Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.
This journal is © The Royal Society of Chemistry and Owner Societies 2020

Electronic Supplementary Information

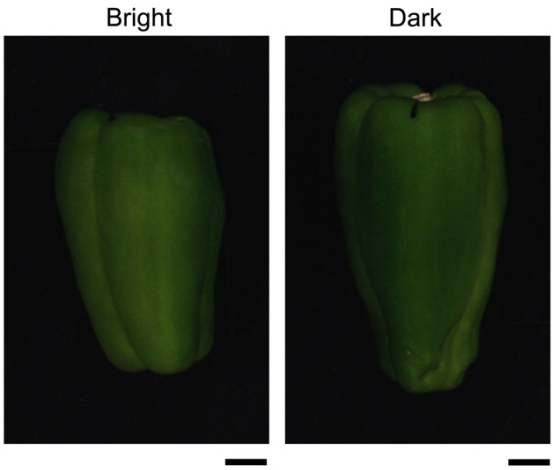

Fig. S1 Colour photographs of green pepper fruits. The corresponding fluorescence photographs are shown in Fig. 1A. Bar = 10 mm.

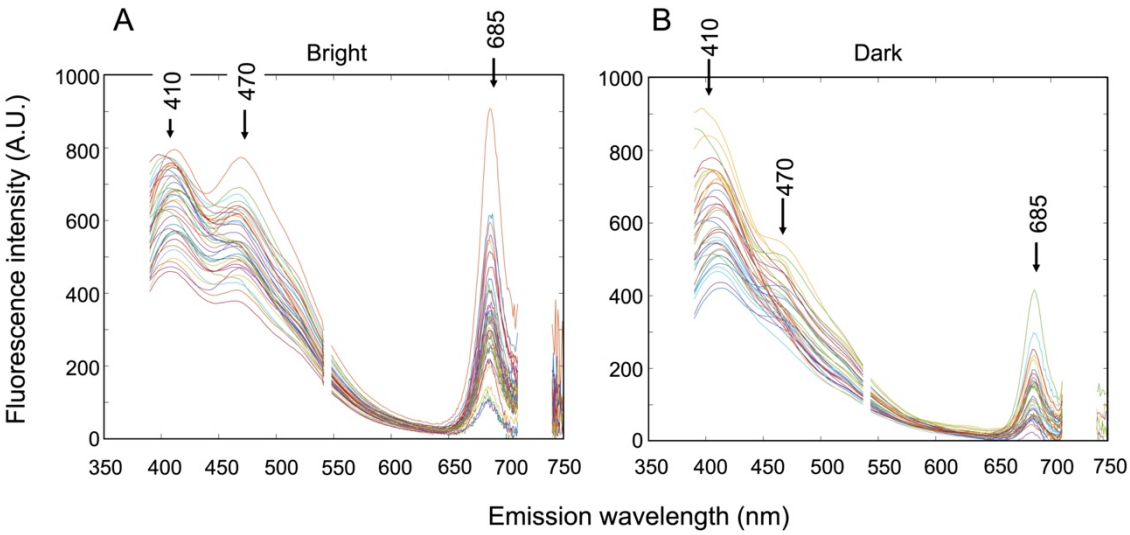

Fig. S2 Fluorescence emission spectra of pericarp in green pepper fruits (excitation at 365 nm). The spectra at around 550 and 730 nm were removed due to the distortions and the second order diffractions ($2\lambda_{ex}$) in the device. Two groups are shown − bright (n = 39) and dark (n = 40) groups.

§ The corresponding fluorescence intensities at 470 and 685 nm are shown in Fig. 1B.

