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Research article

Effect of modified and controlled atmosphere storage on enzyme activity and senescence of *Dendrobium* orchids

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ABSTRACT

This research investigated the effect of different atmosphere storage conditions on 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase enzyme activity; and senescence of cut *Dendrobium* orchid flowers. The atmosphere storage conditions under study were normal atmosphere, modified atmosphere packaging, and controlled atmosphere. Under the normal atmosphere, carbon dioxide (CO_2) and oxygen (O_2) concentrations were 0.03 and 21 %, respectively. For the modified atmosphere packaging, cut orchid flowers were wrapped in polypropylene film prior to filling with 5 % CO_2 and 2 % O_2, while under the controlled atmosphere, CO_2 and O_2 concentrations were maintained at 5 and 2 %, respectively. The storage temperature and relative humidity were 13 °C and 95 %, respectively. The ACC synthase and ACC oxidase activity and ethylene-induced electrolyte leakage were determined and results compared. The controlled atmosphere substantially lowered ACC synthase and ACC oxidase activity and was effective in delaying senescence of cut orchid flowers, as indicated by the longest storage life of 28.33 days, followed by the modified atmosphere packaging (18.15 days) and normal atmosphere (11.67 days). The longer storage life enables suppliers of orchid flowers to efficiently manage the demand and supply and also provides exporters with new opportunities to expand into distant overseas markets. The novelty of this research lies in the use of different storage environments to investigate the senescence mechanisms at tissue level of *Dendrobium* orchid flowers in response to ACC synthase and ACC oxidase enzyme activity.

1. Introduction

The orchid family (*Orchidaceae*) is a diverse and wide spread family of flowering plants, with blooms that are often colorful and fragrant. Orchid flowers are sensitive to ethylene (Ketsa and Luangsuwalai, 1996; Ketsa and Rugkong, 1999, 2000a; 2000b). According to van Doorn (2001), endogenous ethylene induces petal wilting in orchid flowers.

In transportation of orchid inflorescences, atmosphere storage conditions and distance to the destination market play an important role in ethylene production. According to Uthaichay et al. (2007), normal atmosphere storage is conducive to 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase enzyme activity that promote ethylene synthesis and shorten the vase life. Improper handling of postharvest orchid inflorescences accelerates ethylene production and induces premature senescence of the cut flowers, resulting in shorter storage life.

Postharvest physiological changes during storage could be delayed by modifying the atmosphere inside the packaging. According to (Poonsri, 2017), elevated CO_2/lower O_2 atmosphere storage delayed undesirable postharvest changes of fresh flowers, such as wilting and senescence. The storage life of cut *Red Gala* rose flowers stored in the controlled atmosphere (5 % CO_2 and 4 % O_2) at 2 °C was 45 days, with a slightly longer vase life than the air-stored rose flowers (Poonsri, 2015).

Zeltzer et al. (2001) experimentally stored cut rose cultivars in modified atmosphere plastic containers (7.09 kPa CO_2 and 13.17 kPa O_2) at 2 °C for 10 days; and reported that the flowers could retain 3.5 times more fresh weight than those stored under normal atmosphere. In addition, modified atmosphere delays ethylene biosynthesis and the senescence of flowers and leaves. Under controlled atmosphere storage, changes in the metabolism patterns, respiratory enzymes, and membrane were substantially delayed, inhibiting the premature senescence of cut flowers (Defilippi et al., 2006).

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Figure 1. *Dendrobium* pink stripe orchid flower.

In Thailand, the storage condition currently used to prolong the shelf life of cut orchid flowers is normal atmosphere condition (21 % O_2 + 0.03 % CO_2; 95 % RH and 13 °C). The shelf life of cut orchid flowers stored under normal atmosphere condition is about 10–11 days. ACC synthase and ACC oxidase are the enzymes responsible for ethylene synthesis and action and senescence of cut orchid flowers. Thailand is a leading exporter of cut orchid flowers, especially *Dendrobium* pink stripe variety whose annual export value was in excess of USD 100 million (Department of Agriculture, 2018).

