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Effects of Low Temperature on Flowering in Tuberose (*Polianthes tuberosa* L.)

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Flower initiation and development of tuberose (*Polianthes tuberosa* L.) corms did not occur during storage at either 5°C or 25°C. Storage at 5°C did not result in the advantages of early flowering or increasing flower yields and quality, when compared with storage at 25°C. The increase in endogenous gibberellin activity in the corms was greater at 5°C than at 25°C, but there was no relationship between gibberellin activity increase and growth after planting.

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) is one of the most important cut flowers in tropical and subtropical areas (Benschop, 1993). In Taiwan, production area for tuberose is reported to be 1‘20 ha which is 3.1% of the total area of all cut flower production in 1991. Number of tuberose cut flowers harvested there was about 20 million, which is 1.9% of the total production of all the cut flowers (Anonymous, 1992).

Normally, corms are harvested in March, dried for one month and planted in outdoor field in April. The peak of the harvest for cut flowers is July. The highest demand for the flowers in Taiwan, however, is concentrated in New Year season (by lunar calendar ; this corresponds to a period between late January and early February in the Gregorian calendar, and it varies from year to year). To satisfy the demand, corms are stored at 5°C for six months followed by one month storage at outdoor temperature after harvest in March, and planted in September to be grown outdoors.

As a tropical plant which is believed to have originated in Mexico (Bailey, 1919), tuberose does not live in an environment where temperatures as low as 5°C occur for any great duration, and therefore it does not need low temperature in any part of its life cycle, including flower formation, for normal growth to occur. There are some reports available that tuberose grows best at a minimum temperature of 21°C (Post, 1952), that it requires high temperature for flower bud initiation, a daily minimum greater than 13°C for inflorescence initiation and higher than 19°C for individual floret initiation (Kosugi and Kimura, 1960) and that high temperature treatment of the corms at 30°C for two weeks or longer shortened the period between planting and sprouting and increased flowering and the rate of bud formation (Mori et ***al.***, 1990). Therefore, it may well be said that the 5°C treatment and its duration have been established

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empirically without physiological understanding.

The endogenous gibberellin increase, which is induced by low temperature, and its relationship with the rapid elongation growth which follows cold treatment, have been perceived in some geophytes (Aung et *al.*, 1969 ; Ohkawa, 1976) as well as in radish (Suge and Rappaport, 1968), chrysanthemum (Harada and Nitsch, 1959) and aster (Ishida and Takano, 1971). It is also known that exogenous gibberellin treatment can increase the length of flower stalk and spike and the number of tuberose florets (Jana and Biswas, 1979 ; Mukhopadhyay and Bankar, 1983 ; Dhua et *al.*, 1987).

This study was carried out to clarify the effect of low temperature on the growth and flowering of the plant and on the changes in endogenous gibberellins during corm storage.

The term "corm" is used in this paper throughout, whereas "tuber", "rhizome" and "bulb" are also used in other literature.

MATERIALS AND METHODS

Plant materials, temperature treatment and culture

Graded corms of tuberose cv. Double, 2.1 to 2.5 cm in diameter, a harvest of Taiwan in late February 1992, were shipped by air to Kyushu University and stored at 25°C on 5 April. On 30 April, they were divided into two groups, One group was stored at 5°C and another was kept at 25°C. Humidity was not controlled. They were taken from the storage rooms, planted at two week intervals in sands in clay pots (20 cm in diameter) and grown in a plastic-film greenhouse at Kyushu University until 17 September. Ten corms for each treatment were planted. Compound liquid fertilizer, OK-F-l (Otsuka Chemical Co.), was applied in a dilution of 1,000 times with watering.

Measurement

During the corm storage, water loss, flower initiation and development and changes in endogenous gibberellins in the corms were examined at two week intervals on the days corms were planted. In addition to recording the number of days between planting and sprouting, and between planting and flowering, flower characteristics were observed at flowering.

Observation of flower bud development

Ten corms for each treatment were dissected and observed under light microscope for determination of the stage of flower initiation and development. The stages from vegetative to flowering were divided into eight as I =vegetative, II =onset of flower initiation (apical meristem becomes dome shaped), III =elongation of inflorescence apex, IV=node formation, V=elongation of inflorescence, VI=flower stalk elongation, VII=bolting and VIII=flowering.