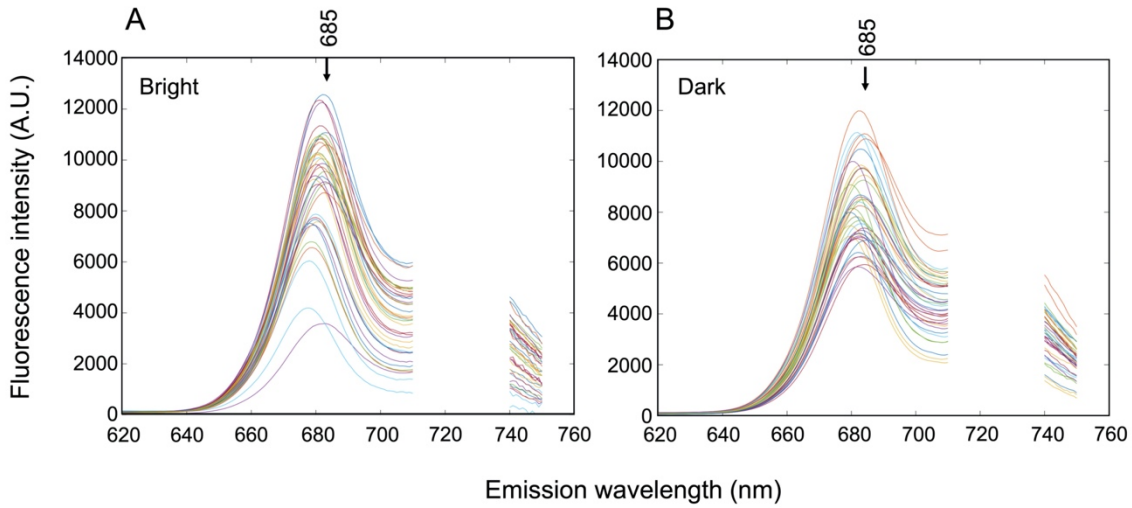

Fig. S3 Red and NIR fluorescence emission spectra of the dried epidermis membrane of green pepper (excitation at 365 nm) for bright (A, n = 39) and dark (B, n = 40) fluorescence groups. Data for the emission at around 730 nm were excluded due to the second order diffractions ($2\lambda_{ex}$) in the device.

§§ These spectra are the same as those in Fig. 3A but rescaled to better present the peak at 685 nm.

Table S1 Traits of fruit, pericarp and epidermis. †

Var. No.	Parts	Traits	Abbr.
1	Fruit	Fruit length	length
2	Fruit	Fruit width	width
3	Fruit	Weight	weight
4	Fruit	Fruits L/W ratio	L/W
5	Fruit	Firmness at pedicel end	firm_p
6	Fruit	Firmness at blossom end	firm_b
7	Fruit	Moisture content on a fresh weight basis	moisture
8	Fruit	Absorption of red light	r
9	Fruit	Absorption of green light	g
10	Fruit	Absorption of blue light	b
11	Pericarp	Absorption of red light, inner side	r_in
12	Pericarp	Absorption of green light, inner side	g_in
13	Pericarp	Absorption of blue light, inner side	b_in
14	Pericarp	Fresh weight per unit surface area	weight_per
15	Pericarp	Dry weight per unit surface area	dry weight_per
16	Pericarp	Thickness	thick
17	Epidermis	Absorption of red light	r_epi
18	Epidermis	Absorption of green light	g_epi
19	Epidermis	Absorption of blue light	b_epi
20	Epidermis	Weight per unit surface area	weight_epi
21	Epidermis	thickness	thick_epi
22	Epidermis	410 nm	F410_epi
23	Epidermis	470nm	F470_epi
24	Epidermis	685 nm	F685_epi

† All variables are mean centered and normalized before PLS-DA, thus having no unit.

