Effects of Temperature and Shade Treatment on Flower Colors and Characteristics in Newly Established Reddish−Purple Tuberose (Polianthes)

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Effects of Temperature and Shade Treatment on Flower Colors and Characteristics in Newly Established Reddish–Purple Tuberose (*Polianthes*)

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Effects of temperature and light condition on flower colors and flowering characteristics of newly established reddish–purple flowered tuberose '77A05' were investigated. Days to anthesis from planting of corms at 20 °C were significantly longer than at 25 or 30 °C. Floret diameter and petal length was significantly larger at 30 °C than at 20 or 25 °C.

Flower color was almost white at 30 °C, whereas the flowers cultivated at 20 °C showed reddish–purple. The flowers cultivated at 25 °C were almost white by 45% shading, but pale reddish–purple under 25 or 0% shade treatment. Anthocyanin content was extremely low at 30 °C. These results seem to relate to the enzyme activity which participate the biosynthesis of anthocyanins.

Environmental conditions for the cultivation of the anthocyanin–containing hybrid tuberose must be carefully considered.

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) is an ornamental bulbous plant native to Mexico and it is one of the most important cut flowers in tropical and subtropical areas (Shillo, 1992). There are a few cultivars in the world, and in Taiwan only two cultivars 'Single' and 'Double' are cultivated (Shen *et al.*, 1987; Shen *et al.*, 1997). All the existing cultivars in the world are white and there are no other colors available. *Polianthes howardii* having reddish–purple flowers was introduced to Taiwan from Mexico in 1985 (Shen *et al.*,1993) and we started cross breeding between the two species. Finally many hybrids having various flower colors such as pink, reddish–purple, purple, orange and yellow were bred as previously reported (Shen *et al.*, 1997).

In the previous report (Huang *et al.*, 1998), we clarified the pigment composition of the petals of these newly established hybrids, among which anthocyanins were the most important substances for the expression of the flower colors of pink, red, reddish–purple and purple. The colored tuberoses are recommended to be cultivated in greenhouses or plastic tunnels to avoid the damage of rain fall, insect attack and some diseases. However, the temperature in these facilities is usually higher and light intensity is lower

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than those in open field.

Temperature and light intensity are two main environmental factors affecting petal pigmentation (Harborne, 1965). Shading treatment to flowers or fruits causes the decrease of anthocyanin accumulation in such organs in many horticultural crops (Biran and Halevy, 1974; Matsuzoe *et al.*, 1999; Siegelman and Hendricks, 1958; Weiss and Halevy, 1991).

In the present study, effects of temperature and shading treatment on several flower characteristics and flower colors of a reddish-purple flowered tuberose, one of the anthocyanin-containing hybrid lines, were investigated.

MATERIALS AND METHODS

Plant materials and growing conditions

Corms of reddish-purple tuberose '77A05', an anthocyanin-containing hybrid line of which the pedigree is shown in Fig. 1, grown in the farm of National Chiayi University,

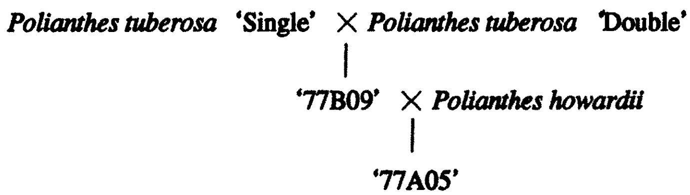

Fig. 1. Pedigree of reddish-purple tuberose '77A05'.

Chiayi, Taiwan, Republic of China were harvested in the end of February 1998 and stored at room temperature. They were planted on 21 May 1998 in plastic container (20×65×22 cm) containing 10 cm deep sand, and grown at 20, 25 or 30 °C under natural light (0% shading), shaded with white (25% shading of natural light) or black lawn cloth (45% shading) in the phytotron of the Biotron Institute, Kyushu University.

