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CHARACTERISTICS OF POSTHARVEST QUALITY OF CHRYSANTHEMUM CUT FLOWERS UNDER PRETREATMENT WITH NITROGENOUS COMPOUNDS

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ABSTRACT

This study was done using three nitrogenous compounds to evaluate their effects on postharvest characteristics of chrysanthemum (*Chrysanthemum indicum*) cut flowers. The study consisted of three separate and parallel experiments, in which ammonium sulfate, calcium nitrate and potassium nitrate were used in different concentrations of zero, 50, 100, 200 and 500 mg dm^{-3} N for pretreatment of stems for 6 hour before transferring to holding d-water solution. The results showed that petal ion leakage and leaf SPAD values were increased by increasing ammonium sulfate, but not calcium or potassium nitrate. Petal carotenoids were increased by pretreatment of ammonium sulfate and potassium nitrate. There was constant increase in water uptake by increasing the levels of calcium nitrate and potassium nitrate, while ammonium sulfate at high concentrations (200 and 500 mg dm^{-3} N) resulted in significant less water uptake compared to control. Ammonium sulfate in 50 and 100 mg dm^{-3} increased shelf life of pretreated stems, but higher concentrations significantly reduced cut flowers shelf life. Increasing concentrations of calcium nitrate and particularly potassium nitrate have led to prolongation of flower shelf life to 12 days compared to 6 days of control. The results indicate that pretreatment of chrysanthemum cut flowers with ammonium sulfate in rather low concentrations or with moderate to high concentrations of calcium nitrate or potassium nitrate can significantly improve shelf life and postharvest flower qualities.

Key words: ammonium, carotenoids, nitrate, shelf life, senescence, water uptake, ion leakage

INTRODUCTION

Quality and shelf life of cut flowers depend on many culture and management factors during plant growth period and after harvest. Improving the quality and shelf life of cut flowers could have significant economic consequences for both producers and consumers.

Despite ambient temperature is the major environmental factor in postharvest quality and shelf life of cut flowers; however, many other factors such as humidity, microbial effect and nutrient elements can also influence cut flower's shelf life [Skutnik et al. 2001, Sairam et al. 2011]. Ethylene, on the other hand, is the main

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compound that mediates many physiological and biochemical functions in plant tissues including aging and senescence [Pun and Ichimura 2003, Souri et al. 2009, Marschner 2011]. Many unsuitable conditions including high temperature, mechanical damage, air pollutions, pests and diseases, as well as nutrient shortages can intensify ethylene production and senescence progress of plant tissues. Various postharvest treatments have been evolved to improve the quality including shelf life of cut flower in many ornamental plants, mainly due to control of ethylene production or its action [Ichimura et al. 2003, Ahmadi et al. 2009]. Accelerated postharvest senescence is a major limitation to the marketing of many species of cut flowers and considerable effort has been devoted to develop postharvest treatments to extend the marketing period of cut flowers [Nergi and Ahmadi 2014]. Carbohydrates and especially sucrose have been shown to reduce ageing rates of cut flowers resulting in their longer shelf life [Doi and Reid 1995, Ichimura et al. 2003].

Nitrogen forms and the ammonium to nitrate ratios are key important factors on many morphological and physiological features in plants. Ammonium nutrition of plants can improve micronutrients especially manganese content of plant tissues, especially under soil and water alkalinity conditions [Kronzucker et al. 2001, Souri et al. 2009]. Chlorophyll content and efficiency of photosynthesis of plant leaves have positive correlations with N levels [Marschner 2011]. This can increase carbohydrate and sugar production in plant tissues. On the other hand, nitrogenous compounds can affect ethylene production, chlorophyll degradation and ion leakage in plant tissues [Druge 2000]. In fact, any internal or external factors that promote ethylene production can also decrease cut flower shelf life. So, treatments which delay leaf senescence can also increase the life of flowers. In this respect, water conductivity of stem vessels is very important. Any reduction in leaf turgor pressure, promotes ethylene production or activation, resulting in chlorophyll degradation [Souri et al. 2009]. Cell membrane permeability and activity of antioxidant enzymes are also important in leaf senescence [Sairam et al. 2011].

