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Flowering and inflorescence development of *Lachenalia aloides* 'Pearsonii' as influenced by bulb storage and forcing temperature

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Abstract

The effect of bulb storage and forcing temperatures on growth, flowering, and inflorescence development and blast of *Lachenalia aloides* Engl. 'Pearsonii' was investigated. Bulb temperature treatments began when about five florets were developed. Bulbs were stored at 10 °C, 12.5 °C, 15 °C, 20 °C, and 25 °C for 15, 30, or 45 days and forced in greenhouses at 17/15 °C and 21/19 °C, day (D)/night (N) temperature. Flowering was accelerated, and leaf length and floret number were reduced, when bulbs were stored at 10 °C, 12.5 °C, or 15 °C for 45 days compared with storing at 20 °C or 25 °C. Flowering was further accelerated by forcing at 17/15 °C compared with 21/19 °C (Experiments 1 and 2). When bulbs were stored at 10 °C, 15 °C, 20 °C, or 25 °C for 4 weeks and grown in greenhouses at 17/15 °C, 21/19 °C, 25/23 °C, and 29/27 °C, D/N temperature, the incidence of inflorescence blast was increased when bulbs were stored at 10° and 15 °C and forced at 25/23 °C compared with low temperatures (Experiment 3). Bulbs were stored at 10 °C, 15 °C, 20 °C, or 25 °C for 4 weeks and forced in greenhouses maintained at 18/16 °C, 22/20 °C, or 26/24 °C, D/N temperature, for 12 weeks. During forcing, plants were subjected to a constant 18/16 °C or temperatures were changed after 4 and 8 weeks (e.g., 18/16 °C–22/20 °C–18/16 °C) (Experiment 4). Inflorescence blast occurred when the temperature was 26/24 °C during the first 4 weeks after potting of bulbs that were stored at 15 °C (83%) and 10 °C (50%). Plants from bulbs stored at higher temperatures did not show inflorescence blast. To produce quality plants with short leaves, many

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florets, and short floral stems (scape plus inflorescence), it is recommended to store bulbs at 10–15 °C before potting for 30 days and to force at 17/15 °C to accelerate flowering. Inflorescence development during bulb storage at 10 °C and inflorescence blast that occurred after only 3 days of 30 °C was demonstrated using scanning electron microscopy and magnetic resonance imaging techniques.

Keywords: New floral crop; Potted plant; Bulb; Physiological disorder; Scanning electron microscopy; Magnetic resonance imaging

1. Introduction

Lachenalia is a bulbous geophyte belonging to the Liliaceae, and is native primarily to the southwestern Cape and Namaqualand in South Africa (Duncan, 1988). *Lachenalia aloides* (L.f.) Engl. 'Pearsonii' has been cultivated for nearly two centuries as a garden plant (Jansen van Vuuren et al., 1993). Several cultivars with flower colors ranging from red to yellow have been developed in the last 15 years at the Roodeplaat Vegetable and Ornamental Plant Institute of the Agricultural Research Center (Lubbinge et al., 1983; Hancke and Coertze, 1988). However, bulbs were not readily available for commercialization until 1997 (Kleynhans et al., 2002) and *Lachenalia* is still considered a new crop due to the limited availability of new hybrids to the industry and lack of information on controlled flowering.

Inflorescence initiation in *Lachenalia* hybrid 'Romelia' occurs at 15°–25 °C (Louw, 1991) or 10 weeks at 20 °C (Louw, 1993). Roh et al. (1995), however, reported that the shoot apex of *Lachenalia* 'Ronina' and 'Pearsonii' remained vegetative for more than 6 months when bulbs were stored at 15 °C. Inflorescence initiation is influenced by growing temperature before bulb harvest and is accelerated by growing plants at 23–27 °C after flowering and storing bulbs at 25 °C after harvest (Roh et al., 1995). Previously recommended greenhouse forcing temperatures were 30/15 °C for 4 weeks followed by 25/8 °C until flowering (Louw, 1991). Flowering was delayed or plants did not even flower, and the incidence of abnormal development of inflorescence and florets (Fig. 1) increased when *L.* 'Pearsonii' and *L. aloides* var. *quadricolor* (Jacq.) Engl. were forced at temperatures exceeding 23 °C during summer or when bulbs stored at 10–15 °C were forced (Roh, unpublished data).

Failure to flower, low flowering percentage, and a lack of uniformity and periodicity of flowering in many geophytes depends on many factors, such as bulb size and periodicity of inflorescence initiation. Failure of scape elongation due to a death of florets (inflorescence blast) is caused by unfavorable external factors, such as temperature during bulb production, storage, and forcing (Rees, 1992; Roh et al., 1998) or the internal source-sink relationship between inflorescence and other organs (Theron and Jacobs, 1996). In *Lachenalia*, failure of flowering after inflorescence initiation and development is observed that are similar to a physiological disorder observed in *Tulipa* (Rees, 1992). Typical disorders caused after initiation of the inflorescence include a near-normal extension of scape with dead florets, malformation of some florets formed at the base of the inflorescence, cessation of scape elongation above the center of the plant, and dried and

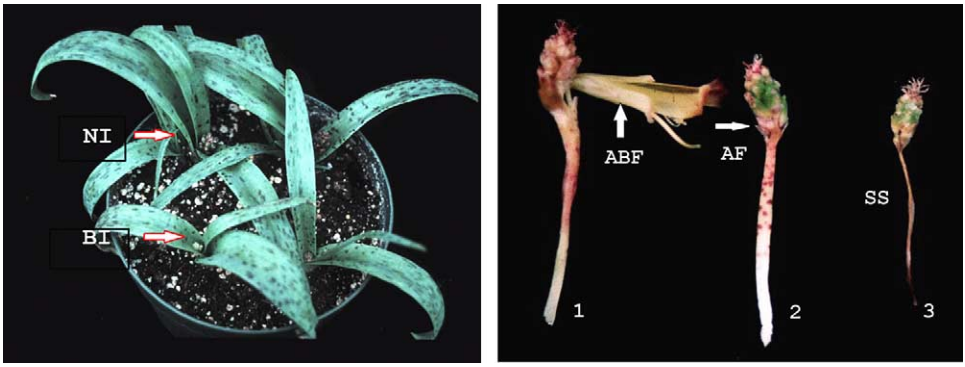

Fig. 1. Observation of inflorescence blast in *L. aloides*, 'Pearsonii', showing normal (NI) and blasted (BI) inflorescence (left). Effect of high temperatures on inflorescence and floret development, showing a abnormally developed floret (ABF) (1), normal scape development with reduced number of florets and aborted florets (AF) (2), or shriveled scape (SS) (3) (right).

dead florets. These physiological disorders are frequently observed when the scape starts to emerge during forcing.