However, studies on the activity of both enzymes and their effects on the storage life of cut orchid flowers are very limited. As a result, this research investigates the effect of different storage environments on ACC synthase and ACC oxidase enzyme activity and senescence of cut *Dendrobium* pink stripe orchid flowers, as indicated by storage life.

Specifically, this research focuses on ACC synthase and ACC oxidase activity and ethylene-induced electrolyte leakage of cut *Dendrobium* pink stripe orchid flowers. The experimental storage conditions included normal atmosphere, modified atmosphere packaging, and controlled atmosphere environment. Under the normal atmosphere, CO_2 and O_2 concentrations were 0.03 % and 21 %. For modified atmosphere packaging, cut orchid flowers were wrapped in polypropylene (PP) film before filling with 5 % CO_2 and 2 % O_2. Under the controlled atmosphere, CO_2 and O_2 concentrations were maintained at 5 and 2 %, respectively.

The advantage of the proposed modified atmosphere packaging (PP-wrapped) and controlled atmosphere (5 % CO_2 + 2 % O_2) lies in the significantly extended shelf life of cut orchid flowers ($p < 0.05$). By comparison, the storage life of cut orchid flowers under normal atmosphere, modified atmosphere packaging, and controlled atmosphere were 11.67, 18.15, and 28.33 days, respectively.

2. Materials and methods

Mature cut *Dendrobium* pink stripe orchid flowers were from an orchid plantation in Thailand's *Pathumthani* province. A total of 900 orchid inflorescences (80 % flower blooming) were used in the experiments under three atmospheric conditions. The orchid flowers were harvested from one same orchard, and the harvesting started at 6 o'clock (in the morning) and lasted about half an hour.

The cut orchid flowers were cleaned with water and those with irregular size and injuries removed. The stem ends of orchid inflorescences were trimmed at 45-degree angle and individually inserted into a plastic tube filled with 30 ml distilled water. Figure 1 shows cut *Dendrobium* pink stripe orchid flowers.

The orchid flowers were carefully placed in cardboard boxes, with 10 orchid inflorescences per box. To control the microclimate, the inflorescences subjected to controlled atmosphere were transferred to acrylic containers (40 × 40 × 60 cm), where the microclimate (i.e., oxygen (O_2), carbon dioxide (CO_2), and nitrogen (N_2)) was regulated by a gas flow system. The atmospheric composition inside the acrylic containers was analyzed on a daily basis using a gas analyzer (PBI-Dansensor; Checkmate II, Denmark).

The experiments were carried out under three conditions: normal atmosphere, modified atmosphere packaging, and controlled atmosphere environment. Under the normal atmosphere (the control), were 0.03 % and 21 %, respectively with nitrogen (N_2) making up for the rest. For the modified atmosphere packaging, cut orchid flowers were wrapped in PP film (25 μm in thickness) before filling with 5 % CO_2 and 2 % O_2, with N_2 accounting for the rest. Under the controlled atmosphere, CO_2 and oxygen O_2 concentrations were maintained at 5 and 2 %, respectively (with N_2 accounting for the rest) until end of storage life. According to Poonsri (2015), 5 % CO_2 + 2 % O_2 was the optimal atmosphere for storage of cut *Dendrobium* red bomjo orchid flowers.

The storage temperature and relative humidity (RH) of all treatments were 13 °C and 95%, respectively. According to Poonsri (2017), the optimal storage temperature and RH of *Dendrobium* orchid flowers are 13 °C and 95%, respectively. All experiments were carried out in triplicate, and results were averaged. The enzyme activity and electrolyte leakage were determined every 3 days. The experimental period lasted 30 days, given the longest storage life of 28.33 days under 5 % CO_2 + 2 % O_2 atmosphere condition (Poonsri, 2015).

