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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

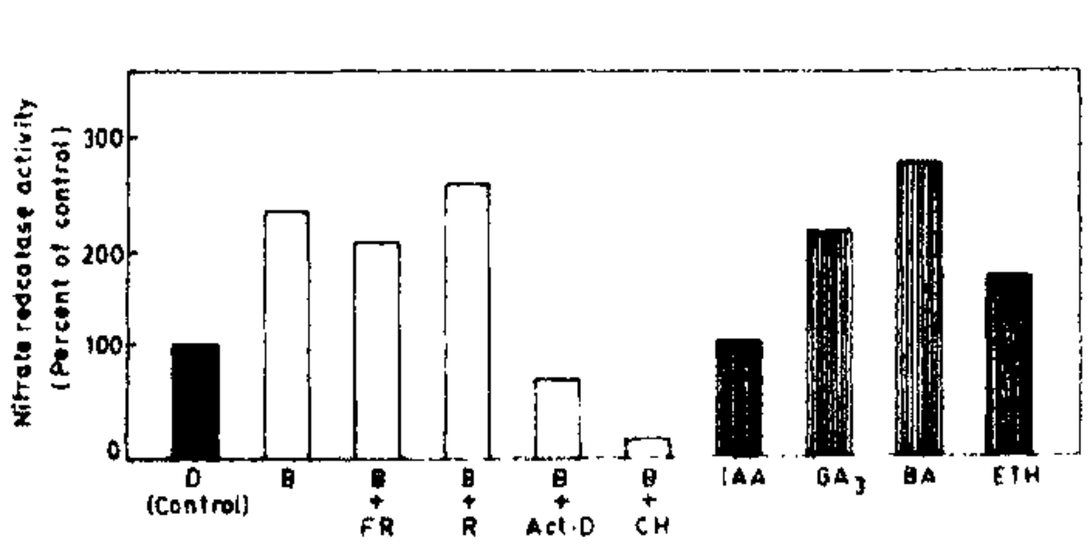

Figure 1. Effects of light, phytohormones and inhibitors of RNA and protein synthesis on nitrate reductase activity. B, blue light; R, red light; FR, far-red light.

seeds[14]. Blue light has been suggested to amplify the fresh synthesis of RNA[11] and soluble proteins[17, 18] The inhibition by cycloheximide suggests that mostly pre-formed mRNAs are translated on the polybribosomes to yield the active enzyme. It has been shown that light-mediated increase in the amount of polyribosomes consequently promotes nitrate reductase activity[19].

Exogenous supply of hormonal substances in the incubation medium of embryos in the dark affected enzyme activity (figure 1). GA_3 enhanced nitrate reductase activity to the same effectiveness as on 5 min irradiation with blue light while benzyladenine was more effective. Ethrel slightly enhanced the activity while IAA had no effect. Cytokinins *per se* enhance the activity of nitrate reductase in *Agrostemma* embryos[20] caused by *de novo* synthesis[21] and markedly enhance the efficiency of nitrate reductase induction by substrate in many plant species[12]. In tobacco leaves, GA_3 and a combination of GA_3 and kinetin enhanced the activity of nitrate reductase[22] and replaced the light requirement for its induction[13].

The question as to whether light effect on nitrate reductase activity is mediated via changes in hormone concentrations or balances or via membrane(s) changes or functions affecting responsivity to hormones, is still open.

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BIPOLARIS SPICIFERA AND *EXSEROHILUM ROSTRATUM* CAUSING LEAF SPOTS OF *EUCALYPTUS TERETICORNIS* — NEW RECORD FROM INDIA

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FOLIAR infection of 3-month-old *Eucalyptus tereticornis* Sm seedlings was observed in forest nurseries at Onkar, Mysore, Karnataka during April/May 1984. Infection occurred usually at the margin and tips of mature leaves as minute greyish brown specks. The spots coalesced to form large necrotic areas. Two fungi, *Bipolaris spicifera* (Bain) Subram, anamorph of *Cochliobolus spcifer* Nelson (IMI 288286) and *Exserohilum rostratum* (Drechsler) Leonard and Suggs, anamorph of *Setosphaeria rostrata* Leonard (IMI 288285, 288287, 288288,