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RESEARCH ARTICLE

Effect of dry and wet storage at cool temperatures on the post-harvest performance of *Ranunculus asiaticus* L. flowers

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Abstract **A study was undertaken to assess the effect of different storage temperatures on senescence and postharvest performance of isolated flowers of *Ranunculus asiaticus* L.. The main aim of the work was to develop a cost-effective storage protocol to reduce the postharvest losses and to bring out the transportation of cut flowers of *R. asiaticus*. The flowers were subjected to two different storage treatments, dry storage and wet storage, and their postharvest performance was compared under laboratory conditions. For this purpose, the buds were harvested at 8:00 AM at loose bud stage (Stage II of flower development). The harvested buds were cut to a uniform size of 15 cm and processed for dry or wet storage. For dry storage, the buds were packed after wrapping them in moistened filter papers and kept at 5ºC and 10ºC. For wet storage, the buds were held in distilled water in separate glass beakers kept at 5ºC and 10ºC, respectively. A separate set of buds each for dry and wet storage was kept at room temperature (15±2)ºC. After 72 h storage, the buds were kept at room temperature in distilled water. The average life of an individual flower that opened fully was about 4–5 days. The buds kept under wet storage at 5ºC and 10ºC for 72 h maintained their premature status, while the buds held at room temperature for 72 h generally bloomed. All the buds stored dry maintained their premature status irrespective of storage temperature. Storage of buds for 72 h at 5ºC, followed by transferring to distilled water improved the longevity by about 5 to 6 days. Cold storage treatment before transferring to holding solution improved floral diameter, membrane integrity besides maintaining higher fresh and dry mass of flowers, sugar content, soluble proteins, and phenols. Our results suggested that wet and dry storage of premature buds of *R. asiaticus* for 72 h at 5ºC, followed by placing them in distilled water, improved the cut flower longevity and can be used as effective postharvest storage treatments for this beautiful cut flower.**

Keywords longevity, sugars, phenols, cold storage, membrane permeability

Introduction

Senescence though a terminal developmental stage can be accelerated by an array of both biotic and abiotic factors, such as light, temperature, nutrients, ethylene, pathogens, pollination, etc. (Taverner et al., 1999; van Doorn and Woltering, 2005; Wagstaff et al., 2005; Jones, 2008; Zhou et al., 2008). Temperature is considered to be an important abiotic factor that can influence the respiration rate, response to ethylene, moisture loss, and physical damage in various flowers (Cevallos and Reid, 2001; Leonard et al., 2001; Celikel and Reid, 2002; Gul et al., 2007). The optimal harvest maturity stage and postharvest temperature management are important for the maintenance of quality with respect to cut-flower marketing. The flowers harvested at an immature stage may not open fully, and the petals of flowers harvested at fully open stage are prone to damage and increased packaging costs (Cevallos and Reid, 2001; Leonard et al., 2001; Celikel and Reid, 2002; Gul et al., 2007). Cold storage allows regulation of market supply when there is surplus production, "holding over" to achieve higher prices or accumulation of sufficient stock for commercial consignments when supplies are limited and utilization of cost-effective refrigerated transport becomes necessary (Nowak and Rudnicki, 1990). *Ranunculus asiaticus* L. commonly known as 'butter cup' blooms from April to June in Kashmir. It possesses dark red terminal flowers, having a cluster of stamens with brownish

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anthers at the center surrounding the pistil. It is widely grown as a garden plant, cut flower, and flowering potted plants. The present study was undertaken to study the effect of storage temperature under dry or wet conditions on senescence and postharvest performance of *R. asiaticus* flowers in order to develop an efficient and cost-effective storage protocol for this beautiful flower.

Materials and methods

Plant material

Uniform floral buds of *Ranunculus asiaticus* L. growing in the University Botanic Garden were used for the present study. The buds were harvested at 8:00 AM at loose bud stage (Stage II of flower development). The harvested buds were immediately brought to the laboratory, cut to a uniform size of 15 cm, and processed for dry and wet storage. For dry storage, the buds were wrapped in moistened filter papers, packed in perforated polyethylene flower sleeves (25 cm long and 15 cm wide top), and kept in cooling incubators under dark conditions at 5ºC and 10ºC. For wet storage, the buds were held in 500-mL glass borosil beakers containing distilled water. The beakers were kept in cooling incubators in dark at 5ºC and 10ºC. A separate set of buds each for dry and wet storage was kept at room temperature (15±2)ºC under simulated conditions. After 72 h dry or wet storage, the buds were kept at room temperature after transferring them to 100-mL Erlenmeyer flasks containing 75 mL distilled water (DW). The samples were kept under cool white fluorescent light with a mix of diffused natural light (10 W/m^2) for 12 h a day and relative humidity (RH) of 60%±10%. The day of transfer of buds to DW was designated as day zero.