2.1. ACC synthase activity

Prior to the analysis, 1 g of column and ovary tissues of orchid flowers was homogenized in 6 mL HEPPS buffer, containing 100 mM N-(2-hydroxyethyl) piperazine-N′-(3-propanesulfonic acid), 4 mM dithiothreitol (DTT), and 0.5 μM pyridoxal phosphate. The buffer pH was adjusted to 8.5 with potassium hydroxide (KOH). The homogenates were centrifuged at 12,000 g for 20 min at 4 °C. The supernatants were resuspended for 24 h in dialysis buffer, containing 2 mM HEPPS buffer, 0.1 mM DTT, and 0.2 μM pyridoxal phosphate (1:10 v/v).

The ACC synthase activity was assayed by incubating 0.4 mL of extract in 6 mL vial, containing 50 μL of 0.5 mM S-adenosyl methionine and 90 μL distilled water, at 30 °C for 3 h (Hoffman and Yang, 1982). Afterward, 100 μL of 10 mM $HgCl_2$ and 600 μL distilled water were added into the vial containing 200 μL of extract. The vial was capped and placed in an ice tub for 1 h. In the analysis, 200 μL of extract was used to determine the ACC content (Lizada and Yang, 1979). A 100 μL mixture of 5.25 % NaOCl and saturated NaOH (2:1 v/v) was then introduced into the vial. The mixture was vortexed (Scientific Industries; Vortex-Genie 2, USA) for 15 min and incubated in ice for 3 min. Afterward, 1 mL of gas sample was drawn from the vial, and the ethylene concentration was determined using gas chromatography (Agilent Technologies; 6820, USA). The ethylene production was converted into nmol ACC/mg protein/h, and the protein content was determined using bovine serum albumin as standard (Bradford, 1976).

2.2. ACC oxidase activity

In the analysis, 1 g of column and ovary tissues of orchid flowers was homogenized in 6 mL vial filled with extraction buffer (pH 7.2),

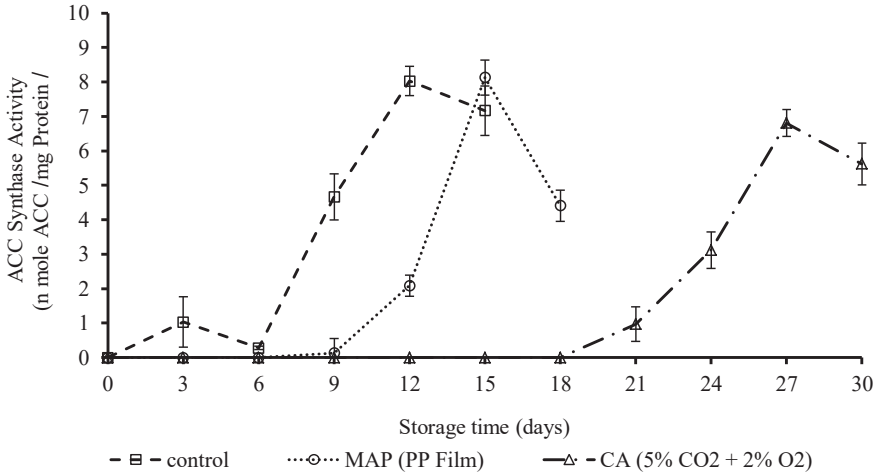

Figure 2. Effect of controlled atmosphere (5 % CO_2 + 2 % O_2) and modified atmosphere packaging (PP film) on ACC synthase activity of cut *Dendrobium* orchids, given the respective storage temperature and RH of 13 °C and 95 %. The error bars represent standard deviations.

containing 0.1 M Tris-HCl, 30 mM Na-Ascorbate, 5 mM DTT, and 30 % glycerol (w/v). The homogenates were centrifuged at 12,000 g for 20 min at 4 °C, and the supernatants were collected for ACC oxidase assay. The ACC oxidase activity was assayed by incubating the extract in 400 μL buffer (pH 7.2), containing 0.1 M Tris-HCl, 30 mM Na-Ascorbate, 100 μL FeSO4, and 30 % glycerol; and 100 μL of 10 mM ACC was then added into the vial. The mixture was incubated at 30 °C for 1 h prior to placing in an ice tub for 3 min.

