International Journal of Botany

ISSN: 1811-9700

International Journal of Botany 7 (1): 113-117, 2011
ISSN 1811-9700 / DOI: 10.3923/ijb.2011.113.117

The Effect of Circularly Polarized Light on the Growth of Plants

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Abstract: The growth of lentil and pea under left-handed and right-handed Circularly Polarized (CP) light was studied. It was found that the shoots of both plants grow faster under left-handed CP light. The state of polarization of light penetrating the outer layer of stem and leaf was analyzed by means of polarizing optical microscopy and polarimetry that allows to quintify the changes in the state of light polarization. The birefringence of the outer layer of the leaf (epidermis) and stem was found to have a negligible effect on polarization state of light. In both plants, lentil and pea, circular polarization of light does not change significantly when light penetrates the outer layer (epidermis) of stem or leaf. The birefringence of the outer layer of leaf and stem is small; therefore, the accelerated growth of shoots under left-handed CP light results from its absorption in the interior of the leaves or stems. The observed effect can be used to promote the growth of plants.

Key words: Pea, lentil, circular polarization, light absorption, birefringence

INTRODUCTION

Several recent experiments indicated that the growth of plants and cells is indeed sensitive to polarization of light. However, mostly linearly polarized light was studied. In an article about bimodal polarotropism, Kataoka *et al.* (2000) demonstrated that Vaucheria becomes oriented normal to the E-vector at an optimal intensity of light (Kataoka *et al.*, 2000). The influence of linearly polarized light on rooting of olive cuttings was also demonstrated in an article by Al-Bachir (1995). It was shown by Lamparter *et al.* that the tip cells of the moss Ceratodon grow in the direction perpendicular to the electric vector of polarized light (Lamparter *et al.*, 2004). It was also demonstrated that plants' leaves themselves can change the state of polarization of incoming light (Voshchula *et al.*, 2007) and that polarization of reflected light includes the information for canopy structure, such as leaf inclination (Michio, 2004). The high degree of orientation of microfibrils was established along the stems and roots of cleaver, but orientation was different in different cell types of stems (Goodman, 2005). All these studies dealt with linearly polarized light.

The role of CP light was studied mostly on the molecular level, namely how light interacts with chlorophyll-containing cells' parts, the light harvesting complex. During the last three decades, significant progress was achieved in understanding the details of light harvesting processes on molecular levels (Deisenhofer *et al.*, 1985; Hatch (Hal), 1992; Gussakovsky *et al.*, 2000, 2007; Ruban *et al.*, 1997). Recently, it was shown that the intensity of light and the wavelength of illumination affect the light harvesting complex and its chiral organization in pea leaves (Gussakovsky *et al.*, 2007). It was also found that the absorption of CP light depends on aggregation of light harvesting complex of photosystem II (LHCII) (Ruban *et al.*, 1997). Macroorganization of chromophores with a long range chiral order was found by analyzing CP luminescence in pea chloroplasts (Gussakovsky *et al.*, 2000). It was shown that the light color is not only affected by LHCII aggregates in pea leaves, but also led to different numbers of left-handed and right-handed microaggregates. It is important to note that the above data considered only the interaction of CP light with plants' chlorophyll containing parts, ignoring its interaction with the amino acids.

There is a knowledge gap in the scientific picture of how CP light affects the growth and development of the plant as a whole and also how it affects the development of individual cells.

The experimental study and analysis of light interaction with the whole plant seems to be easier when CP light is considered. Indeed, the different orientation of leaves and stems with respect to the electrical vector of incoming light is not important in this case, since the electrical vector of light is not fixed and rotates in space. However, care must be taken when one analyzes how circular polarization of light changes when light propagates inside the leaves. The outer parts of the

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leaves and stems that are irradiated with polarized light can change the state of polarization when light penetrates their interior. It is related to the fact that the cellulose microfibrils in stems and roots are oriented and therefore, might be birefringent (Goodman, 2005). Birefringent materials are able to change the state of light polarization (Scharf, 2006) if the birefringence is significant or light travels a long distance in a birefringent medium. If microfibrils of stem are predominantly oriented along one direction, then the state of light polarization can change and CP light can become elliptically or linearly polarized after passing through them. Therefore, care must be taken in order to analyze the results of experiments in which plants are irradiated by polarized light.

The purpose of this research is twofold. The first objective is to study how circular polarization of light affects the process of growth of lentil and pea under well-controlled, reproducible conditions. The second is to study how the state of polarization of CP light may change when the light passes the outer layer of the pea and lentil leaves.