Assessment of vase life and solution uptake

The average vase life of the flowers was counted from the day of transfer of buds to holding solutions and assessed to be terminated when flowers lost their ornamental/display value (underwent color change, wilted, and lost turgidity). The volume of holding solution absorbed by the buds was calculated by measuring the volume of solution on a particular day and subtracting it from the initial quantity of the vase solution kept in the flasks, taking into account the volume of solutions evaporated by using blank flasks in triplicate (containing particular vase solutions without buds) alongside the flasks with buds.

Conductivity of leachates, floral diameter, and fresh and dry masses

Conductivity of leachates, floral diameter, and fresh and dry mass of the flowers were determined on day 6 after harvest (transfer of buds to the test solutions). Dry mass was determined by drying the material in an oven for 48 h at 70ºC. The changes in membrane permeability were estimated by measuring the conductivity of leachates (µS) of petal discs (5 mm in diameter) incubated in dark in 15 mL glass distilled water for 15 h at 20ºC.

Determination of tissue constituents

At each stage, 0.5 g chopped material of perianth tissue was fixed in triplicate in hot 80% ethanol. The material was macerated and centrifuged three times. The supernatants were pooled and used for the estimation of sugars and total phenols. Reducing sugars were estimated by the method of Nelson (1944) using glucose as the standard. Total soluble sugars were estimated after enzymatic conversion of nonreducing sugars into reducing sugars with invertase. Nonreducing sugars were calculated as the difference between total and reducing sugars. Total phenols were estimated by the method of Swain and Hillis (1959) using gallic acid as the standard. Proteins were extracted from 0.5 g petal tissue drawn separately from five different flowers. The tissue was homogenized into 5 mL of 5% sodium sulphite (w/v), adding 0.1 g of polyvinylpyrrolidone and then centrifuged. Proteins were precipitated from a suitable volume of cleared supernatant with equal volume of 20% trichloroacetic acid, centrifuged at 1000 × g for 15 min, and the pellet redissolved in 0.1 N NaOH. Proteins were estimated from a suitable aliquot by the method of Lowry et al. (1951) using Bovine Serum Albumin (BSA) as the standard.

Statistical analysis

Each treatment was represented by five replicates (flasks), and each flask contained two buds. Each value represents the mean of five replicates. The data has been analyzed statistically and LSD computed at $P_{0.05}$ using MINITAB (v 15. 1.2- EQUINOX_Softddl.net) software.

Results and discussion

The average life of an individual flower after it opened fully was about 4–5 days. Flower senescence was characterized by initial petal wilting (loss of turgor in the petals and color change from dark red to brick red), followed by abscission at the later stages upon slight teasing. The floral buds stored under dry conditions for 72 h maintained their premature status irrespective of storage temperature (Fig. 2). However, the buds stored under wet conditions for 72 h at 10ºC and room temperature (RT) bloomed during storage, as compared to corresponding buds stored at 5ºC (Fig. 3). The buds previously wet stored for 72 h at 10ºC and RT showed pedicel bending at day 2 after their transfer to DW (Fig. 5). The average vase life of buds dry stored for 72 h at 5ºC was about 6.8 days and that of buds wet stored under similar conditions