Afterward, 1 mL of gas sample was drawn from the vial, and the ethylene concentration was determined using gas chromatography (Agilent Technologies; 6820, USA). The ethylene production was converted into nL C_2H_4/mg protein/h, and the protein content was determined using bovine serum albumin as standard (Bradford, 1976).

2.3. Electrolyte leakage

Electrolyte leakage was determined according to McCollum and McDonald (1991), with minor modifications. Prior to analysis, orchid petals were cut into discs of 1 cm in diameter (weighing approximately 1 g each) using stainless steel cork borer and rinsed three times with deionized distilled water and dried with tissue paper. In the analysis, 10 orchid petal discs were placed in 30 mL 0.4 M mannitol solution and shaken at 100 cycles per min (Hettich Universal; 320R, Germany).

The mixture was incubated for 3 h at room temperature before determining the initial electrolyte leakage (initial reading). The electrical conductivity measurement (i.e., electrolyte leakage) was carried out using conductivity meter (HANNA instruments; EC 214, Portugal). The total electrical conductivity (final reading) was measured after complete destruction of the petal tissues in autoclave (Gemmy Industrial; Speedy Autoclave Vertical Type HL341, Taiwan) at 121 °C and 15 psi for 30 min and cooled down to room temperature. The percentage of electrolyte leakage is calculated as the ratio of initial reading to final reading (Eq. (1)).

$$\text{Electrolyte leakage } (\%) = \frac{\text{Initial conductivity reading}}{\text{Total conductivity reading}} \times 100 \quad (1)$$

One-way analysis of variance (ANOVA) was used to determine the statistical differences between treatments, given the 5% significance level ($p < 0.05$).

3. Results and discussion

3.1. ACC synthase and ACC oxidase activity

In Figures 2 and 3, the ACC synthase and ACC oxidase activity of orchid flowers in modified atmosphere packaging and controlled atmosphere were significantly lower than under normal atmosphere ($p <$

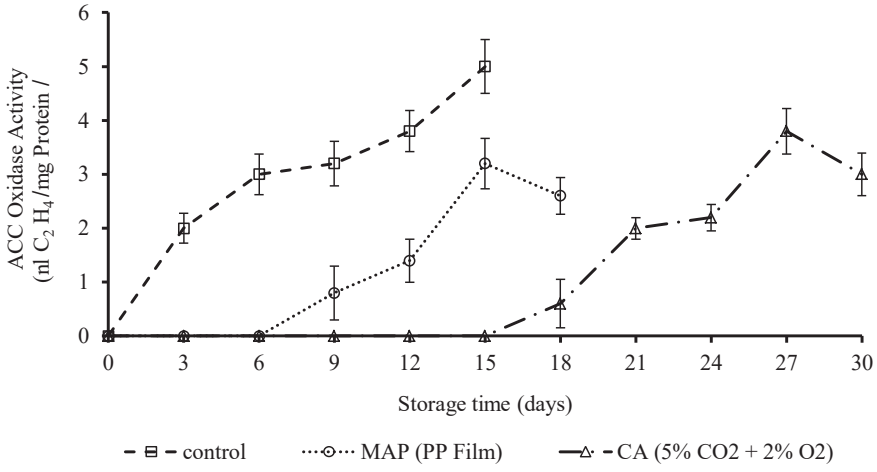

Figure 3. Effect of controlled atmosphere (5 % CO_2 + 2 % O_2) and modified atmosphere packaging (PP film) on ACC oxidase activity of cut *Dendrobium* orchids, given the respective storage temperature and RH of 13 °C. and 95 %. The error bars represent standard deviations.

