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Effects of higher temperature on the coloration in flowers of tulip※ (*Tulipa gesneriana*)

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It is well known that there have been generally three different fields for the study on the coloration in flowers, that is, the morphological, colorimetrical and biochemical. These studies, however, have been so far unconnected, and the literature which deals with the exact relationship among these studies is scanty, while the mechanism of coloration seems to be clarified by the synthesis from the results of these studies.

The present study was designed to throw some light on the interrelationship between the results of colorimetrical and biochemical investigations.

It was shown in a previous paper (Hiura & Nakaji, 1967) that there were colorimetrical and physiological differences in the reaction for the higher temperature (23°C) between the outside and the inside of the same petal.

In the present report, further observations on the influence of temperature on coloration by using five cultivars are described, together with some connections between the colorimetrical and biochemical investigations.

Materials and Methods

The materials used consisted of five cultivars (*Tulipa gesneriana*) listed in table 1. The bulbs planted at the ratio of three per one pot were managed under the outdoor condition from the beginning of November in 1966 to the end of February in 1967, and at the sprouting time they were moved to the growth cabinet in the 15°C constant room, which is the optimum for growth of tulips.

The treatment methods used were essentially the same as that described by

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the previous report (1967), and only a brief outline will be given here. The experimental plot was classified according to the time switched over in the condition of 25°C from 15°C, namely the V_1 plot was before pigmentation of petals and the V_2 plot was before flowering, while the Vc plot as the check was managed till flowering under the condition of 15°C.

Table 1. Flower color and variety name of cultivars

Cultivar No.	Variety name	Petal color name by I. S. C. C.-N. B. S.	
		Outside	Inside
2	Red Matador	strong purplish red	vivid reddish orange
3	Cramoisi Brillant	dark red	dark red
5	Van der Eerden	moderate purplish red	strong purplish red
6	Feu Brillant	moderate purplish red	strong red
7	Utopia	moderate purplish red	strong red

MEASURING COLORS :

The color of one outer petal per plant was measured by the color-meter of the four elements type. The value of Hunter's coordinate a_L and b_L was calculated by the Hunter's formula :

$$a_L = 175(1.02\ X - Y)/Y$$
$$b_L = 70(Y - 0.847\ Z)/Y$$

ANALYTICAL TECHNIQUES :

For the analysis of the anthocyanins present, the outside and the inside of the same petal were teared off separately by the tweezers and the part was macerated respectively. The crude extracts with 1 % hydrochloric metanol were developed by the ascending method of the paper chromatography. Chromatograms were run on Toyo No. 51 (40×40) paper and solvents used were as follows :

normal-butanol : gl. acetic acid : water = 4 : 1 : 5 (v/v)
〃 : 2N HCl = 1 : 1 (v/v)
iso-amylalcohol : conc. HCl : water = 5 : 1 : 1 (v/v)
m-cresol : gl. acetic acid : water = 50 : 2 : 48 (v/v)
formic acid : conc. HCl : water = 5 : 1 : 4 (v/v)
acetic ester : gl. acetic acid : water = 4 : 1 : 2 (v/v)

Results

MEASURING COLORS :

Table 2 summarizes the results of color measurements according to the C. I. E. and Hunter's system. The effect of temperature is illustrated graphically in figure 1 in comparison of the two kinds of treatment plot (V_1 & V_2) with the check plot (Vc).

