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Postharvest Longevity and Viability of Cooler-stored Lotus Propagules[1]

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Abstract

Storage of enlarged lotus rhizomes used as a vegetable crop has been extensively studied, but little is known about the viability of lotus propagules (rhizomes with shoots) during storage. In this study, ornamental lotus, *Nelumbo nucifera* 'Embolene', was used to evaluate the effects of gum acacia, sphagnum moss and Terra-Sorb® hydrogel on the physiology and postharvest longevity or viability of stored lotus propagules. After 45 days of storage at 5C (42F) and 95% RH, there were no decayed propagules, and 94% of total propagules maintained their viability after planting. Water retention and shelf-life of rhizomes were similar among all treatments during storage. However, more water loss occurred with treatments at higher concentrations of gum acacia. After harvest, large differences in total sugar were found among individual samples, while starch content remained unchanged. A strong quadratic relationship was observed between total sugar and storage time for all treatments, but there was no significant effect of treatment on total carbohydrate change in lotus propagules after 45 days of storage.

Index words: *Nelumbo nucifera*; storage; carbohydrate; aquatic plants; gum acacia; hydrogel.

Species used in this study: Lotus (*Nelumbo nucifera* Gaertn.).

Chemical used in this study: ZeroTol™ (Hydrogen Peroxide), Terra-Sorb® (Hydrogel), Potassium Polyacrylamide Acrylate Copolymer; Gum acacia (a complex mixture of saccharides and glycoproteins).

Significance to the Nursery Industry

Ornamental lotus, *Nelumbo* nucifera 'Embolene', was used to evaluate the effects of gum acacia, sphagnum peat moss and hydrogel on the longevity and quality of cooler-stored lotus propagules. Nurseries often deal daily with potentially hundreds of plants with varying cultural requirements. Such diverse crops need intensive scheduling to meet specific production requirements. If some plants can offer more flexibility in their scheduling through the implementation of simple effective postharvest storage, shipping and handling techniques, it is a great help to the growers. Lotus propagules in this study retained strong viability after 45 days of storage in the cooler at 5C (42F) and 95% RH. Surface sterilization by soaking propagules in 1% ZeroTol™, or another surface sterilant, followed by low temperature storage is required to maintain the viability of lotus rhizomes during storage.

Introduction

Lotus (*Nelumbo*, Nelumbonaceae) is a well-known perennial aquatic plant with edible, ornamental, medicinal and ecological uses. Only two species exist within genus *Nelumbo*, *N. lutea* Willd. native to North America and *N. nucifera* Gaertn. native to China. However, more than 1,000 cultivars have been introduced from these two species. Sacred lotus (*N. nucifera*) has been a popular crop in China for more than 6,000 years (19). Lotus is grown and consumed throughout Asia, with all parts of the plant (including seed, rhizome, leaf, stalk, petal, anther, pericarp, and fruit receptacle) used as food or for medicinal purposes (9, 10). Besides its typical ornamental uses, lotus flowers are also used in religious ceremonies (9). Lotus seeds are among the oldest in the world and have been noted to maintain their viability for more than a thousand years (13, 15). In contrast, rhizomes survived poorly in long-term storage because of continued water loss, shrinkage, browning and decay (21, 22). Maintenance of freshness of edible lotus rhizomes has been well studied in the food storage industry (8, 18, 19, 20, 21). Enlarged rhizomes used as vegetables can be stored up to 150 days at 6–8C (43–46F) with 95–100% RH (2), whereas, rhizomes usually have a shelf life of only 2 weeks at room temperatures (11) and can be stored in soil for only 10–30 days (5). Lotus rhizomes for propagation remain viable through winter in containers and ponds without additional protection in Southern China (17). However, post-harvested propagules are quite difficult to store for extended durations and little research is available on longevity of liner propagules for shipping and production purposes.

