Introduction

Roses are among the most important ornamental plants used as potted and cut flowers, as well as in decoration of urban landscapes. They are at the top of the cut flowers’ market and represent a good economic choice in many parts of the world (Fanourakis et al. 2013b). Many environmental stress factors interact to reduce the quality and vase life of produced roses (Ahmadi et al. 2009; Fanourakis et al. 2013b). Management practices during cultivation period and postharvest handling can also affect the quality and vase life of cut rose flowers (Starkey and Pedersen 1997; Druge 2000). It has been shown that higher carbohydrate levels of plant tissues or postharvest application of sucrose can improve the vase life of cut flowers in many ornamental plants (Doi and Reid 1995; Ichimura et al. 2003). Various cultivation strategies and postharvest treatments have been proposed to improve the quality of cut flowers (Ichimura et al. 2003; Ahmadi et al. 2009). Nutrient elements such as N, K, Ca, and their availability to plant roots can significantly improve the quality and vase life of cut roses (Roude et al. 1991; Starkey and Pedersen 1997; Druge 2000; Zamani et al. 2011). Proper application of nutrient elements plays an important role in overall plant photosynthesis and carbohydrate production. Nitrogen, potassium and calcium are among those nutrients with the highest effect on plant physiological processes and metabolite production (Marschner 2011). Chlorophyll content and efficiency of photosynthesis of plant leaves have positive correlations with nitrogen and potassium levels (Marschner 2011; Souri 2016). While in practice there is no negative effects of extra calcium and potassium concentrations on plant growth and quality; however, application of nitrogen more than plant’s need, could negatively influence many
quality factors in agricultural plants. On the other hand, different forms of nitrogen, and the ammonium to nitrate ratios can significantly affect morphology and physiology of plants (Souri et al. 2009). Better absorption and internal translocation of metal micronutrients generally occur with stabilized ammonium nutrition in alkaline soil conditions (Souri et al. 2009; Kronzucker et al. 2001). In a study by Pineda et al. (2010), application of ammonium sulfate (3%) with sucrose (0.2%) significantly increased vase life of chrysanthemum (Chrysanthemum indicum) flowers. Improvement of pot or cut flowers’ quality by application of various chemical fertilizers including urea calcium nitrate or amino acids at pre- or post-harvest has been reported (Roude and Terril 1991; Druge 2000; Zamani et al. 2011). Nitrogen has a vital role in plant cell metabolism, and preharvest application of N-forms and N-levels has a great impact on growth and quality of crops (Druge 2000; Souri and Roemheld 2009; Marschner 2011). Such effects may also exist for postharvest N application. Application of nitrogen forms to increase cut flowers’ life is safer than application of chemical solutions. Therefore, in the present study, effects of various nitrogen concentrations in holding solution from different sources were evaluated on postharvest quality of rose cut flowers.

2 Material and Methods

Rose flowers (Rosa hybrida cv. “Utopia”) were obtained from a greenhouse located in the Pakdasht region in the Southwest of Tehran-Iran. The plants were grown under a hydroponic system, supplied with Hoagland nutrient solution. The flower shoots were harvested in the early morning and immediately transferred to the postharvest laboratory at Dept. of Horticultural Sciences, Tarbiat Modares University, Tehran-Iran (October, 2016). Partially opened flowers with similar maturity were used, and for better uniformity regarding the size and appearance, flower stems were trimmed under water to a stem length of about 50 cm with five leaves on each stem.

Various treatments were assigned in a completely randomized design with three replications. Four flower stems were included per replication (in an Erlenmeyer). Different concentrations of ammonium sulfate, calcium nitrate and potassium nitrate of 0, 5, 10, 15 and 20 mg N L⁻¹ were prepared using distilled water, in a half liter Erlenmeyer. Distilled water was used for control treatment (zero concentration of nitrogen). The samples were placed in a growth chamber with average temperature of 18±2°C, a photoperiod of day/night (12/12 h), and a light intensity of 250 µmolm⁻²s⁻¹ for better uptake of water and nutrients. The light was supplied using white fluorescent lamps and relative humidity was kept about 70%.