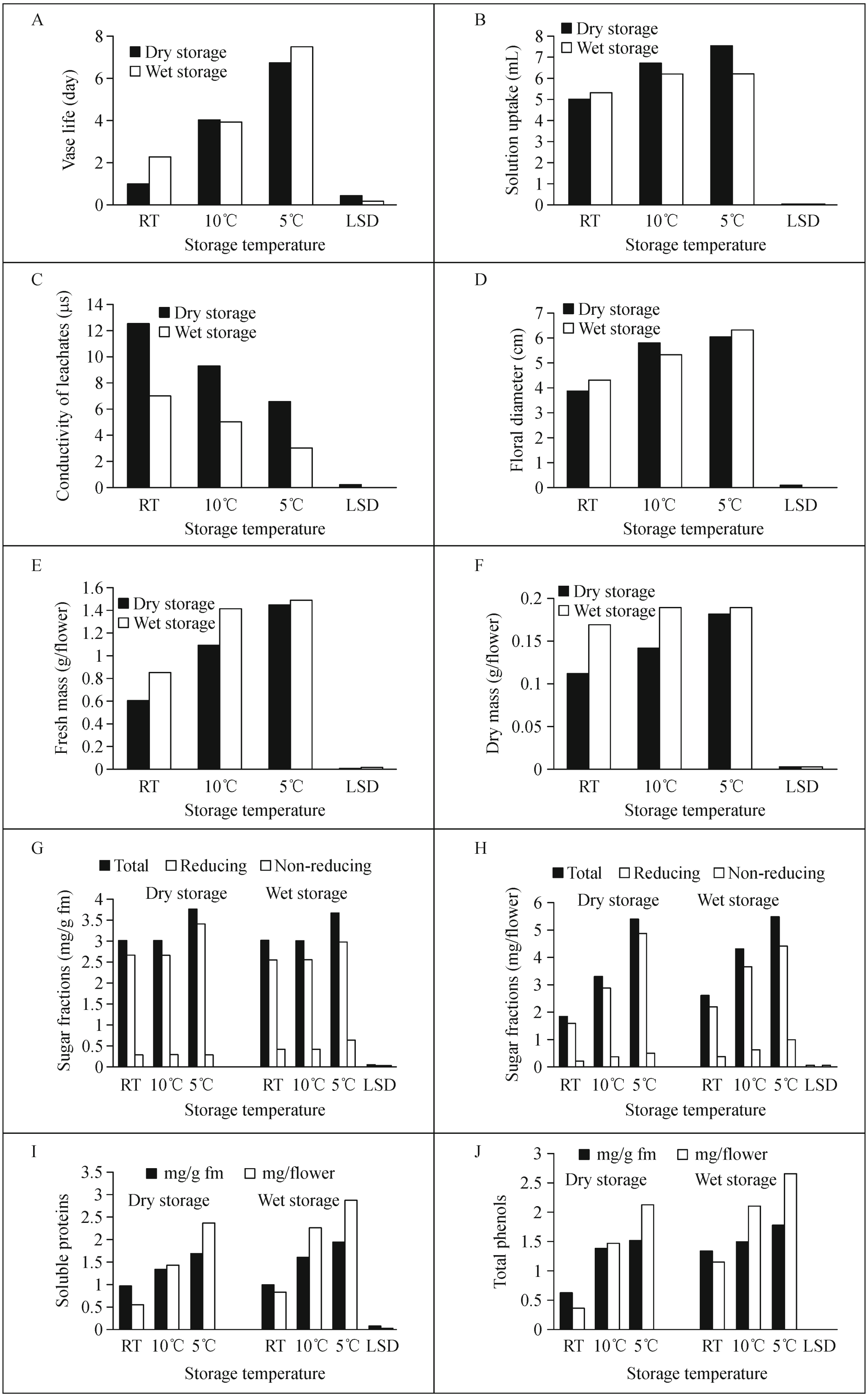

Figure 1 Effect of dry and wet storage for 72 h at different storage temperatures [Room temperature, RT ((15±2)ºC), 10ºC and 5ºC] on different traits of flowers. Figures 1A–J represent vase life, solution uptake, conductivity of leachates, floral diameter, fresh mass, dry mass, sugar fractions (1G and 1H), soluble proteins, and total phenols at day 6 after transferring to (DW) in isolated flowers of *Ranunculus asiaticus*, respectively. LSD is computed at $P_{0.05}$.

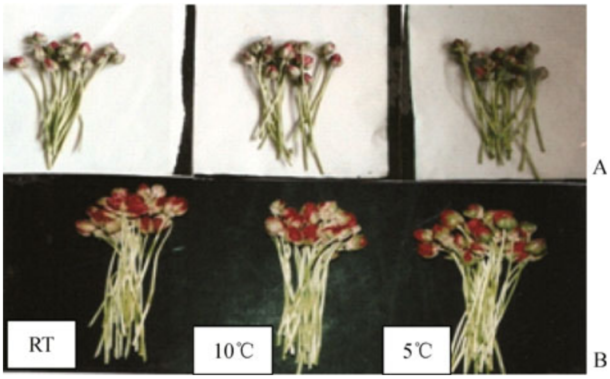

Figure 2 Flower buds of *Ranunculus asiaticus* before (A) and after (B) 72 h postharvest dry storage at different temperature regimes. From left to right, the arrangement buds kept at room temperature (RT, (15±2)ºC), 10ºC, and 5ºC is shown.