Lotus propagules (usually rhizomes with shoots) are mainly used for propagation to maintain the homogenous genotypes. Shelf-life extension of propagules during shipping and planting season will benefit both lotus producers and consumers. Although low temperature storage is the most practical method of prolonging the shelf-life of lotus rhizomes (19, 20), application of anti-desiccant materials may benefit long-term storage. Gum acacia (gum arabic, from *Acacia* species), an edible coating, has been used to limit water loss (14) and to form a protective film in encapsulation (3). Gum arabic has a significant effect on protection of probiotic cultures during drying, storage and gastric transit (4). This biopolymer may also improve water retention when applied to the surface of plant tissue during storage. Hydrogel, a hydrophilic polymer has also been reported to increase water retention when applied to soil or planting media (1).

In this study, Terra-Sorb® fine Hydrogel™, peat moss and gum acacia were investigated to determine the effect on the longevity of lotus propagules under low temperature stor-

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age [5C (42F), 95% RH]. Changes in moisture retention and sugar and starch content of lotus propagules in cold storage were also examined.

Materials and Methods

Containerized rhizomes of lotus 'Embolene' were harvested at Paterson Greenhouse at Auburn University on February 13, 2005. Healthy propagules (rhizomes with shoots) were cut with two or three internodes remaining and soaked in 1% ZeroTol™ (CropKing, Inc., Serville, OH) for three seconds for surface sterilization and placed into two-gallon (7.6 liter) zip-lock bags (1 treatment/bag) after the following treatments were applied: (a) control — stored as is; (b) wrapped with moist sphagnum moss; (c) dipped in tap water saturated with Terra-Sorb® fine Hydrogel™ (Plant Health Care, Inc., Pittsburgh, PA) for three seconds; and (d, e, f) dipped in 10, 20, and 30% (w/v) gum acacia (Colloides Natural International, Rouen Cedex, France) for three seconds, respectively. Treated materials were placed in a walk-in cooler at 5C (42F) and 95% RH. Ten samples were collected initially, five of which were weighed and dried in a 70C (158F) oven for 72 hr and then weighed for dry mass. After weighing, dried samples were ground in a Thomas® Wiley® mini-mill and stored dry at room temperature in sealed containers for analysis. Rhizome moisture content (%) was determined by 100 × (mass of fresh rhizomes – mass of dry rhizomes)/mass of fresh rhizomes. The remaining five samples were individually planted in 29 liter (#7) plastic containers to evaluate the viability of propagules. Thereafter, ten samples of each treatment were randomly collected every 15 days during 45 days of storage, five for carbohydrate analysis and five for planting. Samples treated with hydrogel and gum acacia were gently surface-wiped with tissue before weighing. A total of 190 propagules were used in this experiment, 95 for planting and 95 for lab analysis.

Total soluble sugar and starch contents were determined by using the Anthrone Reagent and Nelson methods, as described by Li et al. (7) and Owens et al. (12). Sugars were extracted from 20 mg of ground rhizome tissue with 1 mL 80% ethanol in 2 mL micro-centrifuge tubes. Tubes were shaken occasionally for 10 min at 25C (77F) and centrifuged at 14,000 rpm for 5 min at 5C (42F). The supernatant was retained. Ethanol extraction was repeated twice and the combined supernatants diluted to a final volume of 5 mL with 80% ethanol. Sugar concentrations in the ethanol extracts were determined with anthrone (16) and glucose as a standard. Optical density of supernatants was read at 625 nm in a Bio-Tek SynergyHT with software KC4^{TB} V3.1 (Bio-Tek® Instruments, Inc., Winooski, VT). The ethanol extracted residue was placed in a 60C (140F) oven for 12 h to evaporate any residual ethanol before starch hydrolysis. Water (500 µL) was added to each tube, and the tubes were heated in a boiling water bath for 10 min to gelatinize the starch. The pH of the solution was adjusted to 5.1 by adding 400 µL 0.2 M sodium acetate buffer. Starch was digested by adding 0.2 U of amyloglucosidase (Sigma-Aldrich, Inc., St. Louis, MO; product A1602-25MG from *Aspergillus niger)* and 40 U of α-amylase (Sigma-Aldrich, Inc., St. Louis, MO; product A2643*)* in 100 µL of 0.2 M Na acetate buffer (pH 5.1). Tubes were incubated at 55C (133F) for 24 hr with occasional shaking. Tubes were centrifuged at 14,000 rpm for 5 min and the supernatant diluted to 1:100 of dilution in 15 mL glass tubes. Tubes were then incubated at 37C (99F) for 30 min in a water bath, afterwards 500 µL of copper reagent (reagent D) was added. The solution was heated for 15 min in an 80C (176F) water bath and then cooled down to room temperature for 15 min. 500 µL of arsonomolybate reagent (reagent C) was added and vortexed until all foaming ceased. Finally, 3.5 mL of water was added and vortexed. The samples along with standards containing 0, 20, 40, 60, 80, and 100 µg/mL of glucose solution were read at 540 nm in a Bio-Tek SynergyHT. Starch concentration was calculated as 0.9 × glucose concentration. For each treatment, four samples and four replicates were analyzed. For each sample, 20 mg of ground dried lotus rhizome was used for analysis.

