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Identity of normal and mutant flower-color pigments in four different *Tradescantia* clones confirmed by means of microspectrophotometry

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ABSTRACT

As a step to investigate the genetic identity of normal blue and mutant pink flower-color pigments in four different *Tradescantia* clones (BNL 02, KU 27, KU 9 and KU 20), which are heterozygous for flower color (blue/pink), the light absorption spectra of the normal blue and mutant pink cells in their stamen hairs were measured microspectrophotometrically. It was found that the blue cells of all the four clones showed the maximum absorption peak at 574 nm, the second peak at 618 nm, and a shoulder at around 544 nm. It was also found that the pink cells of all these clones had two absorption peaks at 546 and 586 nm and a shoulder at around 512 nm. These findings prove that all the four clones examined produce the identical blue-color pigment normally, and also the identical pink-color pigment when the dominant gene for the blue color underwent mutation. One leaky mutant (intermediately colored) hair cell of clone BNL 02 was shown to be producing both the blue- and pink-color pigments.

1. INTRODUCTION

The stamen-hair system of *Tradescantia* heterozygous for flower color has proven to be one of the most suitable materials to detect the genetic effects of ionizing radiations and various chemicals at such low levels as human beings may actually be exposed, and also to study the variation in spontaneous mutation frequency, as reviewed earlier (Underbrink et al., 1973; Ichikawa, 1981, 1992; Schairer et al., 1983). Several blue/pink heterozygous clones such as BNL 02 and BNL 4430 established at Brookhaven National Laboratory, USA, and KU 9, KU 20 and KU 27 established at Kyoto University, Japan, have been often used in the studies of somatic mutations in their stamen hairs, as reviewed earlier (Ichikawa, 1992).

The genetic nature of flower colors of *Tradescantia* has been studied in clones BNL 02 (Mericle and Mericle, 1967, 1971; Mericle et al., 1974; Christianson, 1975; Sanda-Kamigawara et al., 1991), BNL 4430 (Emmerling-Thompson and Nawrocky, 1980), and some other hybrids (Emmerling-Thompson and Nawrocky,

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1979). In *Tradescantia* clones heterozygous for flower color, which are mostly interspecific hybrids, however, it is difficult to conduct usual allelism tests by crosses, although the segregation ratios which were close to a normal Mendelian monofactor 3:1 ratio of blue to pink have been reported in the progeny of clone BNL 02 (Mericle and Mericle, 1967; Sanda-Kamigawara et al., 1991). The dominant normal blue-color and recessive mutant pink-color pigments of clone BNL 02 have been studied spectrophotometrically, and their light absorption patterns have been reported (Mericle and Mericle, 1971; Mericle et al., 1974).

The present study was performed as a step to study the genetic identity of the normal blue and mutant pink flower-color pigments of four different *Tradescantia* clones (two diploid and one triploid stable clones and one highly mutable triploid clone), using a microspectrophotometrical method.

2. MATERIALS AND METHODS

Clones studied

In the present study, clones BNL 02, KU 27, KU 9 and KU 20 were examined. All of them are blue/pink heterozygotes possessing a single dominant gene for the blue color.

Clone BNL 02 is a diploid hybrid ($2n=2x=12$) thought to be derived from a natural cross between *T. occidentalis* (Britt.) Smyth. and *T. ohiensis* Raf. (Mericle and Mericle, 1971, 1973). This clone has been most often used in earlier studies of somatic mutations (Ichikawa, 1992), and is one of the most stable clones in terms of spontaneous somatic mutation frequency in the stamen hairs (Sparrow and Sparrow, 1976; Ichikawa, 1984, 1992).

Clone KU 27 is a diploid segregant ($2n=2x=12$) from clone BNL 02 (Sanda-Kamigawara et al., 1991), and is as stable as clone BNL 02 in terms of spontaneous mutation frequency in the stamen hairs (Sanda-Kamigawara et al., 1991; Ichikawa, 1992).

Clone KU 9 is a triploid hybrid ($3x=18$) between a blue-flowered diploid clone of *T. paludosa* And. et Woods. ($2n=2x=12$) and a pink-flowered tetraploid clone of *T. ohiensis* ($2n=4x=24$) (Ichikawa, 1972; Ichikawa and Takahashi, 1977). This clone shows a relatively stable spontaneous mutation frequency in the stamen hairs, which is about two times higher than those in clones BNL 02 and KU 27 (Takahashi and Ichikawa, 1976; Ichikawa, 1984, 1992).

