Relationships Between Xanthoxin, Phototropism, and Elongation Growth in the Sunflower Seedling Helianthus annuus L.

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Abstract. For phototropic curvature of a green sunflower seedling, only the hypocotyl has to be illuminated; the tip and cotyledons are not involved in stimulus perception. The etiolated seedling is phototropically insensitive, illumination of only the hypocotyl renders it sensitive. It is concluded that the photoreceptor is located within the responding organ. In curving seedlings, the endogenous indoleacetic acid (IAA) remains evenly distributed. However, the inhibitor, xanthoxin (Xa), accumulates on the illuminated side. The degree of phototropic response is generally related to the concentration of Xa. The amount of phototropic curvature is independent of the rate of elongation growth, the former can be changed without affecting the latter, and vice versa. The data conflict with the Cholodny-Went theory, whereas they support the hypothesis of Blaauw that the phototropic reaction is caused by the local accumulation of a growth-inhibiting substance on the irradiated side.

Key words: Auxin (indole-3-acetic acid) – Elongation growth – Helianthus – Hormones – Phototropism – Xanthoxin

Introduction

The prevailing theory used to describe the mechanism of tropistic curvatures is that of Cholodny (1927) and Went (1928), which essentially ascribes the bending response to a lateral auxin gradient in the curving tissue. For phototropicism of the stems, Blaauw (1915) proposed that the bending toward the light source may be caused by a relative inhibition of cell elongation at the irradiated side of the curving organ. Brauner (1922) further corroborated this view by demonstrating that unilateral illumination of a decapitated, etiolated coleoptile causes curvature, after replacement of the non-illuminated tip. He suggested a growth-inhibiting substance to be involved.

Auxin bioassays cannot distinguish between differences caused by growth-promoting or -inhibiting substances in plant extracts. Spectrofluorometric determination of the distribution of indoleacetic acid (IAA) in phototropically responding, green sunflower seedlings revealed the absence of a lateral gradient in extractable or diffusable endogenous auxin (Bruinsma et al. 1975). Instead, the presence of a gradient of growth-inhibiting substance(s) was suggested. Thompson and Bruinsma (1977) demonstrated the presence of the abscisin, xanthoxin (Xa), in light-grown sunflower seedlings and were unable to detect this growth inhibitor in etiolated seedlings that fail to respond to a phototropic stimulus.

In the present paper, the degree to which Xa may be involved in the phototropic response of the sunflower seedling is investigated. Also, the relationship between the amount of curvature and the rate of elongation growth of the hypocotyl is studied.

Material and Methods

Plant Material. Seeds of Helianthus annuus L., cv. Giganteus, were surface-sterilized by 3% (w/v) NaClO solution for 45 min, rinsed in tap water, and placed between wet filter paper for approximately 20 h. Small and ungerminated seeds were discarded and the remaining, germinated seeds were individually transferred to small pots using a soil mixture of humus soil: sand: clay = 1:1:1. The seedlings were grown in darkness or with a photoperiodicity of 14 h light per day from high pressure mercury vapor lamps (Philips HP 17T, 400 W), producing 130, 145, and 90 µW cm⁻² in the blue, red, and far red, respectively, at plant height. Seedlings with a hypocotyl length of 40 to 60 mm were used on the 5th or 6th day.

Abbreviations: CCC = chlormequat, (2-chloroethyl)trimethylammonium chloride; GA₃ = gibberellic acid; IAA = indole-3-acetic acid; Xa = xanthoxin
after germination, because these plants gave the best and most uniform response (Franssen 1980). The seedlings were grown and the experiments carried out at 25 ± 2°C, 60 ± 10% relative humidity.

**Phototropic Experiments.** Unilateral irradiation was done in a non-reflecting black box. Up to 100 seedlings of the appropriate age and length were placed in the box with one cotyledon pointing toward a 5-cm wide, horizontal slit, through which they were unilaterally illuminated.

White light was obtained from a fluorescent tube (Philips 40 W/33) which gave, at plant distance, 70 and 64 μW cm⁻² in the blue and red, respectively (no far-red). Blue light was obtained from a blue fluorescent tube (Philips 40 W/18) with two layers of Cinamoid blue, No. 62, giving 67 μW cm⁻² blue light. Red light was obtained from a red fluorescent tube (Philips 40 W/15) with one layer of Cinamoid red, No. 14, producing 86 μW cm⁻² red light (no blue, no far-red). For irradiation with far-red, 8 incandescent tubes (Philinea, Osram linestra, 80 W/40) with one layer of Cinamoid red, No. 14, producing a far-red/red ratio of 7.3. Cinamoid filters were obtained from Strand Electric, London, U.K., and plexiglass from Rohm and Haas, Darmstadt, FRG.

In covering experiments, the cotyledons (and the tip) or the hypocotyl were covered with aluminium foil. Because of growth of the hypocotyl during the treatment, the covering had to be expanded every 4 to 5 h. This treatment, if done with care, did not affect the bending response (Franssen 1980).

The bending of each hypocotyl was determined every 20 min for 2 h, by measuring the angle between its upper and lower parts with a protractor. Because of the individual variability of the seedlings, the average of the maximal curvatures of all hypocotyls was determined as the maximal curvature of an experimental group, disregarding the differences in bending rate. The bending of a seedling after its first maximal response is also influenced by a geotropic reaction (Franssen 1980). The length of the hypocotyl was measured with a ruler to the nearest mm, at the beginning and end of each experiment.

All experiments were repeated at least twice; some tables contain the combined results of the experiments. The statistics were carried out by the nonparametric method (Lehman 1975).

**Hormone Determinations.** Straight, curving, and curved parts of the hypocotyls were bisected, immediately frozen in liquid nitrogen to avoid enzymatic reactions at the cut surface, and stored at −20°C. IAA was determined as described by Knegt and Bruinsma (1973). Xa was extracted, as described by Thompson and Bruinsma (1977), with a modification in some experiments, i.e., filtration through a Sep-pack C18 cartridge before the first evaporation in vacuo. This modification allowed for the omission of the paperchromatography step (Franssen 1980). Thin-layer chromatography was carried out on 0.2-mm silica plates using hexane:ethyl acetate (1:3, v/v). In this solvent system Xa has an Rf value of 0.2-0.4. This abscisic acid was determined in the cress seed bioassay as described by Franssen et al. (1979).

**Results**

**The Hypocotyl as Site of Stimulus Perception.** To investigate whether the perception of the phototropic stimulus is located in the tip (apex and the still undeveloped first leaves) and the cotyledons, as suggested by Darwin (1880) and the Cholodny-Went theory, or in the hypocotyl itself, according to Blaauw and Brauner, the tip and cotyledons of green sunflower seedlings were either removed or covered with aluminium foil immediately before unilateral illumination. The results in Table 1 show no statistically significant difference in bending with the intact control plants, indicating that perception occurs within the hypocotyl.

In contrast to green seedlings, etiolated ones fail to respond to a phototropic stimulus. To render dark-grown seedlings phototropically sensitive, illumination from above with white or blue light for 18 h is necessary (Fig. 1). During this pretreatment only illumination of the hypocotyl is relevant, illumination of the tip and cotyledons is ineffective in the development of phototropic sensitivity (Table 2). In addition,