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POSTHARVEST EXOGENOUS APPLICATION OF VARIOUS BACTERIAL STRAINS IMPROVES THE LONGEVITY OF CUT 'ROYAL VIRGIN' TULIP FLOWERS

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Tulip (*Tulipa gesneriana* L.) holds spectacular and colorful bright floral buds that are widely adorned like no other flower, across the globe. In cut flowers, persistent and prolonged display life is the matter of concern which decides quality index for florists and consumers. Endophytic bacteria, taken up by the plants when colonized, can act as sink for 1-aminocyclopropane-1-carboxylate (ACC), hence delay senescence. These bacteria, when used as suspension solution for cut flowers, increase the vase life manifolds which signify their application to commercial floriculture. Keeping in view, the exalted status and market value of cut tulips, an experiment was conducted to study the response of four bacterial strains viz. *Burkholderia phytofirmans* (PsJN), *Caulobacter* sp. (FA-13), *Enterobacter* sp. (MN-17) and *Bacillus* sp. (MN-54) along with control treatment (non-treated stems) in concentrated and diluted forms to estimate the postharvest longevity. 15 mL of suspension solution of bacterial culture was sprayed on each observational unit under controlled conditions in laboratory. In this experiment, (PsJN) in concentrated form, performed best and resulted in the longest vase life (11.2 d), delayed leaf yellowing (8.8 d), more water uptake (70.33 mL), less stem elongation (5.14 cm), more days to open flower bud (2.47 d), maximum flower diameter (50.79 mm) and . Whereas (MN-17) in concentrated form performed best in fresh and dry mass ratio and reduced stem bending. In conclusion, use of plant growth promoting bacteria (PGPB) is a useful tool in escalating vase life of cut tulips with improved floral attributes.

Keywords: PGPB, senescence, leaf yellowing, vase life, cut flower, water uptake

INTRODUCTION

Over the last few years, global exports of cut flower have grown exponentially, especially in the developing countries which are transforming into diversification strategy to change production of low value and traditional agriculture into high value non-traditional agriculture. One of the major reasons for this paradigm shift is irrational pricing pattern that jeopardize the earning of growers of small land holdings in particular (UNCTAD, 2008). Tulip as spring blooming bulbous plant, possess exorbitant charming buds in different hues and colors that has acquired significant stature in flower trade by gaining the status of a delicacy as a bedding plant in garden landscape (Bukhari, 2005) and as a cut flower. Tulip, being moderately ethylene sensitive has to cope with tepal senescence due to endogenous ethylene production which is a major factor in abridging display life and quality. Although concentration of endogenously produced ethylene in tulips is meager, even this low concentration pacifies rapid senescence of tepals. Senescence is exceptionally a regulated process having an effect on single cells, organs and the whole plant which eventually leads to death during the developmental stages of plant species that enables them to survive (Lim *et al*., 2007). It may occur at final stage in leaf development and precedes cell death. This process is initiated by chloroplast degeneration, followed by the catabolism of macromolecules, such as nucleic acids, proteins, and lipids and eventually the degradation of mitochondria and nuclei. The released nutrients are transferred to actively growing young leaves and developing fruits and seeds, leading to increased reproductive success (van Doorn and Woltering, 2004; Rogers, 2006).

Postharvest life of flowers must be endowed with prolonged freshness to fetch consumer appreciation and acceptance in local as well as global cut flower market (Collier, 1997). They are promptly sensitive, as their degradation starts soon after detachment from parent plant because of induced senescence by ethylene (Figueroa *et al*., 2005). Plant growth-promoting bacteria are recognized to slow down leaf yellowing and control many factors of cut flowers such as breaking dormancy, delaying senescence and ethylene activity as well as quality flower initiation and improved quality traits (Hunter *et al*., 2004). Certain bacterial strains are biocontrol agents, capable of killing other microorganism present in the vase solution. Plant growth-promoting bacteria (PGPB) have

substantial tendency to slow down the senescence of cut flowers and acts as an anti-aging (Nayani *et al*., 1998). ACC deaminase-containing PGPB can sequester and subsequently cleave ACC, thus preventing detrimental concentrations of ethylene to accrue inside plant tissues (Ali *et al*., 2012).