Various physiological and quality parameters were measured during the experiment, as well as at the end date of flower life for each sample. The holding solutions were renewed every three days, after recording its pH and the water uptake rates in each vase. Holding solution pH was recorded using a pH meter (model Metrohm 827) and the average pH of holding solution during the three days was recorded in the results. With sealing of Erlenmeyer flasks the evaporation was prevented and the amount of water uptake in holding solution by four flower stems was recorded by measuring the consumed mL of solution using a graduated cylinder. The amount of water uptake has been presented as the amount (mL) for four stems during three days in holding solution. The number of days to visible petal wilting was recorded as the vase life. Leaf chlorophyll index was measured using SPAD meter (model S02 Plus, Illinois, USA) six days after beginning the experiment and for those with shorter life, leaf SPAD was measured at their final day of vase life.

For determination of relative water content of petals and leaves, whole petals or leaf blade discs were first weighed to determine their fresh weight (FW) and then were hydrated to full turgidity for 24 h on de-ionized water in a closed Petri dish. The samples were taken out and dried off with tissue paper and immediately weighed to obtain fully turgid weight (TW). The samples were oven-dried at 70°C and weighed to determine dry weight (DW). The RWC was estimated from the following equation (Zamani et al. 2011):

$$RWC\% = \frac{(FW-DW)}{(TW-DW)} \times 100.$$  

Petal electrolyte leakage was determined following (Emongor 2004) after placement of 1 g of fresh petal tissues into a glass beaker containing double-distilled water. Then the electrical conductivity (EC) of the solution (EC₁) was measured using table EC meter (model Metrohm 827). After boiling the sample for 2 min and then cooling to room temperature, the electrical conductivity of the solution was measured again (EC₂). The percentage of electrolyte leakage was calculated as:

$$EL\% = \frac{(EC₁/EC₂)}{100}.$$  

Leaf catalase activity was measured by grinding one gram of leaf tissue in liquid nitrogen and 50 mM buffer of potassium sulfate (pH 7.0) following Ezhilmathi et al. (2007). Leaf proline concentration was determined with the ninhydrin method and by spectrophotometer
absorption at 520 nm following method of Paquin and Lechasseur (1979).

Data were analyzed using SPSS 16 and comparison of means was done using Duncan multiple range test at 5% level.

**Ethical approval:** The conducted research is not related to either human or animal use.

### 3 Results

The results showed that when different concentrations of nitrogen from various sources were applied in holding solution, the physiological quality traits of cut rose stems showed different responses (Table 1). Leaf SPAD values (Table 1) were highest in 5 mgL⁻¹ N-ammonium and 20 mgL⁻¹ potassium nitrate, which showed a significant difference only compared to 15 and 20 mg L⁻¹ N-ammonium. The relative water content of leaf (RWC) was significantly reduced by ammonium concentrations of 10, 15 and 20 mg L⁻¹ compared to the control (Table 1), whereas concentration of 20 mg L⁻¹ KNO₃ significantly increased the leaf RWC compared to the control. Determination of leaf proline (Table 1) revealed that higher ammonium concentrations increased the amounts of this trait. The highest leaf proline was recorded in those flower stems treated with 15 and 20 mgL⁻¹ N-ammonium that showed significant differences with other treatments. Increasing nitrate concentrations of both nitrate salts resulted in significant lower amounts of leaf proline concentrations compared to the control.

The leaf catalase activity showed a different pattern, in which it was significantly increased with 5 and 10 mgL⁻¹ N-ammonium compared to control, whereas it was significantly decreased with higher ammonium concentrations of 15 and 20 mg L⁻¹ (Table 1). Different concentrations of calcium nitrate and potassium nitrate had no significant effect on leaf catalase activity compared to control.

