

THE ROLE OF CAROTENOIDS IN PLANT PHYSIOLOGY

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Abstract - Plant carotenoids are influenced reversibly and irreversibly by exogenous and endogenous factors. Pesticides, particularly herbicides and plant growth regulators, induce the presence of carotenoids in plant cells. Even degradation products of carotenoids, formed in the plants, show an important influence on the stability of these pigments.

An interesting subject, the physiological importance of carotenoids in connection with the pollution of flowers is mentioned at the end of this paper.

There are no green plants occurring in nature which lack carotenoid pigments. Carotenoids protect the chlorophylls against the light-induced destruction by molecular oxygen. A second function of carotenoids is that of an accessory light-gathering pigment and a third function is probably as a carrier of light-induced reductive deoxygenations. The third function is restricted to epoxy-carotenoids.

It is assumed that carotenoids move through the membrane of the plant cells. Such transport mechanisms have not only a scientific interest. They are of practical importance for farmers and crop producers. The existence of carotenoids and their distribution in cultivated plants or plants for feeding animals is of highest importance. To increase the carotenoid content of plants and to protect carotenoids against many kinds of attacks has to be mentioned in this connexion. In such a practical philosophy the interaction of plant protecting agents with carotenoids is of high importance. Unwanted interactions of plant protection agents or pesticides, particularly irreversible effects in the plant, have to be eliminated. The application of insecticides does not bring many problems in regard to the protection of the plants. Fungicides and herbicides are much more difficult to handle. To kill a pathogenic fungus or in a selected way an unwanted weed without damaging the surface area of a leaf of a plant to be protected is not easy. Everybody is well aware what effects can be caused by so-called total herbicides.

The influence of some plant protecting agents and their influence on carotenoids is mentioned here briefly. Three well known herbicides, amitrol, dichlormate and pyriclor, have been chosen, due to the fact that detailed studies in regard to their influence of carotenoids are described in the literature (1).

For the crop product the visible symptoms in the treated plants are of first interest. Very soon a bleached, chlorotic appearance of tissue is produced subsequent to herbicide application. It seems that the chloroplast structure is disrupted. The work of Krinsky (2), where it is reported that carotenoids can remove oxygen from excited chlorophyll-oxygen complexes via a carotenoid-epoxide cycle, has to be mentioned here. This basic mechanism prevents photooxidation or bleaching of the chlorophyll and could explain the protective action of carotenoids.

If wheat seedlings are grown in the presence of the before-mentioned herbicides, it can easily be shown that the carotenoid synthesis is inhibited and therefore the absence of carotenoids leads to photodestruction of plastid ribosomes and proteins as well as chlorophylls.

The inhibition of carotenoid synthesis is suggested as the primary effect of these herbicides, or of herbicides in general, resulting in chlorosis, plastid disruption, and ultimately, death of the plant.

Naturally occurring degradation products of carotenoids show specific physiological effects in plants. Plants which are resistant to drought can be divided into two groups. There are plants which conserve water and such ones which show an ability to absorb an increased volume of water. The first mechanism is based on biochemical pathways, the latter is physiological.

A way of conserving water is to reduce the time when the leaf stomata are open or to only open the stomata at night-time. A compound which is responsible for such a regulation mechanism is abscisic acid (3).

The concentration of abscisic acid is increased in plants manyfold during wilting. The effect of abscisic acid is reversible and the compound drops to normal low levels when the water supply is replenished. There is evidence that the abscisic acid produced during wilting is not completely degraded but instead stored in a biologically inactivated form in the plant, presumably in the leaves and becomes available again to the plant if a further water stress occurs. Other related compounds, basically sesquiterpenes, can replace the abscisic acid. Some of these compounds show very often an additional insecticidal effect. The question arose to what extent the exogenous application of abscisic acid to plant leaves has practical benefit in reducing transpiration and hence the amount of water needed by a given crop. Unfortunately, it would at present be very expensive in that relative large amounts of abscisic acid are needed to be effective and in view of the rapid turnover of this compound when applied externally to crop plants.

Combinations of abscisic acid and ascorbic acid have been sprayed on citrus trees to get synchronous drop of ripe fruits. The promising results found the enthusiasm of the growers - but not the price of the combination.

There is no doubt that chemical and biochemical relations exist between various carotenoids and the plant growth regulator abscisic acid. In vitro experiments give evidence for the reality of this hypothesis. Unfortunately, there is even today a lack of experimental evidence for this hypothesis in higher plants. It is indeed a fascinating idea that carotenoids after normal degradation in the plant are transformed in metabolites which show specific physiological effects in the same plant.

Besides the importance of carotenoids in photosynthesis and photoprotection there is also the influence of carotenoid metabolites to be taken into consideration. Progress in synthesis of abscisic acid and related compounds (4) will perhaps stimulate further research in this field.

Particularly the almost ubiquitous presence of violaxanthin has to be mentioned and the possible role of this di-epoxide as a precursor of physiologically active degradation products has been discussed for many years (5).

It can be taken for sure that only β -carotene acts as a pure photoprotector besides its well-known role as precursor of vitamin A in animals. What happens to β -carotene in plants was postulated some years ago by Glover and Redfearn (6) in their degradation scheme. It is indeed interesting that only the homologous β -apo-carotenals could be found as degradation products of β -carotene in plants but never the corresponding apo-carotenoic acids. So it can be suggested even β -carotene in many cases as a final product in plants, which in combination with the chlorophylls is an important source of feed.

Why compounds like violaxanthin exist in such a wide variety of plants cannot be answered clearly. The formation of an epoxide can be regarded as a reason with the consequence to be the source of physiologically active compounds like abscisic acid, xanthoxin and others. A postulate at least can be made, that oxidized carotenoids have a function as precursors of physiologically active metabolites influencing the life of plants.