Global cut flower industry is currently using unsafe, potentially harmful and expensive chemicals to inhibit ethylene production for enhancing the display life of cut flowers. In this regard, use of plant-growth-promoting-bacteria (PGPB) that exhibit ACC deaminase activity is an effective replacement for the chemicals that are frequently used in cut flower industry these days (Ali *et al*., 2012) while substantial efforts have been applied in developing protocols for postharvest treatments to ameliorate this issue (Khan *et al*., 2006). Keeping in view the potential of tulips as an unaccustomed crop in Pakistan among the grower community, an experiment was conducted to envisage the response of different bacterial strains in concentrated (10^8 CFU mL^{-1}) and diluted forms (10^7 CFU mL^{-1}) to assess the postharvest longevity.

MATERIALS AND METHODS

***Bacterial strains and plant material*:** Four pre-isolated endophytic bacterial strains viz. *Burkholderia phytofirmans* strain PsJN, *Caulobacter* sp. strain FA-13, *Enterobacter* sp. strain MN-17 and *Bacillus* sp. strain MN-54 were obtained from prepared culture medium containing 10% TSB (Tryptic Soy Broth) in concentrated form (10^8 CFU mL^{-1}) and diluted form (10^7 CFU mL^{-1}) from Environmental Sciences Lab. Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. Cut stems of tulip of 'Royal virgin' cultivar, grown in open field were harvested at tight bud stage early in the morning and shifted to Postharvest and Floriculture Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. Upon arrival, basal part of the stems was cut 1 inch to avoid air embolism along with a basal leaf to fit the stem into the glass vessel. After placing single stem in the vessel containing 300 mL of distilled water (DW), each stem was sprayed with 15 mL of bacterial strain solution, according to the treatments, for the evaluation of display life. The glass vessels containing cut stems were placed on the shelves in the Lab. having temperature, relative humidity and light intensity at 23±2°C, 60±10% and photosynthetically active photon flux of 12 µmol m^{-2} s^{-1} with 12 h photoperiod from cool white florescence tubes respectively was maintained.

***Measurements*:** Data were collected for various parameters like vase life (duration from keeping the stems in DW in Postharvest floriculture Lab. to the time when individual cut stem was ended), days to start senescence, days to start leaf yellowing, water uptake (measured in mL from vases when first cut stem was ended), stem bending, stem elongation, fresh mass, dry mass, fresh and dry mass ratio, bud opening, flower diameter and flower quality. Criterion for ending included necrosis and wilting of petals and bending of stems

***Statistical analysis*:** Experiment was designed according to Completely Randomized Design with three replications, having five observational units in each treatment. The data collected were statistically analyzed by using Tukey's HSD of variance technique, and treatment means were compared at 5% level of probability.

RESULTS

***Vase life (days)*:** Vase life is an essential aspect to evaluate the quality of cut flower. It is the time duration, in which any cut stem or foliage retains its undeniable appearance in vase. Foliar application of PsJN, followed by MN-54 in concentrated form, performed best and resulted in maximum vase life (11.2 d) and (9.53 d), respectively (Fig. 1). In cut tulips, days to start senescence, an attribute for assessing the longevity of cut stems, was delayed when concentrated PsJN was applied. It supported to maintain the tulip quality and appearance by providing maximum days (12.1 d) of healthy stems, followed by concentrated MN-54 which delayed senescence for 9.53 days (Fig. 2).

***Leaf yellowing (days)*:** It is hastened in cut stems, when placed in the environment favoring less photo-respiration process or with more ethylene production. This phenomenon was detained up to (8.8 d) when PsJN in concentrated form was used, while concentrated MN-54 on an average hampered this process up to (7.73 d) (Fig. 3).