It has been shown that a mixture of ammonium sulfate (3%) and sucrose (0.2%) has significantly increased shelf life of chrysanthemum flowers [Pineda et al. 2010]. Treatment of plants by ammonium sulfate and ammonium nitrate can improve their product's quality [Roude et al. 1991, Druge 2000, Pineda et al. 2010, Zamani et al. 2011]. Chrysanthemum is an important cut flower after roses at the market, and represents the second economical important flower in the world. The shelf life of cut flowers in chrysanthemum is generally 10 days, and cut flowers with longer life are in interest of all producer, exporter and consumers. Nitrogen, in various forms, is a key player in many metabolic processes of plant cells. Despite the role of preharvest applications of N forms and N levels have been studied; however the role of postharvest treatment with nitrogen sources of cut flowers stems has not been evaluated yet. Therefore, in present study the effects of various nitrogenous compounds were evaluated on postharvest of chrysanthemum cut flowers.

MATERIAL AND METHODS

Chrysanthemum flowers (*Chrysanthemum indicum* cv. 'Yellow Engineering') were obtained from farms located in Pakdasht region in Southwest of Tehran-Iran. The flower shoots were harvested in early morning and immediately were transferred to laboratory with 15°C, and the cut shoots were trimmed regarding the size and appearance for better uniformity, while stem cut was in water to prevent entrance of bubbles into the stem vessels. Stems with partially opened flowers were used, and those of similar maturity were further selected.

Various pretreatments with nitrogen forms and nitrogen levels were assigned in a completely randomized design with three replications, in which four uniform flower stems were included in each replicate. Three nitrogenous compounds of ammonium sulfate, calcium nitrate and potassium nitrate, each in five concentrations of zero, 50, 100, 200 and 500 mg dm^{-3} as pure N was used for 6 hour pretreatments of cut stems (from 8 o'clock at the morning until 14 at afternoon). Solutions of nitrogenous compounds were prepared using distilled water. Sole distilled water without and N- compound application was used for pretreatment of control plants (zero concentration of nitrogenous compounds). Pretreatment of stems was

done with transfer of stems to Erlenmeyer flasks containing 500 cm^3 solution of given concentrations of respected salt. Thereafter, stems were transferred to containers containing only distilled water. The holding distilled water was replaced after three days and until day 12^{th}, which was the end of experiment.

The laboratory had an average temperature of 18 ±2°C and a photoperiod of 10/14 hour light/dark with a light intensity of 150–200 µmol m^{-2} s^{-1}. The light was supplied using white fluorescent lamps and relative humidity was about 70%.

Various physiological and quality parameters of stems were measured. The changes in pH of holding distilled water solution were recorded after three days using pH meter (model Metrohm 827). The volume of water uptake was recorded using a graduated cylinder, while with sailing of Erlenmeyer flasks the evaporation was prevented. The cumulative water uptake of stems was recorded for each replicate. Days to initiation of visible petal wilting were recorded as shelf life of cut flowers.

For determination of petal carotenoid concentrations, one g of fresh petals was grinded using liquid N. The powdered material received 10 cm^3 acetone 80% while grinding was continuously operated in mortar and parcel. The mixture was centrifuged at 4000 rpm for 20 min and thereafter the absorption was recorded by spectrophotometer.

The relative water content of petal, leaf and stem were determined with whole petals, leaf blade discs, and stem chips, respectively. The samples were first weighed to determine their fresh weigh (FW) and afterwards were hydrated to full turgidity for 24 h on distilled water in a closed petri dish. The sample was then well dried off with tissue paper and immediately weighed to obtain fully turgid weight (TW). The sample was oven-dried at 70°C and weighed to determine its dry weight (DW). The relative water content (RWC) was estimated from the following equation: RWC = (FW−DW)/(TW−DW).

