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https://doi.org/10.5109/9320

出版情報：九州大学大学院農学研究院紀要. 52 (2), pp.315-319, 2007-10-29. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **52** (2), 315–319 (2007)

Changes in Carbohydrate and ABA Content in Tulip Bulbs during Storage

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(*Received June 29, 2007 and accepted July 17, 2007*)

Changes in carbohydrate and ABA content in tulip bulbs during storage at 20 and 5˚C were investigated in relation to the response to gibberellin treatment after planting. The breakdown of starch and the accumulation of soluble carbohydrate in the scales were observed during storage. Cold (5˚C) treatment promoted the metabolic changes of carbohydrate, but the similar changes proceeded slowly at 20˚C. ABA content in the scales gradually decreased similarly as the duration of storage was extended regardless of storage temperature. Decrease in ABA in the scales during storage is time dependent but not temperature dependent and the decrease may promote the sensitivity to gibberellin after planting.

INTRODUCTION

A period of 12–16 weeks of low temperature is required for rapid flower stalk elongation and flowering in tulip after flower initiation at moderate temperature (De Hertogh, 1974). Gibberellin application cannot completely substitute for the required cold treatment of uncooled tulip bulbs but does for partly cooled bulbs (van Bragt and Zijlstra, 1971; Hanks, 1982). Saniewski *et al*. (1999b) reported that exogenously applied gibberellic acid (GA_3) induced shoot growth and flowering of non pre–cooled bulbs when the roots were removed, although the stem was not long enough in comparison with that of fully cooled bulbs. Geng *et al*. (2005) found that it took 83 and 80 days for flowering when the non–cooled bulbs of 'Oxford' were planted with GA_3, respectively with and without root primordia in September, 57/41days (with/without root primordia) in November, 37/28 days in December and 30/23 days in January. They suggested that some endogenous metabolic changes but not depending on low temperature, proceed in the bulbs during storage at 20˚C or it may be the same phenomenon promoted by low temperature but proceeded slowly at higher temperature.

Reserve carbohydrates stored in bulb scales are the major source of energy and carbon for early shoot growth in bulbous plant (Miller, 1992; De Hertogh and Le Nard, 1993). Among carbohydrates, starch is the major storage carbohydrate in tulip bulbs, and α–amylase is regarded as the key enzyme initiating starch degradation in most tissues (Manners, 1985). Sucrose is a non–reducing sugar, and probably serves as the primary transport carbohydrate in tulip bulbs, and its breakdown and metabolism is a critical step for growth of the plant. In tulip bulbs, it has been suggested that low–temperature treatment during storage influences mainly the conversion of starch to soluble sugars, and these soluble constituents are transported to the shoot and used for elongation growth after planting (Charles–Edwards and Rees, 1974, 1975; Davies and Kempton, 1975; Ohyama *et al*., 1988).

The most considerable inhibiting factor for sprouting of tulip bulbs is ABA, and the presence of ABA has been documented (Syrtanova *et al*., 1975; Aung and De Hertogh, 1979; Terry *et al*., 1982) in tulip bulbs. ABA inhibited the shoot growth of cooled tulip bulbs (Saniewski, *et al*., 1990) or that induced by gibberellin in non pre–cooled derooted bulbs (Saniewski *et al*., 1999a). Saniewski and Gabryszewska (1983) suggested from their in vitro results that ABA levels should decrease during the cold treatment for proper stem elongation and flowering of tulip after planting.

In this study, changes in carbohydrate and ABA content in tulip bulbs during storage were investigated in relation to the response to gibberellin treatment.

MATERIALS AND METHODS

Plant materials and treatments

Bulbs of tulip (*Tulipa gesneriana* L. cv. Oxford, 10–12 cm in circumference, a product of Niigata Prefecture, Japan), upon arrival at the laboratory on 10 September and 1 October 2004, were stored at 20˚C (non–cooled bulbs), and those arrived on 1 October were transferred to 5˚C on 4 October (cooled bulbs). The bulbs were disinfected with 1.0% Benlate (Du Pont) for 1 hour and air–dried before the temperature treatments.

During the storage, 10 bulbs in each analysis were taken and divided into scales and central buds on 22 September, 22 October, 22 November and 27 December. After measuring fresh weights, they were frozen in liquid nitrogen and kept at −80˚C. They were freeze–dried and weighed before analysis.