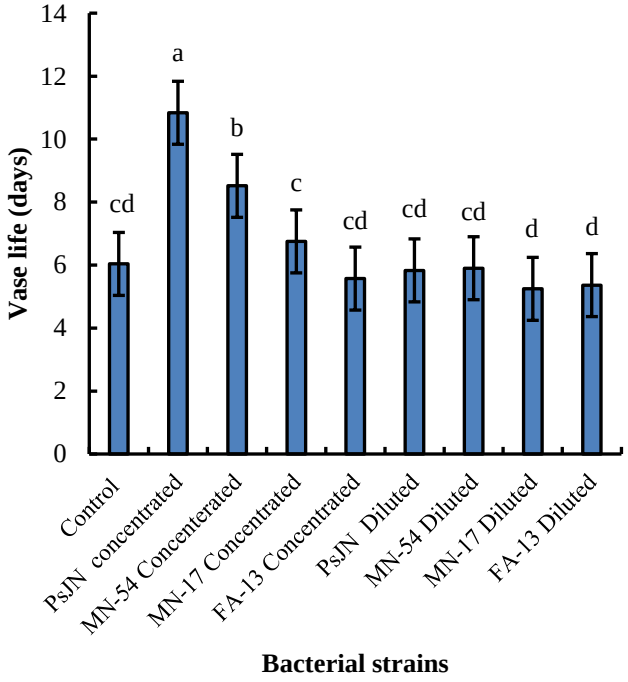

Figure 1. Effect of bacterial strains on vase life of cut tulips

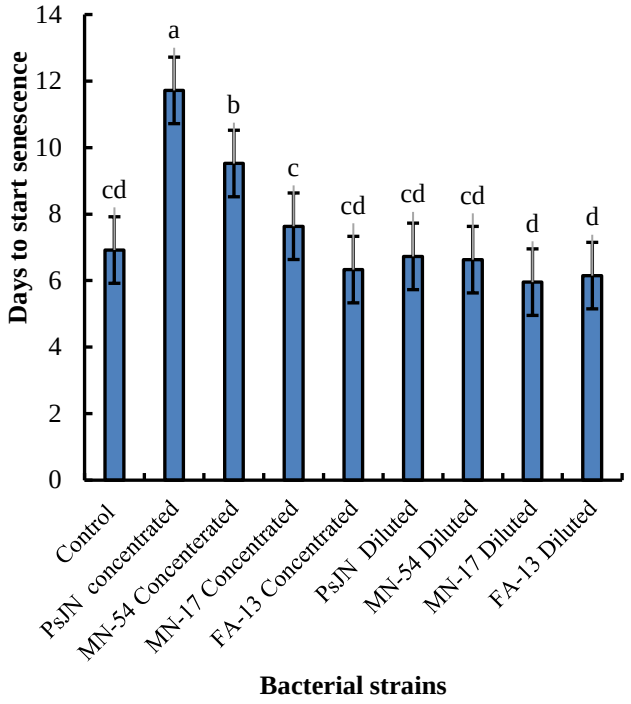

Figure 2. Effect of bacterial strains on days to start senescence of cut tulips

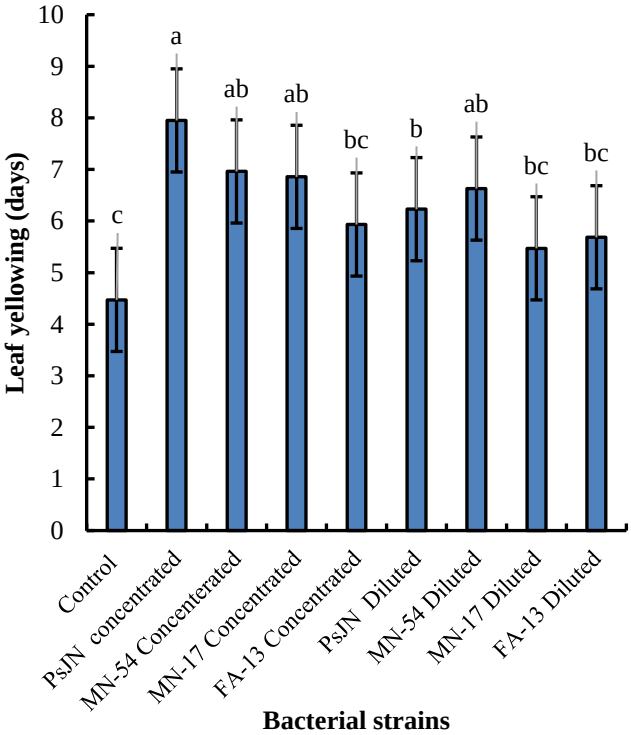

Figure 3. Effect of bacterial strains on days to leaf yellowing of cut tulips

***Water uptake (mL)*:** Lower water uptake may be due to the stem blockage caused by microbe accumulation. Maximum water uptake (70.33 mL) was recorded when PsJN in concentrated form was applied, which helped the cut stems to maintain its turgidity and fresh appearance. It was followed by MN-54 in concentrated form, where water uptake of 9.53 mL on an average was recorded (Fig. 4).

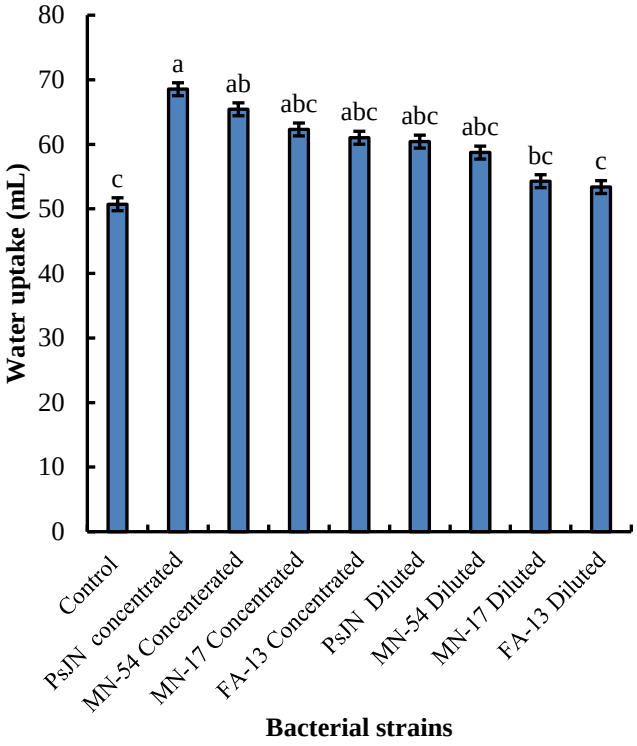

Figure 4. Effect of bacterial strains on water uptake of cut tulips

***Stem elongation (cm)*:** Cut stems became scraggy and start wilting, that happens due to higher rate of transpiration than that of the uptake volume. Foliar application of PsJN, followed by MN-54 in concentrated form resulted in minimum stem elongation (5.14 and 9.84 cm elongated tulip stem respectively) (Fig. 5).

***Bud diameter (mm)*:** In cut flower trade, bud diameter play decisive role regarding quality attributes. Maximum bud diameter of 50.79 mm was retained when PsJN in concentrated form was exogenously applied followed by concentrated MN-54 where flowers attained bud diameter of 45.05 mm (Fig. 6).

Cut tulips show a physiological disorder of stem curving or bending to varying degree of angle after harvest, if placed horizontally or placed without certain vase solution for a certain time period. Minimum stem bending was observed in MN-54 in diluted form (5.13˚), which was followed by concentrated FA-13 which resulted in maximum stem bending (10.3˚) but the treatments remained non-significant.

However, maximum fresh and dry mass ratio (9.74) was recorded by use of concentrated MN-17 spray and the results were non-significant to all other treatments. PsJN in concentrated form allowed the floral buds to open after a few more number of days (2.47 d) followed by (FA-13 1.40 d), where all the treatment results were non-significant.