Clone KU 20 is also a triploid ($3x=18$), but the origin of this clone remains unknown. It possesses, however, part of the characteristics of *T. ohiensis*, and thus appears to be a hybrid between this and an unidentified species (Ichikawa and Takahashi, 1977; Ichikawa, 1984). This clone is highly mutable especially at lower temperature, showing as much as about 23- (Takahashi and Ichikawa, 1976) to 40-fold (Imai et al., 1991) differences in spontaneous mutation frequency in the stamen hairs in the temperature range of 17.5 to 28.3°C, and is therefore regarded

as a temperature-sensitive mutable clone (Ichikawa and Takahashi, 1977; Ichikawa, 1984, 1992; Imai et al., 1991).

Microspectrophotometry

Microspectrophotometrical analyses were performed in the Exhibition Room of Olympus Optical Industry Co. Ltd., Kanda-Surugadai, Chiyoda-ku, Tokyo. Fresh flowers of clones BNL 02, KU 27, KU 9 and KU 20 were carried to the Exhibition Room, storing them in a thermos together with ice (i.e., keeping them at 0°C).

Six stamens were taken from each flower, and were placed in a small amount of liquid paraffin dropped on a glass plate. Observing them under a stereoscope with a white fluorescent lamp and against a milky-white stage plate at a magnification of 20 times, pink mutant hair cells which had occurred spontaneously were sought.

The stamens which had pink mutant cells in their stamen hairs were then placed under another stereoscope equipped with the Olympus MMSP-TU microspectrophotometrical apparatus, and the light absorption spectra of the normal blue cells and the mutant pink cells in the stamen hairs were measured. The microspectrophotometrical apparatus was equipped with a computer, and the absorption of every 2 nm of light wave length in the range of 400 to 700 nm was read automatically and computed. The light absorption pattern (curve) of each of the cells examined was drawn also automatically.

3. RESULTS AND DISCUSSION

The results of the microspectrophotometry on individual normal blue cells in the stamen hairs of clones BNL 02, KU 27, KU 9 and KU 20 are shown in Fig. 1. It is obvious in this figure that the normal blue hair cells of all the four clones showed essentially identical light absorption patterns. All the absorption curves obtained from the four different clones had the maximum absorption peak at 574 nm, the second peak at 618 nm, and a shoulder at around 544 nm.

The results obtained from individual mutant pink cells in the stamen hairs of the four clones are shown in Fig. 2. It is also clear that the pink hair cells of all the four clones exhibited essentially identical light absorption spectra, although the pink cells of clone KU 20 showed a relatively higher absorbance in the short-wave range than those of other clones. All the absorption curves of the four clones commonly had two absorption peaks at 546 and 586 nm and a shoulder at around 512 nm.

These results demonstrate that all of the four different clones examined produce the identical blue-color pigment normally, and also the identical pink-color pigment when the dominant gene for the blue color mutated or was deleted.

The absorption curves of the blue and pink stamen-hair cells of these four clones

