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EFFECTS OF PRE-STORAGE TREATMENT WITH ETHANOL AND CO_2 ON ONION DORMANCY

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Abstract

Onion (*Allium cepa* L.) cvs. 'Tazan' and 'Iyomante' were pre-treated either with ethanol (at a dose of 0.34, 0.68 and 0.91g.kg^{-1}) and stored at 0, 10, 20 and 30ºC or with 100% CO_2 (for 3.5, 7 and 14 days) and stored at 20ºC. Treatment of onion with ethanol delayed rooting, sprouting and reduced decay in both cultivars. Chemical analysis and organoleptic tests showed that the treatments had no adverse effect on the quality of onion. Pre-storage treatment with 100% CO_2 for 3.5 and 7 days enhanced rooting and sprouting, while 14-days treatment exhibited a slight inhibitory effect.

Key words: Onion, storage, ethanol, CO_2

Introduction

Sprouting and rooting are the major factors limiting storage life of onion. After harvest, onions are in a natural state of dormancy (Jones and Mann 1963). Length of the dormant period varies with cultivar and storage temperatures (Miedema 1994a) and is usually not affected by the external stimuli (Abdalla and Mann 1963, Thomas 1969). Scientifically, dormancy terminates with the beginning of inner sprout growth (Abdalla and Mann 1963); the commercial storage life and market value, however, is not affected unless there is visible sprout or root growth.

The optimum temperature for onion sprouting ranges from 10 to 20ºC (Miedema 1994a). Roots appear first followed by sprouts (Miedema and Kamminga 1994). It seems that sprouting is initiated by two factors (1) induction of cytokinine with the depletion of abscissic acid (Miedema and Kamminga 1994, Abeles *et al.* 1992) and (2) stress such as wound (Miedema 1994b), cold (Benkeblia and Selselet 1999) or heat shock (Miedema 1994a). Successful postharvest treatments have often shown inhibitory effect on cytokinine. For example, removal of new roots retarded sprout elongation. This is because roots supply cytokinine to the bulb and removal of roots inhibited the induction of cytokinine (Miedema 1994b). Similarly sprout inhibition at high temperature (30ºC) is considered to be due to thermo-dormancy (Yoo *et al.* 1997) because storing onion continuously above 25ºC inhibits the induction of cytokinine (Miedema and Kamminga 1994). While the suppression of sprout at low temperature (0ºC) (Miedema 1994a, Ramin 1999) or in controlled atmosphere (CA) (Weichmann 1986) is due to reduced metabolic reactions within the bulb.

Conventional methods of storing onions are costly. Storing onions in CA gives encouraging results but the gains usually do not justify the commercial application. Similarly, storing onions at 0 or 30ºC requires expensive refrigeration or heating systems. It is therefore, necessary to explore alternate low cost and environment-friendly method to store onion without compromising the quality. Treatment with anti-ethylene compounds such as silver thiosulfate (STS) (Benkeblia and Selselet 1999) and nitrous oxide (N_2O) (Qadir *et al.* 2000) have shown to delay sprouting. Ethanol has also exhibited the ability to inhibit the synthesis and

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action of ethylene (Saltveit and Mencarelli 1988). Ethanol is a natural metabolite produced by plant tissue under aerobic (Smock 1944) and anaerobic (Saltveit 1989, Kelly and Saltveit 1989) conditions. Like other anti-ethylene compounds, ethanol might also extend the bulb onion dormancy. This possibility was tested by direct application of ethanol or exposing the onion to 100% CO_2.

Materials and Methods

Plant material

Two cultivars of bulb onion (*Allium cepa* L) autumn sown crop 'Tazan', grown in Saga Prefecture (Lat. 33º 16/ N, Long. 130º 18/ E) and spring sown crop 'Iyomante', grown in Furano city, Hokkaido (Lat. 43º 1/ N, Long. 142º 20/ E), were used in this study. The medium sized (170-180 g) bulbs obtained 3 weeks after harvest from a commercial supplier in May and November 1999 were graded for uniformity of size.