The choice of CP light was inspired by studies of light interaction with amino acids, photosynthesis studies cited above, and by the following considerations. As chromophores in chloroplasts and amino acids are all organized in a chiral fashion, this may lead to additional significant sensitivity of plants to the handedness of incoming light in visible and ultraviolet range of the spectrum. Moreover, CP light provides a more uniform illumination of the plants. Indeed, the electrical vector is circularly rotating at the point of entry inside the plant and therefore, the spatial orientation of plant leaves with respect to the light source should not affect the absorption of light.

MATERIALS AND METHODS

The choice of pea and lentil as plants to grow under the left-handed and right-handed CP light was dictated by their well-known ability to develop quickly and also because their chloroplasts were used in some experiments with light (Gussakovsky *et al*., 2000, 2007).

The following setup was used to study the growth of plants. Two plastic boxes of height 15 cm were kept open at an angle of 50 degrees to the horizontal (Fig. 1). A layer of cotton filled with water and nutrition mixture (concentrated solutions consisting of 7% urea nitrogen, 10% soluble potash, and 10% phosphate diluted in water by a factor of 10^4) was placed at the bottom of the box. The openings of the two boxes were covered with left and right circular polarizers (purchased from American Polarizers, Inc.) respectively, side walls were covered with black paint to avoid any additional reflection of light that could affect its polarization state.

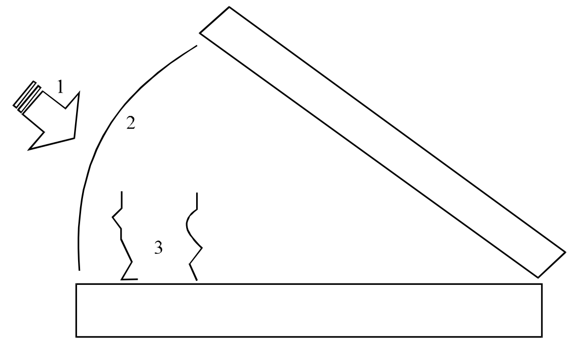

Fig. 1: Schematic representation of the box with 1: Shoots illuminated by circularly polarized light, 2: Left or right-handed circular polarizer through which the light (arrow) penetrates into the box, 3: Shoots

Four experiments were carried out at different times of the year to account for natural light changing conditions from October to March (2009). Plants were kept at room temperature (22°C) and humidity of about 50%. In experiments conducted in October and March the plants in the boxes were irradiated by natural light. Boxes were placed in front of a window of width 1 m, and their position was interchanged once a day in order to maintain similar light irradiation conditions for two boxes. The light flux measured at a maximum sunshine irradiation inside the boxes was 400 lux. The optical densities of left-handed and right-handed polarizers were the same. This was double-checked by measuring the transmission spectra of both polarizers, which were identical within an 0.1% error. The transmission of both polarizers decreased more at shorter wavelengths. At the wavelength of 500 nm transmission decreased by 25%, at 300 nm it decreased by 75% and at 280 nm it decreased by 85%. Artificial light conditions (luminescent irradiation throughout the day with a light intensity of 450 lux) were used in the experiment conducted in December. In every experiment 35 seeds of each type were planted and approximately 30 shoots were analyzed. The seeds that did not germinate were discarded.

To investigate the birefringence of plants tissue, the cylindrical pieces of the pea and lentil stems were cut and unfolded. Then, the interior was cleaned and washed with distilled water. The quantitative analysis of birefringence of a biological tissue is not a trivial problem. Light scattering and non-uniformity makes it difficult to analyze. To overcome these problems a thin upper layer of leaf (epidermis) was put in index matching fluid (which decreases light scattering) between two glasses, and the absorption of light was measured under an optical microscope on a rotating table between crossed polarizers The intensity of light passing through the sample was

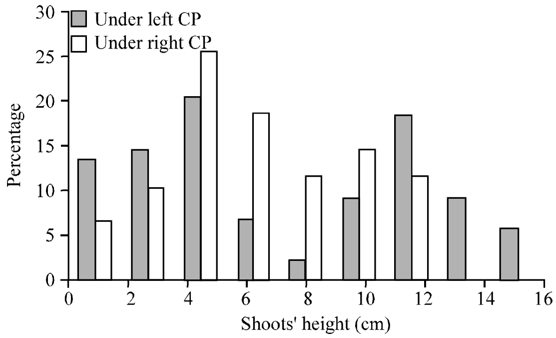

Fig. 2: Distribution of lentil's shoots in experiments conducted in October-December. Gray columns correspond to plants grown under left -handed light; white columns correspond to shoots grown under right-handed light. The maximum length of shoots grown under left-handed CP light is greater