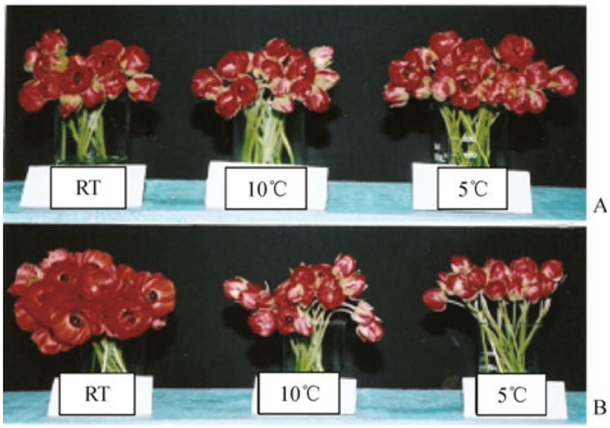

Figure 3 Flower buds of *Ranunculus asiaticus* held in glass beakers containing distilled water (DW). Figures 3A and 3B are carried out before and after 72 h postharvest on wet storage at different temperature regimes, respectively. From left to right, the arrangement of buds kept at room temperature (RT, (15±2)ºC), 10ºC, and 5ºC is shown.

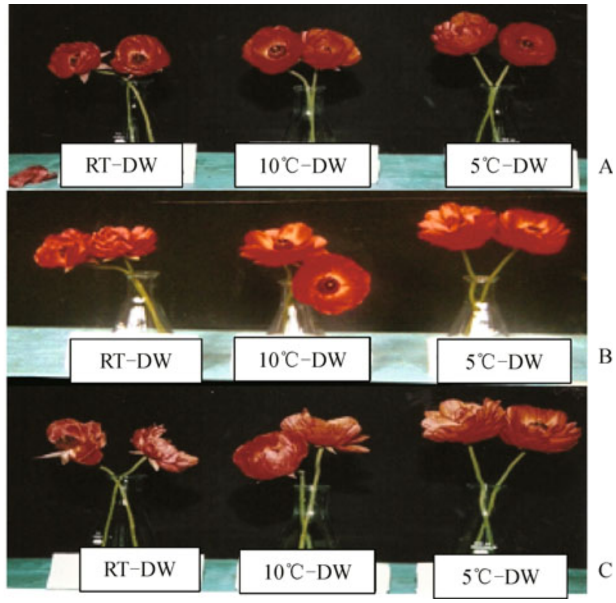

Figure 4 Flowers of *Ranunculus asiaticus* held in distilled water (DW) after 72 h dry storage at different days after transfer to DW. Figures 4A–C represent days 2, 4, and 6 after transfer (Fig. C). From left to right, the arrangement of the flasks containing buds previously held at different temperatures, namely, (RT, (15±2)ºC), 10ºC and 5ºC is shown.

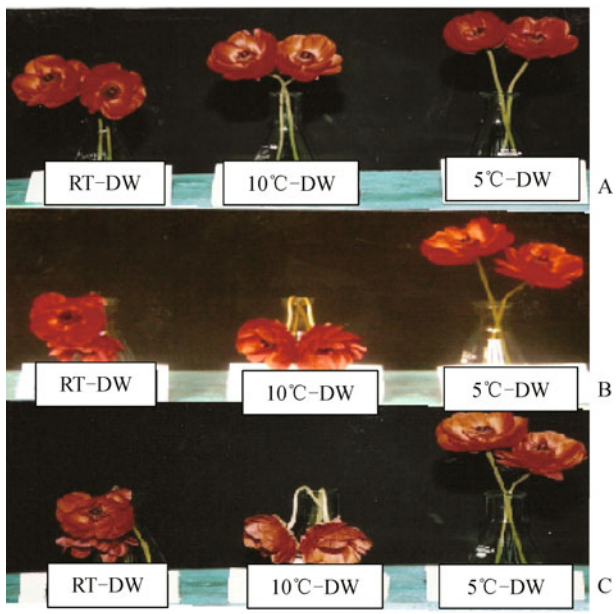

Figure 5 Flowers of *Ranunculus asiaticus* held in distilled water (DW) after 72 h wet storage at different days after transfer to DW. Figures 5A–C represent days 2, 4, and 6, respectively, after transfer. From left to right, the arrangement of the flasks containing buds previously held at different temperatures, namely, (RT, (15±2)°C), 10°C and 5°C.