Nuclear magnetic resonance (NMR) imaging has successfully provided both physiological and anatomical information on plants (Roh and Joung, 2004; Roh et al., 1996, 2004). Although the interpretation of NMR signals is complicated, and greatly affected by cellular heterogeneity, NMR techniques have been used to study plant water relations, plant metabolism, morphology and inner structures of tissues or organs, and plant stress physiology due to the availability of knowledge as compared to other research techniques (Ide et al., 1998; Ishida et al., 2000; Kerr et al., 1997; Ratcliffe et al., 2001; Robinson et al., 2000; Van der Toorn et al., 2000). The non-destructive nature of magnetic resonance imaging (MRI) can indicate which places in the tissues respond to endogenous physiological changes and external stimulants prior to visual morphological changes.

To produce compact plants at flowering (Roh et al., 1995) and to increase post-harvest life and floret numbers (du Toit et al., 2003), optimum environments must be provided during bulb storage and the greenhouse forcing period. However, information on the temperature requirement during bulb storage, greenhouse forcing in relation to flowering, and inflorescence death of *Lachenalia* is still lacking. As a part of the ''new flower crop research program'' at the Unit supported by the Society of American Florists and American Floral Endowment, *Lachenalia* was evaluated. In order to establish the critical temperature during bulb storage and forcing period and to examine floret development at an early growth stage, research was initiated to (a) investigate the effect of bulb storage temperatures and storage duration on the flowering of *Lachenalia* 'Pearsonii', (b) assess the effect of bulb storage and forcing temperatures on flowering and inflorescence blast, and (c) study the floral development during bulb temperature treatment period and high forcing temperature by scanning electron microscopy and MRI techniques.

2. Materials and methods

2.1. The effect of bulb storage and forcing temperature on flowering

Experiment 1 (1991–1992): 'Pearsonii' bulbs, 6–7 cm circumference (circum.), had developed about five florets showing the outer perianth when received from Israel (Agrexco Co., Jamaica, NY) on August 20, 1991. Bulbs were kept at 21 °C until September 11, at which time storage treatments commenced. Bulbs were stored at constant 10 °C, 12.5 °C, 15 °C, 20 °C and 25 °C for 15, 30, or 45 days in dark and potted on November 8, November 23 and December 8, respectively. Three bulbs were potted per 15 cm bulb pan filled with Pro-Mix BX (Premier Brands, Inc., Stamford, CT) and this growing medium was used through the investigation. Plants were initially forced in a 17/15 °C (D/N) greenhouse for 30 days, after which were divided into two groups and one group was maintained at 17/15 °C and another group transferred to a 21/19 °C greenhouse and forced until flowering. Data were collected from 30 plants per treatment.

Experiment 2 (1992–1993): 'Pearsonii' bulbs, 6–7 cm in circum., had developed about five florets showing the outer perianth when received from Israel (Agrexco Co., Jamaica, NY) were received on August 21, 1992 and kept at 20 °C. On Sept. 7, bulbs were stored at constant 10 °C, 12.5 °C, 15 °C, 20 °C, and 25 °C for 15, 30, and 45 days. Bulbs were potted singly in 10 cm pots filled with the same growing medium as above and forced at 17/15 °C or at 21/19 °C until flowering. Data were collected from 12 plants per treatment.

2.2. The effect of bulb storage and forcing temperature on inflorescence blast

Experiment 3 (1991–1992): 'Pearsonii' bulbs (7–8 cm circum.) were stored at constant 10 °C, 15 °C, 20 °C, and 25 °C for 4 weeks, potted (3 bulbs/pot), and forced in greenhouses maintained at 17/15 °C, 21/19 °C, 25/23 °C, or 29/27 °C for 8 weeks ($4 \times 4 = 16$ treatments). On December 4, all plants were moved to a 17/15 °C greenhouse until flowering. Data were collected from 27 plants per treatment.

Experiment 4 (1992–1993): 'Pearsonii' bulbs (6–7 cm circum.) were stored at constant 10 °C, 15 °C, 20 °C, and 25 °C for 4 weeks, potted, and forced in greenhouses maintained at 18/16 °C, 22/20 °C, or 26/24 °C. During a 12-week greenhouse forcing period, plants were either subjected to the same forcing temperature (18/16 °C, 22/20 °C, or 26/24 °C) throughout, or plants were moved to another temperature at 4-week interval (Table 3). For example, some plants were forced continuously at 18/16 °C (coded as 16°–16°–16°) or initially for 4 weeks at 18/16 °C, followed by 4 weeks at 22/20 °C, and finally 4 weeks at 18/16 °C (16°–20°–16°).

On 2 December 1992, developmental stages of the inflorescence were scored: (1) no visible inflorescence, (2) inflorescence visible, (3) scape length <2 cm, (4) scape length ≥ 2 cm, and (5) florets reaching anthesis. Due to a mixture of cultivar with *L. aloides* var. *quadricolor* (Jacq.) Engl. which was $<6\%$ and a loss of about 2%, a mean per pot was calculated for statistical analysis. Data were collected from 36 plants per treatment.

In both experiments, greenhouse light levels were maintained between 32–35 $\mu m\ s^{-1}\ m^{-2}$ level by supplemental lighting from 400 W high pressure sodium lamps (07:00–22:00 h) and shade cloth. The date of flowering was recorded when the first three florets opened. At flowering, data from each plant were collected on floral stem length (scape plus inflorescence), the longest leaf length, and the number of florets. The incidence of inflorescence blast was recorded throughout the forcing period after emergence by checking visually for the presence of a dried scape and inflorescence. The number of days to flowering was counted from the date bulb storage treatments were initiated. During greenhouse forcing, all pots were completely randomized. Data were analyzed by the general linear model (GLM) (SAS Institute Inc., 1988). Experimental error (variations among pots within treatments) and sampling error (variations among plants within pots) was not statistically significant. Average values per pot were calculated and data were analyzed again. Flowering percentage was analyzed after arcsine transformation.