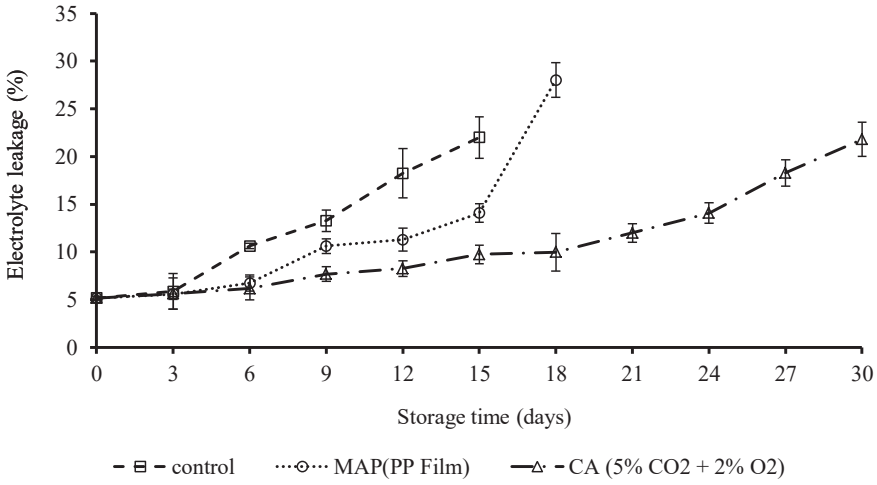

Figure 4. Effect of controlled atmosphere (5 % CO_2 + 2 % O_2) and modified atmosphere packaging (PP film) on electrolyte leakage (membrane permeability) of cut *Dendrobium* orchids, given the respective storage temperature and RH of 13 °C and 95 %. The error bars represent standard deviations.

0.05). The ACC synthase activity under normal atmosphere increased rapidly after day 6, while that of modified atmosphere started to rise after day 9. The ACC synthase activity under controlled atmosphere steadily increased after day 21.

The ACC oxidase activity under normal atmosphere rose steadily since the beginning. On the other hand, the ACC oxidase activity under modified atmosphere packaging and controlled atmosphere were not observable until after days 6 and 15, respectively. Higher levels of ACC synthase and ACC oxidase activity under normal atmosphere in early stages resulted in premature petal senescence and shorter storage life due to accelerated ethylene production. The visible symptoms of senescence and ethylene sensitivity during storage of orchid flowers were closely correlated to increased ethylene production. The lower ACC synthase and ACC oxidase activity under the PP-wrapped and controlled atmosphere conditions result in lower ethylene production, thus delaying flower senescence and prolonging the storage life.

Ethylene is a natural product of plant metabolism where amino acid methionine is converted into S-adenosyl methionine (SAM). SAM is a precursor for ACC, which is an immediate precursor for ethylene. ACC synthase is an enzyme that converts SAM to ACC and plays a significant role in ethylene synthesis. Meanwhile, ACC oxidase is the enzyme that regulates the conversion of ACC into ethylene. Ethylene is an important hormone for ripening and germination. According to Kader (2002), ACC synthase and ACC oxidase activity and ethylene synthesis were influenced by plant genetic factors and the environment, e.g., temperature, O_2 and, CO_2 concentrations.

The experimental results also demonstrated that modified atmosphere packaging and controlled atmosphere inhibited ethylene action and delayed senescence of cut *Dendrobium* orchid flowers. The lower O_2 and higher CO_2 condition in the modified atmosphere packaging and controlled atmosphere bound with ethylene receptors and inhibited ethylene action, delaying the premature senescence of flowers. The finding is consistent with (Serek et al., 1994; Kader, 1989). The ethylene production was reduced as low O_2 inhibited the conversion of ACC into ethylene (Yang and Hoffman, 1984; Wang, 1990). In addition, CO_2 treatment inhibited the ACC synthase and ACC oxidase activity in tomatoes (Rothan et al., 1997; de Wild et al., 2005) and in peaches (Mathooko et al., 2001). Essentially, elevated CO_2 inhibited ACC synthase and ACC oxidase activity, resulting in lower ethylene production.