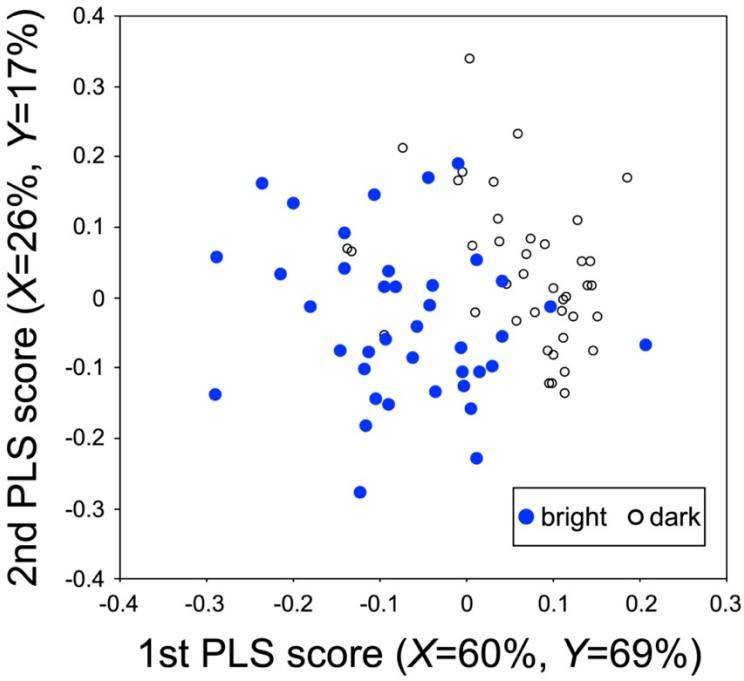

Fig. S4 (A) PLS-DA score plot. Component 1 vs. 2. (A) The percentage in the axis means the explanation power of each PLS component for the *X*- and *Y*-variables (24 traits and binary vectors of the bright (n = 39) and dark (n = 40) fluorescence groups (−1 and 1), respectively).