2.3. Scanning electron microscopy (SEM), nuclear magnetic resonance imaging (MRI), and floret development (Expt5).

Bulbs grown in a greenhouse maintained at temperatures described previously (Roh, 1990b) were harvested in April, and then stored at 25 °C until 9 December, 1994 when the lower 6–8 florets were developed. A total of 20 bulbs were then stored at 10 °C for 6 weeks and potted in 15 cm pots, five bulbs per pot. At planting, shoots were 2 to 3 cm long. Pots were placed in growth chambers maintained at either a constant 20 °C and 30 °C under 12 h of light/dark cycle for 0 and 3 days. Light was provided by cool white fluorescent and incandescent light at photosynthetic photo flux density at 22 $\mu m\ s^{-1}\ m^{-2}$ during the day (0600–1800 h). Tissues for SEM were sampled after 0 and 3 days at 30 °C following 10 °C storage, and fixed in 4% (v/v) gluteraldehyde and 1.5% (v/v) acrolein in a 0.05 M $Na_2PO_4 + KH_2PO_4$ buffer (pH 7.0). Samples were dehydrated in a graded series of ethanol solutions, critically point dried, and coated with gold-paladium. Samples were observed in a JEOL 6100 scanning electron microscope (Peabody, MA).

Samples for MRI were collected before and after 6 weeks of 10 °C treatment and 3 days of 30 °C after 6 weeks of 10 °C. Magnetic resonance images were measured using a Bruker 400 MHS NMR spectrometer (Bruker Instruments, Billerica, MA). Multi echo spin echo images were collected using a standard pulse acquisition sequence. Image collection matrix size was 64 (bulb axis) × 256 × 256 (frequency encode direction) and frequency encoded data were collected with a sweep width of 50 kHz and later digitized into 256 complex points. The field view was 30 mm × 20 mm × 20 mm with a 0.5 mm slice thickness giving a resolution of 98 μm × 391 μm × 500 μm. Eight echoes were acquired at echo times in 9.12 ms intervals with a repetition time of about 1 s and with a slide thickness of 0.5 mm. Two scans were used giving a total image acquisition time of approximately 4.5 h. The raw data was trapezoidal multiplied in the frequency direction, base line corrected then reconstructed into images using a 2d fourier transform. The raw images were median filtered, then the best-fit curve was fitted to each pixel location in the sequence of images. A simplex routine was used to find the values of t_2 and standard deviation (S.D.). Images were then constructed using the parameters t_2 and sd, as well as

the variance of the raw data to the best fit equation (Faust et al., 1997; Ishida et al., 2000; Ratcliffe et al., 2001). MRI measurement was repeated using three bulbs, but only one typical data set is presented because similar images were obtained for the three bulbs.

3. Results

3.1. The effect of bulb storage and forcing temperature on flowering (Experiments 1 and2)

In Experiment 1, bulbs stored at 10 °C, 12.5 °C, or 15 °C flowered in 75–82 days when forced at 17/15 °C, regardless of the storage duration. Leaf length was significantly shortened and flowering was accelerated when bulbs were stored at 10 °C for 45 days (Fig. 2). Bulbs stored at 20 °C or 25 °C and forced at 17/15 °C took longer than 95 days to flower. Forcing at 21/19 °C delayed flowering compared to forcing at 17/15 °C, regardless of bulb storage temperature. Floral stem length was less than 20 cm when bulbs were stored at $\leq$20 °C, regardless of the storage durations. Bulbs stored at 25 °C and forced at 21/19 °C produced floral stems >20 cm. The number of florets decreased from >18 to <13 as the duration of bulb storage at 10 °C, 12.5 °C, or 15 °C increased from 15 to 45 days. More than 24 florets were produced when bulbs were stored at 20 °C or 25 °C, regardless of storage duration. The number of florets and leaf length increased as forcing temperature was increased. These data are not presented, as they can be illustrated by those of Experiment 2, which were essentially similar.

Fig. 2. Appearance of *L. aloides*, 'Pearsonii' at flowering when bulbs were stored at 10 °C for 15 (left), 30 (center), and 45 (right) days and forced at 17/15 °C greenhouse.

Table 1
The effect of bulb storage temperature, storage duration, and forcing temperature on the growth and flowering of *Lachenalia* 'Pearsonii' (Experiment 2)

Bulb storage		Forcing temperature[a] (°C)	No. of days to flower	Length (cm) of			No. of florets
Temperature (°C)	Duration (days)			Leaf	Scape	Inflorescence	
10[b]	**15**	**17/15**	**102**	**25**	**12**	**19**	**20**
		21/19	**121**	**29**	**12**	**17**	**19**
	30	17/15	96	20	11	15	10
		21/19	98	28	12	15	11
	45	17/15	95	17	13	18	8
		21/19	84	20	12	16	8
12.5	**15**	**17/15**	**101**	**25**	**10**	**16**	**18**
		21/19	116	29	14	19	18
	30	17/15	97	22	11	16	12
		21/19	106	29	13	16	12
	45	17/15	101	18	12	17	9
		21/19	90	22	11	16	8
15	**15**	**17/15**	**108**	**25**	**11**	**16**	**17**
		21/19	121	30	15	20	18
	30	17/15	108	23	11	15	12
		21/19	127	32	13	17	12
	45	17/15	110	19	13	17	9
		21/19	101	26	12	15	10
20	15	17/15	111	26	11	17	18
		21/19	133	29	15	21	18
	30	17/15	116	26	12	17	15
		21/19	133	34	17	22	16
	45	17/15	119	21	14	19	13
		21/19	121	30	10	13	14
25	15	17/15	120	27	12	19	24
		21/19	138	35	16	23	32
	30	17/15	133	25	13	20	26
		21/19	148	33	15	23	27
	45	17/15	144	21	13	19	30
		21/19	161	28	15	20	29
Level of significance[c]							
Storage temperature (ST)							
Lin[d]			**	**	**	**	**
Quad			*	*	*	*	**
Storage duration (SD)							
Lin			**	*	ns	**	**
Quad			*	*	*	*	**
Forcing temperature (FT)			**	**	*	**	ns

Table 1 (*Continued*)

Bulb storage		Forcing temperature[a] (°C)	No. of days to flower	Length (cm) of			No. of florets
Temperature (°C)	Duration (days)			Leaf	Scape	Inflorescence	
ST × SD			**	**	*	*	**
SD × FT			**	*	**	**	ns
ST × FT			**	*	*	*	ns
ST × SD × FT			**	ns	ns	**	ns

[a] Day/night.