Measurement of growth, flower characteristics and flower color

Days to sprouting and anthesis from planting were recorded. Flower characteristics of flower stalk length, flower stalk diameter, number of florets, floret diameter and petal length were measured at anthesis. Fully expanded and full-colored six fresh flowers were collected randomly from the plants in each treatment and flower colors of a central portion of a petal per flower were measured by colorimeter (NR-3000, Nippon Denshoku Co., Japan).

Measurement of the amount of anthocyanins and analysis of the anthocyanidins

The dried and weighed petals (about 1g) were soaked overnight in 0.1% hydrochloric acid-methanol. The crude extracts were filtered and filled up to 50 ml and the absorbance were measured at 530 nm by a spectrophotometer (UV-160A, Shimadzu Co.

Ltd., Japan). The residue of the extracts was concentrated to a small amount and hydrolyzed with 2 ml of 2N hydrochloric acid at 100 °C for 1 h. The hydrolysates were adsorbed on a Sep–Pak C_{18} cartridge (Waters). The cartridge was washed with distilled water twice to eliminate the water–soluble or hydrophilic contaminants, then the anthocyanidin were eluted by 0.1% hydrochloric acid–methanol. A small portion of the filtrate was passed through membrane filter, and the filtrates were analyzed by high performance liquid chromatography (HPLC) (LC–6A pump, Shimadzu), using Cosmosil $5C_{18}$–MS column (Nakarai Tesque, 4.6 mm i.d.×250 mm) under 40 °C condition (CTO–10A column oven, Shimadzu) and SPD–6AV spectrophotometric detector (Shimadzu) set at 530 nm. A flow rate of 1.0 ml·min^{-1} was maintained and a mixture of acetonitrile–4% phosphoric acid (15:85, V/V) was employed as the eluent.

RESULTS

Days to sprouting from planting were not different among the treatments (Table 1). Those to anthesis were significantly two weeks longer at 20 °C than at 25 and 30 °C, but there was no shade effect on flowering days within the same temperature treatments.

Table 1. Effect of temperature and shade treatment on flowering of reddish–purple tuberose '77A05'.

Treatment		Days to sprouting	Days to flowering
Temp. (°C)	Shading (%)[z]		
20	0	5.0 a[y]	87.3 b
	25	5.7 a	87.8 b
	45	5.8 a	88.5 b
25	0	5.8 a	71.3 a
	25	5.7 a	71.8 a
	45	5.7 a	73.2 a
30	0	5.4 a	70.6 a
	25	5.4 a	72.4 a
	45	5.6 a	72.0 a

[z] % shading of natural light.
[y] Mean separation within columns by Duncan's multiple range test, 5%.

Floret diameter under natural light (0% shading) and petal length under 0 and 25% shading were significantly longer at 30 °C than those at lower temperatures (Table 2). Number of florets decreased by decreasing the light intensity, and in the same light intensity, it was largest at 25 °C. Flower stalk length and flower stalk diameter were not remarkably different among the treatments.

The values of brightness (L*) of flowers were higher than 80 at 30 °C in any light intensity, whereas they were 50–60 at 20 and 25 °C (Fig. 2). The values of chroma (C*)

Table 2. Effects of temperature and shade treatment on flowering performance of reddish-purple tuberose '77A05'.

Treatment Temp. (°C)	Shading (%)[z]	Flower stalk length (cm)	Flower stalk diameter (cm)	No. of florets	Floret diameter (cm)	Petal length (cm)
20	0	126.0 a[y]	0.52 c	28.0 bc	2.35 ab	1.05 ab
	25	131.0 ab	0.46 b	26.0 b	2.27 a	1.02 a
	45	135.7 bc	0.40 a	21.2 a	2.27 a	1.03 a
25	0	134.8 bc	0.52 c	31.2 d	2.33 ab	1.07 ab
	25	136.7 bc	0.52 c	30.2 cd	2.34 ab	1.08 ab
	45	139.2 c	0.46 b	26.2 b	2.47 c	1.11 bc
30	0	125.4 a	0.55 c	26.8 b	2.70 d	1.24 d
	25	124.8 a	0.51 bc	22.2 a	2.42 bc	1.22 d
	45	130.0 ab	0.50 bc	21.0 a	2.39 bc	1.15 c

[z] % shading of natural light.
[y] Mean separation within columns by Duncan's multiple range test, 5%.