The average water consumption (of 3 days) for four stems in each Erlenmeyer was significantly decreased with the two highest ammonium concentrations of 15 and 20 mgL⁻¹ N (Table 2), whereas the two lowest levels of 5 and 10 mg L⁻¹ N-ammonium had no significant difference from the control (Table 2). In contrast, the two highest concentrations of potassium nitrate (15 and 20 mg L⁻¹) and

| Con. (mg L⁻¹) | Leaf SPAD value | Leaf relative water content (%) | Leaf prolinecon. (mg kg⁻¹ fresh weight) | Leaf catalase activity (µmol H₂O₂ red. min⁻¹ mg⁻¹ protein) |
|---------------|-----------------|-------------------------------|----------------------------------------|-----------------------------------------------------|
| Control 0     | 44.2ab          | 73.2b                         | 225c                                   | 0.84b                                               |
| 5             | 49.4a           | 75.3b                         | 214c                                   | 1.60a                                               |
| Ammonium 10   | 41.5ab          | 71.6bc                        | 285b                                   | 1.42a                                               |
| Sulfate 15    | 34.6b           | 64.1c                         | 360a                                   | 0.53c                                               |
| 20            | 32.4b           | 62.2c                         | 356a                                   | 0.31c                                               |
| Calcium 10    | 44.7ab          | 76.6ab                        | 185c                                   | 0.77b                                               |
| Nitrate 15    | 46.3ab          | 76.8ab                        | 165cd                                  | 0.88a                                               |
| 20            | 46.4ab          | 80.7ab                        | 155d                                   | 0.92b                                               |
| Potassium 10  | 46.5ab          | 77.3ab                        | 190c                                   | 0.80a                                               |
| Nitrate 15    | 45.3ab          | 79.6ab                        | 150d                                   | 0.96a                                               |
| 20            | 48.7a           | 85.4a                         | 145d                                   | 1.04a                                               |

Quality factors of cut flowers were measured at 6 days after applying treatments or at the final day of their vase life for those with a short life.
the highest concentration of calcium nitrate (20 mg L\(^{-1}\)) significantly increased the water uptake by flower stems. The holding solution pH (Table 2) was significantly increased by the highest concentration of both calcium nitrate and potassium nitrate relative to the control. The two highest ammonium concentrations have resulted in a significant reduction in pH compared to the control (Table 2). The relative water content of petals was significantly reduced by the two highest ammonium concentrations (15 and 20 mg L\(^{-1}\)) of holding solution (Table 2). There was no significant effect of different nitrate concentrations (from both salts) on this trait. Petal ion leakage (Table 2) was significantly increased by the two highest ammonium concentrations (15 and 20 mg L\(^{-1}\)) compared to the control, whereas there was an increasing trend for this trait with increasing nitrate concentrations of both salts as well as in 5 mg L\(^{-1}\) N-ammonium treatment.

The most evident effects of nitrogen forms and their concentrations were observed on flower vase life (Table 2). The three highest concentrations of potassium nitrate (10, 15 and 20 mg L\(^{-1}\)), two highest concentrations of calcium nitrate (15 and 20 mg L\(^{-1}\)) and the lowest concentration of ammonium sulfate (5 mg L\(^{-1}\)) have resulted in significantly longer flower vase life compared to the control. Ammonium in the two highest concentrations (15 and 20 mg L\(^{-1}\)) has resulted in significant shorter vase life.