[b] Desirable temperatures and the duration of bulb storage and forcing temperature which produced more than 17 florets and flowered in less than 110 days are indicated in bold.

[c] **, *, and ns: significant at 1% and 5% level and non-significant, respectively.

[d] Lin: linear; Quad: quadratic.

In Experiment 2, plants were forced continuously at 17/15 °C or 21/19 °C after potting (Table 1). When bulbs stored at 10 °C or 12.5° were forced at 17/15 °C, flowering was accelerated as the storage duration was increased from 15 to 45 days. As the storage duration at >15 °C was increased, flowering was delayed when plants were forced at 21/19 °C. Flowering was earliest (84 days) and latest (161 days) when bulbs were stored at 10 °C and 25 °C for 45 days, respectively.

Leaf length was <20 cm when bulbs stored at 10 °C or 12.5 °C for 30 days, or at 10 °C for 45 days, and then were forced at 17/15 °C (Table 1). Scape length ranged from 10 to 16 cm in all treatments. Inflorescences were ≤18 cm and scapes were 15–16 cm when flowering was accelerated by bulb storage and forcing temperatures. The number of florets ranged from 8 to 12 when bulbs were stored for 30 or 45 days at 10 °C, 12.5 °C, or 15 °C and forced either at 17/15 °C or 21/19 °C. When bulbs were stored at 10 °C, 12.5 °C, or 15 °C for 15 days and forced at 17/15 °C, the number of florets was ≥17. In Experiments 1 and 2, inflorescence blast was not observed, however, number of florets was reduced to less than 10, when bulbs were stored longer at 10 °C, 12.5 °C, and 15 °C.

3.2. The effect of bulb storage and forcing temperature on inflorescence blast (Experiment 3 and 4)

In Experiment 3, flowering was earliest (97 days) when bulbs were stored at 10 °C and forced at 17/15 °C, and latest (159 days) when bulbs were stored at 25 °C and forced at 29/27 °C (Table 2). Leaf length increased as bulb storage temperatures increased. When plants were forced at 17/15 °C or 21/19 °C, 14–16 florets were formed on the scape from bulbs stored at 10 °C and 15 °C. Bulbs stored at 20 °C or 25 °C formed >21 florets. Scape and inflorescence lengths were <12 cm and <17 cm, respectively, when bulbs were stored at 10 °C, regardless of the forcing temperatures (Table 2). When bulbs stored at 10 °C were forced, flowering decreased from 100% to 23% as forcing temperatures increased from 17/15 °C to 29/27 °C. Similar trends were observed when bulbs were stored at 15 °C. However, when bulbs were stored at 20 °C or 25 °C, flowering was higher than 92%, regardless of forcing temperatures.

In Experiment 4, when bulbs were stored at 10 °C and forced initially for 4 weeks at 18/16 °C, followed by 4 weeks at 22/20 °C, and finally for 4 weeks at 18/16 °C (16–20–16 °C),

Table 2
The effect of bulb storage and forcing temperatures on the growth and flowering of *Lachenalia* 'Pearsonii' (Experiment 3)

Temperature (°C)		No. of days to flower	Length (cm) of			No. of florests	Flowering (%)
Storage	Forcing[a]		Leaf	Scape	Inflorescence		
10	17/15	97	21	12	17	16	100
	21/19	98	26	12	15	14	100
	25/23	111	25	12	15	13	77
	29/27	116	23	10	14	15	23
15	17/15	109	25	15	20	16	100
	21/19	121	30	15	20	15	100
	25/23	143	28	19	25	14	55
	29/27	150	25	17	22	10	23
20	17/15	117	27	15	21	21	100
	21/19	135	33	20	26	21	100
	25/23	150	32	23	31	20	96
	29/27	152	31	24	32	19	100
25	17/15	135	27	16	24	35	100
	21/19	143	34	21	29	30	100
	25/23	155	31	23	32	33	92
	29/27	159	30	23	31	25	100
Level of significance[b]							
Storage temperature (ST)							
	Lin[c]	**	*	*	**	**	*
	Quad	*	**	**	*	**	ns
Forcing temperature (FT)							
	Lin	**	ns	**	**	ns	*
	Quad	*	ns	*	*	ns	*
ST × FT	Lin	**	*	*	*	**	*

[a] Day/night.
[b] **, *, and ns: significant at 1% and 5% level and non-significant, respectively.
[c] Lin: linear; Quad: quadratic.

the growth and developmental stage (score 4.9) on December 2 was more advanced than all other treatments. Subsequently, flowering was the earliest (87 days) and all plants flowered (Table 3). However, when plants were forced continuously for 12 weeks at 26/24 °C (24–24–24 °C), the growth and developmental stage was not advanced (score 1.1) and only 50% of the plants flowered in 117 days.

When bulbs stored at 10 °C or 15 °C were forced at 18/16 °C during the first 4 weeks flowering was ≥80%, regardless of temperatures given during the second 4 weeks (Table 3). However, when bulbs stored at 10 °C or 15 °C were forced initially at 26/24 °C, flowering was ≤63% and ≤37%, respectively, regardless of forcing temperatures during the following 8-week period. When bulbs were stored at 20 °C or 25 °C, 100% and ≥87% of plants flowered, respectively, regardless of the forcing temperatures.

