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FLUORESCENCE SPECTRAL ANALYSIS OF PLANT LEAVES FOR DETECTION OF HEALTHY STATUS OF PLANT LEAVES

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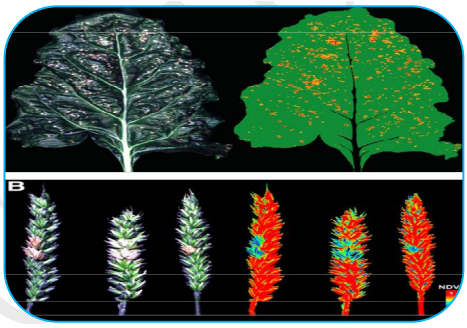
ABSTRACT



UV-B radiation induced blue fluorescence and red chlorophyll fluorescence spectra of green leaves of plants with different leaf structures were determined and potential forms and candidates for blue fluorescence emission were investigated. The blue fluorescence blue fluorescence is characterized by a main maximum in the 450 nm region and a second maximum/shoulder in the 530 nm region. The latter is called green fluorescence. The red chlorophyll fluorescence spectra, in turn, exhibits two maxima in the 690 and 730 nm regions. In general, the intensities of blue fluorescence, green fluorescence and red chlorophyll fluorescence spectra emissions are significantly higher on the lower side of the leaf than on the upper side. The ratio of blue fluorescence to red chlorophyll fluorescence spectra emissions varies from plant species to plant species. The blue fluorescence and green fluorescence emission spectra appear to be a mixed signal composed of fluorescence emission of several plant vacuole and cell wall materials, which may originate primarily in the epidermis. Leaves with removed epidermis and chlorophyll-free leaves, however, still exhibited blue fluorescence and green fluorescence emissions.

KEYWORDS: *blue fluorescence and red chlorophyll fluorescence, green leaves of plants.*

INTRODUCTION

Green leaves of higher plants are composed of a chlorophyll-free epidermal layer that surrounds photosynthetically active, green mesophyll cells. In bifacial leaves of C3-plants, green mesophyll cells are arranged in densely packed parallel rows of cylindrical cells and a network of randomly distributed green cells in large air interstices and cavities on lower leaf sides. As a result, the number of chloroplasts and photosynthetic pigments in the upper half of the leaf is several times higher than that of the lower leaf. In C4-plants the green mesophyll cells with their specific photosynthetic pathway are usually arranged in a semi concentric arrangement of mesophyll and bundle-sheet cells called a Kranz-type leaf structure. Hence the characteristic differences between upper and lower leaf sides of C3-plants are not seen in C4-plants.

In contrast to green mesophyll cells with chloroplasts, epidermal cells contain leucoplasts that are devoid of chlorophyll and carotenoids. The vacuoles of upper and lower epidermis cells contain various phenolic compounds, which vary from plant to plant, such as cinnamic acid, stilbenes, coumarins, various flavonoids, etc., some of which exhibit blue fluorescence. Fluorescence emission spectra have not yet been recorded. Phenolic plant substances and alkaloids as well as catechins and other secondary plant products are not only bound to the vacuole but some of them may also be present

in the cell wall. It is generally accepted that the bulk of these specific plant products are localized in the epidermal cells, where they may act as a barrier to protect the chlorophyll containing mesophyll cells from UV-damage.

Most of the visible light absorbed by photosynthetic pigments is used for photochemical quantum conversions in photosynthesis. A small portion of the absorbed light energy, however, is re-emitted as either heat or red chlorophyll fluorescence red chlorophyll fluorescence. The latter is well investigated and exhibits two maxima in the region of 690 and 735 nm. The red fluorescence emission ratio F690/F735 can be used as an indicator of in vivo chlorophyll content as well as an indicator of short-term or long-term stress. The blue fluorescence blue fluorescence, induced by UV-light and apparently known already in the last century, was recently rediscovered by Chappell. blue fluorescence shows a maximum in the 450 nm region and a second maximum in the green region near 530 nm. The origin and physiological significance of the blue fluorescence of green plants, however, has not yet been elucidated.

To better understand blue fluorescence, in the present paper we investigate the blue, green and red fluorescence signatures of green leaves with different leaf structures due to UV-B radiation, report on the differences between upper and lower leaf sides, and describe the fluorescence. Spectra of various endogenous organic plant compounds that are potential candidates for in vivo blue-green fluorescence emission.

METHOD:

UV-B radiation (339 nm induced fluorescence-emission spectra of plant leaves between 410 and 810 nm were measured using either a) Shimadzu spectrofluorometer red chlorophyll fluorescence5001PC (with red sensitive photomultiplier R928; excitation or continuous light source: X502) b) a pulsed UV- A laser/OMA III system consisting of an optical multichannel analyzer with a nitrogen laser and diode array system. The laser frequency was 20 Hz and the integration time was 2's for a fluorescence-emission spectrum. Leaf fluorescence was excited and sensed on the leaf surface at an angle of 47°, on the upper or lower leaf side. Leaf pigments (chlorophyll a + b and carotenoids x + c) were determined using re-extinction coefficients in acetone.

