Cause of blue petal colour

SIR — The question of how blue flower colour develops is a fascinating problem which has long attracted attention[1,2]. Early this century Willstätter proposed the pH theory[3], and soon after, Shibata and Shibata put forward their metal complex theory[4] on the basis of the finding that the vacuolar pH (pH_v) in plant cells is generally around 5, and more acidic than that of the cytosol[5]. We were earlier able to confirm the validity of the metal complex theory for commelinin, the blue pigment of *Commelina communis*, by structural determination[6]. However, pH microspectrophotometric measurements, by Asen *et al.*, of blue morning glory petals indicated an alkaline cell sap, which could be responsible for the blue flower colour[7]. Their methods were not able to assess pH_v directly in living cells and therefore we have measured the pH_v of individual blue and red coloured living cells, of morning glory using a proton selective microelectrode[8].

VACUOLAR pH OF MORNING GLORY PETALS

Flower	Cell location	pH_v (no. of experiments)
Blue open flower	Abaxial epidermis	7.7 ± 0.2*,# (26)
Purplish red bud	Abaxial epidermis	6.6 ± 0.4**,# (22)
Purple flower (CO_2, treated)	Abaxial epidermis	6.9 ±0.3**,# (7)
Blue open flower	Parenchyma	6.0 ±0.4 ## (16)
Purplish red bud	Parenchyma	5.8 ±0.5 ## (7)

Data fluctuated within ± 0.1 pH unit per min. Values are means ± s. d. (no. of experiments). Significant differences ($P < 0.001$) were obtained between the * and **, and # and ##.

The open flower of the morning glory, *Ipomoea tricolor* cv. heavenly blue, is light blue, although the buds are purplish red (Fig. 1*a*). We determined the structure of heavenly blue anthocyanin (HBA, Fig. 1*b*)[9] and confirmed using HPLC that both red buds and blue open flowers contain only HBA as a pigment. In aqueous solutions of various pH the colour of this pigment varied widely with variation in pH[1]. The spectra of HBA in aqueous solutions at pH values of 7.68 and 6.37 corresponded to the reflective spectra of the open petal and the bud, respectively, and their colours were stabilized by intramolecular stacking of caffeic acid residues (sandwich-type tacking)[1,10]. Addition of Al^{3+} or Fe^{3+} ions to the HBA solution at pH 6.40 did not result in any bathochromic shift, suggesting no participation of metal complexation.

Light microscopy of transverse sections of petals confirmed that HBA was distributed homogeneously in the vacuoles of both types of epidermal cells, but not in the vacuoles of the parenchyma cells. For measuring pH_v a cut petal was set on a Plexiglas vessel with its abaxial side uppermost and the tip of a double-barrelled pH microelectrode[11] was inserted into the epidermal cells. The pH profile gained by gradually advancing the electrode tips exhibited three stable pH stages (Fig. 2). The pH value of the first inserted abaxial epidermal cell of a blue petal was higher than 7.5 and that of its redepidermis was around 6.6. After the first advancement (Fig. 2, first arrow), the pH suddenly decreased to around 6 and with a further advancement (second arrow) increased to 7.7 in blue petals and to 6.9 in the red petal case. The low pH value of the second stage can be assigned to the pH_v of the colourless parenchyma cells and, thus, the electrodes can be concluded to have been consistently inserted into vacuoles (table). When flowers were exposed to, high ambient concentration of CO_2 gas, the petal colour changed to purple immediately. This colour change proved reversible upon return to air. The pH_v of the CO_2-treated cells was found to be 6.9 (see table), and thus the colour change correlated with the pH_v just as in the *in vitro* case with isolated pigment.

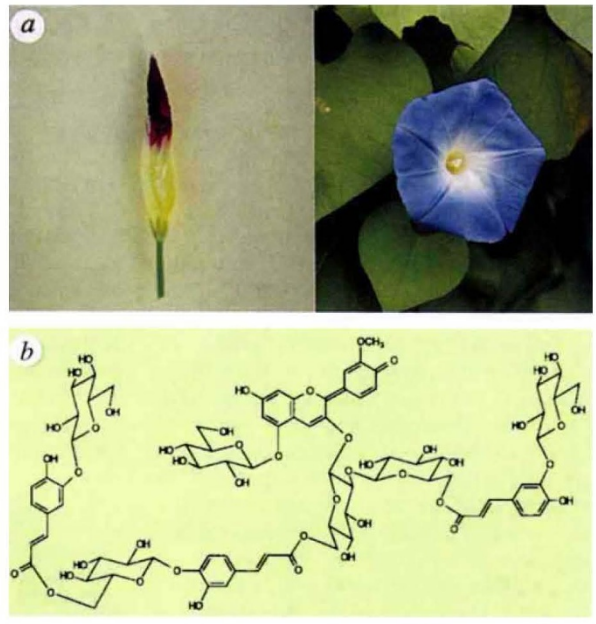

FIG. 1*a*, Blue blooming flower (right) and the bud (left) of *Ipomoea tricolor* cv. heavenly blue. *b*, Structure of heavenly blue anthocyanin (HBA)[9].

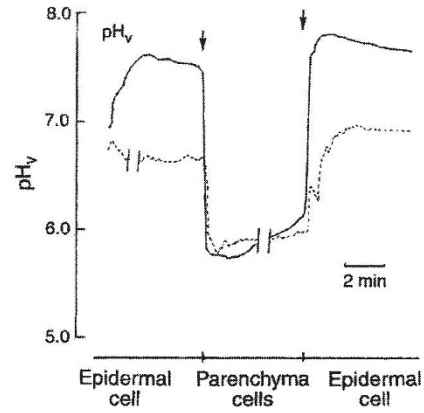

FIG. 2 Profiles of the vacuolar pH (pH_v) of blue open flower-petal (solid line) and red bud-petal (dotted lines) cells using double-barrelled pH-sensitive microelectrodes[11]. The tip was inserted at an angle of 45° into the cells under microscopic observation. A three-step change in pH values was observed when the tip was advanced stepwise. Arrows, advance of the electrode.

The present study provides clear evidence that colour variation in the blue flower petals of *Ipomoea tricolor* cv. heavenly blue is caused by the unusually high vacuolar pH. Independent of whether metal complexation[5] or a high vacuolar pH is the causal stimulus, the mechanism of blue-colour development involves an anionic anhydrobase, the quinonoidal anion of anthocyanin[2].

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