Petal electrolyte leakage was determined after placement of 1 g of fresh petals into a glass beaker containing double-distilled water. The electrical conductivity of the solution (EC1) was measured using table EC meter. After boiling the sample for two 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 (%) = (EC1/EC2) × 100.

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

RESULTS

The results showed that various nitrogen sources had different effects on physiological quality traits of chrysanthemum cut flowers. Petal ion leakage was increased by pretreatment of flower stems with ammonium sulfate concentrations (tab. 1), as the lowest and highest ion leakage was in 0 and 500 mg dm^{-3}, respectively. Pretreatment with various concentrations of calcium nitrate (tab. 2) and potassium nitrate (tab. 3) revealed that there was no significant effect of different concentrations of these two salts on petal ion leakage.

Determination of carotenoids content of petals showed that pretreatment of cut flowers with ammonium sulfate significantly increased their carotenoids content, as the highest amount (15.7 mg g^{-1} FW) was observed in 500 mg dm^{-3} treatment (tab. 1). There was also a similar increasing trend of carotenoids concentrations with increasing calcium nitrate (9.5 mg g^{-1} FW) and potassium nitrate (10.5 mg g^{-1} FW) levels (tabs 2 and 3), however the promoting effects of potassium nitrate concentrations on petal cartenoids was higher than calcium nitrate.

SPAD index of leaves were measured at last day of flower shelf life. Ammonium sulfate particularly in higher concentrations increased the leaf SPAD values (tab. 1). The significant highest SPAD index of leaves on cut stems was recorded for those which were treated with 500 mg dm^{-3} ammonium sulfate. However, various concentrations of calcium nitrate and potassium nitrate had no significant effects of SPAD value of stem leaves (tabs 2 and 3).

Petal and leaf relative water content were decreased with increasing ammonium sulfate concentrations (tab. 1), while there was a constant increase for these two traits by increasing potassium nitrate and particularly calcium nitrate concentrations (tabs 2 and 3). A similar trend was observed for stem relative water content.

Table 1. Physiological characteristics of cut flowers after pretreatment with different concentrations of ammonium sulfate

	Ammonium sulfate[a]				
	0 (control)	50 mg dm^{-3}	100 mg dm^{-3}	200 mg dm^{-3}	500 mg dm^{-3}
Petal ion leakage[b] (%)	14.7d	13.6d	15.3c	19.6b	25.0a
Petal carotenoids (mg g^{-1} FW)	8.2c	11.5b	11.8b	12.3b	15.7a
Leaf SPAD index[c]	50.1b	51.2b	52.8ab	53.4ab	56.3a
Petal relative water content (%)	83.6a	84.2a	82.7a	80.3ab	78.1b
Leaf relative water content (%)	43.2a	45.3a	41.6ab	39.1b	39.4b
Stem relative water content (%)	33.4a	33.7a	31.4ab	29.4ab	28.6b
Total water consumption (cm^3)[d]	93.5a	95.2a	86.0b	65.6c	35.7d
Solution pH[e]	6.3a	6.4a	6.0a	6.0a	6.2a
Flower shelf life[f]	6.5b	7.5ab	8.5a	6.0bc	5.0c

[a] Stems were placed in pretreatment solutions of different concentrations of ammonium sulfate as pure N
[b] Quality factors of flower stem were measured at 4 days after pretreatment
[c] Plant chlorophyll index at final day of their shelf life
[d] Amounts of total water uptake of vase cut flowers
[e] Average pH of holding solution of two-four times solution replacement
[f] Number of days that cut flowers were fresh without petal senescence
*Data with similar letter in each row have no significant difference at 5% level of Duncan test

Table 2. Physiological characteristics of cut flowers after pretreatment with different concentrations of calcium nitrate

