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Improved keeping quality of *Dendrobium* "Bom" orchids using nutrients entrapped in a biodegradable hydrogel

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ABSTRACT

Poly-γ-glutamic acid (γ-PGA) produced by *Bacillus licheniformis* TISTR 1010 was blended with polyvinyl alcohol (PVA) and irradiated with cobalt-60 γ-rays to produce hydrogels. The most absorbent hydrogels were formed using a γ-PGA to PVA mass ratio of 90:10 and an irradiation dose of 40 kGy. The optimally made hydrogel had a gelation of 60.3 ± 2.4% and a deionized water absorption of 13.6 ± 0.4 g g^{-1}. These hydrogels filled to capacity with a liquid fertilizer solution were used to make a 'solid' fertilizer for potting the *Dendrobium* "Bom" cut orchids for improving the shelf-life for export. The fertilizer absorption by the hydrogel was 2.0 ± 0.8 g g^{-1}. In deionized water, the prepared solid fertilizer released the nutrient sucrose with average release rates of 0.92 ± 0.01 g L^{-1} h^{-1} (measured over a 0–10 h interval) and 0.08 ± 0.00 g L^{-1} h^{-1} (measured over a 10–168 h interval). The hydrogel solid fertilizer kept the orchids fresh for ~8 days at 4 °C and ~7 days at 25 °C. Relative to liquid fertilizer, the solid fertilizer delayed flower opening by 4 days at 25 °C. Use of a γ-PGA (0.5 g L^{-1}) solution alone also delayed flower senescence and opening. Simple empirical models were shown to satisfactorily predict opening and senescence of flowers with cut stems potted in the solid fertilizer and other media.

1. Introduction

Cut-flowers are an important global industry. Often the flowers are produced in tropical regions and exported as cut bouquets to markets far afield. Keeping quality of the flower has a major impact on this industry. *Dendrobium* orchids are a popular source of cut-flowers because of the many colors, sizes and shapes of their flowers. Thailand is the world's largest exporter of tropical cut orchids, mainly *Dendrobium* orchids (Supnithi et al., 2011). China, Japan and the United States are key importers of Thai cut orchids, but the market is global. Around 1600 species of *Dendrobium* exist, but commercial flowers are generally hybrids.

The longevity of cut-orchid depends on cultivar and postharvest handling (De et al., 2014). Transport and vase life of cut orchids is reduced by endogenously produced plant hormone ethylene, resulting in wilting or abscission of the petals (Woltering and Van Doorn, 1988). Providing the cut-flower with nutrients (fertilizers) and ethylene-suppressor chemicals can enhance the shelf-life and other attributes of the flowers.

Hydrogels are cross-linked polymers that are generally soft, elastic and have a good capacity to absorb fluids (Ajji et al., 2005). Hydrogels containing absorbed fertilizers and other preservatives can be used to improve the keeping quality of cut-flowers by supplying them with nutrients during shipment and in the vase. Hydrogel 'solid fertilizers' overcome problems such as spillage and rapid drying associated with the use of liquid fertilizers. Toxicologically benign biodegradable hydrogels are of particular interest as they do not leave behind any harmful residue once degraded.

Hydrogels can be readily produced by γ-irradiation (Choi and Kunioka, 1995) of biopolymers such as poly-γ-glutamic acid (γ-PGA)

Abbreviations: 1-MCP, 1-methylcyclopropene; C_S, sucrose release from hydrogel absorbent in deionized water (g L^{-1}); K_{TB}, flower opening rate constant (day^{-1}); K_{TS}, flower senescence rate constant (day^{-1}); N_{BO}, number of flowers opened; N_{FS}, number of flowers senesced; P_B, fraction of flowers opened (%); P_{Bmax}, maximum fraction of flowers opened (%); P_S, fraction of flowers senesced (%); P_{Smax}, maximum fraction of flowers senesced (%); γ-PGA, poly-γ-glutamic acid; PVA, polyvinyl alcohol; Q_R, sucrose release rate from hydrogel absorbent to deionized water (g L^{-1} h^{-1}); R^2, regression coefficient; RH, relative humidity (%); RMS, root mean square; S_{BO}, flower opening score; S_{FS}, flower senescence score; T_{BO}, total number of flowers opened; T_{FS}, total number of fresh flowers; t, incubation time (h); t_{0B}, time for initial flower opening (day); t_{0S}, time for initial flower senescence (day); W_c, weight of the hydrogel before immersion; W_d, dry weight of the hydrogel after extraction; W_o, dry weight of the hydrogel before extraction; W_s, weight of the swollen hydrogel