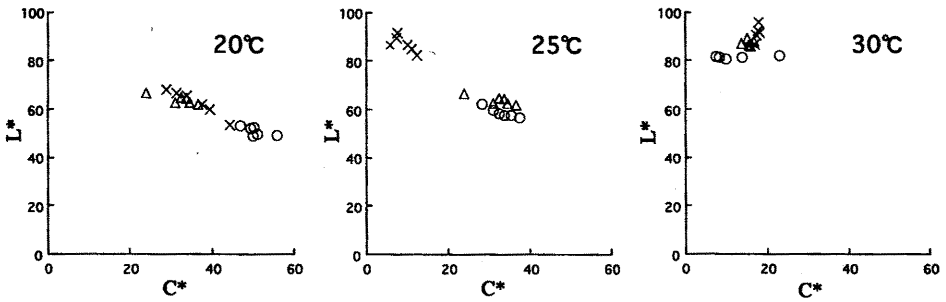

Fig. 2. Effects of temperature and shade treatment on the values of brightness (L*) and chroma (C*) of the petals of reddish-purple tuberose '77A05'.
○: 0% shading. △: 25% shading. ×: 45% shading.

were between the range from 5 to 22 at 30 °C with all shade treatments but with 45% shading at 25 °C. They were between 20 and 40 at 25 °C with 0 and 25% shading but at 20 °C with 25 and 45% shading, and 40–60 at 20 °C without shading (Fig. 2). These indicate that the flower color was almost white at 30 °C. The flowers cultivated at 20 °C showed reddish-purple under any light intensity (Figs. 2 and 3). Those cultivated under 45% shading were almost white, but showed pale reddish-purple under 0 or 25% shading at 25 °C.

The anthocyanin content was extremely low at 30 °C, but not different between the

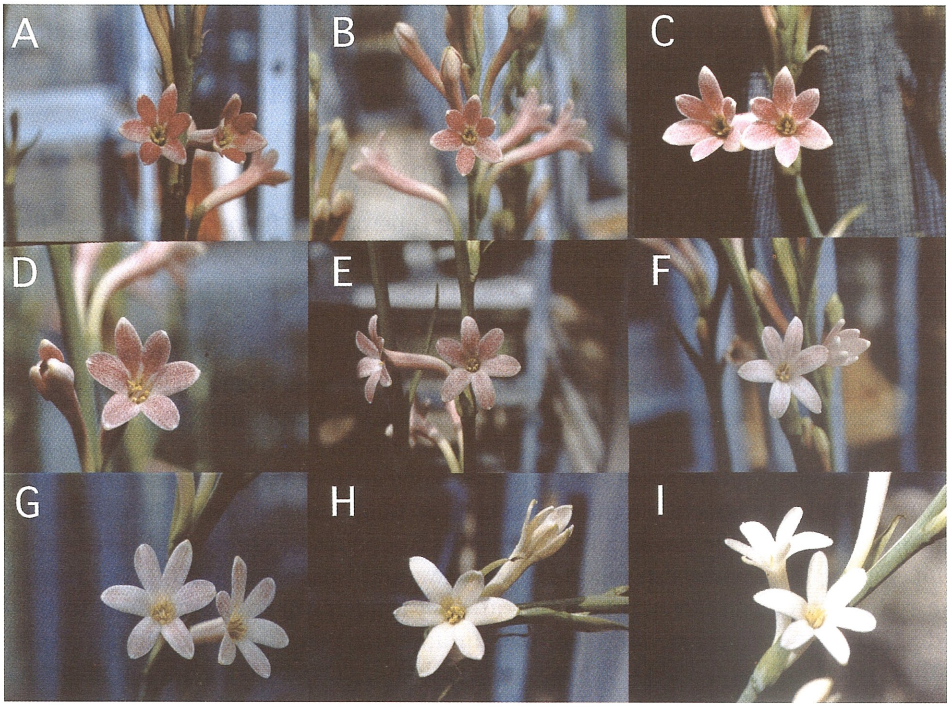

Fig. 3. Effects of temperature and shade treatment on flower colors and floret morphology of reddish–purple tuberose '77A05'.
A: 20 °C+0% shading, B: 20 °C+25% shading, C: 20 °C+45% shading,
D: 25 °C+0% shading, E: 25 °C+25% shading, F: 25 °C+45% shading,
G: 30 °C+0% shading, H: 30 °C+25% shading and I: 30 °C+45% shading.

