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Full Length Research Paper

Interaction of lycorine with UV-B and kinetin in cucumber (*Cucumis sativus* L.) Cotyledons

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Interaction of lycorine, a strong inhibitor of ascorbic acid biosynthesis with kinetin and UV-B radiation has been studied in cucumber (*Cucumis sativus* L.) cotyledons. Lycorine inhibited hypocotyl growth of seedlings as well as kinetin induced expansion growth of cotyledons in darkness. Exposure to UV-B radiation has similar inhibitory effect on growth. Inhibition of growth by lycorine was accompanied by inhibition of ascorbic acid biosynthesis but UV-B radiation promoted ascorbic acid synthesis while inhibiting growth. The inhibitory effect of lycorine on ascorbic acid synthesis could be completely recovered by kinetin. The role of ascorbic acid as a growth promoter and as an antioxidant is discussed in the light of lycorine effect.

Key words: Ascorbic acid, growth, kinetin, lycorine, ultraviolet-B radiation.

INTRODUCTION

Lycorine is a pyrrolophenanthridine alkaloid extracted from amarillidaceae (Arrigoni et al., 1975; Liso et al., 1984). Lycorine is a strong inhibitor of biosynthesis of ascorbic acid in plant tissues and inhibits 90% of synthesis in pea (*Pisum sativum*) seedlings and potato *(Solanum tuberosum*) slices (Arrigoni et al., 1975) at 2 x 10^{-5} and 3 x 10^{-6} M concentrations respectively. A similar reduction has also been observed in *Vicia faba* and *Allium cepa* seedlings (Arrigoni, 1994). The effect of lycorine varies with the plant tissues and at millimolar concentration, the synthesis of ascorbic acid (AA) was inhibited only to an extent of 18% in *Cilvia miniata* leaves (Arrigoni et al., 1975) and 14% in maize (*Zea mays*) embryos (De Tullio et al., 1998).

Lycorine is also a powerful inhibitor of growth in higher plants, algae and yeast (Arrigoni et al., 1975). Lycorine inhibits the germination and elongation in *V. faba* (Arrigoni, 1994) and expansion growth in higher plants (De Leo et al., 1973). The physiological changes brought about by lycorine are related to its ability to inhibit ascorbic acid biosynthesis. These inhibitory effects of lycorine are ascribed to suppression of the biosynthesis of AA by blocking conversion of the precursor L-galactonic acid- γ-lactone to ascorbate (De Gara et al., 1994), and in lycorine treated tissues, addition of galactone–lactose induce ascorbic acid biosynthesis comparable to controls (De Gara et al., 1992).

Ascorbic acid (AA) has been found to act as an electron donor in plasma membrane redox reactions (Asard et al., 1995), a cofactor of enzyme controlling cell metabolism (De Gara et al., 1991) and a key regulator of cell division (Liso et al., 1984; Innocenti et al., 1990; Citterio et al., 1994) and elongation (Cordoba-Pedregosa et al., 1996; Arrigoni et al., 1997). When the endogenous AA level is experimentally lowered by lycorine, an inhibitor of the AA de novo biosynthesis (Arrigoni et al., 1975) both cell division (Liso et al., 1984) and cell elongation (Cordoba Pedregosa et al., 1996) are inhibited.

Expansion growth in cucumber cotyledons is promoted by cytokinins (Narain and Laloraya, 1974) and inhibited by UV-B radiation (Takeuchi et al., 1995; Tekchandani and Guruprasad, 1998). UV-B radiation is known to cause an oxidative stress in plants and in defense the plant tissues synthesize antioxidants like ascorbic acid

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Abbreviations: **APX**, Ascorbic acid peroxidase; **DCPIP**, di chlorophenol indophenol; **EDTA**, ethylene diamine tetra acetic acid sodium salt; **PVP**, polyvinyl pyrrolidone; **UV-B**, Ultraviolet – B radiation.