Extraction procedure for gibberellins

Extraction of gibberellins followed standard procedures described in many journals and books (for example, Yokota et *al.*, 1980), with some modification. The corms were lyophilized and kept at -40°C until analysis. They were homogenized and extracted for 24 h with 80% methanol (MeOH) containing 200 mgl^{-1} butylated hydroxytoluene as an antioxidant at 5°C. The MeOH extract was filtrated three times by

centrifuging and evaporated under reduced pressure at 37°C. Distilled water was added to the residue to make a volume of 100 ml and pH was adjusted to 3.0 with 1N HCl. The water phase was extracted four times with 50 ml ethyl acetate (EtAc). The EtAc phase was extracted four times with 50 ml of 2% sodium bicarbonate ($NaHCO_3$). After reacidification of the $NaHCO_3$ phase to pH 3.0 with 6N HCl, it was extracted again four times with 50 ml EtAc, and the EtAc phase was evaporated to dryness. The dried sample was adjusted to 5% MeOH with distilled water and passed through a Sep-Pak C_{18} cartridge (Waters Associates). The C_{18} cartridge attached with 0.45 μm PTFE filter (Kanto Chemical Co. Inc.) was then washed with 80% MeOH, evaporated to dryness and stored frozen at -40°C until further use.

Separation and assays of gibberellins

Separation and analysis of the extracts also followed standard procedures (Yokota *et al., 1980* ; etc.), with some modification. The crude extracts were purified by high performance liquid chromatography (HPLC)(Shimadzu LC-GA) on a column of Cosmosil $5C_{18}$-ODS (250 mmx4.6 mm in diameter). The column temperature was maintained at 35°C. The solvent was programmed from 30 to 80% methanol in 0.1% acetic acid with a flow rate of 1 $ml min^{-1}$. Each of 30 fractions separated by HPLC was collected with a fraction collector after 2 min flow. One fraction volume was 2 min flow. Each fraction was evaporated to dryness. These solutions were then tested for gibberellin activity.

Bioassays for gibberellins were conducted following the method of Murakami (1968) and Nishijima and Katsura (1989). Seeds of dwarf rice (*Oryza sativa cv.* Tanginbozu) were sterilized with 0.1% (w/v) Benlate (Du Pont) for 60 min, washed with water and soaked in 20 $ml l^{-1}$ uniconazol for 24 h in darkness at 30°C. The uniconazol treatment has been proved to increase the sensitivity of dwarf rice seedlings to minute amounts of gibberellins and to counteract the effects of growth retarding substances contained in the extracts (Nishijima and Katsura, 1989). Seeds were then washed and germinated in water in darkness at 30°C. Seeds having about 3-4 mm long coleoptile were selected and transplanted in vials (23 mm in diameter *x* 50 mm in height) filled with 0.8% agar (w/v), and incubated at 30°C under continuous light. Three days after the transplantation, one μl of each fraction dissolved into 100 μl of 50% acetone was applied to the region between the coleoptile and the first leaf of a seedling with a microsyringe. Length of the second leaf sheath was measured 3 days later.

RESULTS

Water loss

Water loss during storage either at 5°C or 25°C was only 3% in the first 14 weeks. In the corms stored at 25°C water loss rapidly increased in the last six weeks, reaching 7% at the end of storage ; at 5°C less than 5% water loss occurred (Fig. 1). None of the corms stored either at 25°C or 5°C suffered severe loss in quality for planting.

Flower development

Throughout the storage one or two corms at 5°C and one to three corms at **25°C** were at Stage II, the stage of the onset of flower initiation (Fig. 2). One or two corms at Stage III were also observed during the storage either at 5°C or 25°C. At either

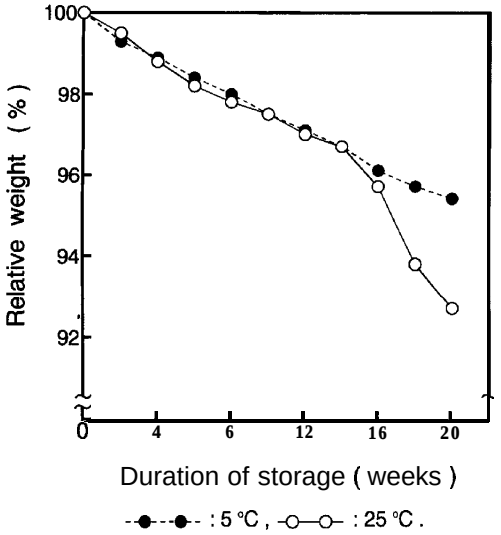

Fig. 1. Effects of storage temperature on water loss in tuberose corms.

temperature most of the corms remained at the vegetative stage throughout the storage period, and further flower initiation or development was not observed.