Table 2. Summary of measuring colors

Plots	Cultivar No.	Side of Petal	Coordinates							
			C. I. E. System					Hunter's System		
			Y	x	y	Pe	nm	L	a	b
Vc	No. 2	outside	16.11	0.47	0.31	41.0	622	39.83	38.58	10.88
		inside	15.55	0.58	0.35	81.3	603	39.02	50.05	22.96
V_1		outside	13.55	0.50	0.29	46.0	− 492	36.81	48.04	9.50
		inside	15.70	0.60	0.35	87.0	604	39.63	51.25	24.44
V_2		outside	15.39	0.46	0.31	39.0	622	38.88	38.05	9.98
		inside	15.31	0.57	0.34	76.0	604	38.97	48.93	21.89
Vc	No. 3	outside	7.35	0.50	0.32	49.3	622	27.04	27.54	9.83
		inside	9.21	0.57	0.33	73.3	610	30.07	41.57	15.45
V_1		outside	8.31	0.51	0.32	54.8	614	28.74	32.20	10.63
		inside	7.55	0.60	0.29	70.8	642	29.01	44.61	15.42
V_2		outside	7.91	0.50	0.33	54.0	608	28.16	28.67	11.10
		inside	9.41	0.58	0.33	67.3	645	30.18	42.16	16.80
Vc	No. 5	outside	13.16	0.43	0.30	29.0	− 492	35.87	29.46	5.98
		inside	13.27	0.49	0.30	44.0	640	35.85	45.08	10.43
V_1		outside	19.49	0.41	0.32	28.0	610	41.07	33.54	5.62
		inside	16.33	0.47	0.30	38.5	645	40.19	42.68	9.34
V_2		outside	13.11	0.45	0.28	40.0	− 494	37.23	40.00	4.80
		inside	10.35	0.53	0.26	64.0	− 494	35.86	44.44	11.10
Vc	No. 6	outside	14.12	0.44	0.30	31.0	700	37.58	34.94	39.87
		inside	10.22	0.53	0.31	58.0	620	31.97	6.59	12.92
V_1		outside	10.72	0.51	0.34	60.0	604	32.50	30.67	14.01
		inside	13.17	0.53	0.31	58.0	619	35.92	42.93	14.69
V_2		outside	11.04	0.49	0.32	49.0	613	33.06	33.54	11.56
		inside	12.41	0.53	0.31	58.0	620	34.66	44.85	15.18
Vc	No. 7	outside	15.81	0.45	0.29	57.2	608	39.75	41.66	6.10
		inside	14.57	0.51	0.33	31.0	700	38.16	40.71	15.10
V_1		outside	20.72	0.42	0.30	27.0	− 492	45.46	34.32	7.32
		inside	16.77	0.50	0.32	51.8	613	40.94	40.43	15.04
V_2		outside	16.16	0.44	0.29	35.0	− 493	40.12	37.41	6.21
		inside	15.87	0.53	0.33	62.0	608	39.83	44.07	17.34

Red Matador (Cultivar No. 2) :

Outside color,

In the V_1 plot, a_L gives the positive and b_L gives the negative relation in comparison with the check plot, and the red color becomes strong.

In the V_2 plot, both a_L and b_L reveal a slight relation for the check plot, and there are no influences of temperature on the coloration.

These results with each of plots may be taken to indicate that the red color becomes strong with the increase of the term of higher temperature condition.

Inside color,

Both a_L and b_L reveal a slight relation for the check plot in the V_1 and V_2 plot, and there are no influences of temperature on the coloration.

In view of the above results it may be concluded that there are differences of physiological reactions for temperature in the outside and the inside of the same petal.

Cramoisi Brillant (Cultivar No. 3) :

Outside color,

Both a_L and b_L give the positive reaction in comparison with the check plot in the V_1 and V_2 plots, showing the relation of $V_1 > V_2$.

These results with each of plots may be taken to indicate that the red color becomes strong with the increase of the term of higher temperature condition.

Inside color,

This is in agreement with the outside color in the view that the red color becomes strong. But the degree of its additional color makes differences between the outside and the inside, and the outside shows more remakable in physiological reactions than the inside.

In view of the above results it may be concluded that there are differences of physiological reactions for temperature in the outside and the inside of the same petal.

Van der Eerden (Cultivar No.5) :

Outside color,

In V_1 and V_2 plots, a_L gives the positive and b_L gives the negative relation in comparison with the check plot, and the red color becomes strong. But the degree of its additional color shows the relation of $V_1 < V_2$, and these results with each of plots may be taken to indicate that the red color relatively becomes strong with the decrease of the term of higher temperature condition.

Inside color,

In the V_1 plot, both a_L and b_L give the negative relation in comparison with the check plot, and the red color becomes weak.

In the V_2 plot, a_L gives the negative, and b_L gives the positive in comparison with the check plot, and the red color becomes weak. The degree of its subtractive color shows the relation of $V_1 > V_2$, and these results with each of plots may be taken to indicate that the red color becomes weak with the increase of the term of higher temperature condition.

In view of the above results it may be concluded that there are differences of physiological reactions for temperature in the outside and the inside of the same petal.

Feu Brillant (Cultivar No. 6) :

Outside color,

Both V_1 and V_2 plots in comparison with the check plot reveal the rela-

tion that a_L gives the negative and b_L gives the positive, and the red color becomes weak, showing the relation of $V_1 > V_2$, and these results with each of plots may be taken to indicate that the red color becomes weak with the increase of the term of higher temperature condition.

Inside color,

Both V_1 and V_2 plots in comparison with the check plot reveal the relation that both a_L and b_L give the positive, and the red color becomes strong. But the degree of its additional color shows the relation of $V_1 < V_2$, and these results with each of plots may be taken to indicate that the red color relatively becomes strong with the decrease of the term of higher temperature.

In view of the above results it may be concluded that there are differences of physiological reactions for temperature in the outside and the inside of the same petal.