	Calcium nitrate[a]				
	0 (control)	50 mg dm^{-3}	100 mg dm^{-3}	200 mg dm^{-3}	500 mg dm^{-3}
Petal ion leakage[b] (%)	14.7a	12.9a	12.2a	13.3a	14.8a
Petal carotenoids (mg g^{-1} FW)	8.2b	8.7ab	8.6ab	9.1ab	9.5a
Leaf SPAD index[c]	50.1a	50.4a	49.6a	51.3a	50.8a
Petal relative water content (%)	83.6b	84.8b	86.3b	89.1ab	93.9a
Leaf relative water content (%)	43.2c	49.6b	52.5ab	58.2a	60.1a
Stem relative water content (%)	33.4b	35.1ab	38.2a	41.5a	40.8a
Total water consumption (cm^3)[d]	93.5c	97.6bc	99.3bc	108.6b	135.7a
Solution pH[e]	6.3a	6.4a	6.4a	6.5a	6.6a
Flower shelf life[f]	6.5b	7.5b	10.0a	11.0a	11.0a

[a] Stems were placed in pretreatment solutions of different concentrations of calcium nitrate
[b] Quality factors of flower stem were measured at 4 days after pretreatment
[c] Plant chlorophyll index at final day of their shelf life
[d] Amounts of total water uptake of vase cut flowers
[e] Average pH of holding solution of two-four times solution replacement
[f] Number of days that cut flowers were fresh without petal senescence
*Data with similar letter in each row have no significant difference at 5 % level of Duncan test

Table 3. Physiological characteristics of cut flowers after pretreatment with different concentrations of potassium nitrate

	Potassium nitrate[a]				
	0 (control)	50 mg dm^{-3}	100 mg dm^{-3}	200 mg dm^{-3}	500 mg dm^{-3}
Petal ion leakage[b] (%)	14.7a	12.6a	13.2a	14.0a	15.3a
Petal carotenoids (mg g^{-1} FW)	8.2b	9.8ab	9.7ab	10.8a	10.5a
Leaf SPAD index[c]	50.1a	49.3a	50.8a	51.3a	51.7a
Petal relative water content (%)	83.6b	84.2ab	85.7ab	87.4a	89.5a
Leaf relative water content (%)	43.2b	47.5ab	49.2ab	48.1ab	55.3a
Stem relative water content (%)	33.4b	33.8b	36.9ab	38.4a	39.1a
Total water consumption (cm^3)[d]	93.5c	93.2c	102.4bc	111.1b	127.5a
Solution pH[e]	6.3a	6.25a	6.4a	6.3a	6.2a
Flower shelf life[f]	6.5c	7.0c	9.0b	10.0b	12.0a

[a] Stems were placed in pretreatment solutions of different concentrations of potassium nitrate
[b] Quality factors of flower stem were measured at 4 days after pretreatment
[c] Plant chlorophyll index at final day of their shelf life
[d] Amounts of total water consumption of vase cut flowers
[e] Average pH of holding solution of two-four times solution replacement
[f] Number of days that cut flowers were fresh without petal senescence
*Data with similar letter in each row have no significant difference at 5% level of Duncan test

The amount of water uptake in pretreated of 50 mg dm^{-3} ammonium sulfate showed no significant difference with control, but there was constant reduction in water uptake of cut flowers by increasing ammonium sulfate levels to 500 mg dm^{-3}. Those cut flowers which were treated with 500 mg dm^{-3} ammonium sulfate showed nearly 38% water uptake of control treatment (tab. 1). Nevertheless, water uptake of cut flowers was significantly increased when calcium nitrate and potassium nitrate especially in higher concentrations were used. Treatment of cut flowers with 500 mg dm^{-3} calcium nitrate and potassium nitrate resulted in about 45% and 36% higher water uptake, respectively (tabs 2 and 3).

Determination of pH of holding solution showed that pretreatment of cut stems with ammonium sulfate resulted in pH reduction of holding solution (tab. 1). However, there was a slight increase in holding solution pH when calcium nitrate was used (tab. 2). Pretreatment with potassium nitrate had no significant effect on holding solution pH (tab. 3).