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(Kunioka, 2004; Choi et al., 2005) as well as synthetic polymers such as polyvinyl alcohol (PVA) (Ajji, 2005; Abd El-Mohdy and Ghanem, 2009). γ-PGA is a natural polymer of D-/L-glutamic acids (Sirisansaneeyakul et al., 2017). It is produced by bacteria such as *Bacillus subtilis* (Choi et al., 2005; Kongklom et al., 2017). This cationic polymer is biodegradable, water soluble, non-toxic and edible (Sirisansaneeyakul et al., 2017). Polyvinyl alcohol (PVA) is a hydrophilic, biodegradable, non-toxic and biocompatible synthetic polymer (Jiang et al., 2011). PVA has been used in pharmaceutical and biomedical applications for controlled release of drugs (Peppas et al., 2000). PVA is known to form hydrogels if its solution are irradiated with ionizing radiation (Ajji, 2005).

This work used irradiation of γ-PGA/PVA blends with cobalt-60 γ-rays to produce hydrogels and assessed their suitability for use in floriculture products. The miscibility of γ-PGA/PVA blends, gelation characteristics, and uptake of water and fertilizer solution were studied. Optimally produced hydrogel blends of γ-PGA/PVA were used in attempts to extend the shelf-life of cut flowers of the orchid *Dendrobium* "Bom". The impact of nutrient supply through hydrogels on flower opening and senescence was examined.

2. Materials and methods

2.1. Materials

Crude culture broth of *Bacillus licheniformis* TISTR 1010 was produced as previously reported (Kongklom et al., 2017). Poly-γ-glutamic acid (γ-PGA) was purified from the cell-free broth according to the method of Ashiuchi et al. (1999). This provided the γ-PGA powder for the preparation of polymer hydrogels. The molecular weight of the γ-PGA used was in the approximate range of 60 kDa–135 kDa (Kongklom et al., 2017). Commercial PVA (JF-17; purity = 94%, molecular weight ~80 kDa) was purchased from Japan Vam & Poval Co., Ltd., Osaka, Japan.

The liquid fertilizer comprised of the following (per L of water): sucrose 120 g, sodium benzoate 400 mg (as a preservative) and 0.65 g 1-methylcyclopropene (1-MCP; AnsiP®-G, Lytone Enterprise, Inc., New Taipei City, Taiwan; www.lytone.com).

The mature *Dendrobium* orchids (Sonia 'Earsakul'; Sonia is BOM (Bangkok Orchid Mericlone) grex epithet and Earsakul is the cultivar) with purple flowers were a gift of Bangkok Flowers Centre Co., Ltd., Thailand. Each orchid cutting (peduncle length of ~40 cm) had either 4–7 bud flowers or 5–7 bloomed flowers (Fig. 1), for different experiments. The peduncles were immersed in distilled water and stored at 4 ± 2 °C, 80–95% relative humidity (RH), for further experiments. The storage period did not exceed 8 h.

2.2. Preparation of γ-PGA/PVA hydrogels

The γ-PGA/PVA hydrogels were prepared by ^{60}Co γ-irradiation. Hydrogel formulations with the following γ-PGA:PVA mass ratios were tested: 100:0, 90:10, 80:20, 70:30, 60:40, 50:50 and 0:100. Effects of the various doses of γ-rays on the formation of polymer hydrogels were investigated. The following γ-ray doses were tested (kGy): 40, 60 and 80.