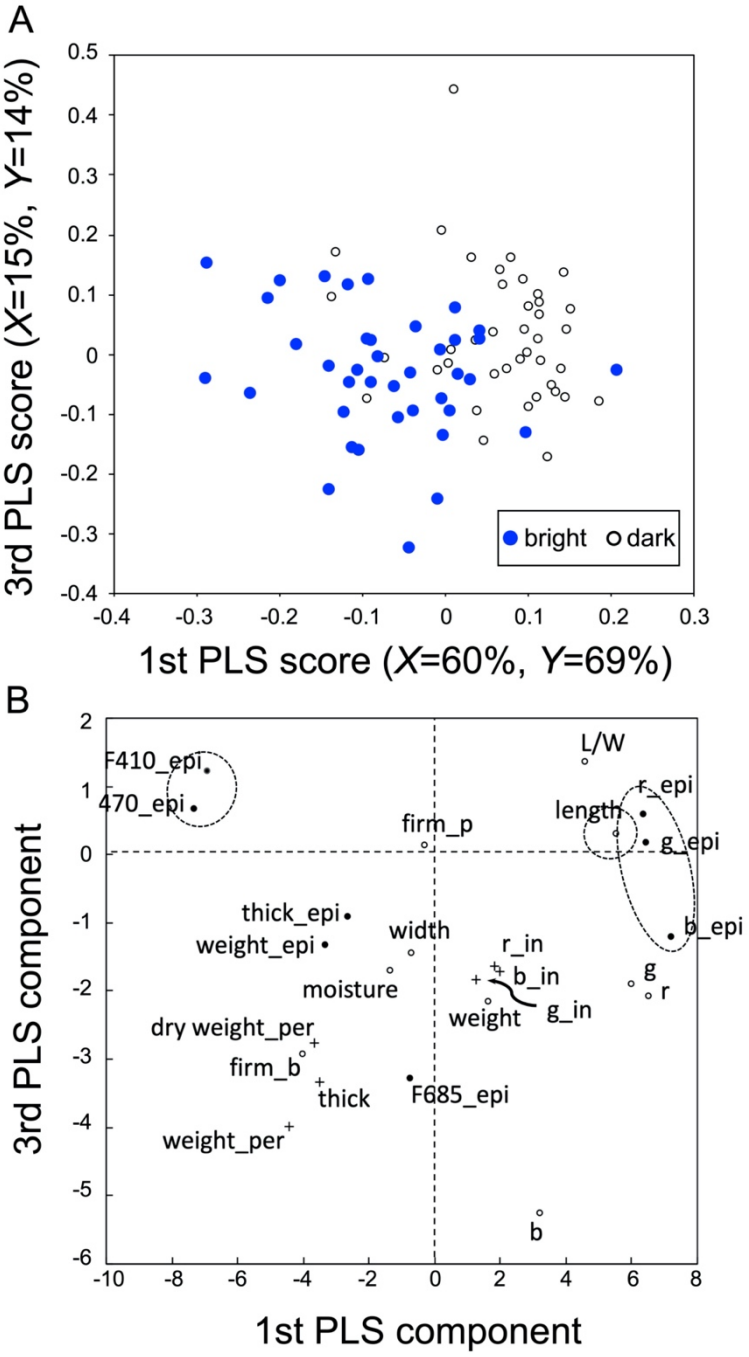

Fig. S5 (A) PLS-DA score and (B) loading plots. Component 1 vs. 3. (A) The percentage in the panel A axis means the explanation power of each PLS component for the *X*- and *Y*-variables (24 traits and binary vectors of the bright (n = 39) and dark (n = 40) fluorescence groups (−1 and 1), respectively). (B) The positive value of the1st loading is related with the dark group. The abbreviations F410, F470, F685 stand for fluorescence of the dried epidermis (epi) at 410, 470, and 685 nm, respectively (excitation at 365 nm). r, g, and b indicate the absorption of red, green, and blue light, respectively, at the fruit

surface, inner (in) pericarp, and dried epidermis (epi). The other variables can be referred to Table S1. The open, cross, and filled circles indicate the traits of the fruit, pericarp, and epidermis, respectively.

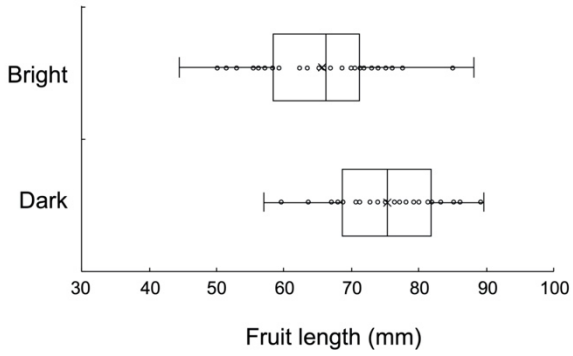

Fig. S6 Fruit length of green pepper fruits. The bright (n = 39) and dark (n = 40) groups are shown.

Experimental section

Fruit material

Green peppers (250 fruits) were harvested in Koyu, Miyazaki Prefecture on March 2, 2020. The samples were shipped on March 3, 2020 and arrived at Kyoto University on March 5, 2020. During the shipment, the temperature varied from 15 to 25 °C. After arrival, they were stored at 18 ° C and 60 ± 10 % RH for four days prior to the experiment. On March 9, 120 fruits that had no injury were selected. Out of these, 40 bright-fluorescent and 40 dark-fluorescent samples were manually selected under UV LED illumination at 365 nm (10 nm width) (CCS Inc., Japan). The sample-source distance was 300 ± 50 mm. Twenty fruits were used each day during the experiment from March 10 to 13, 2020.

Autofluorescence image

The green peppers were classified into two groups based on their blue autofluorescence (i.e. bright and dark groups). To quantitatively confirm the fluorescence brightness, we captured fluorescence images using a camera under the same 365-nm LED (CCS Inc., Japan) illumination used for the classification. A long-pass filter (50% cut at 430 nm) was attached to the camera lens to eliminate the reflection of the excitation light, as described in our previous study.[1] To capture the images, we used a CCD camera AD-080CL (JAI Corp., Ltd., Japan) with F-1.8, shutter exposure 1/30 seconds (fluorescence), and 1/250 s (colour). The distance between the sample and the light source was 200 mm, and the distance between the sample and the camera was 420 mm.

Autofluorescence microscopic images

To investigate the tissues in which fluorophores existed, we captured the fluorescence microscopic images. A fruit was cut into two fractions along the peduncle axis, and one of them was used for this experiment after non-destructive imaging. The half-cut fruit was further cross-sectioned at the equator using a razor. Three fruits were imaged to analyse the two fluorescent groups, and at least two images were recorded per fruit. For excitation, 365 nm UV LED (CCS Inc., Japan) (10 nm width, irradiance at focal plane 0.6 mW cm^{-2}) was used. The distance between the sample and the LED was 40 mm. To capture the images, we used a DFK 23U445 CCD camera (Imaging Source Asia Co.,

Ltd.). A macro lens (1×) and a long-pass filter (50% cut at 430 nm) were attached to the camera lens. The field of view and pixel size were 2,830 × 2,120 μm^2 and 2.21 μm, respectively. The exposure times were set to 1/9–1/6 s and 1/6–1/4 s for the fluorescence and colour images, respectively.