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Starch and soluble carbohydrate content

Freeze–dried powder (25–35 mg) was extracted at 60 °C in 2 mL of 80% (v/v) ethanol (three times, 1 hour per extraction). After each extraction the suspension was centrifuged at 2,000 g for 5 minutes. The supernatants were combined and the ethanol was evaporated at 50 °C. The residue was submitted to a chloroform: water extraction (1.25 mL: 2 mL) and kept on the ice for 30 minutes. Then 800 μL of the water phase was pipetted and submitted to 500 μL of chloroform. The water phases were also used for analysis for sucrose, glucose and fructose. The content was determined by the procedure of Saccharose/D–Glucose/D–fructose UV–test made by J. K. International.

The ethanol–insoluble pellet was dissolved in 5 mL of DMSO and 1.25 mL of HCl (8 M) at 60 °C for 1 h in 50–mL volumetric tube. After cooling to room temperature, 1.1 mL of NaOH (8 M) was added, subsequently, the solution was pipetted to 100–mL volumetric beaker, the tube was washed with about 20 mL of demineralized water three time and the water was combined and pipetted to the beaker, then the combined solution was adjusted to pH 4.0–5.0 and adjusted to 100 mL, then used for analysis of starch.

Solubilized starch was determined by the procedure of F–kit of J. K. International, which is for the determination of native starch and of partially hydrolyzed starch.

ABA content

Scales of 10 bulbs in each treatment were prepared in the same manner as described above for ABA analysis. The freeze–dried powder (1 g of fresh weight equivalent) was homogenized and extracted with 80% methanol containing 200 mgl^{-1} BHT (butylated hydroxytoluene) at 5 °C in the dark with constant shaking on a gyratory shaker for 24 hours. The supernatant was centrifuged at 10,000 rpm for 10 minutes at 5°C, then the residue was re–extracted with 80% methanol and centrifuged two times. The combined and filtered supernatant was evaporated *in vacuo* at 37–40 °C to eliminate methanol. The remaining aqueous solution was adjusted to pH 3.0 with 1N HCl and extracted three times with ethyl acetate. The ethyl acetate fraction was extracted three times with 2% $NaHCO_3$, and the aqueous phase was acidified at pH 3.0 with 6N HCl. It was extracted three times with ethyl acetate. The ethyl acetate fraction was concentrated at 37–40 °C, and the concentrated extract was dissolved with 5% methanol. The methanol solution was passed through Sep Pak C_{18} columns and eluted with 80% methanol. The methanol eluate was evaporated to dryness and kept at –20 °C. ABA content was determined using Phytodetek ABA Enzyme Immunoassay Test Kit according to the instruction of the manufacturer. The experiment was conducted repeatedly in 2005.

RESULTS

Shoot growth and scale weight

Shoot growth of the cooled bulbs was not different

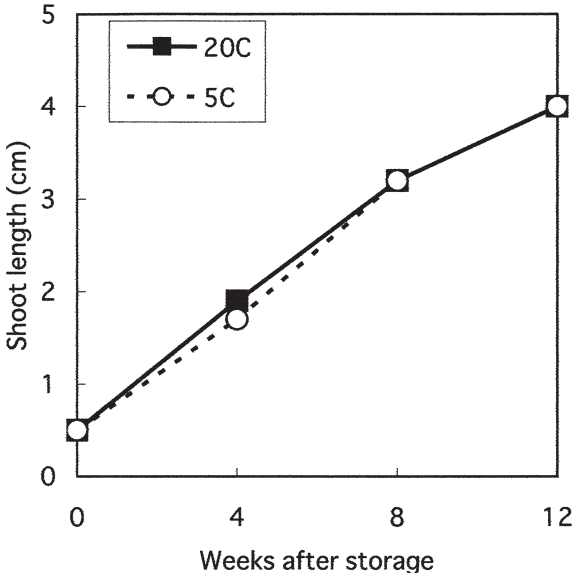

Fig. 1. Shoot growth inside the bulbs during storage.