### 4 Discussion

In this study, various nitrogen compounds in vase holding solution had differently affected the physiological quality traits of cut rose flowers. Application of potassium nitrate resulted in better effects on quality and vase life of flowers; however, there were similarities in calcium nitrate and potassium nitrate effects on many traits. The effect of ammonium sulfate was quite different, in which the lowest ammonium level (5 mg L\(^{-1}\)) improved, but higher concentrations (15 and 20 mg L\(^{-1}\)) reduced cut flower quality and vase life. There were generally positive effects of increasing calcium nitrate and particularly potassium nitrate concentrations on physiological quality factors of cut rose flowers including vase life. Nitrogen is one of the most important nutrient elements in the yield and quality production of plants. The effects of preharvest management of nitrogen on postharvest quality of cut flowers have been shown (Druge, 2000); however, the results of the present study indicated that there are also strong effects of postharvest nitrogen treatments on quality and vase life of rose cut flowers. In our study, it seems that nitrogen forms and their corresponding concentrations induced their effects mainly through changes in water status and probably phytohormone levels of plant tissues. Changes in holding solution pH support this argument,
as there was a 0.9 decrease in holding solution pH with highest ammonium level (20 mg L⁻¹), and about 0.4 increase in holding solution pH with highest levels of nitrate (20 mg L⁻¹) in both forms. It has been shown that there is generally a lower solution pH with ammonium absorption and higher solution pH with nitrate absorption (Souri and Roemheld 2009). There might be an interaction between nitrogen and carbohydrates, photosynthesis and plant hormones on the role of N in postharvest of flowers (Druge 2000; Skutnik et al. 2001). Carbohydrates and plant hormones are vital internal factors regarding postharvest quality of ornamentals, which are strongly affected by nitrogen levels and N sources.

Maintaining water balance in leaves and petals of cut flowers regulates their vase life and quality (Fanourakis et al. 2013a; Fanourakis et al. 2013b; van Doorn 2012). Water content of plant tissues is vital in their related senescence processes (Emongor 2004; Fanourakis et al. 2013a). Similarly, in the present study, the higher concentration of nitrate (20 mg L⁻¹) from both salts (calcium nitrate and potassium nitrate) and the lowest concentration of ammonium (5 mg L⁻¹) improved water status of leaf and petals, and vase life of cut flowers. Different mechanisms may be involved in improved water relations of leaf and petal tissues of roses in the present study. Nitrate is an important osmotic compound in plant cells that generally accumulates in the vacuoles and plays an important role in water uptake and the tissue’s turgidity (Marschner 2011). In applied calcium nitrate and potassium nitrate, the companion ions of Ca and K have roles in stomata opening, as well as in hydraulic conductance of different tissues and may improve leaf and petal water content. These conditions probably have resulted in better quality and flower vase life (Zieslin 1989; Roude et al. 1991). In addition, phytohormones play important roles in water uptake and water status of plant tissues. Cytokinins and giberlines are the two main phytohormones associated with longer life of plant tissues, while ethylene generally is associated with senescence and shortened vase life (Rahayu et al. 2005; Ahmadi et al. 2009; Marschner 2011). It has been shown that postharvest application of cytokinins and giberlines improves cut flower quality and particularly their vase life (Skutnik et al. 2001; Emongor 2004). On the other hand, there is generally a good correlation between nitrogen forms and nitrogen status of plants with level of cytokinines (Paull and Chanrachit 2001; Rahayu et al. 2005). So, despite cytokinines not being determined in the present study, they probably have been increased by higher nitrate treatments (Walch-Liu et al. 2000; Rahayu et al. 2005). Nitrate can also function as a semi-hormonal compound in cell and tissue metabolism (Marschner 2011), affecting growth characteristics including leaf greenness and senescence (Souri 2016).