Pericarp and epidermal materials

The pericarp and epidermal materials were created from the equator of the fruit. The pericarp disc was created using a 18 mm punch. The larger sized epidermal disc was created using a 30 mm punch to cope with potential breakage after isolation. The epidermis was isolated using the protocol of Shafer and Bukovac[2] and Li *et al*.[3] A fruit disc of 30 mm diameter was immersed in an enzyme cocktail. The solution contained pectinase 4.0% (w/v) and cellulase 0.4% (w/v) in 50 mM sodium citrate buffer (pH 4.0) with 1 mM NaN_2. The solution was incubated at 35 °C for four days. After incubation, the inner cellular materials were removed with distilled water, and the cutinised epidermal wall was obtained. The epidermal wall was further air-dried at 35 °C overnight. No cellular structures remained as confirmed by an optical microscope. We have referred to this sample as the dried epidermis to differentiate from the intact epidermis.

Fluorescence emission spectra

To correlate the pericarp autofluorescence with the epidermal autofluorescence, we measured the fluorescence emission spectra of the two tissue materials by using a spectrofluorometer FP-8300 (JASCO Corp., Japan). The excitation and emission wavelengths were 360–370 nm and 390–750 nm, respectively. The emission spectra at the excitation of 365 nm were analysed to compare with the fluorescence images. Both slit widths were set as 5 nm. The sensitivity of the photomultiplier tube was adjusted to prevent saturation. The response time and scan speed were set as 50 ms and 5000 nm min^{-1}, respectively. The tissue materials were mounted on a sample holder (20 mm diameter quartz window) in a front-face geometry. The incident and detection angles were 30° and 60°, respectively. The signal-to-noise ratio was more than 50 at the emission wavelength of 470 nm. The excitation light did not reach the detector due to the long-pass-filter used in the device. The second-order diffraction light ($2\lambda_{ex}$) was manually excluded by replacing the corresponding curves around 730 nm with blanks.

Fruit characteristics: Size, weight, shape, firmness, moisture content, and colour

To investigate the correlation between the surface autofluorescence and fruit characteristics, we measured the fruit size, weight, shape, firmness, moisture content, and colour. As fruit size parameters, the width and length were measured using a caliper (Mitutoyo Corp., Japan). The ratio of the length to width (L/W) was used as a shape parameter. The firmness was measured by a Magness–Taylor firmness meter KM-1 (Fujiwara Scientific Co., Ltd, Japan) using a conical plunger (base diameter 12 mm and cone height 10 mm). This measurement was conducted three times for the two ends (i.e. blossom and pedicel ends), respectively, and averaged. The moisture content was calculated on a fresh weight basis using an oven-dry method. The weight of the pericarp disc was measured from the equator by a balance (Shimadzu Corp., Japan) before and after oven-drying at 105 °C for 24 h. The colour at the equator was measured by a colour meter TES135A plus (TES Electrical Electronic Corp., Taiwan). Center wavelengths and the half-widths of typical spectral sensitivities of R, G, B are 600, 550, 450 nm and 90, 110, 70 nm, respectively.[4] The colour value was shown as the logarithm of the reciprocal of the reflectance at each colour (i.e. log $(1/R_r)$, log $(1/R_g)$, log $(1/R_b)$ for red, green, and blue, respectively) to discuss the accumulation of pigments.[5] The quality parameters that were used for further analysis are summarised in Table S1.

Dried epidermis and pericarp characteristics: Colour, thickness, and weight

To investigate the cause of variation in the surface autofluorescence characteristics, we measured the colour, thickness, and weight of the dried epidermis and pericarp discs. The inner colour of the pericarp discs and the outer colour of the dried epidermis were additionally measured using the colour meter used for the fruit characteristics. The colour value was also shown similar to that of the fruit. The thickness of the fresh pericarp and dried epidermis were measured using a micrometer (Mitutoyo Corp., Japan). The average of three records at three different positions was used for analysis. The weight was recorded using an electronic balance (Shimadzu Corp., Japan) and converted to the weight per unit surface area (1 cm^2).

Cuticular structure

To correlate the surface autofluorescence with the cuticular structure of the epidermis, we obtained the microscopic images of the cuticle at the cross section. The pericarp samples for the microscopic images were prepared by fixing a pericarp cube, and subsequent cryo-sectioning and staining using an Oil Red O solution. The protocol of Isaacson *et al*.[6] was used.