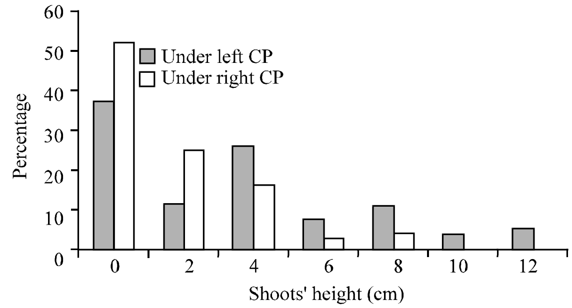

Fig. 3: Distribution of pea's shoots in experiments conducted in October-December. Gray columns correspond to plants grown under left-handed light; white columns correspond to shoots grown under right-handed light. The maximum length of shoots grown under left-handed CP light is greater

recorded by the Ocean Optics spectrometer at 550 nm. The use of this wavelength allowed to discard the absorption of radiation by chlorophyll molecules (absorption peaks of chlorophyll lie beyond 550 nm). This method also allows to check whether or not the sample is birefringent (optically anisotropic with two or three refractive indexes), but does not allow to estimate the degree of birefringence.

A polarimeter (Polatronic NH8) was used in order to get additional insight into the problem of light propagation through the outer part of the stem and leaf. The sample was mounted inside the polarimeter chamber at an angle α with respect to the polarization plane of incoming light. The rotation of the polarization plane was studied at different angles α. Such a setup allowed estimating how linear polarization of light that passes the outer layer of the leaf can be affected by its birefringence. If linear polarization of light does not change much, then it could be concluded that both the effect of birefringence and the change of circular polarization are small.

As a measure of plants' development, the height of the shoots was used. In the three experiments conducted in October and December the height of each shoot was measured on the 8th day of the experiment; in the fourth experiment conducted in March the height of the shoots was measured on the 8th and 16th days. The seeds were subsequently taken out of the boxes and placed on a piece of graph paper where the length of the stretched shoots was measured.

RESULTS

For lentil seeds, the results averaged over the first three experiments are shown in Fig. 2. In this graph the distribution of shoot lengths (percentage of shoots in a certain height range) is plotted. It is easy to see that the length of the tallest shoots in the group grown under left-handed CP light exceeds the maximum shoot length in the group grown under right-handed CP light. In the group that grew under left-handed CP light the maximum length of the shoots reached 17 cm, while in the group growing under right-handed CP light the maximum length reached only 11 cm. In the experiment conducted in artificial light conditions the shoots were in general shorter than in the groups grown under natural light conditions. However, the maximum length of the lentil shoots was approximately 50% greater in the group grown under left-handed CP light than that of the group grown under right-handed CP light. Therefore, the whole distribution of lentil shoot heights that grew under left-handed CP light is shifted towards longer shoots with respect to the distribution of lentil shoot heights that grew under right-handed CP light.

A similar effect was observed for pea seeds. Figure 3 represents the data averaged over the first three experiments conducted in October and December. The shoots' height in the group grown under left-handed CP light reached 12 cm, while the height of the shoots grown under right-handed CP light was only 8 cm. The analysis of shoots heights in each particular experiment shows the following. In the first experiment in October the difference in average length between pea plants under left-handed and those under right-handed CP light was 5.3-3.9 cm = 1.4 cm or 36%. In the second experiment in October the difference in average length reached 2.7-1.2 cm = 1.5 cm or 125%, and in the third experiment in December the difference was 2.0-1.4 cm = 0.6 cm or 43%. In the third experiment the average length of the shoots

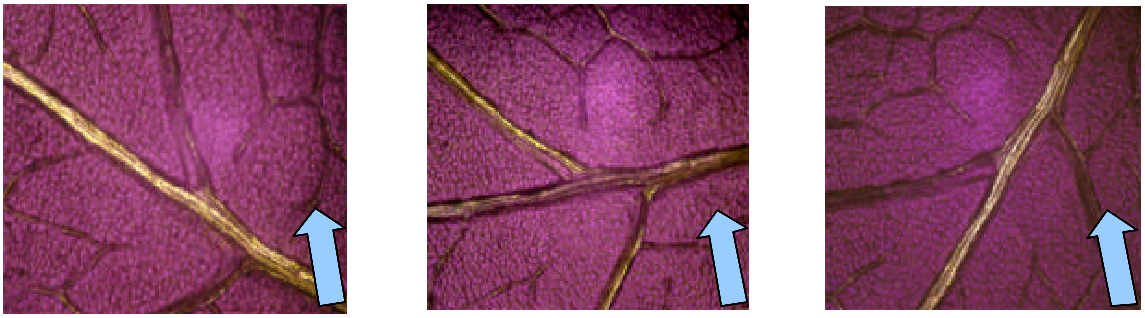

Fig. 4: Parts of a pea leaf viewed through crossed polarizers. Rotation of the leaf by 45 degrees (from the left photo to the right one) changes the intensity of light coming through the veins of the leaf. Direction of electric vector of polarizer A covering the light source is shown as an arrow

was shorter, but in all three experiments with pea shoots, the distribution of heights of shoots that grew under left-handed CP light is shifted towards larger values.