Leaf and scape length and the number of florets (when 100% of plants flowered) responded similarly to bulb storage temperatures (Table 3) as was observed in the previous

Table 3
The effect of bulb storage temperatures and different forcing temperatures given various period of forcing on the growth and flowering of *Lachenalia* 'Pearsonii'

Bulb storage temperature (°C)	Forcing temperature (°C)[a] between week			G and D stage[b]	No. of days to flower	Length (cm) of			No. of florets	Flowering (%)
	0–4	5–8	9–12			Leaf (December 2)	Scape	Inflorescence		
10	16	16	16	4.3	98	22	11	15	10	100
	16	20	16	4.9	87	25	12	17	7	100
	16	24	16	4.6	90	24	12	16	7	93
	20	16	20	3.5	99	26	11	15	9	87
	20	20	20	4.1	95	25	12	16	10	100
	20	24	20	3.5	99	27	13	17	6	83
	24	16	24	2.5	103	24	11	14	8	60
	24	20	24	1.7	107	28	9	11	4	63
	24	24	24	1.1	117	26	15	17	8	50
15	16	16	16	3.0	111	26	12	16	9	100
	16	20	16	2.2	108	27	9	12	9	100
	16	24	16	2.4	109	27	11	14	6	80
	20	16	20	1.7	114	29	12	16	9	80
	20	20	20	1.7	116	30	14	18	9	87
	20	24	20	1.1	133	30	11	14	4	43
	24	16	24	1.2	130	25	9	12	10	37
	24	20	24	1.0	143	27	13	18	10	17
	24	24	24	1.0	166	26	11	13	3	33
20	16	16	16	2.0	121	27	11	15	10	100
	16	20	16	2.5	119	27	9	13	12	100
	16	24	16	2.1	125	27	7	9	10	100
	20	16	20	2.0	120	28	8	13	14	100
	20	20	20	1.7	122	37	11	14	12	87
	20	24	20	1.2	144	29	7	11	13	87
	24	16	24	1.6	130	29	9	13	9	93
	24	20	24	1.4	142	34	12	16	13	100
	24	24	24	1.0	168	31	14	19	16	93

25	16	16	16	1.9	138	26	12	17	19	100
	16	20	16	1.9	139	23	11	16	20	100
	16	24	16	1.1	152	26	11	16	19	100
	20	16	20	1.9	137	28	10	15	23	100
	20	20	20	1.5	146	32	14	17	25	100
	20	24	20	1.0	168	32	11	21	22	100
	24	16	24	1.2	152	28	12	18	22	100
	24	20	24	1.2	167	31	12	17	22	100
	24	24	24	1.2	180	30	15	21	21	100
Level of significance[c]										
Bulb storage (BS)				**	**	**	**	**	**	**
Forcing temperature										
Weeks 0–4 (FTW04) lin[d]				**	**	**	**	**	**	**
Weeks 5–8 (FTW58) lin				**	**	*	*	*	**	ns
BS × FTW04				*	*	ns	*	ns	*	*
BS × FTW58				ns	*	ns	ns	*	*	ns
FTW04 × FTW58				ns	*	ns	*	*	**	**
BS × FTW04 × FTW58				*	**	*	*	ns	*	**

[a] Greenhouse forcing temperatures are 16 (18/16 °C), 20 (22/20 °C), and 24 (26/24 °C).

[b] Growth and development stages recorded on 2 December, were: (1) no visible inflorescence, (2) inflorescence visible, (3) scape length <2 cm, (4) scape length ≥2 cm, and (5) the floret reaching anthesis.

[c] **, *, and ns: significant at 1% and 5% level and non-significant, respectively.

[d] Lin: linear.

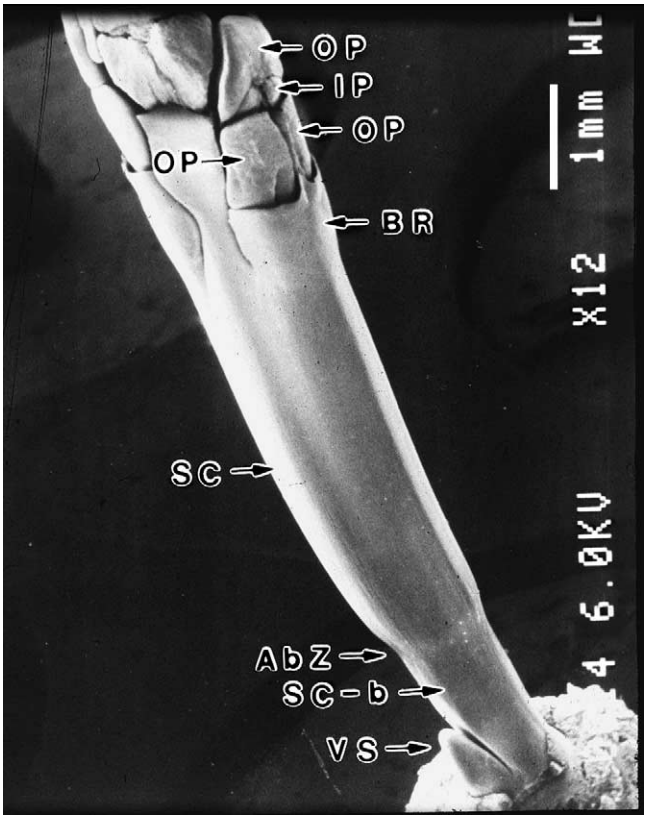

Fig. 3. Scape and inflorescence of *L. aloides* 'Pearsonii' upon completion of floral development before bulb low temperature treatment. Vegetative shoot (VS), scape (SC-b) below the abscission zone (AbZ) and above the zone (SC), bract (BR), outer perianth (OP), and inner perianth (IP).

experiment (Table 2). However, only three to four florets were produced when the flowering percentage was low, particularly when bulbs stored at 15 °C were forced at 24–24–24 °C or at 22–24–24 °C.

3.3. Scanning electron microscopy (SEM), nuclear magnetic resonance imaging (MRI), and floret development (Experiment 5)

When forced at 20 °C all bulbs flowered (data not presented) and scape development became normal, showing bracts sustaining florets and outer and inner perianths on scape (SC) which was turgid in SEM image. A vegetative shoot (VS) was also observed at the base of the scape (SC-b). Abscission zone (AbZ) was also observed which separated SC and SC-b (Fig. 3). Cross sectional view of scape and inflorescence of bulbs showed well developed vascular bundles (V), turgid scape (SC), and a vegetative shoot (VS) adjacent to the base of the scape in MRI (Fig. 4). Successive formation and development of florets with petioles was clearly visible and leaves (L) that surrounded scape,