Application of ethanol

About 3 kg of bulb onion were placed in 8 L glass jar. A glass petri plate (90 mm) containing filter paper was placed on top of the onion bulbs. Absolute ethanol at a dose of 0.34, 0.68 and 0.91 $g \cdot kg^{-1}$, was applied on the filter paper and jars were closed with airtight lids and kept for 24 hours at 20ºC. In the control, water was used instead of ethanol. After opening the jars, bulbs from the same treatment were mixed; 45-50 bulbs per replicate and three replicate per treatment were placed in a cartoon and stored at 0?1, 10±1, 20±1 and 30±1ºC in the dark for 3 months. Relative humidity in the storage room was not controlled.

Enrichment with CO_2

The jars containing onion were ventilated with a continuous flow of pure CO_2 for 3.5, 7 and 14 days using CO_2 regulator (Yamamoto Sangyo YR-509) with 1-25 L flow controller. The control onion was ventilated with fresh air. Flow rate of gasses was about 150 $ml.min^{-1}$. CO_2 level inside the jars was determined by GC using a thermal conductivity detector. After opening the jars, bulbs from the same treatment were mixed; 45-50 bulbs per replicate and three replicate per treatment were placed in a cartoon and stored at 20±1ºC in the dark for 3 months.

Measurement of sprouting, rooting and decay

The bulbs were graded at one-month intervals for sprouting, rooting and decay. At each interval all the bulb onion were visually assessed for sprouting and rooting. A bulb was considered to have sprouted when the sprout leaves had emerged from the neck. The emergence of a fresh root was regarded as rooting. After recording the data decayed bulbs were removed.

Chemical analysis

For chemical analysis, six bulbs per treatment were skinned and cut longitudinally into four wedges at the end of storage period. One wedge from each bulb was diced and the juice was extracted by an electric juicer. The juice was filtered through 4 layers of cheese cloth and used for determination of pH, titratable acidity and total soluble solids. pH was determined by using a pH meter (Horiba pH meter F-12). Total soluble solids were determined by a refractometer (Kikuchi, Tokyo, Japan). Titratable acidity was determined by the method of AOAC (1980). Sugar analysis was done as described by Salama *et al.* (1990) on four bulbs per treatment, with some modifications. Fresh tissue (5 g/bulb) were cut into small pieces and extracted with 50 ml of 80% ethanol at about 50ºC, centrifuged at 8,000 x g for 20 min, supernatant collected and the residue re-extracted twice. The combined extract was evaporated at 40ºC to remove ethanol, passed through Amberlite IR-120; (12 x 70 mm) and elution volume was brought to 100 ml by washing the column with distilled water. The elute was dried under reduced pressure and dissolved in 2 ml of water and filtered. For analysis, 20 µl sample was injected in an (Shimadzu LC-10AS) HPLC system (Shodex SC-125S column, ? 4.6 x 500 mm) and eluted with Milli Q water at 55ºC (0.3 ml / min) and detected at 192 nm.

Pyruvic acid (PA) was determined according to Benkeblia (2000) from four bulbs per treatment. Internal fresh tissues (50 g/bulb) were sliced into 150 ml of water and blended in a juicer for 4 min. After 1 h maceration, the mixture was filtered and the filtrate was diluted 10 times. A control was prepared by blending the tissues with 5% TCA to inactivate the alliinase enzyme that produces PA in macerated onion. For PA analysis, the reaction mixture contained 1 ml of diluted filtrate, 1 ml of distilled water and 1 ml of 2, 4 dinitrophenylhydrazine (630 µM in 2 M HCl). Reaction mixtures were vortexed and incubated at 37ºC for 10 min. After incubation, 5 ml of 0.6 M NaOH was added and vortexed for 5 min. Pyruvic acid was measured by spectrophotometer (Shimadzu UV 160A model) at 490 nm and the amount was calculated from standard PA graph. The PA content of control (tissue level pyruvate) was subtracted from that of the test.

Organoleptic test

For sensory evaluation, wedges (one from each bulb) were diced, mixed and presented to a panel (twenty people) for rating on pungency, sweetness and preference. Rating was on a scale of 1 to 5, where 5 scored for the least pungent, most sweet and most preferred (Smittle 1988).

