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Red-light-induced changes in the distribution of xanthoxin in pea seedlings

H. KATO-NOGUCHI

Department of Bioresource Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-07, Japan

Abstract

The distribution of xanthoxin (Xan), was determined in light-grown, 20-d-old pea (*Pisum sativum* L. cv. Progress No. 9) seedlings. The *cis,trans*-xanthoxin (*c,t*-Xan) and the *trans,trans*-xanthoxin (*t,t*-Xan) were more abundant in the young leaves and terminal bud; their concentrations in leaves were 2 - 3 times those in internodes of the same nodes. After the onset of red-light-irradiation, the concentration of both Xan isomers in 7-d-old dark-grown pea seedlings increased after a 12-h lag time. The increased level of Xan was greatest in the terminal bud and decreased to lower parts of the seedlings. The ratio of *c,t*-Xan to *t,t*-Xan concentration in the seedlings was about 2:3.

Additional key words: legume, *Pisum sativum*, violaxanthin.

Introduction

Xanthoxin (Xan) was purified and identified as a growth inhibitor (Taylor and Burden 1970a, 1972), and it was synthesized *in vitro* by chemical or photochemical oxidation of plant xanthophylls (Burden and Taylor 1970, Taylor and Burden 1970b). Since Xan was rapidly converted to abscisic acid when fed to plants, it was concluded that Xan is a precursor of abscisic acid (Nonhebel and Milborrow 1987, Parry *et al.* 1988, Sindhu *et al.* 1990). However, its significance as an endogenous intermediate in the pathway of abscisic acid biosynthesis is uncertain (Sindhu *et al.* 1990). Xan is a naturally occurring compound apparently and has been implicated as a regulator of stem extension (Anstis *et al.* 1975), seed germination (Taylor and Burden 1970a), stomatal closure (Raschke *et al.* 1975), phototropism (Franssen and Bruinsma 1981, Shen-Miller *et al.* 1982), and gravitropism (Feldman *et al.* 1985). Xan has been detected in a wide range of plant species (Firn *et al.* 1972, Zeevaart 1974, Böttger 1978, Dörffling *et al.* 1978), however, endogenous distribution of Xan

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Abbreviations: f.m. - fresh mass; *c.t*-Xan - *cis.trans*-xanthoxin; *t.t*-Xan - *trans.trans*-xanthoxin.
Fax: (+81) 878 987295, e-mail: hisashi@ag.Kagawa-u.a.c.jP

in plants has not yet been established. When dark-grown plant seedlings are exposed to red-light, the endogenous levels of Xan increased rapidly with a lag time. But the change in its distribution in the seedlings was still unknown. Thus, the present paper describes the distribution of *cis,trans*-xanthoxin (*c,t*-Xan) and *trans,trans*-xanthoxin (*t,t*-Xan) in light-grown pea seedlings and the changes in their distribution in dark-grown pea seedlings after the onset of red-light irradiation.

Materials and methods

Plants and treatments: Pea (*Pisum sativum* L. cv. Progress No. 9) seeds were soaked in running tap water for 6 h, and sown in soil in a greenhouse under natural day-light condition for 20 d. After harvesting, the seedlings were divided into segments by a razor blade (Fig. 1), and the segments were immediately frozen in liquid nitrogen, and stored at -80 °C until extraction of Xan. At the time of harvest, the seedlings were on an average 4.8 cm tall with a fresh mass of 3.2 g per seedling, and had 6 internodes.

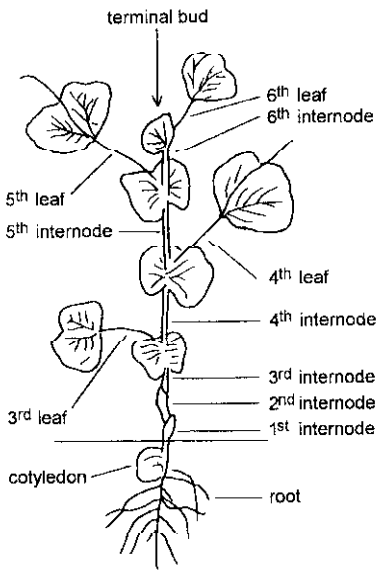

Fig. 1. Diagram of a light-grown, 20-d-old pea seedling. Leaf blades, petioles and stipules were included in leaves.