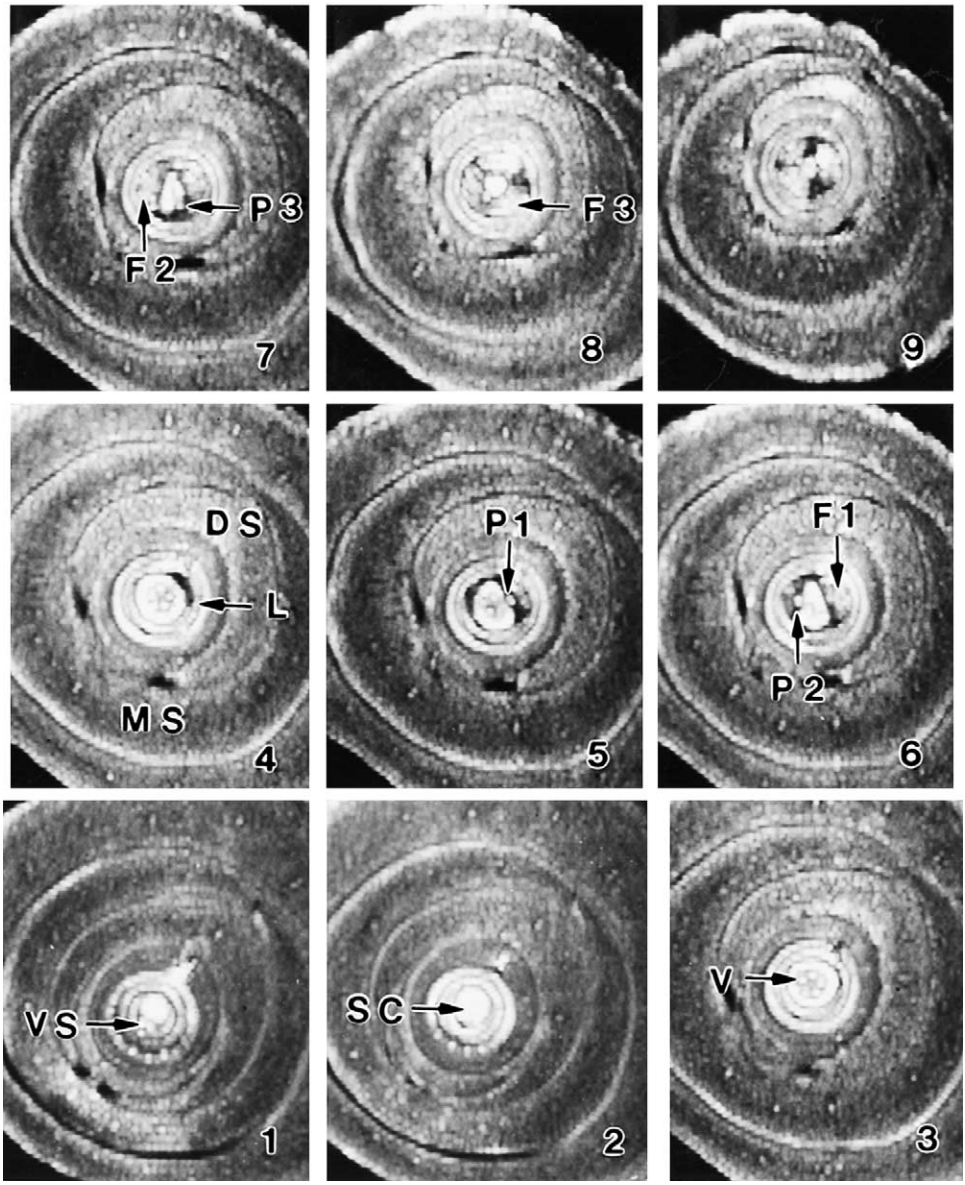

Fig. 4. Magnetic resonance imaging images showing the development of the scape, inflorescence, and florets of *L. aloides* 'Pearsonii' showing vegetative shoot adjacent to the base of scape (VS, frame 1), scape (SC, frame 2), vascular tissues (V, frame 3), mother scales (MS), daughter scales (DS), and leaves (L) (frame 4), pedicel (P1, frame 5) for the first floret (F1, frame 6), pedicel for the second floret (P2, frame 6), and pedicel (P3) for the third floret (F3, frame 8). Frames 1 through 9 represent sequential images at 0.5 mm increment through a single bulb treated at 10 °C for 6 weeks.

inflorescence, and florets were also visualized. The color of scape, leaves, inflorescence, and florets were brighter than that of mother scales (MS) and also daughter scales (DS).

The scape was short and thin before 10 °C treatment, but was long and enlarged after 6 weeks of 10 °C in MRI (Fig. 5). When forced at 30 °C for 3, 6, and 9 days, only two plants