Statistical analysis

Data were analyzed by using ANOVA. Means were then subjected to Duncan's multiple range tests (DMRT) or LSD.

Results and Discussion

Effect of ethanol-treatment on rooting, sprouting and decay

Treatment with ethanol delayed rooting, sprouting and reduced decay in both cultivars (Table 1). The inhibitory effect of ethanol increased with its increasing dose. As Like ethanol, treatment with other anti-ethylene compounds, STS (Benkeblia and Selselet 1999) and N_2O (Qadir *et al*. 2000), have also shown to delay sprouting in bulb onion. Since cytokinine and abscissic acid are known to control the rooting and sprouting in bulb onion (Miedema and Kamminga 1994, Abeles *et al*. 1992), it is possible that ethanol might have either inhibited the cytokinine induction and / or extended the activity of abscissic acid. Rooting and sprouting were more effectively controlled on 'Tazan' than on 'Iyomante' whereas decay was better controlled on 'Iyomante' than on 'Tazan'. This difference in response to ethanol treatment might be due to varietal differences and / or differences in their growing seasons or to some other reasons.

Table 1. Effect of pre-storage treatment with ethanol on storage life of onion at 20ºC.

Variety	Parameters	Storage (month)	Ethanol treatments (g.kg^{-1})			
			0	0.34	0.68	0.91
'Tazan'	Rooting	1	15.5[z] f	1.9 h	1.8 h	0 i
		2	34.6 d	1.9 h	5.5 g	0 i
		3	70.0 a	60.0 b	52.0 c	24.7 e
	Sprouting	1	0 f	1.7 e	1.4 e	0 f
		2	2.0 d	3.5 c	1.7 e	0 f
		3	4.5 b	6.4 a	3.6 c	0 f
	Decay	1	1.8 f	0 g	1.9 f	0 g
		2	6.5 c	1.6 f	3.8 d	0 g
		3	10.6 a	3.2 de	8.7 b	6.6 c
'Iyomante'	Rooting	1	21.0 f	6.2 j	11.4 hi	0 k
		2	45.6 bc	13.2 h	24.2 e	6.8 i
		3	81.3 a	47.3 b	30.4 d	18.3 g
	Sprouting	1	3.6 ef	0 g	0 g	0 g
		2	4.4 e	3.6 ef	3.0 f	3.3 f
		3	13.2 ab	14.8 a	11.0 c	7.3 d
	Decay	1	6.8 d	0 g	0 g	0 g
		2	13.0 ab	3.6 e	3.5 e	0 g
		3	14.2 a	14.7 a	8.6 c	1.8 f

[z] Values show percentage. Means were separated according to DMRT.

Effect of CO_2-treatment on rooting, sprouting and decay

Pre-storage treatment with 100% CO_2 for 3.5 and 7 days enhanced rooting and sprouting while 14 days treatment showed a slight inhibitory effect (Table 2). The short exposure to CO_2 (3.5 and 7 days) might have induced a stress-related response in bulbs. Other stresses such as wound (Miedema 1994b); transferring onion from low to high (Benkeblia and Selselet 1999) or high to low temperature, have been shown to break dormancy, which is probably independent of cytokinine (Miedema 1994a). Since hypoxia is known to induce ethanol production (Saltveit 1989, Kelly and Saltveit 1989), that might have extend the dormancy in 14 days-treated onions. The amount of ethanol produced under anaerobic conditions up to 7 days was possibly not enough to overcome the negative effect of CO_2. However, 14 days exposure to CO_2 might have induced sufficient amount of ethanol that could overcome this negative effect and hence extended dormancy period. Although 14-days treatment showed slightly better results, long term storage under high CO_2 atmosphere have shown devastating effect such as softening and water core (Schouten 1976); therefore, this treatment may not have any practical importance.

Effect of ethanol-treatment on quality parameters of onion

Apart from sprouting, the major quality attributes that need to be retained during storage are flavor and taste. Sugar, pungency and organic acids contribute to these quality attributes. In this study, ethanol treatment diminished the sugar level at higher temperature, independent of ethanol dose (Fig 1). In the control, sucrose level increased remarkably with increasing temperature while fructose level decreased. Consequently, there was a slight increase in total sugar content. This increase in sugar level might be attributed to the conversion of complex oligosaccharides into soluble sugars at higher temperature (Hurst *et al*. 1985). However, these changes in sugar level were lesser in treated bulb than in control. Therefore, it is evident that ethanol treatment could suppress the metabolic activity in onion thereby prolonging dormancy.