Survival rates of propagules in containers were recorded on April 1, 2005, one month after the last sampling and planting day.

Means of moisture and carbohydrates were examined by Tukey's Multiple Comparisons Test (HSD, $\alpha = 0.05$). A two-way ANOVA test was used for determination of interactions. Models of the relationship between moisture or carbohydrates and storage time were analyzed by regression analysis using SAS 9.1 (SAS Institute, Cary, NC).

Results and Discussion

Following 45 days of storage, no propagules exhibited decay or any visible diseases regardless of the treatment used. No effect of treatment or time on viability of lotus propagules was detected during storage. Between 80 to 100% of the planted propagules survived in all treatments. Total survival rate was 94%. Therefore, lotus propagules could remain highly viable for at least 45 days when stored in a 5C (42F), 95% RH cooler. Death of a few propagules might be attributed to damage of primary leaf shoots prior to planting or stress from environmental factors (low temperature and possible disease) after planting.

Moisture in rhizome samples ranged between 73.5 and 77.4%, and the difference was not significant among treatments during storage based on Tukey Multiple Comparison procedure (HSD, $\alpha = 0.05$, Table 1). An average water content change of –0.2 to 4.8% was observed in this study, which was similar to water change of –1.7 to 3.65% in edible lotus rhizomes recorded by Zhan and He (22) during 9 days of storage in plastic bags at 5C (42F). Wang and Li (18) reported only 0.59 to 0.98% of water loss in edible lotus after 30 days of storage in vacuum polythene bags at 4 to 10C (39 to 50F), and Wang and Zhang (19) recorded 0.71 to 0.74% of water loss in cooler at 3 to 5C (37 to 45F) (80 to 85% RH) and 2.5 to 4.9% of water loss at room temperature (8 to 20C (46 to 68F), 70 to 80% RH) after 30 days of storage. Xu et al. (20) observed water loss between 5.27 and 10.23% for four cultivars of edible lotus stored for 15 days at 15C (59F). These studies indicated low temperature played a critical role on water maintenance and decay resistance of stored lotus rhizomes (9).

In our study, except for treatments of 30% gum acacia and sphagnum moss, water content of lotus rhizomes generally decreased initially and then increased slightly, but not statistical significantly (Table 1). Increased moisture content in the latter storage period was unclear but possibly explained by: (a) fast degradation of carbohydrates or increased respiration rate but less water expenditure; or (b) less water loss from tissue as moisture presence was adequate in the sealed bag. A similar reversal in water content in lotus rhizomes was reported during 9 days of short-term storage in polythene

Table 1. Moisture content percentage in propagules of lotus 'Embolene' following storage at 15-day intervals.