Various phenolic substances or other organic compounds were dissolved in methanol or water and blue and green fluorescence emission was determined by 10-5 molar solutions. Endogenous b-carotene was isolated as the front strip of a TLC plate where the chlorophyll and carotenoid of the leaf pigment extract were separated and then purified by two-dimensional TLC using heptane and CCl4 solvents. The plants examined here were grown in the greenhouse of the Botanical Garden of the University of Karlsruhe. These are bean (Phaseolus vulgaris L.), maize (Zea mays L.), Crassula ovata L., tobacco (Nicotiana tabacum L. aurea mutant Su/su) as well as Ginkgo biloba L. from a tree in the college premises. The spectra presented in the figures are from single, developed leaves, although these were representative of a particular plant, as tested by measuring the fluorescence emission of several other leaves (n = 4). The variation in relative fluorescence intensity of blue fluorescence, green fluorescence and red chlorophyll fluorescence between different fully developed leaves of the upper or lower leaf side of the same plant was less than 7%, when comparing leaves with similar chlorophyll content, as determined by analysis.

OBSERVATION:

The UV-light induced fluorescence-emission spectra of the bifacial leaf of C3-plant bean has a maximum at 450 nm (blue fluorescence), a shoulder near 530 nm (green fluorescence), and two peaks in the red region near 690. 735 nm (red chlorophyll fluorescence). The intensity of fluorescence emission is higher in the blue-green as well as in the red spectral region of the lower leaf side with less photosynthetic pigments. The blue to red fluorescence ratio F450/F690, however, is significantly higher in the upper (0.6) than in the lower leaf side (0.4). This is due to a stronger enhancement of red chlorophyll fluorescence at 690 nm than the blue fluorescence emission of the lower leaf side. Due to the lower chlorophyll content of the lower leaf side (larger air spaces between mesophyll cells), the

reabsorption of emitted chlorophyll fluorescence in the 690 nm region is much lower in the lower half than in the upper leaf half, which is densely packed. and chlorophyll-rich palisade cells. The resulting chlorophyll fluorescence ratio F690/F735, which is inversely correlated with chlorophyll content, is significantly higher in the lower than in the upper leaf.

The CAM-plant Crassula with succulent leaves, which in principle has a bipartite leaf structure, exhibits UV-B radiation-induced fluorescence-emission spectra that are similar to the C3-bean-plant Phaseolus. Blue (450 nm), green (530 nm) and red fluorescence were significantly higher in the lower part of the leaves than in the upper part ($P<0.01$) and this also applied to the chlorophyll fluorescence ratio F690/F735 change. In contrast to bean leaves, the upper leaf side blue fluorescence / red chlorophyll fluorescence ratio F450/F690 in Crassula is much higher (2.3 instead of 0.6) but the lower leaf side exhibits a value of 0.6, which is similar to green bean leaves.

Blue and green fluorescence occurs not only in green leaves but also in chlorophyll-free yellow leaves e.g. of the Ginkgo biloba tree. In leaves measured in November 1990, chlorophyll had already been destroyed by the autumn chlorophyll-breakdown process, while a large fraction of yellow carotenoids still remained. In contrast to green leaves, the blue fluorescence blue fluorescence of yellow ginkgo leaves is, however, lower than the green fluorescence green fluorescence, which here exhibits maxima between 560 and 580 nm. The relative intensities of blue fluorescence and green fluorescence differed significantly ($P<0.02$) for upper and lower leaf sides, as documented by the different ratios of blue fluorescence / green fluorescence of upper (0.46) and lower leaf sides (0.8).

Blue and green fluorescence is a mixed signal composed of the fluorescence emission of different parts of the leaf, as shown in a tobacco leaf, where the lower epidermis of the leaf has been removed. The fluorescence spectrum of intact leaves exhibits a blue fluorescence maximum near 460 nm and a shoulder of green fluorescence between 525 and 535 nm. The removed epidermal cell layer, in turn, shows only a blue fluorescence (2 maximum near 435 nm). The underside of tobacco leaves without epidermis gives a fluorescence spectrum with maxima near 460 and 525 nm. This suggests that the blue and green fluorescence of leaves not only originates from different parts of the leaf, but also from different chemicals bound to different parts of the leaf and having different fluorescence-emission maxima.

To gain more insight into the origin and nature of the blue and green fluorescence of leaves, we measured the UV-B radiation induced fluorescence emission spectra of various organic compounds known to be endogenous components of plant leaves. Cinnamic acids, such as caffeic, ferulic and sinapic acids, known to be constituents of vacuoles with preferential localization in leaf epidermis, as well as depsides of chlorogenic acid, caffeic and quinin acid, exhibit blue fluorescence emission with maxima near 445 nm. Coumarins, such as aesculetin and scopoletin, also show strong blue fluorescence emission with maxima near 445 nm. stilbenes, e.g. T-stilbene and reappointing exhibit blue fluorescence emission maxima near 385 and 395 nm, respectively. (+)-Catechin, a tan compound from many plants, has a blue fluorescence maximum near 445 nm. The alkaloids berberine, a cell-wall component of some plants, and quercetin, a widespread flavanol, exhibit green fluorescence emission with maxima near 525 to 535 nm. This also applies to riboflavin, a cytoplasmic compound, which exhibits a green fluorescence maximum near 535 nm.