The shelf life of cut flowers of chrysanthemum was significantly influenced by pretreatment of various nitrogenous compounds. In ammonium sulfate (tab. 1), the pretreated stems with 50 and 100 mg dm^{-3} had longer shelf life of flowers compared to control. However, pretreatment of flower stems with 200 and 500 mg dm^{-3} ammonium sulfate resulted in shorter shelf life compared to control plants (tab. 1). Treating stem flowers with different concentrations of calcium nitrate (tab. 2) showed that there was a constant increase in flower shelf life with increasing calcium nitrate concentrations, however there was no significant difference between 100, 200 and 500 mg dm^{-3} concentrations. A similar trend was observed for shelf life of flowers pretreated with different concentrations of potassium nitrate (tab. 3), as the longest flower shelf life was observed in 500 mg dm^{-3} potassium nitrate treatment.

DISCUSSION

In present study various nitrogenous compounds had different effects on physiological quality traits of cut flowers of chrysanthemum. The effects of calcium nitrate and potassium nitrate on many traits were almost similar, while the effect of ammonium sulfate depending on the applied concentrations was quite different. Ammonium sulfate in concentrations of 50 and 100 mg dm^{-3} had positive effects on most

quality traits, while its concentrations of 200 and 500 mg dm^{-3} showed negative effects particularly on shelf life of cut flowers. Ammonium sulfate in concentrations of 50 and 100 mg dm^{-3} has increased flower shelf life, while higher levels significantly reduced this trait compared to control. However, there was a constant increase of flower shelf life with increasing calcium nitrate and particularly potassium nitrate.

Nitrogen is one of the most important nutrient elements affecting the yield and quality production of cut flowers. Nitrogen management during preharvest has also strong effects on postharvest quality of cut flowers [Druge 2000, Pineda et al. 2010]. Similar to vegetative growth, postharvest response to (pre- or postharvest) nitrogen application depends on N levels and N sources. Despite high levels of N in preharvest may impair the quality of cut flowers, but postharvest application of N levels and N sources may result different. Ammonium treatment of plants may impair flower's quality, mainly due to stressful conditions exerted by extra protons produced by ammonium assimilation [Souri et al. 2009]. Symptoms of senescence observed on different organs were more pronounced in the case of high or pure ammonium supply as compared to higher proportions of nitrate [Druge 2000, Souri et al. 2009]. A similar response, but to lesser extent, was also observed in present study with postharvest pretreatment of high ammonium sulfate levels (tab. 1). However, it has been reported that a vase solution containing Physan (a quaternary ammonium disinfectant solution) and sucrose not only prolonged the longevity of individual florets but also promoted bud opening, so that the life of cut inflorescences of hybrid Limonium 'Fantasia' extended to 17 days compared to 4 to 5 days in deionized water [Doi and Reid 1995].

Leaf SPAD values were higher in ammonium sulfate pretreated stems. Carotenoids content of petals were increased by concentrations of ammonium sulfate and potassium nitrate, while it was less affected by calcium nitrate. These could be due to the stressful conditions induced by influx of ammonium in plant tissues. Higher chlorophyll concentrations due to ammonium treatment via nutrient solution have been reported [Souri et al. 2009]. Higher chlorophyll concentrations under ammonium treatment may is an adaptive mechanism of chloroplasts for inactivating extra protons induced by ammonium assimilation. Intermediate substances such as glutamine may have ameliorating effects on ammonium induced stressful conditions. On the other hand, application of glutamine on rose plants significantly improved the flowers vase life, due to affecting petal senescence changes [Zamani et al. 2011]. Leaf glutamine content is improved by low application of ammonium [Kronzucker et al. 2001], and this is probably the reason for quality and shelf life improvement of chrysanthemum by 50 and 100 mg dm^{-3} ammonium sulfate application in present study.