Table 3. Effects of temperature and shade treatment on the anthocyanin content and anthocyanidin constitutions in the petals of reddish–purple tuberose '77A05'.

Treatment		Anthocyanin content[y]	Anthocyanidin constituents (%)	
Temp. (°C)	Shading (%)[z]		Cyanidin	Delphinidin
20	0	3.3 bc[x]	81.1	18.9
	25	3.7 bc	80.4	19.6
	45	3.9 c	81.8	18.2
25	0	3.7 bc	86.0	14.0
	25	3.3 bc	85.2	14.8
	45	2.9 b	83.8	16.2
30	0	0.9 a	88.0	12.0
	25	0.7 a	89.6	10.4
	45	0.5 a	88.6	11.4

[z] % shading of natural light.
[y] Absorbance at 530 nm.
[x] Mean separation within columns by Duncan's multiple range test, 5%.

treatments at 20 and 25 °C (Table 3). Shade treatment did not give significant difference in anthocyanidin content within the same temperatures. Percentage of cyanidin was about 80% of the total anthocyanins at 20 °C, whereas it increased to about 90% at 30 °C, accordingly delphinidin decreased from about 20 % at 20 °C to 10% at 30 °C.

DISCUSSION

Kosugi and Kimura (1960) reported that in tuberose the flower initiation and flower development occur after the planting of corms and ambient air temperature of at least 19 °C is necessary for flower–bud initiation. Thus, the delay for anthesis at 20 °C in the present study seems to be due to low temperature conditions.

Since the flower size was significantly large at 30 °C, cultivation of tuberose at high temperature seems to bring desirable characters. However, in regard to flower colors, the suitable temperature condition for cultivation of reddish–purple tuberose must be below 25 °C.

A rise in temperature results in a lower concentration of anthocyanins in roses (Biran *et al.*, 1973) and pigment production ceases at temperatures above 30 °C (Shisa and Takano, 1964). Reduction of anthocyanins in the petals of cherry and peach at high temperature (30 °C) was also reported (Maekawa and Nakamura, 1979). The reason of the reduction of anthocyanin contents at high temperature is considered to be the reduction of the supply of carbohydrate, an important substance for the structure of anthocyanins (Ratsek, 1944). Thus, suppression of carbohydrate supply might have caused the flower color fading of the reddish–purple tuberose at high temperature.

Light is by far the most extensively studied environmental factors affecting flavonoid production (Beggs and Wellmann, 1994). Anthocyanin biosynthesis in flowers requires a light stimulus (Dong *et al.*, 1998; Katz and Weiss, 1998). Chalcones are flavonoid and known to be precursor of anthocyanin. They are formed from 4–coumaroyl–CoA and three molecules of malonyl–CoA by chalcone synthase (CHS) and this enzyme activity in the petals increased by a light stimulus (Gleitz and Seitz, 1989). Thus, excessive shading for reddish–purple tuberose seems to weaken the CHS activity and cause to decoloration of the flowers.

The ratio of cyanidin and delphinidin in the petals of '77A05' were different between at 20 and 30 °C. Cyanidin and delphinidin are known as the derivatives from dihydroquercetin and dihydromyricetin, both a kind of dihydroflavonols, respectively (Madhuri and Reddy. 1999). Reports on temperature dependent changes in the ratio of anthocyanidin, such as those of cyanidin and delphinidin in the present study, are not available although the presence of many enzyme systems are hypothesized on the process from dihydroflavonol to anthocyanidin.

We are convinced that newly established colored tuberose has great potential for becoming a new promising floral crop. However, the cultivation conditions of the anthocyanin–containing hybrid tuberose, must be carefully considered.

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