Sprouting, growth and flowering

The number of days between planting and sprouting decreased as the duration of the storage increased at both temperatures, and the rate of decrease was greater at 25°C than at 5°C (Table 1). Days to sprouting was delayed by 11 days owing to 5°C treatment for eight weeks.

No flowering occurred in the corms stored at 5°C for 16 weeks, whereas 67% of the corms stored at 25°C for the same duration flowered (Table 1). Some corms flowered when they were stored at 5°C for 14 weeks, and all the corms stored at 25°C for zero to 14 weeks and those stored at 5°C for zero to 12 weeks flowered. In the corms stored at 25°C for four to 16 weeks, the number of days between planting and flowering was lower than the comparable number for corms which had received other treatments. Corms stored at 5°C for six weeks flowered 20 days later than those stored at 25°C for the same period.

Flower characteristics

Differences in storage temperature (between 5°C and 25°C) produced no significant differences in flowering node number and length and width of flower stalk (Table 2). Length of inflorescence was not affected by the storage temperature until six weeks of storage, but shorter inflorescence occurred in the corms stored at 5°C for eight to 14 weeks than in the corms stored at 25°C for the same duration. Storage at 5°C for six to 10 weeks reduced the number of florets, but this number was not affected by cold temperature treatments of less than four weeks or greater than 10 weeks. Number of petals was lessened by the 5°C treatment for 14 weeks, but it was unaffected by temperature in the first 12 weeks.

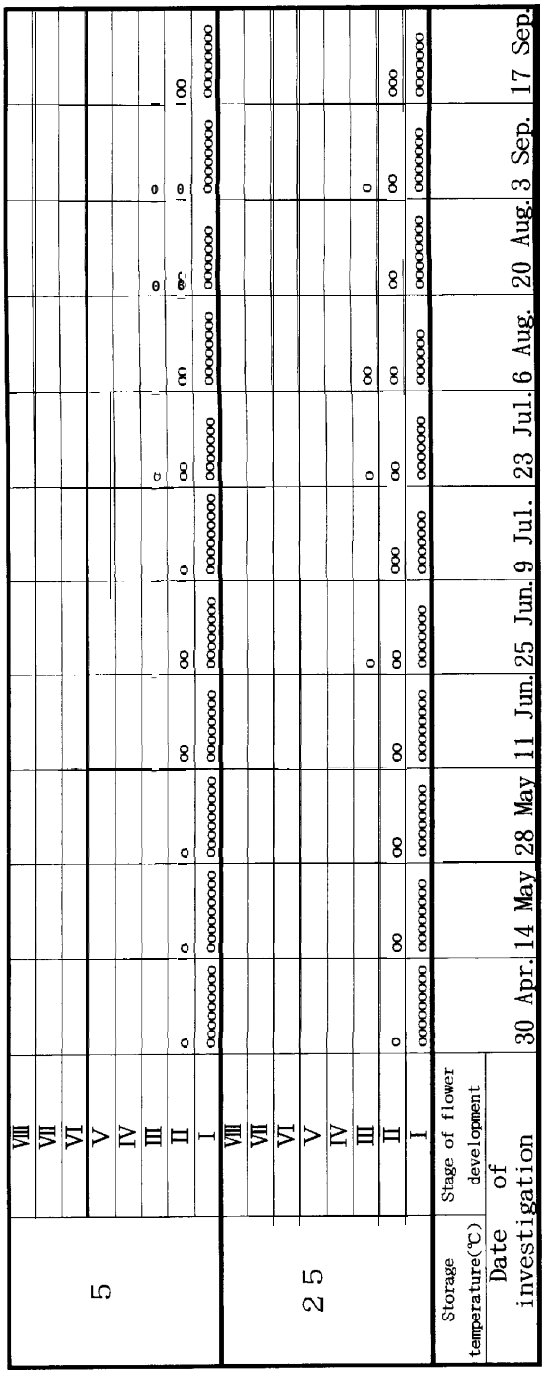

Fig. 2. Effects of storage temperature on flower bud development in tuberose corms.

Table 1. Effects of storage temperature on flowering of tuberose corms.

