

Study of the parameters of anthocyanin supramolecular complexes using derivative reflectance spectroscopy

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Among effective methods for *in vivo* investigation of metalloanthocyanins, the non-destructive approaches are of special interest, particularly, reflectance spectroscopy. Informational capacity of this method can be improved by derivatizing of spectral data.

Our work was aimed at the studying of prospects of reflectance spectra differentiation for estimation of intraspecific flower polychroism, defined by metal-anthocyanin complexes.

The object of our investigation was *Centaurea cyanus* L. flowers with different pigmentation. Reflectance spectra were measured using spectrophotometer equipped with integrating sphere and software for mathematical processing of spectral data.

We established key feature of blue *C. cyanus* flowers – two resolved peaks at 575 and 680 nm, that is characteristic for protocyanin – supramolecular complex of cyanidin, apigenin and Fe^{3+} , Mg^{2+} , Ca^{2+} ions. These peaks in purple flowers were shifted hypsochromically to 550 and 668 nm, respectively. Position of short-wavelength maximum in flowers with other coloration types corresponded to un-associated cyanidin form (523 – 529 nm), while long-wavelength maximum was appeared as shoulder at 660 nm.

Position of the bands of the first and second spectral derivatives suggested superposition of biochromes, which are responsible for specific flower coloration patterns. To estimate this superposition, we used the ratio of the intensities of these bands in derivative spectra. Copigmented form of biochrome corresponds to the band in the first spectral derivative at λ_{max} 665 nm — λ_{min} 708 nm; un-associated form corresponds to the band at λ_{max} 515 nm — λ_{min} 555 nm; in the second spectral derivative, corresponding bands were: λ_{min} 685 nm — λ_{max} 729 nm and λ_{min} 523 nm — λ_{max} 542 nm, respectively. The ratio of these bands was maximal when protocyanin was dominating pigment, and was diminished for purple flowers. The approach introduced here allows discriminating of chromophores with close optical properties.