	Moisture content (%)			
	Sampling time			
Treatment	2/14	3/1	3/16	3/31
C	77.2[z]	76.2	75.9	77.4
M	77.2	75.2	75.7	75.2
H	77.2	76.6	75.5	77.3
G1	77.2	73.9	75.6	76.0
G2	77.2	75.2	74.6	75.5
G3	77.2	74.6	73.6	73.5

[z]Means difference was determined by Tukey's Multiple Comparisons (HSD, $\alpha = 0.05$), No significant difference was found between means of moisture in lotus rhizomes for treatments (C = control, M = sphagnum moss, H = hydrogel, G1 = 10%, G2 = 20%, and G3 = 30% gum acacia).

bags at 5C (42F) (22). In our study, the highest water content and a similar change trend were found in rhizomes that were in either the control or Hydrogel™ treatment groups. A continued decreasing trend was observed in the 30% gum acacia (G3) treatment with a linear relationship observed between moisture (y) in tissue and storage time (x): $y = 76.879 - 1.709 x$ ($P < 0.0001$, $R^2 = 0.6796$). However, no strong linear or quadratic relationships were found between moisture level and time for the other treatments. Gum acacia had no effect on water retention, and, in fact, higher concentrations of gum acacia had a negative effect on moisture level of lotus rhizomes when compared to the control (Table 1).

For carbohydrate analysis, total sugar among the individual samples of lotus propagules differed widely (2.5 to 36.5 mg/g on a dry mass basis, detailed data not shown). Following 45 days of storage, the total soluble sugar decreased drastically from the first to the 30th day (50 to 83% decreased within the first 15 days) then increased from the 30th to the 45th day (Fig. 1). A significant decrease (128.13 to 7.6 mg/g) of total sugar was also recorded by Liu et al. (8) after 11 days of storage for edible lotus at 30.6C (87F). In our study, based on Two-Way ANOVA procedure, the interaction ($P = 0.057$) between treatments ($P = 0.263$) and time ($P < 0.0001$) was not significant on total sugar in lotus rhizomes. A strong quadratic relationship was observed between total sugar (y) and storage time (x) for all treatments through a regression analysis (Table 2). Although total sugar tended to decrease in the 10 to 30% gum acacia treatments within the last 15 days (March 16–31) of treatment, no significant differences were noted in the total sugar concentration of stored lotus rhizomes between the gum acacia treatments (Fig. 1).

There was no intersection ($P = 0.7104$) between time and treatment on starch content in lotus rhizomes. Both treatment ($P = 0.8399$) and time ($P = 0.7977$) were not significant. Starch in dry rhizomes was 27.5 to 38.9% (Fig. 2), which was lower than 62.3 to 68.7% in enlarged edible lotus rhizomes reported by Li et al. (6). Ornamental lotus usually has smaller swollen parts and low starch in rhizomes; therefore, it is not used for vegetable production. The starch content maintained a relatively stable level of between 275 mg/g and 389 mg/g on a dry-weight basis throughout storage, without significant differences (HSD, $\alpha = 0.05$) observed for the same treatment over time as well as for all treatments compared to the control (Fig. 2). Therefore, there was no significant treatment effect on starch concentration in lotus rhizomes in this study. A similar result was reported by Zhan and He (22) following 9 days of storage of edible lotus rhizomes at 5C (42F). The increase of starch during storage compared to the initial day for treatments of sphagnum moss, 10 and 20% gum acacia might be attributed to sampling variation because only four samples per treatment were used for carbohydrate analysis.

This study suggested no additional treatment is needed for a short-term cooler storage of lotus propagules other than good surface sterilization. The 45 days of storage longevity shown in this paper is sufficient to allow production flexibility and the requirements of either native or international shipping. In a later study, surface sterilized propagules of lotus 'Embolene' retained 100% viability (data not shown)