A typical preparation procedure was as follows: γ-PGA powder (0.5 g) was fully dissolved in deionized water (2.5 mL) by stirring for 60 min at room temperature. A PVA (0.5 g) solution was prepared separately by dissolving the polymer in deionized water (2.5 mL) at 90 ± 2 °C while stirring for 60 min. The PVA solution was cooled to ~70 ± 2 °C and held at this temperature. The solutions of γ-PGA and PVA were mixed to obtain the desired mass ratio. For example, 5 mL of each solution was mixed to obtain a solution with a 50:50 mass ratio of the two polymers. The warm mixed solution of the two polymers (5 mL) was poured into a 20-mL glass vial (diameter = 28 mm). The vials were cooled to room temperature and then irradiated with ^{60}Co γ-rays at the required dose. This resulted in the formation of hydrogels. The hydrogels were removed from the glass vials and cut into pieces (1 × 1 cm) and used to measure gelation and absorption of water and fertilizer solution (Abd El-Mohdy and Ghanem, 2009).

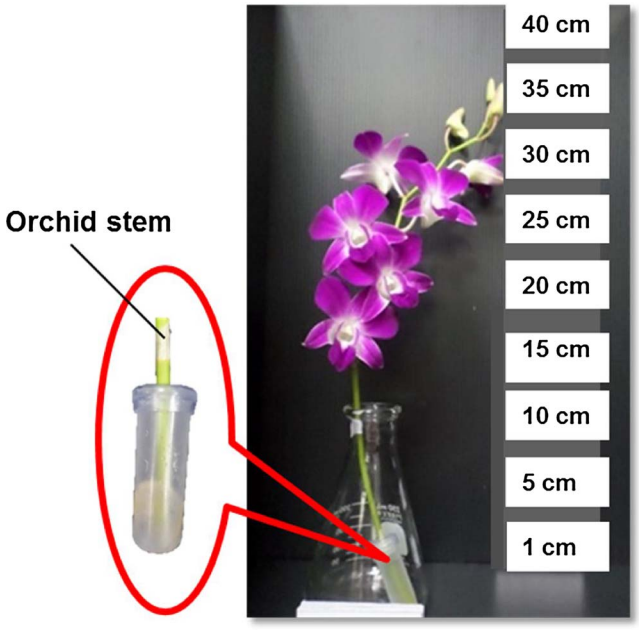

Fig. 1. An orchid potted in nutrient soaked hydrogel solid fertilizer.

The gelation of hydrogels was measured as described by Abd El-Mohdy and Ghanem (2009). Briefly, the hydrogel pieces were weighed, extracted in distilled water at 100 °C for 48 h and dried at 70 °C to constant weight. The gelation was calculated using the following equation:

$$\text{Gelation (\%)} = \frac{W_d}{W_o} \times 100 \quad (1)$$

where W_d was the dry weight of the hydrogel after extraction and W_o was the weight before extraction.

For measuring absorption of liquids, the hydrogel pieces produced after γ-irradiation were dried at 37 ± 2 °C for 1 week. Dry hydrogel pieces (10 pieces; each 1 × 1 cm, 1.0 ± 0.3 g) were immersed in 500 mL of deionized water and kept in a refrigerator (4 ± 2 °C, 60–95% RH) to measure their water absorption. A swollen hydrogel piece was removed from the water every 4–6 h, dabbed with absorbent tissue paper to remove surface moisture and weighed until an equilibrium constant weight was attained (around 3 weeks). This identified the immersion time for attaining absorption equilibrium. A total of ten hydrogel pieces produced under each of the specified conditions were used for measuring absorption. Absorption was calculated using the following equation:

$$\text{Absorption (g g}^{-1}) = \frac{W_s - W_c}{W_c} \quad (2)$$

where W_s was the weight of the swollen gel and W_c was weight of the gel before immersion.

One hydrogel formulation with a high equilibrium absorption of water was used for studies of fertilizer uptake and release. Absorption of fertilizer solution in the hydrogel was measured using exactly the same method as explained above, but with the deionized water replaced with 500 mL of liquid fertilizer. The one hydrogel formulation used in fertilizer absorption studies was also used for shelf-life extension studies with cut orchids.

For studies with cut orchids, one piece of dry hydrogel (1 × 1 cm, 1.0 ± 0.3 g) was soaked in liquid fertilizer (10 mL) for 7 days at 4 °C. This swollen hydrogel piece (3.0 ± 0.7 g) was then removed and placed at the bottom of a 6-mL plastic tube (2 × 5 cm) for potting the cut stem of an orchid for further studies.