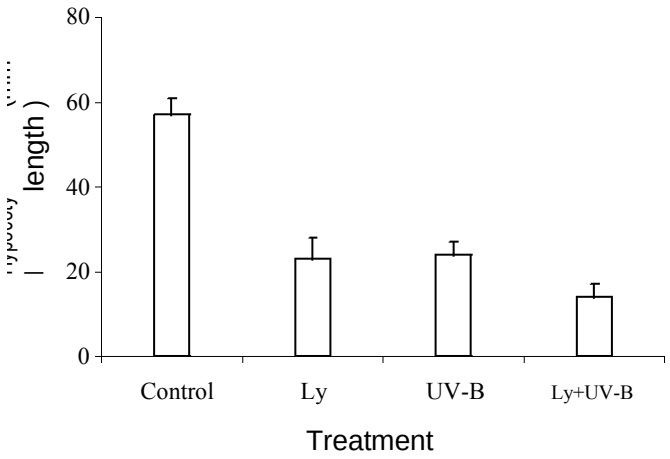

Figure 1. Effect of lycorine (10^{-4} M) on the hypocotyl length of cucumber seedlings grown for 72 h in darkness or exposed to UV-B (1 / 24 h) radiation. Each bar represents the mean of three samples assayed in triplicates and the vertical lines indicates ± SE.

and glutathione, which can detoxify the free radicals by reduction (Takeuchi et al., 1995; Rao et al., 1996; Jain et al., 2003, 2004; Kataria et al., 2007). Although both lycorine and UV- B radiation inhibit elongation and expansion growth; lycorine is known to inhibit ascorbic acid biosynthesis while reports indicate an enhancement in the ascorbic acid synthesis by UV-B radiation. Kinetin, which is known to promote the expansion growth of cotyledons, also promotes ascorbic acid synthesis (Tekchandani and Guruprasad, 1998; Gangwar et al., 2010). An interaction between these three factors is presented here with the objective of understanding the involvement of ascorbic acid in the defense mechanism against UV-B as well as growth.

MATERIAL AND METHODS

Plant material

The seeds of cucumber (*Cucumis sativus* L var long green) were obtained from Suttons and Sons Ltd. Calcutta, India. Seeds of uniform size (11 mm) and shape were selected, rinsed with 0.01 % $HgCl_2$, washed thoroughly under tap water and finally with distilled water. Seeds were spread on moist filter paper in 15 cm Petri dishes and grown in complete darkness at 25 ± 1°C. Cotyledons were excised from seedlings grown for 24, 48 or 72 h in darkness. Cotyledons from these seedlings were excised with the help of sterilized razor blade in such a way that no portion of the hypocotyl tissue remained attached to the cotyledons. The excised cotyledons were floated with their inner surface exposed in 9 cm Petri dishes containing 10 ml of distill water or in 10 ml of lycorine solution (lycorine solution was made by dissolving the lycorine crystals in 1N HCl (Merck) and then adjusting pH to 6.8 with 1N NaOH (Merck). All these manipulations were performed in a dark room (25 ± 1°C) under a green safe lamp (Phillips 25 W covered with 8 layers of green cellophane; irradiation at the level of seedlings being 0.2 W/m^2).

Six cotyledons were floated per Petri dish and grown in complete darkness or exposed to UV-B at 25 ± 1°C. Hypocotyl length was taken by cutting the hypocotyl from the cotyledons and placing the hypocotyl on graph paper and marking the two ends. The mean of ten hypocotyl per Petri dish was taken as the average value. Area of the cotyledon was taken by pressing the blotted dry cotyledon on graph paper and tracing the exact outline. The area was measured by reading to the nearest 0.5 mm square. The mean of the six cotyledons per Petri dish was taken as the average value (Tekchandani and Guruprasad, 1998). Each experiment was run with triplicate sets of cotyledon; the values presented with standard error are the mean of three experiments.

Light source

UV-B (280 to 320 nm) was obtained from FL-20-SE, Toshiba, Tokyo (λ max = 311 nm; 40 W). UV-B was filtered through a polyvinyl chloride film (UV- C-O Mitsuioatsu Ltd, Japan). Irradiance at the level of seedlings was 2.6 mW/cm^2 measured by using radiometer UV-Tex a+b idm, Optix Tex.Inc. Washington D.C.