It is important to underscore a statistical significance of the results, as the average combined number of lentil and pea shoots in each group was 60 and the total number of plants analyzed was more than two hundred. Direct measurements of birefringence were performed using a polarimeter as described in the previous section. The rotation of polarization plane at different positions of the outer layer of pea stem with respect to incoming light did not exceed δ = 0.5 degree. Such a small value of angle cannot change the circular polarization of light.

Similar experiments were conducted with the pea leaves. The surface of a pea leaf was carefully removed with a razor blade and washed with water. However, this procedure leaves the upper part of the leaf with the veins, which cannot be completely removed. Rotation of the sample between crossed polars reveals anisotropy in the vein structure (Fig. 4), but not in the structure of the leaf. Rotation of the plane of polarization by the part of the leaf without wide veins lies within an experimental error of 0.05 degrees, i.e., it was even smaller than that of the stem. The same experiments repeated for lentil leaves and stems gave even smaller or undetectable values of δ.

DISCUSSION

Based on the results described in detail in the previous section, it can be concluded that the irradiation of lentil and pea shoots under left-handed CP light leads to higher shoots and their faster growth. Neither stems nor leaves can change the state of polarization of incoming CP light and the effects related to the accelerated growth of plants under left CP light are all related to light absorption by the interior of the leaf or stem.

Left-handed CP light significantly facilitates the development of pea and lentil shoots. The whole distribution of the shoots' lengths grown under left-handed CP light shifted towards greater values. The tallest shoots and the longest roots were found in the group grown under left-handed CP light. This is a very interesting result, which to the best of our knowledge was observed for the first time. It looks like the influence of light polarization in producing higher shoots is especially strong at earlier stages of growth. The observed effect could be used to stimulate the growth of plants by using left-handed circular polarization.

As it was mentioned in the Introduction, there is not much data on the influence of circular polarization on the growth of plants. However, the indirect confirmations of such influence can be found in the articles that point to the enhanced absorption of left-handed CP light by amino acids. Some of them are related to the asymmetric photolysis of racemic mixtures of amino acids (Norden, 1977; Flores, 1977) that was explained in terms of the enhanced absorption of CP light by molecules with the same handedness. Even light with elliptical polarization could induce asymmetrical photolysis in laboratory experiments (Bonner and Bean, 2000). Of course, photolysis requires high intensity of UV light, and it does not happen in leaves under normal conditions, but one of the prerequisites of photolysis is the larger absorption of CP light with the same handedness. That means one can expect the same enhanced absorption of left-handed blue light by left-handed amino acids in plants. Consequently, in accordance to this hypothesis the increased intensity of blue light irradiation (even non-polarized) must lead to enhanced growth. Interestingly, such an effect of blue (non-polarized) light influence on enhanced branching of *Vaucheria terrestris* and intensified movement of organelles in the cells was already observed (Takahashi *et al*., 2001).

The discussed hypothesis of the mechanism of growth enhancement does not preclude from the existence of another mechanism, namely chiral organization of chlorophyll molecules or stacking of chloroplasts (Gussakovsky *et al*., 2000), or the presence of a twist between light sensitive molecules (Ruban *et al*., 2007). Both may lead to the dominance of one type of chiral organization and thus to the enhancement of the absorption of left-handed light.

A small birefringence that was discovered in the stem cannot change the state of light polarization. The outer layer of leaves is almost isotropic (on a scale larger than the cell size), but the vein structure is anisotropic and clearly birefringent. However, it occupies only a small area of the leaf and therefore, cannot change a state of light polarization significantly.

As for the mechanism of the observed accelerated growth under left-handed CP light, the only explanation that seems to be plausible is the higher absorption of light by either chlorophyll, its chiral aggregates (see the studies of chiral properties of the light harvesting complex discussed in the Introduction), or left-handed amino acids. Indeed, the state of polarization of light passing through the outer part of stem and leaf almost does not change. That means that the interior of the plant containing dyes is also illuminated by the CP light, so the enhanced absorption of left- handed light must be related either to chiral organization of chlorophyll molecules or left-handed amino acids.

CONCLUSION

The shoots of lentil and pea grow faster under left-handed CP light; the state of light polarization that penetrates the outer layer of stem and leaf does not change; therefore, the accelerated growth of shoots under left-handed CP light results from its absorption in the interior of the leaves or stems. The observed effect can be used to promote the growth of plants.

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