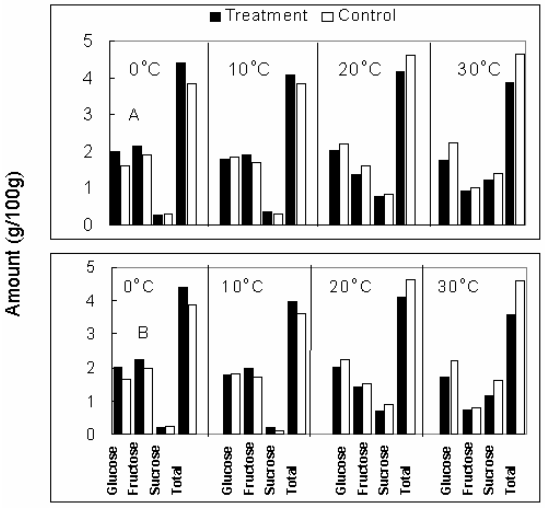

Fig. 1. Changes in sugar levels in ethanol-treated onion (A) Tazan and (B) Iyomante after 3 months of storage at different temperatures.

Table 2. Effect of pre-storage treatment with 100% CO_2 on storage life of onion at 20ºC.

Variety	Parameters	Storage (month)	CO_2 treatments (days)			
			0	3.5	7.0	14
'Tazan'	Rooting	1	40.0[z] hi	43.0 h	42.3 hi	29.3 j
		2	48.7 g	66.7 d	65.7 d	43.0 h
		3	66.7 d	73.7 bc	72.7 bc	62.7 de
	Sprouting	1	2.1 i	3.7 gh	5.0 g	4.3 gh
		2	4.7 gh	6.3 g	7.3 ef	7.0 ef
		3	8.0 e	11.0 cd	14.0 c	13.0 c
	Decay	1	1.3 f	0 g	0 g	0 g
		2	6.7 bc	2.3 e	0.7 g	0 g
		3	8.0 b	5.0 d	4.7 d	1.3 f
'Iyomante'	Rooting	1	48.7 g	66.3 d	57.0 f	40.3 hi
		2	59.7 ef	75 b	65.7 d	47.7 g
		3	70.0 bc	80.0 a	73.3 bc	65.0 d
	Sprouting	1	5.3 g	7.7 ef	8.0 e	7.7 ef
		2	6.7 g	8.7 e	11 cd	10.0 e
		3	16.7 ab	18.3 a	20.0 a	18.7 a
	Decay	1	2.3 e	0 g	0 g	0 g
		2	12.3 a	4.3 d	0 g	0 g
		3	11.7 a	8.3 b	5.7 bc	2.7 e

[z] Values show percentage. Means were separated according to DMRT.

Table 3. Pyruvate development (µM/g fresh weight) in onion after 3 months of storage at 20ºC.

Variety	Ethanol (g kg^{-1})			
	0	0.34	0.68	0.91
'Tazan'	42.3[z]	42.3	43.8	41.8
'Iyomante'	42.0	42.0	52.0	51.5
Significance	NS	NS	*	*

[z] Mean of 48 values. NS = non-significant * F test were significant at $P = 0.05$.

The pyruvate content, which indicates pungency levels in onion, was not affected in 'Tazan', but increased significantly in 'Iyomante' with 0.68 and 0.91 g.kg^{-1} ethanol treatment (Table 3). Degree of pungency is determined by the amount of volatile compounds released and it has been shown that the thiosulfinate volatiles possessed antibiotic activity (Virtanen and Matikkala 1959). As ethanol treatment has shown better performance in controlling decay in Iyomante, it could, therefore, be assumed that this elevated level of pungency might have added to the suppression of bacterial or fungal deterioration. However, in the organoleptic test the elevated pungency was not apparent (Table 4). Our results showed that treatment with ethanol did not induce odd-flavour and have no adverse effect on sugar / pungency ratio. In addition, ethanol treatment did not have any adverse effect on pH, total soluble solid (TSS) and titratable acidity (TA) or organic acid index of onion (Fig. 2). A slight increase in organic acid and a consequent decrease in pH were observed with increasing temperature.