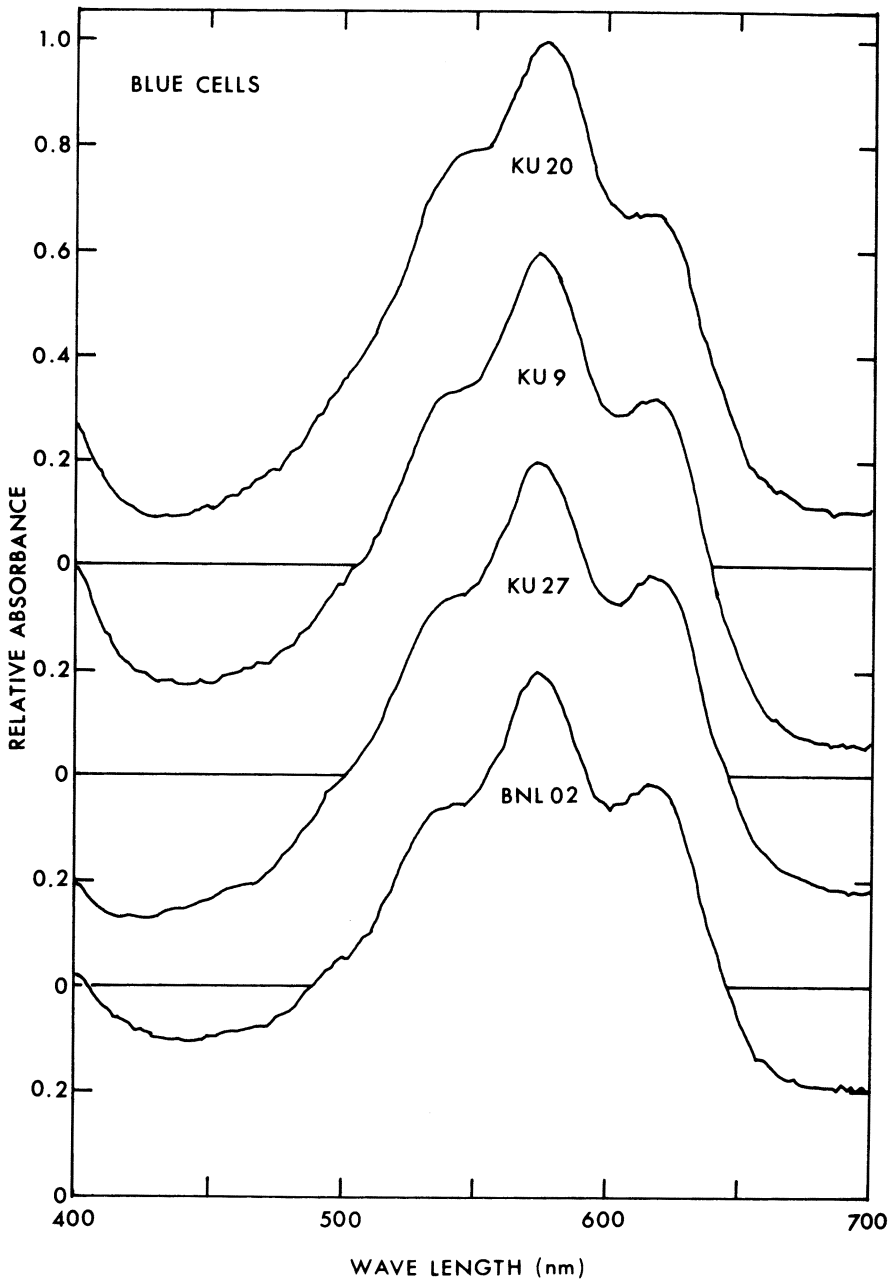

Fig. 1. Light absorption curves obtained microspectrophotometrically from the normal blue stamen-hair cells of clones BNL 02, KU 27, KU 9 and KU 20.