Ascorbic acid (AA) content

Ascorbic acid was extracted from the control and treated cotyledons by the method of Franke (1955). 100 mg cotyledons were ground in mortar and pestle with some NaCl in freshly prepared 10 ml 2% meta-phosphoric acid (2 g in 100 ml) and centrifuged at 6000 g for 10 min at 4°C. The supernatant was kept in dark on ice until use. Ascorbic acid content was determined spectrophotometrically (Shimadzu-UV-1601) at 524 nm by measuring the reduction of DCPIP (Merck). 1 ml of supernatant was mixed with 1 ml water, 1 ml meta-phosphoric acid (Sigma-Aldrich), 0.5 ml sodium citrate buffer (pH 2.3; Merck) and 1 ml DCPIP. The reagents were added in the same order as described. Absorbance was recorded at 524 nm against blank containing water. The amount of ascorbic acid present was calculated with reference to a standard curve (data not shown) ranging from 2 to 20 μg/ml ascorbic acid (Sigma Aldrich).

RESULTS AND DISCUSSION

Growth

Elongation growth of hypocotyls and kinetin induced expansion growth of cotyledons in cucumber are inhibited by treatment with lycorine or by exposure to UV-B radiation. Treatment of cucumber seedlings with lycorine (10^{-4} M) decreased the length of the hypocotyls by 60% when the seedlings were grown in darkness up to 72 h (Figure 1). Exposure of control seedlings (grown in distilled water) to UV- B (1/24 h) also inhibited the hypocotyl length to the same extent (60%). When the lycorine treated seedlings were exposed to UV-B (1h / 24 h) the hypocotyls length was further reduced (Figure 1).

When the cotyledons excised from 48 h grown seedlings were grown in darkness in presence of kinetin (10 g/ml) the cotyledons expanded in the area (Figure 2). Kinetin induced expansion growth was inhibited to the extent of 52% by lycorine (10^{-4} M); alternatively, exposure of kinetin treated cotyledons to UV-B (1 h / 24 h) also caused an inhibition of 64% in the area of cotyledons. Thus there is a similarity in the inhibition of growth by these two factors; and in combination they have an additive effect

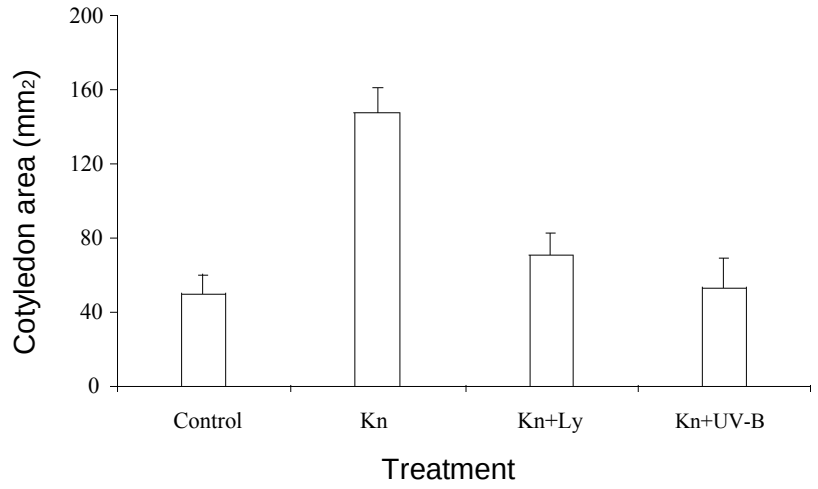

Figure 2. Reduction of kinetin (10 µg/ml) induced expansion growth of excised cotyledons after 72 h by lycorine (10^{-4} M) and UV-B (1/24 h) . Each bar represents the mean of three samples assayed in triplicates and the vertical lines indicates ± SE.