Storage temperature (°C)	Duration of storage (weeks)	Date of planting	Days to sprouting	% flowering	Days to flowering
	0	30 Apr	20.0 f	100	118.4 c
25	2	14 May	15.6 cde	100	109.2 c
5	2		24.1 g	100	115.0 c
25	4	28 May	11.9 c	100	101.0 b
5	4		18.7 ef	100	110.1 c
25	6	11 Jun	7.2 b	100	97.9 a
5	6		17.3 def	100	117.3 c
25	8	25 Jun	4.2 a	100	93.3 a
5	8		15.2 cde	100	109.6 c
25	10	9 Jul	3.3 a	100	95.6 a
5	10		14.0 cd	100	111.6 c
25	12	23 Jul	4.7 a	100	94.0 a
5	12		14.3 cde	100	116.8 c
25	14	6 Aug	5.3 a	100	100.4 a
5	14		15.2 cde	63	140.0 d
25	16	20 Aug	5.1 a	67	114.7 c
5	16		12.1 c	0	

Corm storage at 25°C and 5°C treatments began on 30 April.
Mean separation within columns by Duncan's multiple range test, 5%.

Table 2. Effects of storage temperature on flower characteristics of tuberose corms.

Storage temperature (°C)	Duration of storage (weeks)	Date of planting	Flowering node number	Flower stalk length (cm)	Flower stalk width (cm)	Inflorescence length (cm)	No. of florets	No. of petals per floret
	0	30 Apr	15.1 bc	103.0 a	0.77 a	35.2 def	33.7 c	28.5 bcde
25	2	14 May	14.4 abc	101.9 a	0.81 a	38.1 efg	33.3 c	29.2 de
5	2		12.7 ab	108.2 abc	0.78 a	38.6 efg	30.0 bc	28.9 de
25	4	28 May	15.7 c	108.9 abc	0.78 a	35.9 def	33.3 c	27.6 bcd
5	4		12.8 abc	105.7 ab	0.74 a	36.2 def	31.2 c	27.2 bc
25	6	11 Jun	14.2 abc	102.4 a	0.68 a	35.0 def	30.7 bc	27.6 bcd
5	6		11.9 a	108.0 abc	0.72 a	36.3 def	23.3 a	28.2 bcde
25	8	25 Jun	14.8 bc	114.6 c	0.83 a	42.3 fg	32.3 c	27.0 b
5	8		14.1 abc	112.3 bc	0.76 a	33.4 cde	24.6 ab	28.4 bcde
25	10	9 Jul	14.8 bc	111.9 bc	0.85 a	45.4 g	33.7 c	29.5 e
5	10		13.9 abc	111.7 bc	0.73 a	29.0 bcd	23.8 a	28.6 cde
25	12	23 Jul	12.9 abc	111.9 bc	0.73 a	36.8 def	23.0 a	29.1 de
5	12		14.1 abc	109.2 abc	0.72 a	27.0 abc	23.2 a	28.7 cde
25	14	6 Aug	13.6 abc	111.9 bc	0.91 a	36.6 def	27.8 abc	27.6 bcd
5	14		14.4 abc	109.8 abc	0.74 a	21.4 a	22.4 a	19.6 a
25	16	20 Aug	14.3 abc	101.7 a	0.84 a	25.7 ab	27.8 abc	20.5 a

Corm storage at 25°C and 5°C treatments began on 30 April.
Mean separation within columns by Duncan's multiple range test, 5%.