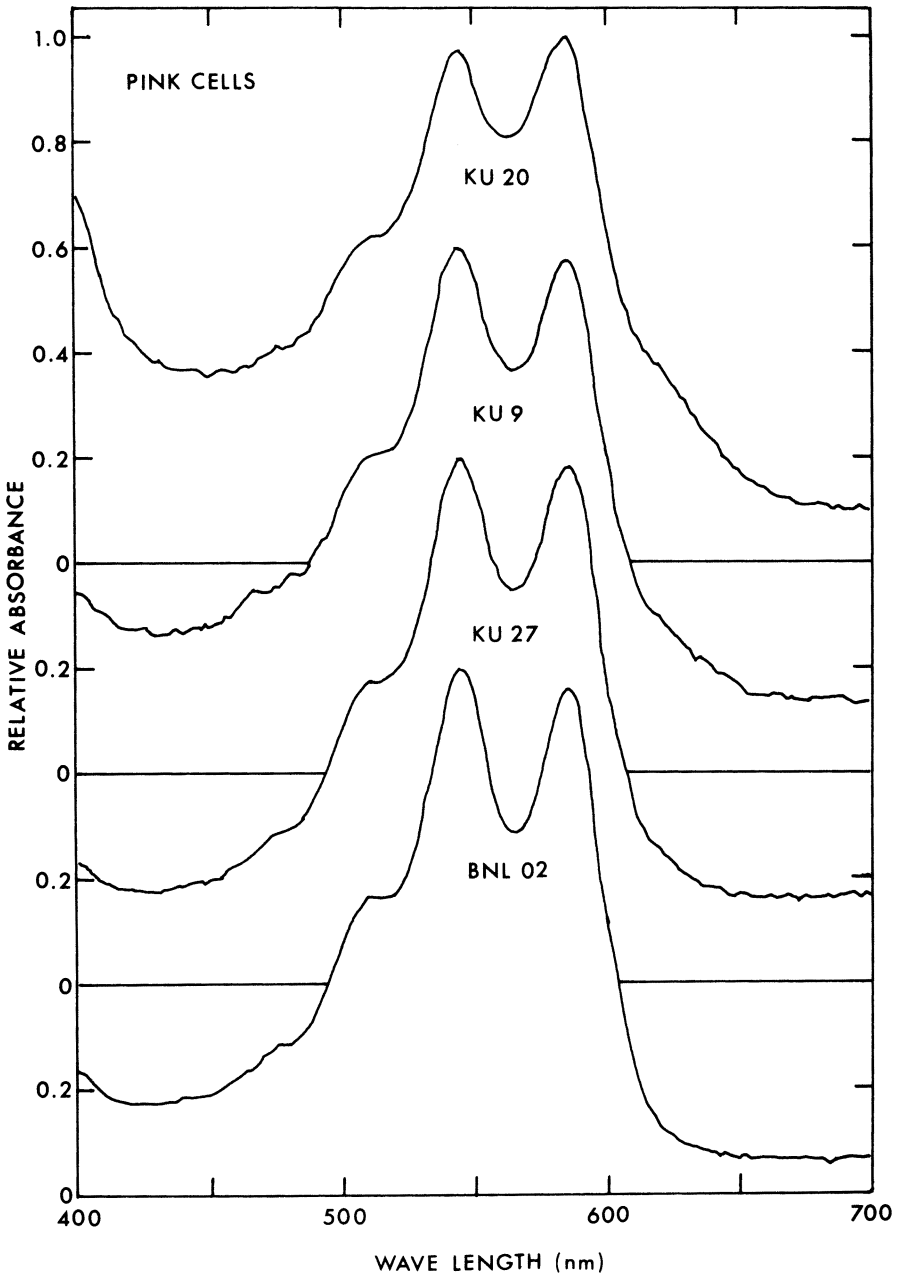

Fig. 2. Light absorption curves obtained microspectrophotometrically from the mutant pink stamen-hair cells of clones BNL 02, KU 27, KU 9 and KU 20.

