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Structural and Physiological Characteristics of the Coloured Spots on the *Leucojum* Perigone

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S u m m a r y

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The structure and physiological activity of coloured spots on the white perigone of *Leucojum vernum* were studied. The spots contained about 20 times less chlorophylls and about 2 times less carotenoids per fresh weight than the leaves. The greenish spots contained chloroplasts with a normally organized thylakoid system and the DAB reaction was positive thus confirming photosynthetic activity. The yellowish spots had a partially developed thylakoid system with single thylakoids occasionally stacking to form two overlapped thylakoid membranes. In some plastids specific complex aggregates of parallel membranes were also observed. Osmiophilic DAB deposits were present at most of the membranes of the single thylakoids, but at the membranes, arranged in aggregates, the DAB reaction was less intense. The photosynthetic activity of spot plastids was on the average 3 times lower than in the leaves but they performed a very efficient photosynthesis. Although the spots probably have ecological significance, their photosynthetically active tissue also suggests a possible physiological role.

I n t r o d u c t i o n

The flowers have an important ecological role in attracting biotic pollinators. The colour of petals or perigone lobes usually results from pigments in chromoplasts (carotenoids) and in the cell sap (flavonoids). Exceptionally, parts of these flower structures can be green as in the case of *Leucojum, Galanthus* or *Ornithogalum*, thus resembling the tissues with photosynthetic activity. Each tip of

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the white perigone of *Leucojum vernum* has a greenish to yellowish spot. In order to examine whether the spots have physiological activity, we studied the structure, pigment content and photosynthetic activity of the spot tissue.

Material and Methods

Leaves and flowers of *Leucojum vernum* L., growing in its natural environment, were used as the experimental object. For ultrastructural analyses, small pieces of flower spot tissue were fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.2), thoroughly washed, and then postfixed in 1% OsO_4 in the same buffer. After dehydration in a graded series of ethanol the tissue was embedded in Araldite. Ultrathin sections were made with a RMC MT 6000-XL ultramicrotome and were stained with uranyl acetate and lead citrate. The sections were examined with an EM 10A Zeiss electron microscope. For the localisation of photosynthetic activity (the activity of Photosystem I), after a short fixation in formaldehyde, the tissue was treated with diaminobenzidine (DAB) in phosphate buffer (WRISCHER 1978) and thin sections were examined in the electron microscope without further staining.

The pigments were extracted by grinding the spots on the perigone of *Leucojum* in 80% aqueous acetone. After centrifugation, the supernatant was decanted and the pellet re-extracted several times with 80% aqueous acetone. Chlorophylls and carotenoids were quantitatively determined according to LICHTENTHALER 1987.

Photosynthetic activity of spot pieces was measured with an O_2 electrode (Hansatech Ltd., England). The reaction mixture contained 0.1 mol phosphate buffer (pH 7.2-7.4) and 0.1 mol sodium bicarbonate (MILES 1980). The samples were illuminated at saturating illumination with a halogen lamp.

Results and Discussion

Although the greenish to yellowish spots on the perigone tips of *Leucojum vernum* resembled photosynthetically active leaf tissue, light microscope observations showed that they differ in anatomical structure. On the other hand, they had a more complex structure than the white part of the perigone tissue. The cytoplasm of the spot cells was rich in endoplasmatic reticulum, Golgi apparatus and ribosomes suggesting considerable physiological activity.

Coloured spots contained about 20 times less chlorophylls per fresh weight than the leaves. In contrast to chromoplasts, the colour of which results from increased accumulation of carotenoids, spot plastids contained about 2 times less carotenoids per fresh weight than the leaves (Table 1). Thus, the characteristic yellowish colour of the spot is only due to a significantly higher level of total carotenoids in relation to chlorophylls.

The tissue of greenish spots had typical chloroplasts. The grana were composed of 2-5 normally arranged thylakoids and they were connected by stroma thylakoids which stretched along the chloroplast (Fig. 1a). The stroma thylakoids and peripheral grana thylakoids reacted positively with DAB thus confirming photosynthetic activity. The stroma was rich in ribosomes and regularly contained accumulations of plastoglobules. Yellowish spots contained plastids which had a partially developed thylakoid system. Normal grana were missing and long single

thylakoids stretched in a parallel fashion through the plastid. Occasionally, these single thylakoids were stacked to form two overlapping thylakoid membranes. Furthermore, in some plastids, specific complex aggregates of parallel membranes were also observed (Fig. 1b). The distance between neighbouring membranes of the aggregate was very constant (on the average 24 nm). These particular membrane aggregates possibly originate from some kind of stacking of long single thylakoids, since intermediate forms were observed. Similar membrane structures were found in some mutant plastids that contain small quantities of chlorophyll (BACHMANN & al. 1969), or in developing chromoplasts (SPURR & HARRIS 1968, LJUBEŠIĆ 1970). As spot plastids contained significantly higher levels of total carotenoids (in relation to chlorophylls) than the leaf chloroplasts, the excess of carotenoids is most likely localized in lipophilic plastoglobules and partly also in these specific membrane aggregates. Osmiophilic DAB deposits were present at most of the membranes of the single thylakoids (Fig. 1c). In the membranes arranged in specific aggregates the DAB reaction was less intense, indicating loss of photosynthetic activity (Fig. 1d). The stroma of these plastids was dense and contained many ribosomes, implying still intensive protein biosynthesis.

Table 1. The amount of pigments and photosynthetic activity in perigone coloured spots and leaves of *Leucojum vernum*.

	chlorophyll a+b (mg/g fr.wt.)	carotenoids (mg/g fr. wt.)	photosynthetic activity (μmol O_2/g fr. wt./h)
greenish spot	0.118	0.141	49.57
yellowish spot	0.050	0.172	30.08
leaf	1.417	0.302	121.05

The spots contained photosynthetically active plastids, although their photosynthetic activity was on the average 3 times lower than in the leaves (Table 1). However, taking into consideration that the level of chlorophylls in the spots was on the average 20 times lower than in the leaves, their plastids performed a very efficient photosynthesis. Similar observations were made on chloroplasts of some aurea mutants (WRISCHER & al. 1986). In yellowish spots, photosynthetic activity was about 1.6 times less than in greenish spots (Table 1).

Although the spots probably have ecological significance, the specific anatomical structure, metabolic activity of the cells and the presence of photosynthetically active plastids also suggest a possible physiological role.

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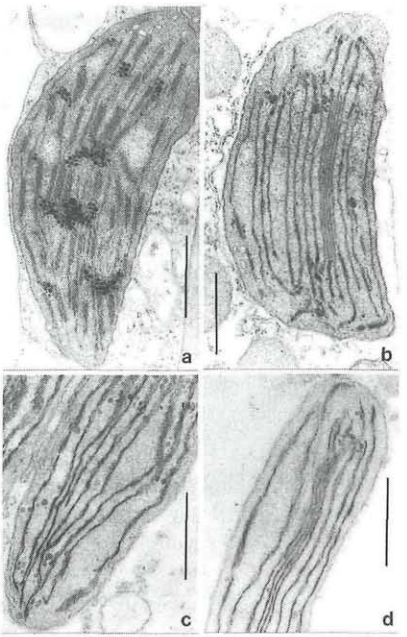

Fig. 1. Plastids from the coloured spots on the perigone of *Leucojum vernum*. Bars = 1 µm. a - chloroplast from greenish spot with a typical thylakoid system and accumulations of plastoglobules; b - plastid from yellowish spot with single thylakoids and specific membrane aggregate; c and d - parts of plastids from yellowish spot - DAB reaction.

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