Utopia (Cultivar No. 7) :

Outside color,

Both V_1 and V_2 plots in comparison with the check plot reveal the relation that a_L gives the negative and b_L gives the positive, and the red color becomes weak, showing the relation of $V_1 > V_2$.

These results with each of plots may be taken to indicate that the red color becomes weak with the increase of the term of higher temperature condition.

Inside color,

Both a_L and b_L reveal a slight relation for the check plot in V_1 plot, and there are no influences of temperature on the coloration.

Both a_L and b_L give the positive relation for the check plot in V_2 plot, and the red color becomes strong, and these results with each of plots may be

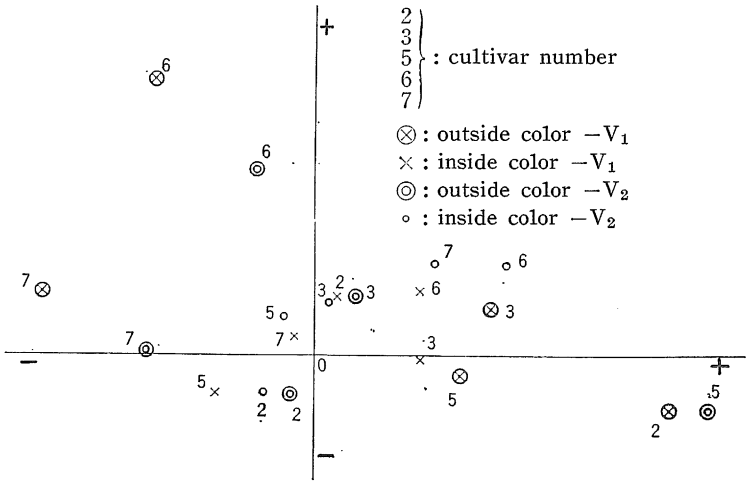

Fig. 1. Coloration in comparison of the V_1 & V_2 plots with Vc plot by Hunter's system.

taken to indicate that the red color becomes strong with the increase of the term of higher temperature condition.

In view of the above results it may be concluded that there are differences of physiological reactions for temperature in the outside and the inside of the same petal.

PIGMENT CONTENTS AND CHARACTERIZATIONS :

Six pigments of anthocyanin are detected in all, and table 3 summarizes the results of appearance of each pigment in the cultivars employed.

Table 3. Appearance of each anthocyanin in the cultivars

Side of petal	Cultivar No.	Spot Number						
		1	2	3	4	4′※※	5	6
Outside	2	+	+	+	−	−	Y※	−
	3	+	+	+	+	+	+	−
	5	+	+	+	+	+	Y	−
	6	+	+	+	−	−	−	−
	7	−	+	+	−	−	−	−
Inside	2	−	+	+	−	−	+	−
	3	−	+	+	+	−	+	−
	5	−	+	+	+	−	+	−
	6	−	+	+	−	−	−	+
	7	−	+	+	−	−	−	+

※ : yellow spot (flavone pigment ?) ※※ : in case of the m-cresol solvents

Two pigments (spot No. 2 and 3) are common to all cultivars, but the appearance of the other four pigments differs with the side of petal. Namely, spot No. 1 is always found in the outside, but not in the inside, in the four cultivars except cultivar No. 7.

Spot No. 4 gives the same relation, but this relation is found in the case of m-cresol solvents and not in the other solvents. Further experiments on this pigment are being carried out.

Spot No. 5 is found in the inside, but not in the outside and yellow spot is found on the same Rf value. This relation is found in the cultivar No. 2 and No. 5, too, but in the cultivar No. 3 the pigment of the same Rf value is found in both sides.

Spot No. 6 is found only in the inside against the spot No. 1.

In three treatments (V_1, V_2 & Vc) these results are in common, and bring out the fact that there are no influences of temperature on the pigment components.

Acknowledgments

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Summary

Studies on the coloration in petals by the colorimetrical and paperchromatographical method have been made of the tulip flower in the condition of higher temperature than the optimum.

The results are as follows :

1. There are differences in the process of physiological reactions for higher temperature between the outside and the inside of the same petal, and the relation is represented in the following three types.

A. The red color becomes strong

a. with the increase of the term of higher temperature condition.
(Cultivar No. 2-outside color, No. 3, and No. 7-inside color)

b. with the decrease of the term of higher temperature condition.
(Cultivar No. 5-outside color, and No. 6-inside color)

B. The red color becomes weak with the increase of the term of higher temperature condition.
(Cultivar No. 5-inside color, No. 6-outside color, and No. 7-outside color)

C. There are no influences of temperature.
(Cultivar No. 2-inside color)

2. The component of pigments in the petal is not influenced by the higher temperature.

3. There are differences in the component of pigments between the inside and the outside of the same petal, that is, some components are found in the outside and not in the inside, vice versa.

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