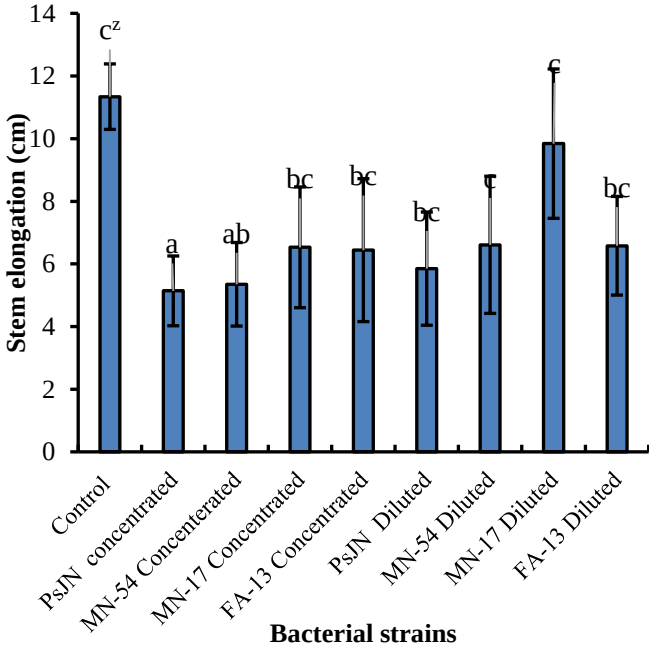

Figure 5. Effect of bacterial strains on stem elongation of cut tulips

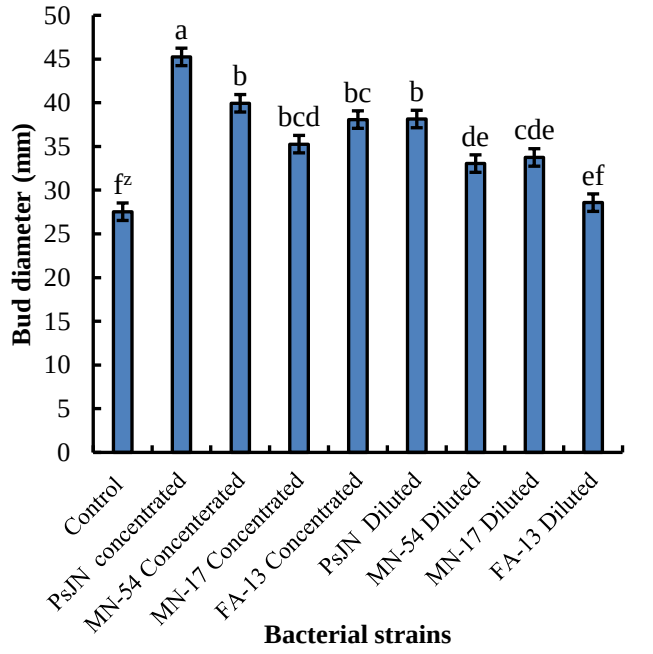

Figure 6. Effect of bacterial strains on bud diameter of cut tulips

DISCUSSION

Our findings confirm that exogenous application of various bacterial strains induced unswerving impact on improving the longevity of cut tulips. Current findings suggest that PsJN in concentrated form had statistically perked up the vase life up to 11.2 days. This might have had been due to ACC deaminase activity exhibited by the bacterial strains. This aspect has been reported by Bandra *et al*. (2006) to be possessed by various other microbial species of endophytes, rhizobia, and fungi. This typical feature is also widely observed in strains of "PGPB" like *Azospirillum lipoferum*, alcaligenes, bacillus, burkholderia, enterobacter, pseudomonas, rhizobium, rhodococcus, and *Variovorax paradoxus* (Glick *et al*., 1998). On the other hand, as postharvest handling of cut flowers is an arduous process affected by many physiological, biotic and abiotic factors that trigger senescence (Reid and Jiang, 2012) must undergo ambient postharvest measures. These findings portray improved vase life in terms of longevity which is a key factor for assessing the quality and standard of cut flower from florist as well as consumer point of view. Moreover, shorter display life, early senescence and bent neck which are the major glitches of cut tulips usually cause abated value (Collier, 1997) were tweaked by exogenous application of the bacterial strains used in this study. They might have played their role in curtailing rapid transpiration, wilting or lower water uptake beyond the homeostatic levels.