Ammonium sulfate in the lowest concentration (5 mg L⁻¹) showed positive effects on many physiological traits of cut roses. However, higher concentrations significantly reduced the quality traits of cut flowers including vase life, leaf and petal RWC, catalase activity and water consumption of flower stems. The values of pH in holding solution indicate that flower stems have absorbed ammonium ions from the holding solution and generally there was a decreasing trend in pH with ammonium concentrations (Table 2). Physical properties of the cut surface of stems and passive movement of ammonium ions probably are involved in the ammonium or nitrate absorption phenomena (Marschner 2011); however, within the stem cells the absorption occurs mainly as active phenomena resulting in reduction of solution pH due to equilibration of ammonium uptake by H⁺ release (Kranzucker et al. 2001; Souri et al. 2009). Despite the ammonium concentrations of flower stems not being measured in this study, it seems that the influx of ammonium into the stem, leaf and petals increased with ammonium concentrations (Walch-Liu et al. 2000; Kranzucker et al. 2001), as ammonium in higher concentrations restricted quality factors. The adverse effects of high ammonium concentrations are probably related to its toxicity and damages to water uptake and particularly its transportation in the stem vessels (Souri et al. 2009). The proline content of leaves support this conclusion. Ammonium nutrition of plants may impair the flower’s quality, mainly due to stressful conditions exerted by extra protons produced by ammonium uptake and assimilation (Kranzucker et al. 2001). Symptoms of senescence on different organs were more pronounced in the case of high or pure ammonium supply as compared to higher proportions of nitrate (Druge 2000). Proton toxicity and physiological calcium deficiency may impair postharvest life following ammonium supply (Starkey and Pedersen 1997; Souri and Roemheld 2009). The xylem vessels and particularly aquaporine proteins are probably very vulnerable to proton toxicity and calcium deficiency. Nevertheless, postharvest treatment of cut flowers with ammonium may respond differently. Despite roses having low sensitivity to ethylene, various rose cultivars may respond quite differently to ethylene concentrations (Fanourakis et al. 2013b). The postharvest behavior of rose flowers is an outcome of physiological processes occurring in the leaves, stem, flower bud, and in peduncle (Zieslin 1989). Any reduction in leaf turgor pressure, promotes ethylene production or activation, resulting in chlorophyll biodegradation (Souri et al. 2009). The higher
ethylen production via ammonium nutrition (Marschner 2011) and higher cytokinin levels via nitrate nutrition of plants (Rahayu et al. 2005) has been reported.

Glutamine as an important intermediate amino acid in the ammonium assimilation pathway has ameliorating effects on ammonium toxicity (Souri and Roemheld 2009). Application of glutamine on rose plants significantly improved the flower’s vase life, due to affecting petal senescence changes (Zamani et al. 2011). In plants, glutamine is increased by low application of ammonium (Kronzucker et al. 2001), and this is probably the reason for quality and vase life improvement of cut rose flowers at 5 mgL⁻¹ N-ammonium in the present study. Application of Physan (an ammonium disinfectant solution) and sucrose has prolonged the bud opening and vase life of cut flowers in Limonium ‘Fantasia’ which extended to 17 days compared to 4 to 5 days vase life in deionized water (Doi and Reid 1995).

In the present study the companion ion also may play a role in postharvest quality of cut flowers. In calcium nitrate and potassium nitrate, apart from nitrate roles, calcium and potassium are two major nutrient elements important for quality behavior of cut flowers (Roude et al. 1991; Starkey and Pedersen 1997). Calcium is a senescence delaying agent and can improve shelf life of flowers. Through higher membrane integrity and cell wall hardness, it can reduce senescence of tissues (Marschner 2011). On the other hand, potassium is a major nutrient element in many quality parameters of plants. It has direct and indirect roles in osmotic adjustment of plant cells particularly in root cells allowing the continuous movement of water and solutions within xylem sap (Marschner 2011). Both calcium and potassium ions together with nitrate are involved in better water uptake and hydraulic conductance of plant tissues, as water stress symptoms were the most important criterion terminating vase life in 46 out of 50 assessed rose cultivars (Fanourakis et al. 2013a). These symptoms appear when water loss exceeds water uptake.

5 Conclusion

In this study, the quality parameters of cut rose flowers including vase life were significantly improved by the lowest ammonium (5 mgL⁻¹ N) and the highest nitrate concentrations (15 and 20 mgL⁻¹ N) from both calcium nitrate and particularly potassium nitrate sources. Higher concentrations of ammonium resulted in significant reduction in quality and vase life of cut rose flowers. The findings indicate that water status of plant tissues and probably phytohormones play a role in the results obtained for N sources and levels, as the literature shows general positive correlations between nitrate status and cytokinins in plant tissues. Data on water uptake, vase solution pH, leaf and petal relative water content support this argument that the nitrate and ammonium treatments via accelerated/restricted stomata control or hydraulic conductivity of xylem vessels affect quality and vase life of roses. The water relations generally are associated with ethylene production and senescence phenomena. Nevertheless, the potential role of the growth environment on the obtained results cannot be ignored.

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