From the data it can be concluded that ammonium in higher concentrations (200 and 500 mg dm^{-3}) has resulted in stressful conditions for flower stems. Stems pretreated with higher ammonium sulfate had less water uptake, as well as less relative water content in petals, leaves and stems. Water content of plant tissues plays important role in senescence process and there have been good correlations between tissue water status and their related senescence processes [Emongor 2004, Doi and Reid 1995]. Phytohormones may also play roles in water uptake and water status of plant tissues. Application of giberlic acid to cut flowers of gerbera improved the water content of flower heads and stems, which has resulted in longer shelf life of cut flowers compared to distilled water control [Emongor 2004]. Improvement in water uptake and water status of flowers is attributed to the application of preservative compounds such as sucrose [Pun and Ichimura 2003]. Maintaining water balance in leaves and petals of cut flowers is vital for improving their shelf life and quality. Ammonium similar to sucrose [Doi and Reid 1995], generally induces the closure of stomata, resulting in reduced water loss by leaves or petals in chrysanthemum cut flowers.

Improvement of the yield and quality of flowers by application of various fertilizers is well known. Nitrogen and calcium nutrition of ornamental plants and their effects on improving quality performance has been well documented [Mascarini et al. 2005, De Capdeville et al. 2005]. Similarly, exogenous treatment of nitric oxide (NO) as a nitrogenous compound

and signaling molecule could significantly extend the vase life of cut carnation flowers [Zeng et al. 2011]. The treatment can also delay petal wilting, maintain water metabolism, the antioxidative enzymes activity and mass-eliminate reactive oxygen species as well as cell membrane stability [Zeng et al. 2011].

There may be an interaction of nitrogen with 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 [Marschner 2011]. There is generally a negative effect of preharvest high nitrogen supply on postharvest quality of plants. This may be attributed to cut flowers, too. Proton toxicity and physiological calcium deficiency may impair postharvest life following ammonium supply [Starkey and Pedersen 1997]; however, postharvest treatments of cut flowers with ammonium may respond different. Nitrogen forms have profound effects on phytohormone levels of plant tissues, as there is generally positive correlation between nitrate status and cytokinins in plant tissues [Rahayu et al. 2005]. There might be at least 1.5–3 time increase in vase life of cut flowers by exogenous application of cytokinins [Paull and Chantrachit 2001]. Nitrogen and cytokinins can delay plant tissue senescence in close or similar way. Benzyladenine in root application or as spray has improved the vase life of anthurium (*Anthurium andraeanum*), Heliconia (*Heliconia psittacorum* cv. 'Andromeda', *H. chartacea* cv. 'Sexy Pink'), red and red ginger inflorescence (*Alpinia* purpurata) [Paull and Chantrachit 2001]. Nevertheless, the plant response to cytokinins may be quite different, as vase life of different anthurium cultivars under benzyladenine treatment changes from a 20% reduction to a 2.5 fold increase [Paull and Chantrachit 2001]. Anthurium cultivars that responded positively to BA and were packed for 8 days had 20 days longer vase life than non-BA treated flowers [Paull and Chantrachit 2001].

In present study the companion ion may has also role in postharvest quality of cut flowers. In calcium nitrate and potassium nitrate, apart from nitrate, calcium and potassium are two major nutrient elements important for quality behavior of cut flowers [Sairam et al. 2011]. 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. On the other hand, potassium is the major nutrient element in many quality parameters of plant. It has direct and indirect roles in osmotic adjustment of plant cells particularly in root and vessel cells allowing the continuous movement of solutions within xylem sap [Marschner 2011].

CONCLUSION

In present study, ammonium sulfate, calcium nitrate and potassium nitrate in various concentrations of 0, 50, 100, 200, 500 mg dm^{-3} N had different effects on postharvest quality of chrysanthemum cut flower. Pretreatment of flower stems with different concentrations of nitrate salts showed improvement in flowers shelf life and quality. Shelf life of cut flowers was improved constantly by increasing nitrate form of both salts. Shelf life was also increased with pretreatment of ammonium sulfate of 50 and 100 mg dm^{-3} N but decreased by higher ammonium sulfate levels. The findings indicate that low concentrations of ammonium sulfate or moderate to high concentrations of calcium nitrate and potassium nitrate can improve chrysanthemum cut flower shelf life and quality.

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