A third more or less unusual role of carotenoids will be mentioned in the third section of this paper.

Insects, bats and birds visit flowers to feed on or to collect for future consumption nectar and pollen. During these activities flowers are pollinated, so that both partners have benefit from this mutual association.

Scent and colour of the flower, and the nutritional value of nectar and pollen are important factors in such an interrelationship. Animals are able to detect terpenes and other volatiles of flower odour at some distance. As the pollinator arrives near the plant, it also receives a visual signal, in the contrasting colour of the flower against the general green leafy background.

The fact that interactions between plant and animal are very complex and mostly happen during night hours explains why pollination ecology and related biochemical aspects have rarely been described. In temperate latitude the casual observer in a flower garden can follow pollination processes mostly during daylight. The much wider range of active pollinators in tropical habitats is often ignored. Humming birds, an enormous variety of tropical butterflies, wasps, beetles, bats, moths, flies and fleas have to be mentioned in this connexion.

Grasses are mostly pollinated by wind but the majority of plants requires animals for their pollination, particularly due to the fact that male and female flowers are separated.

Another phenomenon is the so-called flower constancy. Many pollinators visit only a number of plants and in some cases only one. For lower animals only a little capacity exists to see colours. The bats are mostly colour-blind. Bees can see in UV but do not show any sensitivity to red. The preferred colours of butterflies, moths, flies and wasps are not yet known. Anyhow, vivid colours, particularly reds, purples, pinks and red-yellows seem to be preferred.

The chemical basis of flower colours can give to a certain degree an explanation of the preference by certain animals. Flower colours are present as pigments in chromoplasts or cell vacuoles of floral tissues.

In animal kingdom colours are produced mainly by the reflection and refraction of light from cell surfaces. This is not the case in plants. Flavonoids are the most important group of flower pigments. The other major group are the carotenoids, which provide principally yellow colours, with some oranges and reds, and hopefully but not yet absolutely certain in complex forms also blue to purple.

Most varieties of the yellow colour in flowers are due to carotenoids, i.e. xanthophylls, such as zeaxanthin, auroxanthin and flavoxanthin. Deep orange flowers contain large amounts of β -carotene and/or lycopene. In the petals the carotenoids are concentrated in chromoplasts, mostly in bound form, linked to protein or esterified with different fatty acids.

Mixtures of two unrelated classes of yellow pigment are frequently found in petals, especially of carotenoids and yellow flavonoids in Compositae.

In a variety of forms of bee flowers honey guides or guide marks guide the insects to the centre, where the sex organs and nectar are present. In yellow flowers many of these honey guides can only be detected by insects. For the human eye they are invisible. Thompson et al. (7) published in *Science* the first paper about the evidence that honey guides invisible to the human eye occur in flowers in a variety of *Rudbeckia hirta*. In daylight, the petals of this compositae are uniformly yellow. However, in UV light, the outer parts of the ray are UV-reflecting and bright, while the inner parts are dark-absorbing. Chemical analysis show that carotenoids are responsible for the UV reflection of the outer ray, and also that these pigments are uniformly distributed through the ray. In the inner dark absorbing zones of the ray there is a different kind of pigment. A number of water-soluble yellow flavonols are found, derivatives of patuletin. Thus, in *Rudbeckia*, there is a separation of function of the two types of yellow pigment present. The yellow carotenoids provide the general yellow flower colour in the plant, in order to attract the bee or other insects from a distance.

The water-soluble yellow flavonols, present in the inner ray, act as UV honey guide, directing the landed bee, which is UV-sensitive, to the nectar in the centre of the blossom. It can be concluded that different types of yellow pigments in the flower have different functions. Eisner et al. (8) showed that in the same tribe Heliantheae as *Rudbeckia* besides flavonols a still unidentified number of carotenoids exist. Halborne et al. (3) examined a large number of yellow flowered species with both carotenoids and yellow flavonols. Their work is of outmost interest for this particular role of carotenoids in plant physiology.

There is no doubt that in many other flowers, not yet examined, similar correlations can be found. It is assumed that also the colours of the pollen have a similar importance like this of the flowers. The present analytical work which was undertaken with the constituents of pollen shows that besides flavonoids α - and β -carotene, lutein, zeaxanthin and a number of mono- and di-epoxides can be found (9).

REFERENCES

1. E. R. Burns, G. A. Buchanan and M. S. Carter, *Plant Physiol.* **47**, 144-148 (1971).
2. N. I. Krinsky, Function of Carotenoids, in: *Carotenoids* (editor O. Isler), p. 669-716, Birkhäuser Basel (1971).
3. J. B. Harborne, *Introduction to Ecological Biochemistry*, Academic Press London (1977).
4. F. Kienzle, H. Mayer, R. E. Minder and H. Thommen, *Helv. Chim. Acta* **61**, 2616-2627 (1978).
5. D. I. Sapozhnikov, Investigation of the violaxanthin cycle, *Carotenoids other than Vitamin A*, Vol. **3**, 47-61, Butterworths London (1973).
6. J. Glover and E. R. Redfearn, *Biochem. J.*, **58**, 15-16P (1954); *Vitamins and Hormones* **18**, 371-386 (1960).
7. W. R. Thompson, J. Meinwald, D. Aneshansley and T. Eisner, *Science* **117**, 528-530 (1972).
8. T. Eisner, M. Eisner, P. A. Hyppio, D. Aneshansley and R. E. Silberglied, *Science* **179**, 486-487 (1973).
9. H. Thommen, The carotenoids of various cultivated lily species; not yet published.