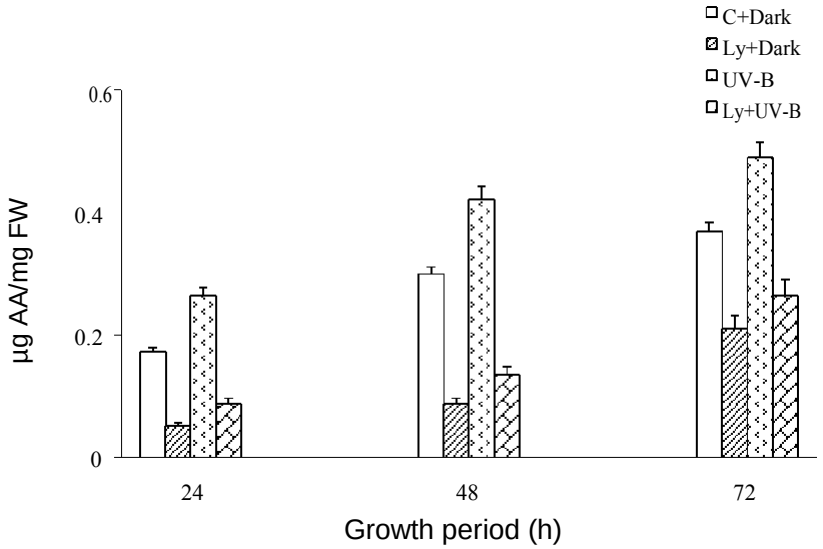

Figure 3. Effect of lycorine (10^{-4} M) on the ascorbic acid content in cotyledons of cucumber seedlings grown for different time periods either in darkness or after exposure to UV-B for 1/24 h. Each bar represents the mean of three samples assayed in triplicates and the vertical lines indicates ± SE.

on the inhibition of growth. Similar observation on inhibition of growth by lycorine and UV-B has earlier been reported (Arrigoni 1994; Takeuchi et al., 1995) although UV-B and lycorine have not been used in combination on the cucumber (*C. sativus*) cotyledons.

Ascorbic acid (AA) content

Inhibition of growth by lycorine has been related to its inhibitory effect on the biosynthesis of ascorbic acid in all the previous studies (Arrigoni et al., 1992; Arrigoni, 1994; De Tullio et al., 1998). In presence of lycorine ascorbic acid content in the growing cotyledons estimated at different growth period was drastically reduced (Figure 3). In contrast to this, exposure of seedlings to UV-B (1/24 h) enhanced the level of ascorbic acid in the cotyledons (Figure 3). Treatment with lycorine reduced the level of ascorbic acid even in the UV-B exposed seedlings (Figure 3). Reduction in the level of ascorbic acid in presence of lycorine was 70% at 24 and 48 h of growth period but was lesser at 72 h (40%) in the cotyledons of