A 5 × 5 mm^2 pericarp square sample was immersed in FFA fixative overnight and then cryo-protected with 10 and 20% sucrose in 100 mM PBS. The fixed pericarp was moulded in OCT medium (Sakura Finetek Japan Co., Ltd.), frozen with isopentane in liquid nitrogen, and then stored at −80 °C until sectioning. The pericarp was cryo-sectioned with a thickness of 5 μm using a microtome HM450 with a dry ice tray unit 715400 (Thermo Fisher Scientific, USA). The section was post-fixed with FFA and then washed with distilled water prior to staining. The section was stained with 60% Oil Red O staining solution for 30 min and observed using an optical microscope Motic BA 200 (Shimadzu Corp., Japan) equipped with an objective PLAN 40×/0.65. Three fruits were sampled from each fluorescent group and 100 images were captured per group.

PLS-DA analysis

To investigate the correlations of the traits with the surface autofluorescence characteristics through complex relations, we used statistical analysis. The correlation was visualised using partial least squares-discriminant analysis (PLS-DA). In PLS-DA, we created a binary vector to represent the two groups (−1 and 1 for the bright and dark fluorescent groups, respectively). Twenty four traits were considered as the *X*-variables (refer to Table S1). The two groups were considered as the *Y*-variables. The data were mean centred and weighted by their standard deviation, ensuring equal variances, thus no unit is presented in Table S1. The PLS-DA was performed using MATLAB R2018a software (MathWorks Inc., USA). The percent contributions of the 24 traits (*X*-variable) and fluorescent groups (*Y*-variable) to the PLS scores were calculated based on the loadings.[7] The statistical differences between these traits among the groups were discussed based on descriptive statistical values *including the mean, median, 1st quartile, 3rd quartile, minimum, and maximum. It may be noted that we excluded the outliers from the ranges defined as* $< Q_1 - 1.5 \times \text{IQR}$ and $> Q_3 + 1.5 \times \text{IQR}$, where the interquartile

range IQR = $Q_3 - Q_1$, and Q_1 and Q_3 represent the 1st and 3rd quartiles, respectively.

References

1 K. Konagaya, D. F. Al Riza, S. Nie, M. Yoneda, T. Hirata, N. Takahashi, M. Kuramoto, Y. Ogawa, T. Suzuki and N. Kondo, Monitoring mature tomato (red stage) quality during storage using ultraviolet-induced visible fluorescence image, *Postharvest Biology and Technology*, 2020, **160**, 111031.

2 W. E. Shafer and M. J. Bukovac, Effect of acid treatment of plant cuticles on sorption of selected auxins, *Plant Physiology*, 1987, **83**, 652–656.

3 Y. Li, Q. Li and B. Chen, Organic pollutant penetration through fruit polyester skin: A modified three-compartment diffusion model, *Scientific Reports*, 2016, **6**, 23554.

4 Konica Minolta, Inc., Color Measurement Learning Center - Konica Minolta Color, Light, and Display Measuring Instruments, https://sensing.konicaminolta.asia/learning-centre/color-measurement-learning-center/general-color-terms/, (accessed June 20, 2020).

5 A. C. Ruifrok and D. A. Johnston, Quantification of histochemical staining by color deconvolution, *Analytical and quantitative cytology and histology*, 2001, **23**, 291–299.

6 T. Isaacson, D. K. Kosma, A. J. Matas, G. J. Buda, Y. He, B. Yu, A. Pravitasari, J. D. Batteas, R. E. Stark, M. A. Jenks and J. K. C. Rose, Cutin deficiency in the tomato fruit cuticle consistently affects resistance to microbial infection and biomechanical properties, but not transpirational water loss, *The Plant Journal*, 2009, **60**, 363–377.

7 G. Oms-Oliu, M. L. A. T. M. Hertog, B. Van de Poel, J. Ampofo-Asiama, A. H. Geeraerd and B. M. Nicolaï, Metabolic characterization of tomato fruit during preharvest development, ripening, and postharvest shelf-life, *Postharvest Biology and Technology*, 2011, **62**, 7–16.