Table 1. Changes of the fresh weight, dry weight and relative water content of tulip bulbs during storage

Organ	Temperature (°C)	Weeks of storage	F.W. (g)	D.W. (g)	Relative water content (%)
Scales	20	0			68.43
		4	25.11	8.10	67.74
		8	23.48	7.85	66.58
		12	22.38	7.47	66.60
	5	0	–	–	–
		4	25.85	8.20	68.26
		8	25.42	7.89	68.96
		12	24.93	7.75	68.91
Shoot	20	0	0.082	0.016	80.48
		4	0.323	0.057	82.46
		8	0.867	0.232	73.24
		12	1.450	0.334	76.97
	5	0	–	–	–
		4	0.163	0.033	79.75
		8	0.683	0.175	74.37
		12	1.017	0.291	71.39

Table 2. Fresh weight, dry weight, relative water content and carbohydrate content in the shoot at the end of storage

Organ	Temperature (°C)	F.W. (g)	D.W. (g)	R.W.T. (%)	Carbohydrate content (mg/g d.w.)			
					Starch	Sucrose	Glucose	Fructose
Flower bud+leaves	20	1.21	0.26	78.4	153	188	16.6	3.2
	5	0.78	0.20	74.1	176	142	8.6	2.8
Floral stem	20	0.24	0.06	76.0	369	161	2.0	0.8
	5	0.24	0.07	69.3	347	178	1.6	2.2

from that of non–cooled bulbs during storage (Fig. 1). Fresh and dry weights of the scales slightly decreased during storage both at 20 and 5°C with little difference between the temperature treatments (Table 1). Those of the shoots at both temperatures increased but the differences were small. A very slight decrease in dry weight in the scales was observed at both temperatures, and instead shoot dry weight increased. Fresh weight of the shoot at 25°C after 12 weeks storage was 1.4 times of that stored at 5°C due to the growth of flower bud and leaves, but not to floral stem growth (Table 2). Increase in dry weight of the shoot at 25°C was also due to the results of the dry weight gain in flower bud and leaves.

Starch and soluble carbohydrates in the scales

Starch degradation occurred in the scales during storage regardless of storage temperature, and the degradation rate in the scales of cooled bulbs was 44%, whereas that of non–cooled bulbs was 15% at the end of cold treatment (12 weeks) (Fig. 2A). Sucrose content increased to 3.5 and 1.6 times of the initial values at 5 and 20°C storages, respectively (Fig. 2B). Glucose and fructose content were small throughout the treatment both in the cooled and non–cooled bulbs (Figs. 2C, D). They increased in the cooled bulbs but no such increase was observed in the non–cooled bulbs.

Starch and soluble carbohydrates in the shoot

Starch content in the flower bud+leaves at 20°C was a little lower than that at 5°C, whereas the opposite was observed in sucrose, glucose and fructose content (Table 2). No such clear difference of the content was found in the floral stem.