Pea seeds were also germinated in moist vermiculite in darkness at 25 °C for 3 d after soaking in running tap water for 6 h. Seedlings having epicotyls 30 - 35 mm in length were transferred, in groups of ten, to plastic containers (5 × 15 × 10 cm) filled with fresh vermiculite. The seedlings were grown in darkness for a further 4 d at

25 °C, and the dark-grown, 7-d-old seedlings (plant height about 70 mm, third internodes length about 10 mm) were irradiated continuously with red-light (20 μmol m^{-2} s^{-1} at plant level; emission peak, 661 nm; half-band width 9 nm) from above at 25 °C as described previously (Kato-Noguchi and Kasai 1991). The seedlings were harvested at 0, 6, 12, 24, 36, 48 h after the onset of the irradiation and were divided into various segments, respectively (Fig. 2), and the segments were stored as described above.

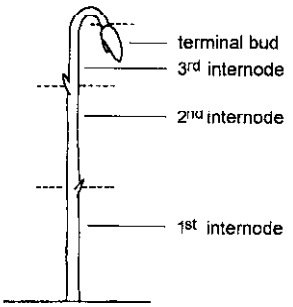

Fig. 2. Diagram of a dark-grown, 7-d-old pea seedling.

Quantification of Xan: Xan was extracted from the frozen segments with acetone, and purified by silica-gel column chromatography and reverse-phase *Sep-Pak* cartridge (*Waters*) as described previously (Noguchi and Hashimoto 1990). The sample thus purified was injected onto a column for high-performance liquid chromatography (column; reverse phase, *TSK Gel ODS-80TM*, 0.46 i.d. × 15 cm, *Toso*, Tokyo), which was eluted at a flow rate of 1 cm^3 min^{-1} with 40 % aqueous methanol (v/v), with detection at 280 nm. The retention times of *c,t*-Xan and *t,t*-Xan was 35.2 and 29.1 min, respectively. Quantification was performed by measuring peak area. During this procedure *c,t*-Xan and *t,t*-Xan were separated as a sharp, single peak from each other and other impurities. It was determined that the peak fractions of the Xan did not contain other substances(s) that might affect the peak area by measuring the ultraviolet absorption spectrum of the peak fractions with a detector (*L-3000*, *Hitachi*, Tokyo) and then comparing the spectrum with that of the pure Xan isolated from bean seedlings (Kato-Noguchi 1992). The overall recovery of *c,t*-Xan and *t,t*-Xan through the entire quantification process was 71.5 ± 2.4 and 73.4 ± 2.7 %, respectively, as calculated from five repetitions of test runs with pure *c,t*-Xan and *t,t*-Xan.

Results and discussion

In light-grown, 20-d-old pea seedlings, *c,t*-Xan and *t,t*-Xan were detected throughout the whole seedlings except in the cotyledons (Table 1). At this stage, the cotyledons were not active in growth. *t,t*-Xan, which was 5-times less active than *c,t*-Xan in

many bioassay systems (Taylor and Burden 1972), was contained more than *c,t*-Xan. Total concentration of *c,t*-Xan and *t,t*-Xan in whole pea seedlings was 34 and 47 ng g^{-1}(f.m.), respectively. Those values and a ratio between *c,t*-Xan and *t,t*-Xan are comparable to those reported for other pea cultivars (Firn *et al.* 1972).

Table 1. Distributions of *c,t*-Xan and *t,t*-Xan [ng g^{-1}(f.m.)] in light-grown, 20-d-old pea seedling. The seedlings were divided into various segments as shown in Fig. 1. Means ± SE from 3 experiments with samples obtained from 50 - 100 segments each.

Plant part	*c,t*-Xan	*t,t*-Xan	Plant part	*c,t*-Xan	*t,t*-Xan
Terminal bud	191 ± 21	271 ± 23	4th internode	24 ± 3	33 ± 4
6th leaf	187 ± 17	259 ± 26	3rd leaf	38 ± 4	26 ± 3
6th internode	54 ± 8	78 ± 8	3rd internode	19 ± 2	12 ± 2
5th leaf	91 ± 9	127 ± 11	2nd internode	8 ± 2	9 ± 2
5th internode	31 ± 3	46 ± 4	1st internode	7 ± 1	9 ± 1
4th leaf	52 ± 5	78 ± 8	roots	4 ± 1	6 ± 2

The *c,t*-Xan and *t,t*-Xan were more abundant in the upper leaves of the seedlings, peaking in the terminal bud which contains the youngest leaf of the seedlings (Table 1). The concentration of *c,t*-Xan and *t,t*-Xan in the leaves was about 2 - 3 times that in the internodes of the same nodes. The ratio between *c,t*-Xan and *t,t*-Xan was about 2:3 in all internodes, leaves and roots. Although Firn *et al.* (1972) did not determine the distribution of Xan in the bean seedlings, they found that young leaves contained more of Xan than older or senescent leaves, and concluded that Xan is produced in young leaves of the bean. Present result might support their conclusion.