was about 7.5 days (Figs. 4 and 5). The flowers dry stored for 72 h at 10 and 5ºC showed an increase in longevity by an increment of about 3–5 days; the buds previously wet stored in distilled water for 72 h at 10 and 5ºC showed improvement in longevity by an increment of about 2–5 days as compared to the corresponding buds stored at room temperature (Fig. 1A). In the present study, the deleterious effects of dry or wet storage at cool temperatures (including chilling injury, bending, bud abortion, petal curling, and color change) as reported earlier in plants, such as *Curcuma alismatifolia* and *Amaryllis belladonna*, were not observed (Bunya-atichart et al., 2004; Gul et al., 2007). The immature floral buds dry or wet stored at 5°C before transferring to DW, registered an increase in vase life as compared to corresponding buds stored at higher temperatures. Harvest at appropriate stage of flower development showed an influence on the appearance, longevity, and vase life of several cut flowers (Ahn and Park, 1996; Lee and Suh, 1996; van der Meulen-Muisers and van Oeveren, 1997; Redman et al., 2002; Gul et al., 2007; Shahri et al., 2009). Low temperature is the most important factor in the successful storage of cut flowers by reducing both metabolic processes and microbial growth rate, besides delaying the symptoms of senescence through regulation at biochemical level (van Doorn and de Witte, 1991; Page et al., 2001). The volume of holding solution absorbed was significantly higher in buds previously dry or wet stored at 5°C and 10°C before transferring to DW, as compared to corresponding buds stored at RT. However, solution uptake was comparatively higher in buds previously dry stored at 5°C and 10°C, as compared to corresponding buds stored under wet conditions (Fig. 1B). The increased water uptake in buds dry or wet stored at 5°C and 10°C was corroborated with the earlier findings on *Amaryllis belladonna* and *Nerine sarniensis* (Gul et al., 2007; Gul and Tahir, 2009). The comparative increased water uptake in buds stored under dry conditions could be probably due to the water stress during 72 h storage. Membrane permeability estimated as ion leakage of petal discs was found to be significantly lower in samples from buds previously dry or wet stored at 5°C before transferring to DW as compared to samples from corresponding buds stored at higher temperatures. The electrical conductivity of ion leachates was found to be comparatively lower in samples from buds previously stored under wet conditions (Fig. 1C). Low temperature was suggested to maintain membrane integrity by decreasing fluidity (Gul and Tahir, 2009). Dry or wet storage of buds at 5°C and 10°C resulted in significant improvement in floral diameter and fresh and dry masses as compared to corresponding buds stored at RT. However, it was comparatively higher in flowers from buds previously stored under wet conditions before transferring to DW (Figs. 1D, 1E, and 1F).

Storage of buds at low temperatures was found to improve diameter and fresh and dry mass of flowers. Low temperature has been reported to enhance the maximum fresh mass achieved in cut rose flowers: besides, a higher storage temperature has been shown to decrease the initial increment in fresh mass of *Grevellia* 'Sylvia' inflorescences (Ichimura et al., 1999; Joyce et al., 2000). Higher fresh mass of flowers from buds that under wet storage conditions is probably due to continuous water supply through xylem during postharvest storage. A higher content of reducing and total sugars was maintained in samples from buds previously dry or wet stored at 5°C as compared to corresponding buds stored at 10°C and RT. The sugar content was comparatively higher in samples from buds previously stored under dry conditions (Fig. 1G). However, when the data was expressed on mg per flower basis, a higher content of reducing and total sugars was maintained in samples from buds stored under wet conditions (Fig. 1H) The higher content of nonreducing sugars was maintained in samples from buds stored under wet conditions before transfer to DW (Figs. 1G and 1H). Storage of buds under dry or wet conditions at 5°C and 10°C resulted in the increase in the content of sugar fractions as compared to corresponding buds stored at RT. The concentration of various sugar fractions, such as glucose, fructose, and sucrose, has been shown to increase at low-temperature regimes in cut roses and *Nerine* plants (Ichimura et al., 1999). Gul and Tahir (2009) attributed the increase in enhancing the influx of water and osmolytes into cells. A significant increase in the content of soluble proteins and phenols was recorded in buds dry or wet stored at 5°C and 10°C. The increase was particularly marked in samples from flowers stored under wet conditions. It has been suggested that low temperatures maintain high protein content in tissues by inhibiting specific proteases responsible for protein degradation (Gul and Tahir, 2009). The differences were evident when the data was expressed on per flower basis (Figs. 1I and 1J). A higher content of phenols has been found to be associated with longer vase life in cut roses (Mwangi et al., 2003).

In conclusion, the present results suggested that the isolated flowers of *Ranunculus asiaticus* L. harvested at the right stage (loose bud stage) can provide a good model for market flexibility as an export cut flower crop. The flowers may be dry or wet stored at 5ºC for 72 h before transferring them into vase solutions without affecting their vase life, which can be used as an effective postharvest storage treatment for this cut flower. Employing the technique, the flowers of *Ranunculus asiaticus* can be transported for a long distance within 72 h after harvest by simply maintaining the temperature as low as 5ºC, which can promote the cut flower trade of this floricultural plant.

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