Similarly, leaf yellowing was also found to be commuted by the exogenous application of PsJN strain which sustained the freshness of foliage to 8.8 days. In case of tulips, both, the flower bud and foliage must complement each other for persistent freshness which is an essential feature of commercial importance to determine the quality gain and significant position (van Doorn *et al*., 2011; Ezhilmathi *et al*., 2007). The diminished leaf yellowing by the PGPB might be due to cleaving of ACC into "alpha-ketobutyrate" and NH_4 ions which helps in reducing the levels of ethylene in ethylene generating and ethylene sensitive parts of the plants (Glick *et al*., 2007). Although, tulip flowers, being moderately ethylene sensitive (Chrysal International, 2010), and its production is influenced by number of physiological factors (Penrose and Glick, 1997) might have been impeded and resulted in ameliorating display life. While, senescence of tepals and leaf yellowing, which is usually followed by abscission (Iwaya-Inoue and Nonami, 2003) was also delayed by the application of bacterial strains.

Similarly, the genus bacillus and pseudomonas are reported to increase the water uptake in chrysanthemum (Kumari *et al*., 2016). In our findings, PsJN resulted in maximum water uptake to 70.33 mL that maintained the turgidity of leaves as well as the tepals, abridging the limping of leaves and flower buds. These PGPB possess ACC deaminase enzyme which play role in delaying senescence and hinders stem bending as

reported in cut carnation (Nayani *et al*., 1998). ACC deaminase-containing bacterial strains successfully delay the flower senescence of ethylene sensitive and moderately sensitive flowers (Ali *et al*., 2012) as reflected in our findings. Postharvest stem elongation which is a characteristic feature of cut tulips coined with stem bending causes dearth in display life and early leaf yellowing (Ferrante *et al*., 2003) was also found to be foreshortened by PsJN application. Usually high rate of stem elongation in cut flowers results in stem bending during display. In majority of the cut flowers, it is mainly associated with the presence of auxins and gibberellins as these growth hormones counteract antagonistically, whereas ethylene production which hastens it (Ferrante *et al*., 2003) might had been in perpetuated limits that retained the actual plant structure to a certain extent.

"PGPB" possessing ACC deaminase activity may help as a valuable replacement of the chemicals like Silver thiosulphate (STS), L-α-(aminoethoxyvinyl)-glycine (AVG) and cyclic olefin norbornadiene (NBD) which is commonly used by the cut flower traders. These compounds are not only carcinogenic but also pose serious environmental threats (Ali *et al*., 2012). On commercial scale, growers use ethephon compounds to inhibit stem elongation but their use result in poor flower opening and deteriorated quality. A finding by Nichols and Kofranek (1982) revealed reverse effect of ethephon by the use of STS (silver thiosulphate), which blocked ethylene receptors, thus indicating ethylene as an active compound in tulips. Similarly, in another finding when cut stems of carnation flowers treated with wild type "PGPB" (*P. fluorescents* strain (YsS6) and *P. migulae* strain (8R6) were treated it showed longer vase life as compared to the flowers treated with AVG (Ali *et al*., 2012) by modulating ethylene synthesis.

It is worth mentioning, some commercially important cut flowers such as, rose, carnation, geranium and zinnia exhibit high level of sensitivity to ethylene while moderately sensitive flowers include (tulip, narcissus, chrysanthemum and anemone), treating all of these flowers with naturally occurring "PGPB" endophytes may enhance their vase life / display life and may attribute to floral diameter to certain extent avoiding the use of potentially harmful chemicals (Woltering and Van Doorn, 1988).

Hence it can be concluded from the above mentioned study that PGPB are not only helpful through their beneficial association when the plants are in soil, but also effective when sprayed on flowering stem and are useful tool for increasing the display life and quality of cut flowers. PsJN in concentrated form proved to be the best in improving quality attributes of cut tulips which extended the vase life for (5.2 d) compared with control while other PGPBs improved flower size, scape length and postharvest attributes.

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