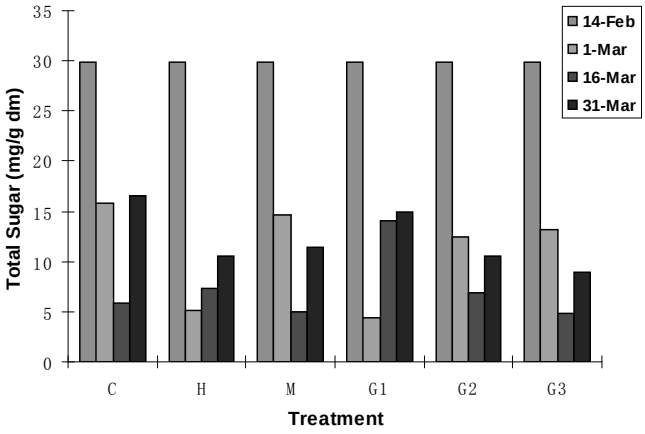

Fig. 1. Changes of total sugar content (on a dry mass basis) in rhizomes of lotus 'Embolene' for six treatments during 45 days of storage. Interaction (P = 0.057) of time and treatment was not significant after analysis of Two-Way ANOVA. Time (P < 0.0001) but treatment (P = 0.263) was the main factor to contribute to change of total sugar in lotus rhizomes during storage (C = control, M = sphagnum moss, H = hydrogel, G1 = 10%, G2 = 20%, G3 = 30% of gum acacia).

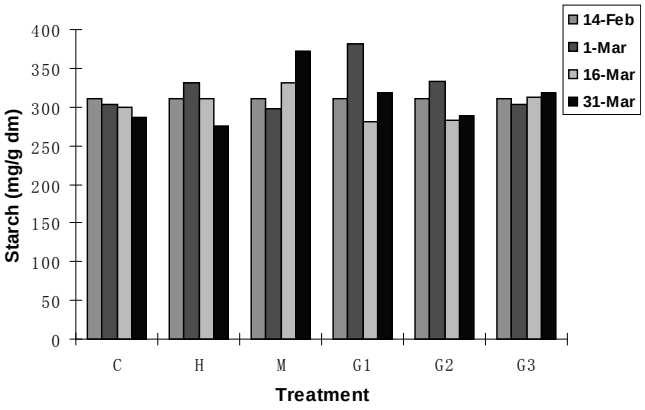

Fig. 2. Changes of starch content (on a dry mass basis) in rhizomes of lotus 'Embolene' for six treatments during 45 days of cooler storage. Interaction (P = 0.7104) of time and treatment was not significant after analysis of Two-Way ANOVA. Both time (P < 0.7977) and treatment (P = 0.8399) were not the main factors to contribute to change of total sugar in lotus rhizomes (C = control, M = sphagnum moss, H = hydrogel, G1 = 10%, G2 = 20%, G3 = 30% of gum acacia).

Table 2. Relationships between total sugar (y, mg/g, dry weight) in rhizomes of lotus 'Embolene' and storage time (x, 1 unit/15 days) during 45 days of cooler storage.

Treatment	Model	
Control	$y = 29.495 – 29.136x + 8.331x^2$	$P < 0.0001$, $R^2 = 0.845$
Hydrogel™	$y = 28.793 – 25.982x + 6.800x^2$	$P < 0.0001$, $R^2 = 0.837$
Sphagnum moss	$y = 30.438 – 22.873x + 5.451x^2$	$P < 0.0001$, $R^2 = 0.850$
10% Gum acacia	$y = 27.733 – 23.652x + 6.777x^2$	$P = 0.0092$, $R^2 = 0.542$
20% Gum acacia	$y = 29.053 – 25.390x + 6.501x^2$	$P < 0.0001$, $R^2 = 0.875$
30% Gum acacia	$y = 30.108 – 22.658x + 5.166x^2$	$P < 0.0001$, $R^2 = 0.891$

in the cooler [5C (42F), 95% RH] for more than six months without additional treatments. However, control propagules without surface sterilization were easily attacked by pathogens and lost viability within 1 to 2 months, indicating low temperature and good sanitation are critical to maintaining the viability of lotus propagules during storage.

Propagules of lotus 'Embolene' retained a high viability after 45 days of cooler storage at 5C (42F) and 95% RH, indicating that lotus propagules can be stored for more than 45 days following harvest. Hydrogel™, sphagnum moss, and three concentrations of gum acacia had no statistically significant effect on storage of lotus propagules in this study. However, this conclusion may be challenged by longer-term storage. Further study is necessary to evaluate effect of biopoloymers on extended storage and viability of lotus rhizomes or propagules and their maximum longevity. An additional treatment using biopolyomers is not necessary for practical shipping and production practices. Since lotus rhizomes are easily perishable material, good sanitation and low temperature are critical for both storage and shipping.

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