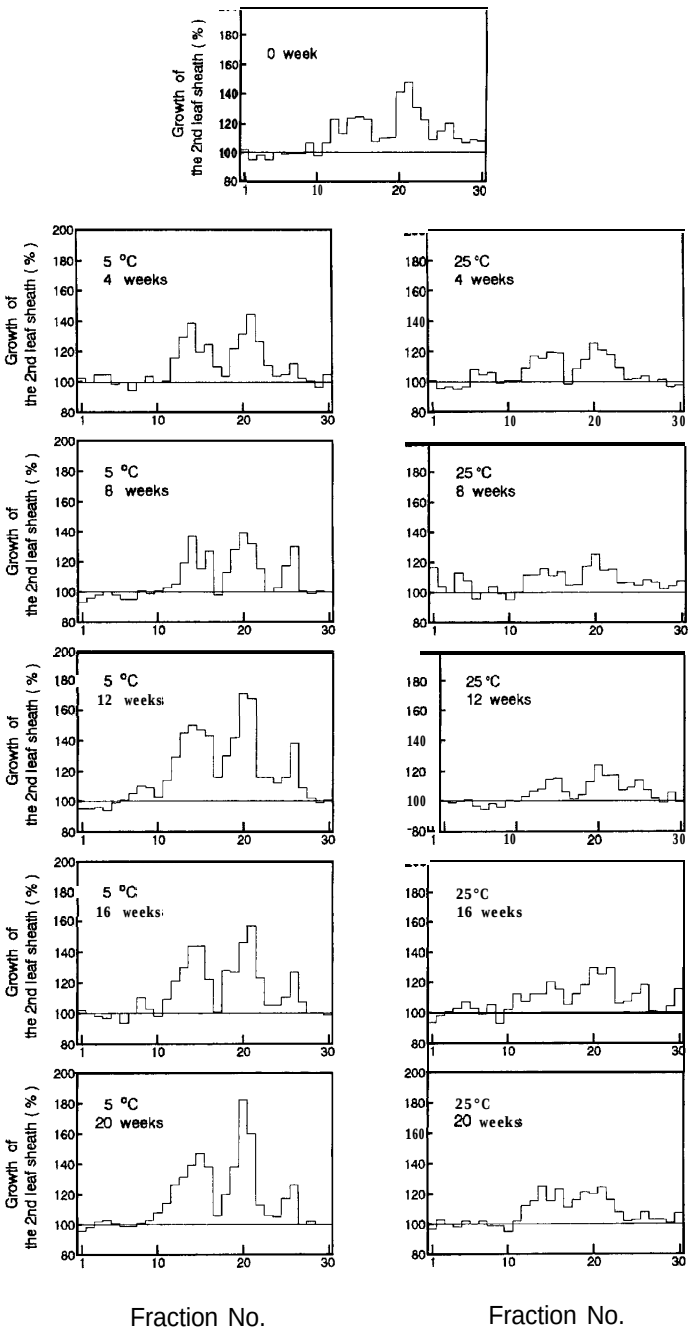

Fig. 3. Effects of storage temperature on changes in endogenous gibberellin activity in tuberose corms.

Gibberellin activity

Figure 3 shows the histograms of gibberellin activity in fractions of the extracts separated by HPLC. Two major regions of the activity were obtained in fractions No. 11-16 and No. 18-22 in the corms at each temperature throughout the storage. The gibberellin activity slightly decreased in corms stored at 25°C, but increased at 5°C. Activity in fractions of No. 11-16 reached the maximum in 12 weeks storage, and in No. 18-22 the maximum was reached in 20 weeks storage.

DISCUSSION

Long term storage of tuberose corms in ambient conditions in Taiwan causes the drying and desiccation of the corms and decreases the number of corms which can be replanted. It is the main reason that the corms are stored at 5°C. This study clearly showed that flower initiation and development does not occur during the storage of tuberose corms, and it is anticipated that flower initiation will occur after planting.

Late sprouting and flowering, as well as the absence of flowering, occurred in proportion to the progress of the storage period at both 5°C and 25°C. This may well be due to the low temperature after planting.

Literature on the influence of storage temperature on flower production in this plant is conflicting. Post (1952) advised 4.5°C storage. Storage at 10°C for 30 days improved the yield of flowers (Dhua *et al.*, 1987), whereas storage at 30°C advanced floral spike yield (Mukhopadhyay and Sadhu, 1987). The discrepancy is inexplicable.

Low temperature treatment delayed flowering and deteriorated flower quality in our experiments. It may be concluded, therefore, that the treatment is unfavorable and it has no advantage for cut flower production. Corms can be stored at higher temperature, which should considerably reduce the cost of electricity used for cooling. Some moisture may be necessary to prevent the corms from drying during storage at higher temperature. Higher but critical temperature and its duration should be examined.

Pathak et *al.* (1980) reported that in tuberose, gibberellin content was high in large bulbs (corms), and it might be possible that gibberellins are involved in early flowering of large bulbs (Benschop, 1993). However, we found that 5°C treatment led to an increase in gibberellin activity in the corms during storage, but when compared with 25°C treatment an effect of growth retardation was evidenced after planting. This suggests that in tuberose plants there is no direct relationship between the increase in gibberellin activity induced by low temperature in the corms during storage and the growth which occurs after planting. It is not in accordance with the general understanding that in many other plants (Harada and Nitsch, 1959 ; Suge and Rappaport, 1968 ; Aung *et al.*, 1969 ; Ishida and Takano, 1971; Ohkawa, 1976) the growth induced by low temperature pretreatment is a result of low temperatures stimulating gibberellin activity. It is not clear whether this is true for tuberose only or more broadly applicable to geophytes of tropical origin. Perhaps it is necessary to reconsider the relationships among low temperature, gibberellin increase, and growth promotion in plants.

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