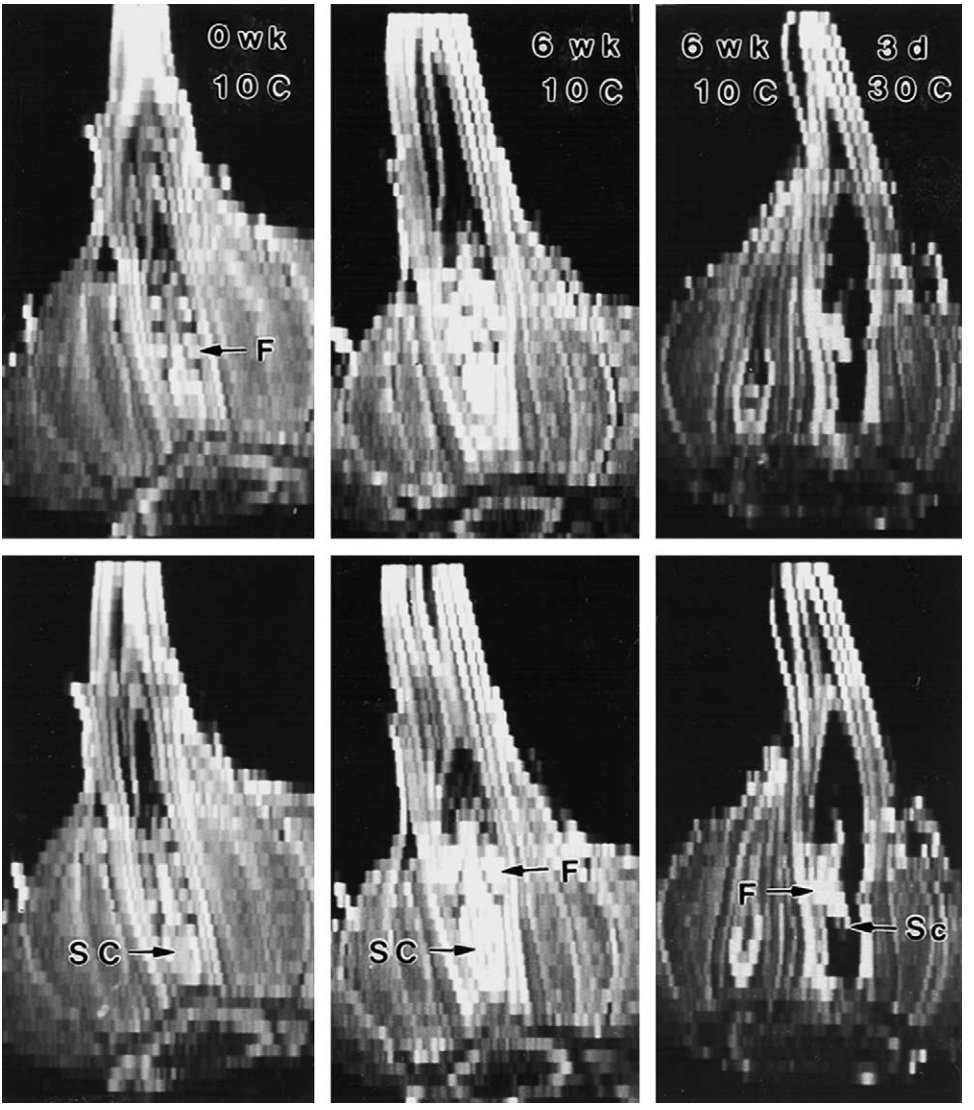

Fig. 5. Magnetic resonance imaging (MRI) images showing scape (SC) and flower (F) development 0 (left) and 6 weeks (middle) of 10 °C bulb treatment (center) and 3 days of 30 °C followed by 6 weeks of 10 °C. Scape was short when bulbs did not receive 10 °C. Scape became long and enlarged after 10 °C, but was dead after 30 °C. Two longitudinal images are shown to visualize organs easily.

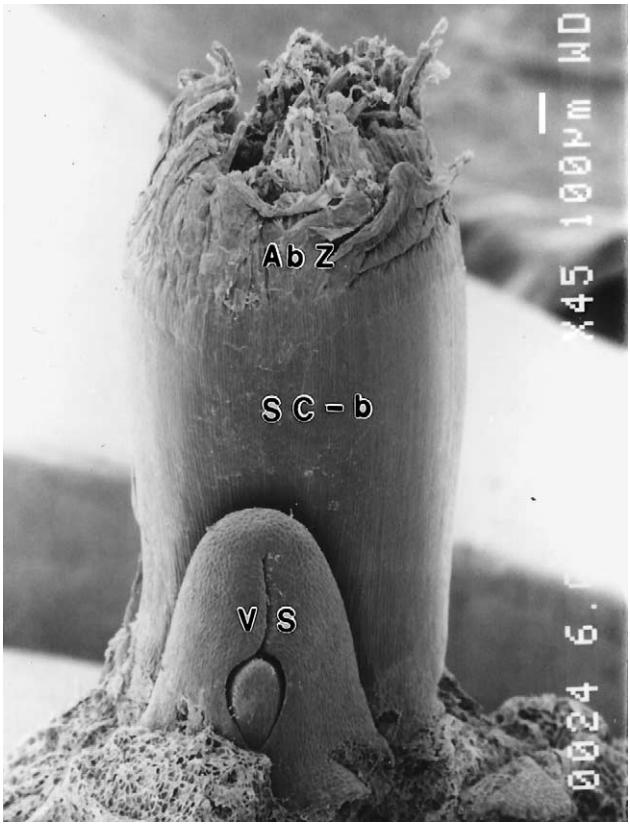

Fig. 6. Scape of *L. aloides* 'Pearsonii' that has a blasted inflorescence. Vegetative shoot (VS), scape (SC-b) below the abscission zone (AbZ) are shown. Scale bar = 100 μm.

that received 3 days at 30 °C did not flower and all plants that received 6 or 9 days did not flower, producing a blasted inflorescence (data not presented). The inflorescence and scape above the abscission zone was shriveled and separated from the abscission zone and was attached to the surface of the leaves (Fig. 4). When the inflorescence blasted (Fig. 6), growth of a vegetative shoot was enlarged and clearly visible at the basal plate (Fig. 7). As mentioned, scape, florets, inflorescence, and leaves were imaged brighter as compared to scales.

4. Discussion and conclusion

4.1. The effect of bulb storage and forcing temperature on flowering

Storage of dormant bulbs of various genera at low temperature is required for rapid scape elongation and flowering (Hartsema, 1961). Once the cold requirement is met, the scape starts to elongate and flowering is accelerated by forcing at optimum temperatures

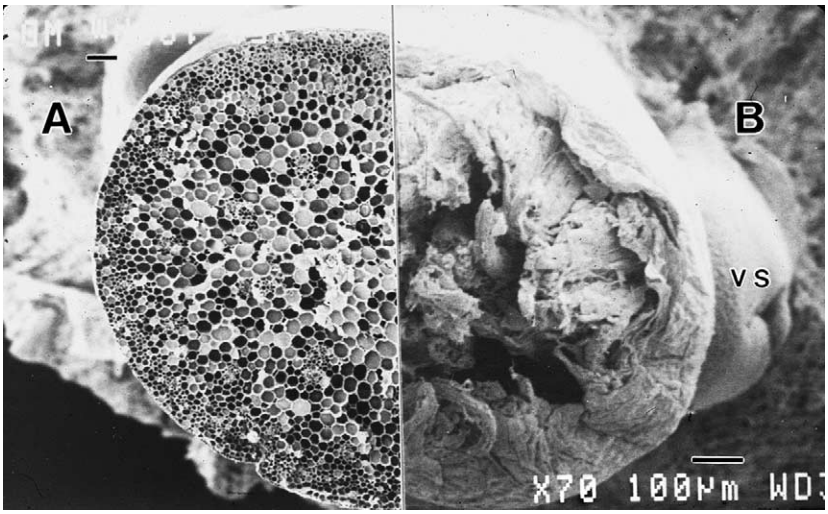

Fig. 7. Cross sections of *L. aloides* 'Pearsonii' scape when inflorescence developed normal (A) or blasted (B). A growing vegetative shoot (VS) is visible at the base of scape. Scale bar in A = 100 μm.