ABA content

ABA decreased rapidly during the first 4 weeks and

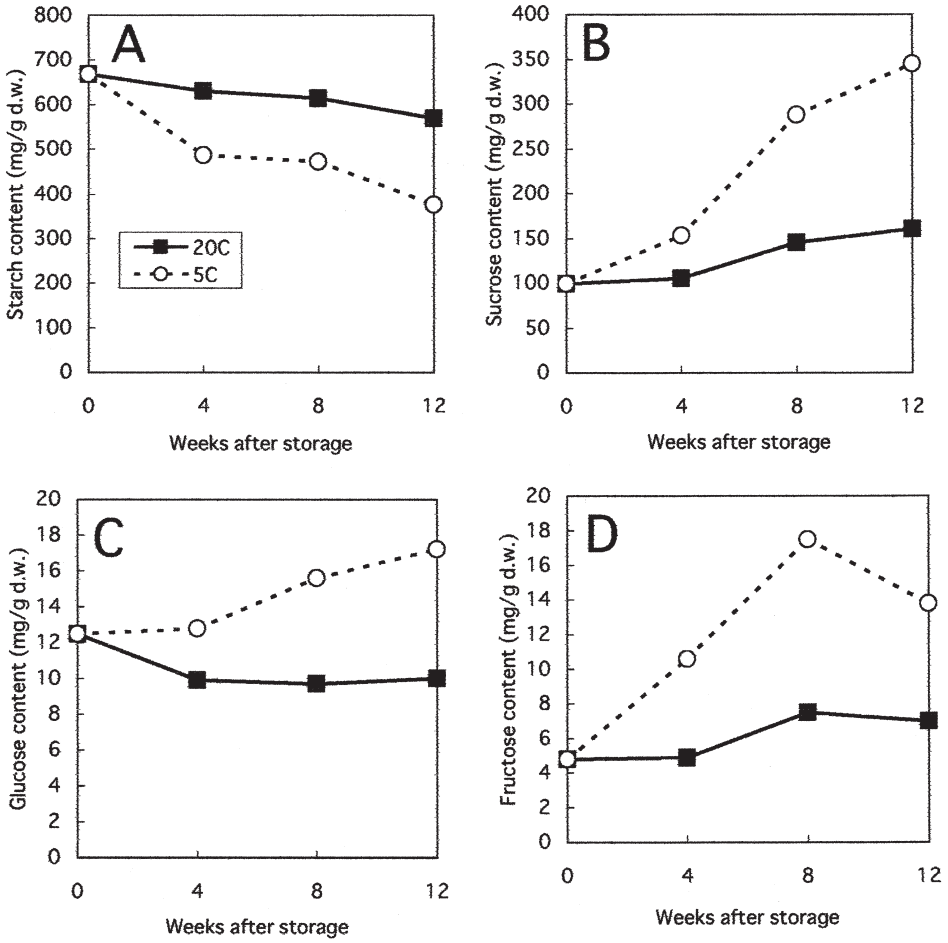

Fig. 2. Changes in the content of starch (A), sucrose (B), glucose (C) and fructose (D) in the scales during storage.

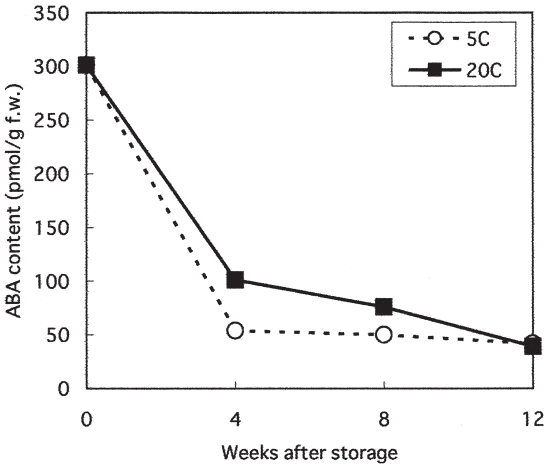

Fig. 3. Changes on ABA content in the scales of the bulbs during storage.

then gradually both in the scales of the cooled and non–cooled bulbs during storage, but there was no significant difference in the content and changes between them (Fig. 3).

DISCUSSION

Similar results on shoot growth and scale weight have been already obtained in 'Apeldoorn' (Davies and Kempton, 1975) and 'Malta' (Ohyama *et al.*, 1996), although some opposite results in shoot dry weight in 'Apeldoorn' have also been reported (Kamenetsky *et al.*, 2003).

Starch breakdown accompanied by the increase in sucrose, glucose and fructose content and the difference by the treated temperatures showed the similar tendency as reported by Kamenetsky *et al.* (2003). It should be noted that the process even occurs but proceeds slowly in non–cooled bulbs.

α–amylase, a key enzyme involved in the hydrolysis of starch into a simpler sugar that can be metabolized, slightly increased during storage in the scales of cooled tulip bulbs, but lowered or remained at its initial level in non–cooled bulbs (Komiyama *et al.*, 1997; Lambrechts *et al.*, 1994). Gibberellins have been suggested to induce expression of α–amylase and the breakdown of starch in the rice seeds, and the mechanisms how gibberellins induce expression of α–amylase and the breakdown of starch have been well documented (Choi *et al.*, 1996; Fincher, 1989). Increase in starch breakdown and accumulation of sucrose in non–cooled bulbs may indicate that α–amylase were also active in non–cooled bulbs. We reported that the later the treatments with GA_3 were given to non–cooled bulbs, the earlier the flowering and longer the flower stalk were obtained (Geng *et al.*, 2005) and suggested some endogenous metabolic changes but not depending on low temperature, proceed in the bulbs during storage at 20 °C. Different responses of cooled and non–cooled bulbs to GA_3 treatment lead Hanks (1982) to suggest that low temperature period increases the sensitivity to gibberellin. Similar results, but with isolated sprout *in vitro*, have been shown by Rebers *et al.* (1994). Gibberellins stimulated floral stalk elongation in the sprouts from non–cooled bulbs only when they were incubated later (late January and February), but the non–cooled sprouts hardly responded when incubated earlier (October and early January). It is difficult to make relations between the time dependent difference in growth response to gibberellin and changes in these carbohydrates during storage. Carbohydrate metabolism may proceed, but slowly, during bulbs storage even at high temperatures.

ABA levels in the scales of 'Apeldoorn' bulbs declined slowly but continuously in 12 weeks of storage at 17 and 5 °C with no differences between the temperature treatments (Franssen and Voskens, 1997). Our results in the present study are similar to this report. Decrease in ABA in the scales during storage may contribute to the response of the bulbs to gibberellin treatment after planting. However, the decrease seems to be time dependent but not temperature dependent.

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