstrated by many workers[2–8]. Evidence also implicates blue light in this control[9–11] exerted probably independent of phytochrome[11]. Phytohormones too can regulate nitrate reductase activity[1,12]. Lips and Roth-Bejerano[13] showed that the effect of light can be replaced by phytohormones in the induction of nitrate reductase. Overall, the information on control of nitrate reductase activity by blue light and phytohormones is yet scarce. In the present communication, the effects of blue light and phytohormones on nitrate reductase have been investigated in excised okra embryos.

Seeds of okra (*Abelmoschus esculentus* L Moench) cv Pusa Sawani were soaked for 6 hr at 27±1°C in the dark. Embryos were then excised from the seeds and kept for 2 hr incubation with 5 ml of 10 mM KNO_3 in the dark. The enzyme activity was induced by this treatment as observed after assaying. The effects of light were studied by giving 5 min irradiation to the embryos at the start of incubation followed by darkness. Light sources were obtained as described earlier[14]. The intensity of blue light was 5 W m^{-2}, red light 5 W m^{-2}, far-red light 1.4 W m^{-2} and of green safe light 0.01W m^{-2}. The inhibitors, actinomycin-D (Act. D) and cycloheximide (CH), and various hormonal substances i.e. Indol-3yl-acetic acid (IAA), gibberellic acid (GA_3), benzyladenine (BA) and ethrel (ETH) were supplied in the incubation medium to observe their effects on nitrate reductase. *In vivo* nitrate reductase activity was assayed by the modified method of Jaworski[15] as detailed earlier[14]. All experiments were repeated at least three times with similar results and representative results are shown.

Irradiation of embryos with blue light for 5 min, followed by 115 min dark, caused a marked increase (135%) in nitrate reductase activity and the effect was not counteracted by subsequent irradiation with far-red light for 5 min (figure 1). On the other hand, enhancement by red light was almost counteracted following far-red light[14]. Thus, red light and blue light could enhance nitrate reductase activity independently. Many effects of blue light on plants involve a photoreceptor, possibly a flavoprotein, other than phytochrome[16]. When irradiation with blue light was followed by red light, the enzyme activity was further enhanced (160%) (figure 1). The effects of inhibitors of RNA and protein synthesis suggested that blue light caused enhancement of nitrate reductase activity was due to *de novo* synthesis (figure 1). The enzyme activity was drastically inhibited by cycloheximide, an inhibitor of protein synthesis in the cytoplasm. Actinomycin-D, an inhibitor of RNA synthesis, also inhibited enzyme activity but to a lesser extent compared with cycloheximide. Thus, the blue light control of nitrate reductase activity may be exerted at the level of transcription and translation. Stored mRNA for nitrate reductase are probably present in the okra

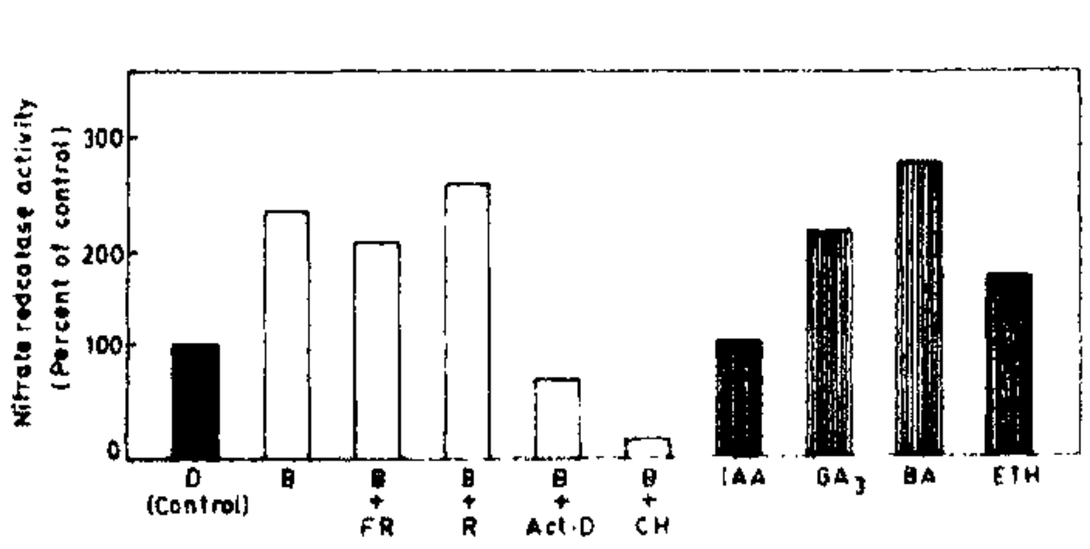

Figure 1. Effects of light, phytohormones and inhibitors of RNA and protein synthesis on nitrate reductase activity. B, blue light; R, red light; FR, far-red light.