(De Hertogh, 1974). Since the low temperature requirement is satisfied after initiation of the inflorescence by storing bulbs at 10 °C, 12.5 °C, and 15 °C, acceleration in flowering of *Lachenalia* 'Pearsonii' may be considered similar to that observed in tulips (*Tulipa* spp.), hyacinths (*Hyacinthus* spp.), and other bulbs (De Hertogh, 1974; Hartsema, 1961).

Storing bulbs at 10–15 °C for 30–45 days satisfied the cold requirement of *Lachenalia* for rapid scape elongation and flowering. However, this requirement was dependent on the forcing temperatures, since flowering of bulbs stored at 10–15 °C for 15 days and forced at 21/19 °C was not accelerated, as compared to bulbs forced at 17/15 °C. Therefore, optimum bulb storage duration at 10–15 °C is considered 30 days.

The differences between Experiments 1 and 2 in the number of days to flower could be due to the environmental conditions during bulb production in Israel or during greenhouse forcing. This could be similar to the observations in *L. longiflorum* Thunb. which showed yearly variations in bulb size, rate of shoot emergence and development, and the number of flowers (Lin and Wilkins, 1975). This clearly indicates that greenhouse forcing performance is influenced by environmental factors during bulb production.

Since the number of florets was not affected by forcing temperature, bulb storage duration and storage temperatures mainly determine the number of florets. Floret initiation, as judged by the number of florets at flowering, is believed to cease during bulb storage at 10–15 °C and to continue at 20 °C and 25 °C (Table 1). Bulbs of *Lachenalia* 'Pearsonii' and the new hybrid 'Ronina' that were stored at 25 °C became reproductive in 110 days, while those at 15 °C were still in a vegetative stage (Roh et al., 1995). A reduction in the number of florets in association with accelerated flowering may, however, be similar to the response observed in *L. longiflorum* (De Hertogh, 1974; Roh and Wilkins, 1977).

Acceleration of flowering, therefore, results from an early completion of floret initiation and development of the initiated florets. Both developmental processes are accelerated by low temperatures during bulb storage and the greenhouse forcing period, particularly 30

days after planting (Table 1) (Roh et al., 1995). It is not known whether the number of florets could be increased without delaying flowering by manipulating bulb storage and forcing temperatures during floret initiation, differentiation, and development. An increase in the number of florets can be achieved by storing bulbs at 10–12.5 °C for 15 days and forcing at 17/15 °C during the first 30 days after potting or until flowering (Table 1). When 'Pearsonii' bulbs are planted without storage and forced to flower at high temperatures, leaves grow to >50 cm in length. The compact appearance of plants that results from a reduced leaf length and the floral stem length is influenced by bulb storage temperature (Roh et al., 1995). Compactness is important since greenhouse space required during forcing is reduced and longer leaves are more prone to damage during packing and shipping.

4.2. The effect of bulb storage and forcing temperature on inflorescence blast

Inflorescence blast was observed when *L. aloides* var. *quadricolor* were grown during summer (Roh et al., 1998). Therefore, 'Pearsonii' was forced at 25/23 °C and 29/27 °C to determine if inflorescence blast is caused by high forcing temperatures. Inflorescence blast was not observed when forced at 17/15 °C or 21/19 °C, regardless of bulb storage temperatures. However, the incidence of blast increased when bulbs stored at 10 °C or 15 °C were forced at 25/23 °C and 29/27 °C for 8 weeks (Table 2). Exposure of bulbs to low temperature during storage and subsequent high temperatures during forcing could be responsible for inflorescence blast.

As judged by the growth and developmental stage of the inflorescence observed after 12 weeks of growth in a greenhouse (Table 3), the inflorescence might have blasted within 4 weeks of forcing (Table 3). The degree of inflorescence blast was not influenced by the temperatures during weeks 5–8 after potting. As discussed above, initiation of florets during bulb storage at 10–15 °C could be completed, resulting in low floret numbers at flowering. Once initiation of florets is completed, during high forcing temperature, development of the initiated florets will proceed and forcing at 18/16 °C and 22/20 °C will ensure normal floret development. The inflorescence blast of *Lachenalia* is a similar response to that reported in flower bud blast of *Lilium* x *elegans* induced by high forcing temperatures for 14 days after potting the cold treated bulbs (Roh, 1990a,b).

Once the cold requirement is satisfied at 10 °C or 15 °C, rapid scape elongation, leaf enlargement, and development of florets, as judged by the growth and development stage (Table 3), may create competition for reserves such as carbohydrates among these organs or tissue. The competition could be severe during the first few weeks after planting since inflorescence blast is induced at a temperature >24 °C during the first 4 weeks after potting (Table 3). The limited supply of carbohydrate to developing florets, which could act as a strong sink, could be responsible for inflorescence blast.

4.3. Scanning electron microscopy (SEM), nuclear magnetic resonance imaging (MRI), and floret development

Inflorescence blast that was observed at the time of data collection was clearly revealed by SEM and MRI. From the image of SEM, abscission was revealed which showed the

mechanism by which bulbs are able to survive in the unfavorable environments, such as high temperatures. Death of the inflorescence stops at the abscission zone, leaving the remaining bulb unit alive. However, the internal structural change cannot be investigated from one bulb during various temperature treatments using SEM. Therefore, the non-destructive MRI technique is employed to study structural changes and physiological and metabolic changes. Development of the inflorescence as influenced in bulbs stored at 10 °C is clearly visualized as was also observed in *Tulipa gesneriana* L. (Van der Toorn et al., 2000). Under these experimental conditions, it is evident that inflorescence blast can occur after only after 3 days of high temperature treatment. Generally, an increase in free water is accompanied by an increase in t_2 relaxation times (Faust et al., 1997; Ishida et al., 2000; Ratcliffe et al., 2001). It has been shown that free water content in actively developing organs, such as the scape, inflorescence, florets, and leaves which are imaged as brighter regions of the bulbs are high as judged by the t_2 relaxation time, as was discussed in tulip (Van der Toorn et al., 2000).

In conclusion, storing *Lachenalia* bulbs at 10–15 °C before potting and forcing at 17/15 °C is essential to produce a high quality plant as judged by short leaves, short floral stem length, and early flowering. Since the number of florets was reduced by storing bulbs at 10–15 °C before potting and forcing at 17/15 °C, methods for temperature manipulation during bulb storage or forcing should be developed to increase the number of florets. Inflorescence blast as shown by SEM and MRI was caused by high forcing temperatures at the beginning of forcing after potting low temperature treated bulbs.

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