Burden *et al.* (1971) found that Xan increased in pea shoots in response to red-light and it has been demonstrated that phytochrome mediates this response (Firn 1974). The levels of both Xan isomers in the dark-grown, 7-d-old pea seedling started to increase after a 12-h lag time in red-light, whereas they remained almost unchanged in darkness (Fig. 3). This lag time prior to the increase is similar to that reported by Firn (1974). Far-red-light-reversibility of the red-light effect was also observed with regard to the increase of Xan concentration when this dark-grown pea seedlings were irradiated with far-red-light immediately after red-light irradiation (Noguchi and Hashimoto 1990).

The increased levels of *c,t*-Xan and *t,t*-Xan were greatest in the terminal bud and decreased in lower parts of the seedlings. After 48-h irradiation, the levels of *c,t*-Xan in the terminal bud, 3rd internode, 2nd internode and 1st internodes were 38, 28, 12 and 8 ng g^{-1}(f.m.), respectively, and were 3.9, 3.5, 1.9, and 1.5 times the level in the non-irradiated control seedlings, respectively. The levels of *t,t*-Xan in the terminal bud, 3rd internode, 2nd internode and 1st internode were 56, 39, 17 and 12 ng g^{-1}(f.m.), respectively, and were 4.1, 3.6, 1.9 and 1.7 times the level in the control seedlings, respectively. The ratio of *c,t*-Xan and *t,t*-Xan concentration was also about 2:3 along the epicotyls of the seedlings.

It has been previously reported that irradiation of dark-grown pea seedlings by red-light induced a 50-fold increase in the level of precursor of Xan, violaxanthin (Anstis *et al.* 1975), and red-light increased the activity of enzyme required for the conversion of precursor to Xan (Burden *et al.* 1971, Feldman *et al.* 1985). These findings together with those described earlier indicate that the red-light effect may involve enhancement of Xan levels, by increasing the availability of the precursor and the activity of the related enzyme, in the terminal bud and younger leaves of the pea seedlings.

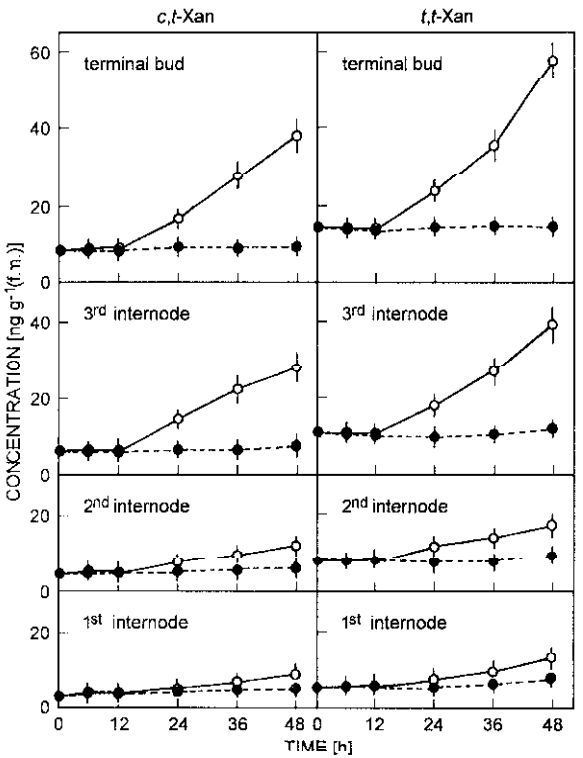

Fig. 3. Red-light-induced changes in the distribution of Xan in dark-grown pea seedlings. Dark-grown, 7-d-old pea seedlings were transferred to continuous red-light, and the levels of *c,t*-Xan and *t,t*-Xan (*open circles*) were determined in segments as shown in Fig. 2. Control plants (*closed circles*) remained in darkness. Means ± SE from 3 experiments with samples obtained from 50 - 100 segments each.

In summary, the distribution of Xan indicates that *c,t*-Xan and *t,t*-Xan were more abundant in the younger leaves and terminal bud in the light-grown pea seedlings. Xan accumulated in the dark-grown seedlings in response to red-light was greatest in the terminal bud and decreased in the lower parts of the seedlings.

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