Lower O_2 and higher CO_2 also helped retain flavors and vitamins by reducing acidity loss, starch to sugar conversion, sugar interconversion, and aromatic volatile biosynthesis (Yahia and Singh, 2009). However, O_2 and CO_2 stress reduced cytoplasmic pH, adenosine triphosphate (ATP), and pyruvate dehydrogenase activity while inducing pyruvate decarboxylase, alcohol dehydrogenase, and lactate dehydrogenase, thus shortening the storage life of fresh produce (Ke et al., 1994, 1995). The effects of severe atmosphere varied, depending on cultivars, ripeness, temperature, exposure time, and ethylene concentration.

In essence, the storage life of cut orchid flowers under normal atmosphere (0.03 % CO_2 + 21 % O_2), modified atmosphere packaging (PP-wrapped), and controlled atmosphere (5 % CO_2 + 2 % O_2) were 11.67, 18.15, and 28.33 days.

3.2. Electrolyte leakage

The electrolyte leakage of cut *Dendrobium* orchid flowers in PP-wrapped modified atmosphere packaging and controlled atmosphere (2 % O_2 + 5 % CO_2) slowly increased, compared with under normal atmosphere (the control), as shown in Figure 4. The extent of senescence of cut orchid flowers corresponded to increase in electrolyte leakage. The gradual increase in electrolyte leakage under PP-wrapped packaging and controlled atmosphere conditions contributed to less senescence and longer storage life, compared with under the normal atmosphere condition. The electrolyte leakage can be calculated by Eq. (1).

Flower senescence could also be attributed to changes in membrane permeability. The permeability of a membrane is the rate of passive diffusion of water and solutes through the membrane. Increased membrane permeability induced interactions between enzymes and the substrate, resulting in senescence, wilting, discoloration, and browning (Faragher et al., 1987). Membrane permeability was determined by the rate of leakage of solutes, including ions from the tissues (Wang, 1990).

Excessive leakage of solutes from plant tissues as a result of increased membrane permeability caused flower petal senescence, loss of turgor, and visible wilting. Senescence of petals of *Tradescantia* was attributable to increased membrane permeability, and ethylene exposure accelerated the membrane permeability. Ethylene accelerated membrane permeability in roses and carnations (Thompson et al., 1982; Sylvestre and Paulin, 1987). Ethylene also accelerated ion and sucrose efflux, resulting in excessive membrane permeability in cells of morning glory flowers (Hanson and Kende, 1975). In addition, elevated CO_2 and lower O_2 atmosphere storage suppressed ethylene action in fresh produce (Woltering et al., 1995). CO_2 attached itself to plant receptors and inhibited ethylene action (Burg and Burg, 1966).

4. Conclusions

This research investigated the effect of storage environments on ACC synthase and ACC oxidase activity and senescence of cut *Dendrobium* pink

stripe orchid flowers. The experiments were carried out under normal atmosphere (0.03 % CO_2 and 21 % O_2), modified atmosphere packaging (PP-wrapped flowers), and controlled atmosphere environment (5 % CO_2 and 2 % O_2). The ACC synthase and ACC oxidase activity and ethylene-induced electrolyte leakage were determined and results compared. The experimental results showed that the controlled atmosphere was effective in delaying senescence of cut *Dendrobium* pink stripe orchid flowers, as evidenced by the longest storage life of 28.33 days, followed by the modified atmosphere packaging (18.15 days) and normal atmosphere (11.67 days). The delayed senescence was attributable to lower ACC synthase and ACC oxidase activity under the controlled atmosphere, thereby reducing electrolyte leakage and extending storage life of *Dendrobium* orchid flowers. The longer storage life enables suppliers of orchid flowers to efficiently manage the demand and supply and also provides exporters with new opportunities to expand into distant overseas markets.

Declarations

Author contribution statement

Warinthon Poonsri: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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