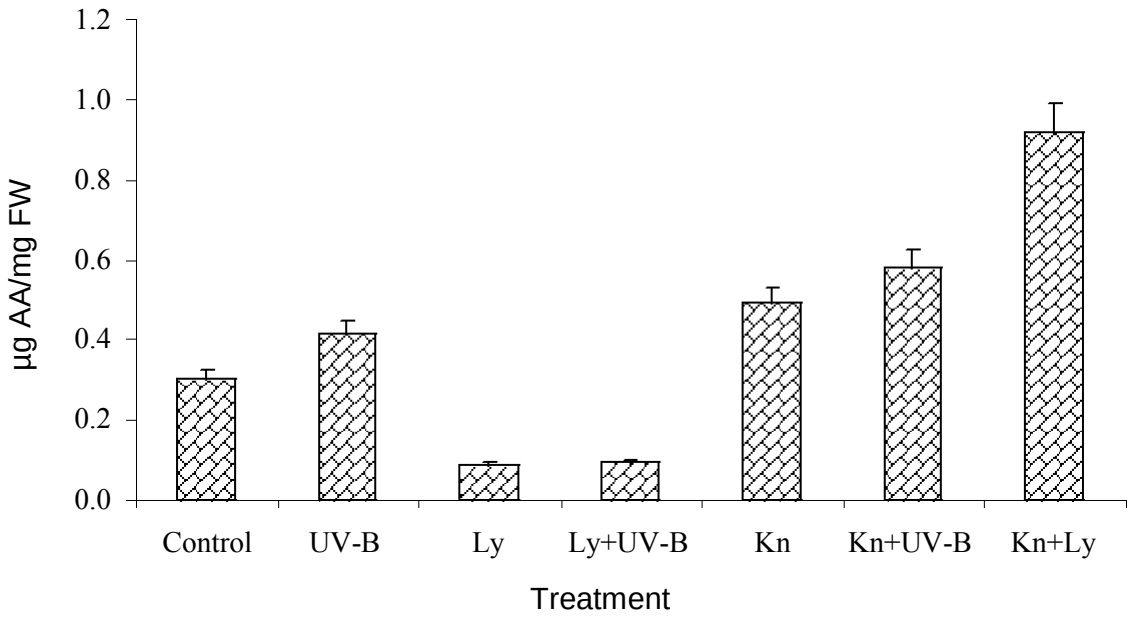

Figure 4. Effect of lycorine (10^{-4} M), UV-B (1 h) and kinetin (10 g/ml) on the ascorbic acid content of excised cotyledons grown in darkness for 48 h. Each bar represents the mean of three samples assayed in triplicates and the vertical lines indicates ± SE.

the seedlings either exposed or unexposed to UV-B (Figure 3).

Treatment of cotyledons with kinetin or exposure to UV-B enhanced the level of ascorbic acid (Figure 4) while lycorine reduced the level of ascorbic acid. When lycorine applied along with UV- B exposure then it reduced the ascorbic level further in the cotyledons (Figure 4); but in the presence of kinetin, lycorine did not inhibit the ascorbic acid level induced by kinetin, rather there was a slight promotion in the level (Figure 4).

In the results presented here, only inhibition of hypocotyl growth of seedlings is correlated with inhibition in the synthesis of ascorbic acid. The inhibition of kinetin-induced expansion of cotyledons is not accompanied by any inhibition in the synthesis of ascorbic acid; on the contrary ascorbic acid was present in higher amounts when lycorine was applied along with kinetin. In addition to this inhibition of growth by UV-B is also not related to decrease in ascorbic acid either, instead there was a raise in the level of ascorbic acid in response to UV-B. A similar raise in the level of ascorbic acid in response to UV- B has earlier been observed in *Arabidopsis* (Rao and Ormrod 1995) and cucumber (*C. sativus*) (Takeuchi et al., 1995; Jain et al., 2004; Kataria et al., 2007). The promotion of ascorbic acid synthesis by UV-B seems to be involved in the antioxidant defense mechanism rather than in the promotion of growth. Thus inhibition of growth is not always accompanied by a decrease in the level of ascorbic acid; these two physiological changes appear to be delinked from each other.

Treatment with kinetin enhanced the endogenous level of ascorbic acid in the cotyledons. Kinetin was able to reverse the inhibitory effect of lycorine on the synthesis of ascorbic acid. There are several different pathways for the natural synthesis of ascorbic acid in the plant tissue (Davey et al., 1999) out of which lycorine is known to inhibit the biosynthetic pathway mediated by galactone-lactose pathway (De Gara et al., 1992). Since kinetin is able to reverse the lycorine induced inhibition of ascorbic acid biosynthesis: there is a possibility that kinetin may activate pathways other than galactone lactose pathway, which results in the accumulation of ascorbic acid. A biochemical evidence for this type of activation is yet to be obtained. However there are two other possibilities for the enhancement of ascorbic acid levels as well an enzymatic or non-enzymatic regeneration and interference in the catabolism.

In this paper, the interaction of lycorine with UV-B and kinetin has been discussed. Lycorine although inhibits kinetin induced expansion growth of cotyledons, the inhibition is not accompanied by reduction in ascorbic acid level. Thus lycorine has a dual effect on ascorbic acid synthesis; induced by UV-B and kinetin, it inhibits the former and promotes the latter. However the action of lycorine on growth is similar-reduction of growth in both the cases, indicating no link between the inhibition of growth and the level of ascorbic acid.

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