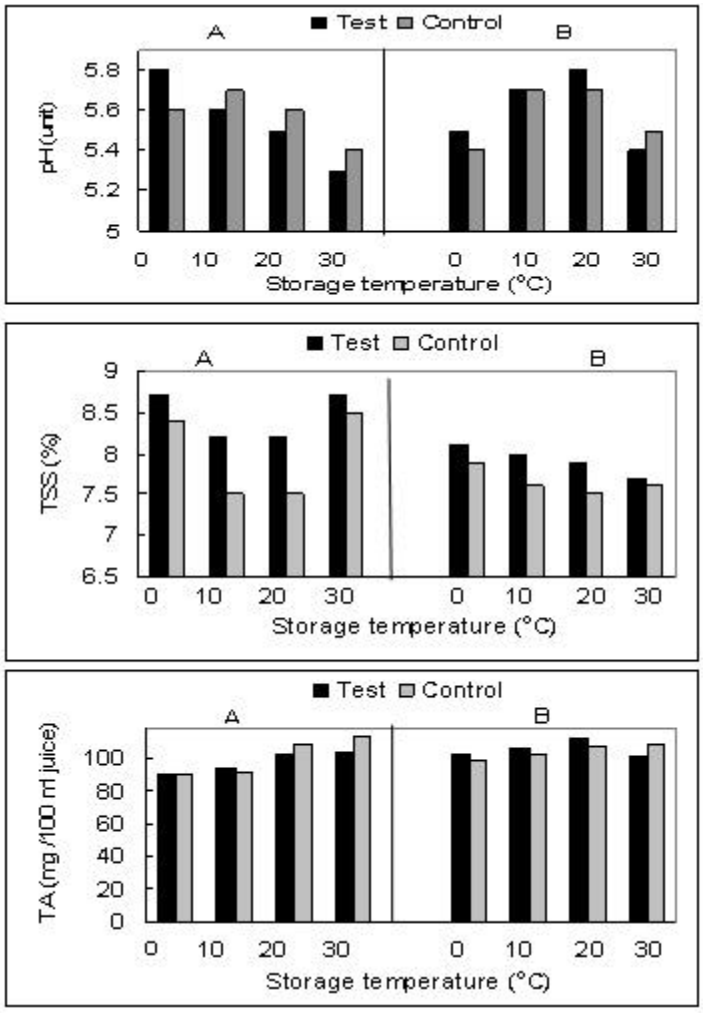

Fig. 2. Changes in pH, TSS and TA in ethanol-treated onion Tazan (A) and Iyomante (B) after 3 months of storage at different temperatures.

Table 4. Organolyptic characters of onion after 3 months of storage at 20ºC.

Variety	Ethanol (g kg^{-1})	Sweetness	Pungency	Overall
'Tazan'	0	2.9 a	3.3 a	2.9 a
	0.34	2.5 ab	2.7 ab	2.5 b
	0.68	3.2 a	3.1 a	3.1 a
	0.91	2.9 a	3.0 a	2.7 b
'Iyomante'	0	3.1 a	2.7 ab	3.3 a
	0.34	2.9 ab	2.8 a	3.0 ab
	0.68	3.5 a	2.8 a	3.5 a
	0.91	2.7 ab	3.0 a	2.8 ab

Mean separation in column according to DMRT at *P= 0.05*.

Over all, pre-storage treatment with ethanol showed promising results. It warrants further investigation of its application on large-scale experiment. Since it is a cost effective system, this treatment may possibly offer substitute to CA system, which is expensive and needs sophisticated facilities. Moreover, in this research, the mechanism of sprout inhibition by ethanol treatment or enhancement by CO_2 treatment is not explored and is not yet understood. Therefore, further research is required to elucidate the physiological backgrounds of the effects of these treatments on rooting and sprouting of onion.

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