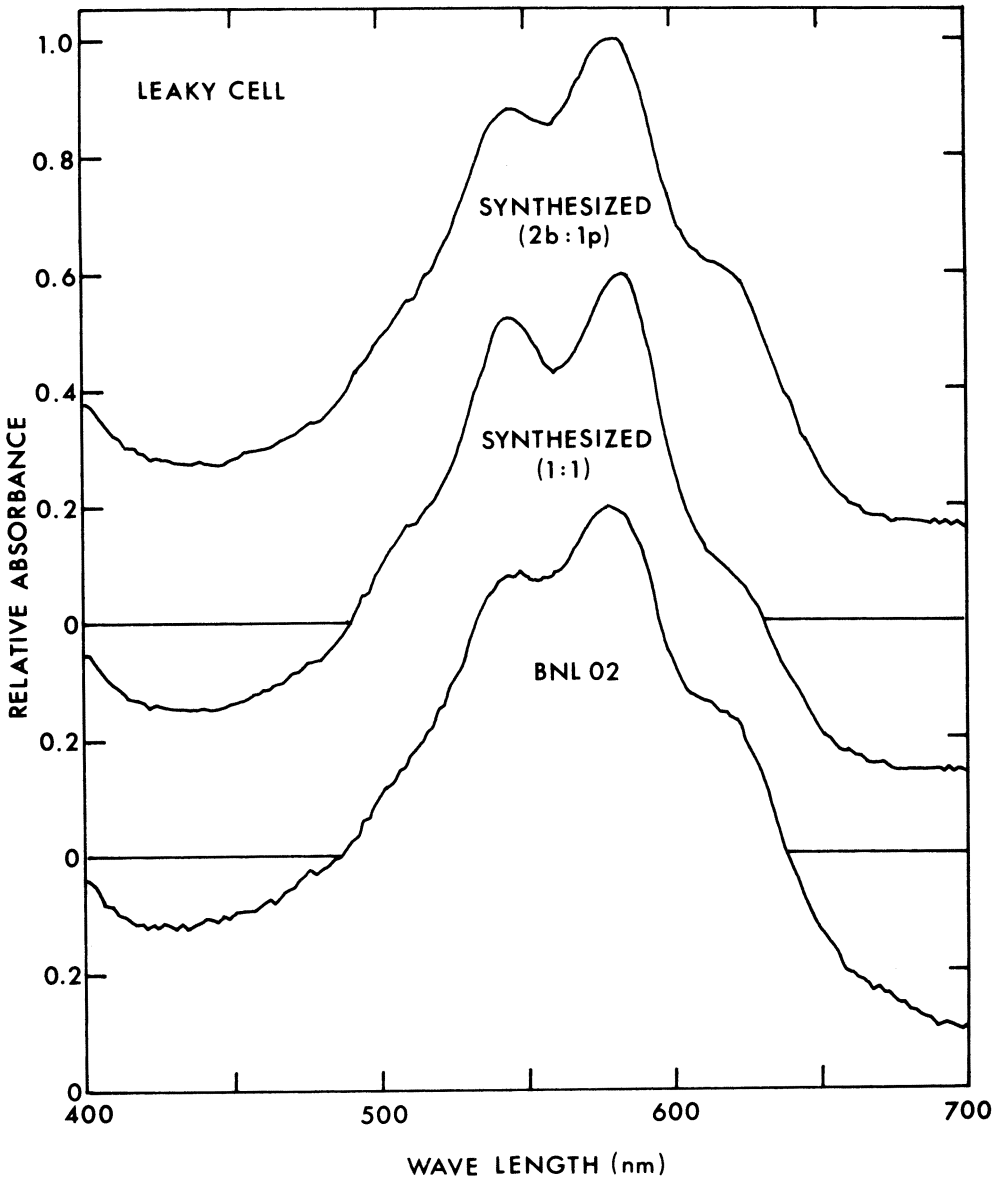

Fig. 3. A light absorption curve obtained micorspectrophotometrically from a leaky mutant stamen-hair cell of clone BNL 02, and two synthesized absorption curves constructed at 1:1 and 2:1 ratios of blue to pink.

are very similar to those reported earlier for the blue petals of clone BNL 02 and for the petals of the pink-flowered progeny of clone BNL 02, respectively (Mericle et al., 1974). One of the minor differences from the earlier report is that the second absorption peak (at 618 nm) of the blue cells in the present study is slightly

higher than the shoulder (at around 620 nm) of the blue petals reported by them. There are also small differences in the values of wave length at peaks, namely, the maximum absorption peak of the blue color at 574 nm differing from 580 nm in the earlier report, and the two peaks of the pink cells at 546 and 586 nm being slightly different from 548 and 590 nm reported earlier.

During the present microspectrophotometrical analyses, one leaky mutant cell which exhibited an intermediate color between the normal blue and mutant pink cells was found in the stamen hairs of clone BNL 02. The light absorption curve obtained from this leaky mutant cell is shown in Fig. 3. The curve had the maximum absorption peak at 578 nm, the second peak at 548 nm, and a shoulder at around 612 nm. When two synthesized absorption curves were constructed based on the curves from the blue and pink cells of this clone (shown in Figs. 1 and 2, respectively), namely, one being constructed at the ratio of 1 blue : 1 pink and the other at the ratio of 2 blues : 1 pink, it was found that the synthesized curve at the latter ratio especially showed a good accordance with the curve for the leaky cell, as seen in Fig. 3. It is therefore concluded that the leaky cell produced both the blue- and pink-color pigments.

The present confirmation of the production of the identical blue- and pink-color pigments in four different clones of *Tradescantia* indicates that all the four clones examined possess, at least, a common biosynthetic pathway of the flower-color pigments, and suggests that these clones have the identical dominant gene to produce the blue-color pigment. It is also suggested that the identical pink-color pigment in these heterozygous clones is produced, when the dominant gene mutated or was deleted, either by the recessive genes having clone-specifically different defects in the biosynthetic pathway or by an identical recessive gene with a certain defect. The present finding therefore gives a strong basis for the feasibility of the direct comparison of the data between these different clones, which have been used in studies of somatic mutation induction in their stamen hairs by ionizing radiations (Ichikawa et al., 1969, 1991; Sparrow et al., 1972; Ichikawa, 1972, 1981, 1992; Underbrink et al., 1973; Ichikawa and Takahashi, 1977; Ichikawa and Ishii, 1991; Sanda-Kamigawara et al., 1991) and chemical mutagens (Ichikawa and Takahashi, 1978; Schairer et al., 1983; Ichikawa et al., 1990; Sanda-Kamigawara et al., 1991; Ichikawa, 1992).

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