We also tested some compounds known to be components of chloroplasts. Reduced NADPH exhibits a blue fluorescence maximum near 46 nm. It is shown here for the first time that reduced phylloquinone has extremely strong blue fluorescence near 445 nm. In contrast, oxidized phylloquinone K1, a component of photosystem I, exhibited no spontaneous fluorescence emission; A weak green fluorescence of K1, which was applied to quantitatively estimate K1, is observed only after prolonged UV-treatment that destroys the naphthoquinone molecule, another chloroplast compound, bound to the chlorophyll carotenoid proteins of photosystems I and II, B - is carotene. B - The carotene fraction extracted from the TLC-plate after one-dimensional separation of leaf pigments exhibited a blue-green fluorescence emission, which, however, completely disappeared upon purification of p-carotene by two-dimensional TLC.

DISCUSSION:

The results of this investigation indicate that UV-B radiation induced blue and green fluorescence emission appears to be a common property of plant leaves and is found not only in green leaves but also in chlorophyll-free yellow leaves, as shown here for the first time. For ginkgo leaves. Green leaves exhibit red chlorophyll fluorescence red chlorophyll fluorescence with two maxima near 695 and 740 nm, in addition to blue fluorescence and green fluorescence emission. Although red chlorophyll fluorescence is commonly induced by blue light or red laser light, results show that it can also be induced by UV-B radiation. Excitation by UV-B e.g. 340 nm, as introduced by Pele, has the advantage of being able to simultaneously measure the entire fluorescence-emission spectra of plant leaves between 410 and 810 nm.

The relative intensity of blue fluorescence, green fluorescence and red chlorophyll fluorescence emissions varies strongly from plant species to plant species and may be plant-specific. The blue fluorescence / red chlorophyll fluorescence ratio (F450/F690) of the upper side of the leaf is low for bean (0.6), but high for Crassula and maize. Natural variation in this fluorescence signature due to stress or developmental stage is not yet known and must first be determined before further statements regarding potential species identification can be made. The chlorophyll fluorescence ratio F690/F735 also varies among plant species, but this variation is due only to differences in chlorophyll content, as shown earlier.

The fluorescence emission of the lower leaf sides of bilateral leaves of C_{3-} plants was significantly higher than that of the upper leaf side over the entire range of blue fluorescence, green fluorescence and red chlorophyll fluorescence emission ($P < 0.005$). Since the lower leaf sides have fewer cells and lower amounts of chlorophyll and carotenoids, this suggests that the intensity of blue fluorescence and green fluorescence emission is strongly influenced by the amount of chlorophyll and carotenoids. The latter can reabsorb a large proportion of the emitted blue and green fluorescence, as this pigment group has broad absorption bands in the blue and green regions. On the underside of the leaf, with fewer cells and larger air spaces, emitted blue fluorescence and green fluorescence emissions have a better chance of being reflected off the cell walls and passing through the leaf without reabsorption than densely packed and pigmented ones. A rich palisade parenchyma on the upper side of the leaves.

In the case of red chlorophyll fluorescence, the high red chlorophyll fluorescence emission of the lower leaf side is controlled only by low reabsorption by chlorophyll, since carotenoids do not exhibit absorption bands in the red region. However, the upper leaf side is higher than the blue fluorescence emission. This may be due to carotenoids, which exhibit their absorption maxima in the blue-green spectral region and which here apart from chlorophyll hinder strong blue fluorescence emission through reabsorption. One can assume that blue fluorescence emission reabsorbed by carotenoids and chlorophyll can be used for photosynthetic quantum conversion or re-emitted as red chlorophyll fluorescence. This transfer of reabsorbed blue fluorescence may be another reason why the increase in red chlorophyll fluorescence emission from the lower leaf sides exceeds that of blue fluorescence emission. This consideration suggests that interactions exist between blue fluorescence and red chlorophyll fluorescence that may influence the shape of the overall fluorescence emission spectra.

On theoretical grounds it can be assumed that the main source of blue/green fluorescence emission of leaves should be substances in the epidermal layer, while fluorescence emission from compounds in green mesophyll cells will contribute much less to the total fluorescence emission of leaves. Due to the reabsorption process. In this context one should be cautious with the interpretation of the fluorescence-emission spectra of tobacco leaves from a young tobacco plant in the greenhouse, where the contribution of the epidermal layer to the blue fluorescence emission was not so high. Probably expected. In old and especially outdoor plants exposed to UV-B radiation and exposed to frequent stress conditions, the contribution of the epidermis to the total blue-green fluorescence emission may be greater. This topic as well as further candidates for blue fluorescence and green fluorescence in leaf exudation and their changes due to development, age and stress is the subject of our current investigation. The main emphasis is on the analysis of blue fluorescence and green fluorescence

emissions of selected plants and their changes in concentration and blue-green fluorescence yield due to environmental stress.

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