On the Pigmented Spherical Bodies and Crystals in Tepals of Cactaceous Species in Reference to the Nature of Betalains or Flavonols

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Red pigmented bodies were found in tepal cells of three Rebutia species, i.e., R. grandiflora, R. hyalacantha and R. krainziana. The pigmented body within the vacuole was spherical and normally one per cell. It contained betalains which were validated from the visible spectra of tepal extracts and microspectrophotometric survey on vacuoles. Such a particular red pigmented body has already been found in some anthocyanin-producing plants, and called “anthocyanoplast”. Now, a similar structure has also been found for the first time in betalain-producing plants. Therefore, it may be called “betalainoplast”. In R. grandiflora, yellow pigmented bodies which were not well characterized due to their minimal size were also present as a betalainoplast.

On the other hand, pigment-crystals were frequently present in the cells having flavonols such as those in pale yellow tepals of four Astrophytum species, namely, A. asterias, A. capricorne, A. myriostigma and A. ornatum, with or without a trace of betalains. Yellowish crystals were determined as the crystal mass of quercetin by the UV spectral analysis and careful comparison of chromatographic properties with an authentic specimen.

Key words: Astrophytum — Betalains — Cactaceae — Pigment crystals — Pigmented spherical body — Rebutia.

It has been known that flower colors of cactaceous species are mainly manifested by betalain pigments, i.e., red-purple betacyanins and yellow betaxanthins (Reznik, 1957; Piattelli and Imperato, 1969; Iwashina et al., 1985). On the other hand, the yellow and red flower colors in several species belonging to Brasiliocactus and Parodia are due to carotenoids, or betalains and carotenoids (Iwashina et al., 1983), and flavonol pigments have been also found in many cacti (Iwashina et al., 1982, 1984, 1986; Iwashina and Ootani, 1986). Among these pigments, betalains are usually dissolved in the cell sap, and particular pigmented solid bodies such as anthocyanophore (Küster, 1939; Blank, 1958) or anthocyanoplast (Pecket and Small, 1980) which were observed in some anthocyanin-producing plants, e.g., Brassica oleracea L., Dianthus caryophyllus L., Pulmonaria rubra Schott, etc., have not been found in cacti as yet (Nozzolillo, 1972; Pecket and Small, 1980).

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In this survey, the red pigmented bodies which are similar to anthocyanoplast were found for the first time in betalain-containing cells of red tepals in three Rebutia species, *R. grandiflora* Backbg., *R. hyalacantha* (Backbg.) Backbg. and *R. krainziana* Kesselr. Also, the yellow pigmented bodies were rarely observed in the tepals of *Rebutia grandiflora*.

Apart from these red and yellow bodies, the yellow crystal mass of flavonol, probably quercetin, was also found in the pale yellow tepal cells in four *Astrophytum* species, i.e., *A. asterias* (Zucc.) Lem., *A. capricorne* (Dietr.) Britton & Rose, *A. myriostigma* Lem. and *A. ornatum* (DC.) Web.

**Materials and Methods**

Fourteen cactaceous species (Table 1) which were used in this experiment, have been cultivated for a long time in Ito Branch Station of the Research Institute of Evolutionary Biology.

The upper epidermis of their mature fresh tepals was quickly stripped off, mounted in water and observed immediately for the presence of particular pigmented bodies under the optical microscope.

**Survey on visible spectra**

Fresh tepals (1 g) were extracted with 50% MeOH (6 ml), and the visible spectra of the filtered extract were measured with a Hitachi recording spectrophotometer (EPS-3T type) according to Iwashina et al. (1985). Also, absorption spectra of pigmented vacuoles in tepal cells were measured with Olympus microspectrophotometer of the MMSP-TU type according to Iwashina et al. (1983).

**Identification of the flavonols**

Quercetin crystals were identified by UV spectral analysis according to Mabry et al. (1970) and also by direct comparison of the chromatographic behavior with that of an authentic specimen.

**Results and Discussion**

**Pigmented bodies of Rebutia species**

Optical microscopic survey was made on a variety of colored tepals of 14 cactaceous species, whereby intensely pigmented bodies were observed only in three *Rebutia* species, i.e., *R. grandiflora*, *R. hyalacantha* and *R. krainziana* (Fig. 1A). The pigmented bodies were red and present in pigmented cells of some limited red flowers only of the species above but not in other colored ones, e.g., yellowish red (*R. senilis* Backbg. and *R. wessneriana* Berg.), reddish orange (*R. minuscula* K. Sch.), red-purple (*R. violaciflora* var. *carminea* (Buin) Don), reddish yellow (*R. chrysacantha* var. *elegans* (Backbg.) Backbg.) or white (*R. minuscula* var. *albiflora* K. Sch.) (Table 1). Typical red and yellow bodies are shown in Fig. 1B and Fig. 2, respectively. In *R. grandiflora*, yellow bodies were rarely found in the vacuoles. The red body was found only as a