



Pre-harvest exogenous application of bacterial strains to assess the flower and bulb quality of cut Tulip (*Tulipa gesneriana* L.) Cv. Clear Water

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This study was planned to investigate the effect of different beneficial microbes to evaluate the quality of flower and bulb of cut tulips (*Tulipa gesneriana* L.) cultivar 'Clear Water' under the environmental conditions of Faisalabad during 2016. Sowing of bulbs was carried out in an open field according to Randomized Complete Block Design (RCBD) having five treatments, significance difference among treatments were tested by using Tukey's test at 5% level of significance among variables. Bacterial strains (treatments) were exogenously sprayed in equal quantity (10^8 CFU mL⁻¹) for treated bulbs and were replicated thrice, T₀ considered as control (no application), T₁*Burkholderia phytofirmans* (PsJN), T₂*Bacillus* sp. (MN-54), T₃*Enterobacter* sp. (MN-17) and T₄*Caulobacter* sp. (FA-13). The results revealed that tulip responded well to bacterial strains and significant improvement was observed in morphological attributes, bulb attributes and other quality parameters. T₁ treatment proved to be the best one regarding morphological and floral traits from commercial point of view. The highest values of plant fresh mass, leaf chlorophyll content, leaf area, flower diameter, scape length, vase life, no. of bulbils and bulbils diameter were observed maximum as compared to control.

INTRODUCTION

Being commercially important ornamental bulbous cut flower, tulip is grown for its huge diversity present in its color, shape and variety. Tulip (*Tulipa gesneriana* L.) is temperate ornamental geophyte, belongs to the family, Liliaceae, grown in beds, borders and pots for garden landscaping and cut flower for aesthetic purpose as well as commercial use. Tulip is popular among top cut flowers due to its conspicuous look and long vase life and always preferred in the global floriculture market (Sajjad *et al.*, 2014). It is known as queen of bulbs owing to its wide range of classes, vibrant colors and shapes. It dominates 3rd rank among top most cut flowers in the world flower market (Anonymous, 2014). In global floriculture world The Netherlands is the dominant grower of tulip bulb production and contribute about 85% (de Hertogh and le Nard, 2012). Pakistan is producing cut flower about 10-12 thousand tons per annum and Pakistan has best climatic and geographical location for the cultivation of cut flower on commercial scale (Bukhari, 2005). Demand of fresh flower is increasing consistently day by day in national market as well as in world market (Labaste, 2005). Consumption of this flower has augmented in most of religious and social events like Christmas, Valentine day, Mother's Day and Happy

New Year festivities (Van Doorn, 1994). Extensive use of tulip in veiled garden landscaping depicts the significance of this flower (Rehman, 2004).

Extended vase life is one of the most indispensable factor that evaluate the quality of cut flowers. Short blooming period, scape length and vase life are one of the major hassle in tulip production that reduce the standard quality of cut flower (Kumar *et al.*, 2013). Being an ethylene sensitive cut flower, tepal senescence in tulip is responsible for the decline of quality and vase life of the flowers (Collier, 1997). Such changes have ominous impact upon its aesthetic look and commercial importance. Ethylene production leads to flower degradation in terms of flower senescence, loss of bright color and shortening of petals life (Jiang, 2002). Postharvest senescence is a major limitation to the marketing of many species of cut flowers and considerable efforts has been devoted to developing postharvest treatments to maintain the aesthetic value and also extend the marketing period. The additions of these bacterial strains are recommended to extend the vase life of cut flowers.

Endophytes, plant growth promoting microbes are beneficial bacteria that increase plant biomass. They stimulate plant tolerance and resistant to environmental stress and denigrate the influence of soil-borne problems (Nelson, 2004). They include an enzyme ACC deaminase that minimizes the ethylene concentration by hydrolyzing ACC into a-ketobutyrate and ammonia (NH₃), (Glick *et al.*, 1998).

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ACC is the immediate precursor of ethylene, therefore, decreasing the levels of ACC will decrease the levels of ethylene and inhibit the growth reduction effect of this plant hormone (Van de Poel and Vander Straeten, 2014). Several endophytes are reported as beneficial bacteria which can be pragmatic as inoculant of bio-fertilizers (Kennedy *et al.*, 2004). Application of microbes is fundamental part of ecological friendly environment and sustainable organic agriculture (Bloemberg *et al.*, 2000). Past findings have demonstrate the remarkable effect of microbes in Gladiolus production as the foliar application of different endophytes like Azotobacter and Azospirillum had ameliorate the morphological and floral traits significantly as compared to control in gladiolus (Srivastava and Govil, 2005). They are useful microbes that are competent to ameliorate plant growth through proper supply of minerals and support to sustain the fertility status of soil (Esitken *et al.*, 2005). Inoculation of plants with PsJN shows maximum number of tiller and tiller height which leads to higher yield in switch grass (Ker *et al.*, 2012). They exert advantageous effect on plant growth and development by altering the root architecture and regulating the production of several plant hormones include Indole acetic acid (IAA), gibberellic acid and cytokinins (Kloepper *et al.*, 2007). They are capable to solubilize mineral phosphate and enhance the availability and supply of phosphorus for effective plant growth. Under field condition, Plants growth promoting bacteria (PGPB) have been reported to solubilize precipitated phosphates to plants (Verma *et al.*, 2001).

Therefore, present study was designed to assess the impact of different bacterial strains application for enhancing the vast life and quality of *Tulipa gesneriana* in a sustainable agricultural production system.

MATERIALS AND METHODS

The experiment was conducted at Floriculture research Area in an open field, Institute of Horticultural Sciences, University of Agriculture, Faisalabad (latitude 31°30 N, longitude 73°10 E and altitude 213 m). Pre-cooled bulbs of a single cultivar of tulip 'Clear water' were planting at the distance of 15 x 15 cm on raised bed at the depth of 3 inches in the month of November, 2015. Prior to planting in the soil, bulbs were treated with fungicide Topsin-M 70WP @ 1 mgL⁻¹ of water (Granneman, 2016). Basal dose of NPK (17:17:17) 250 grams 10 gm⁻² (Ali *et al.*, 2014) were applied by broadcast method to fulfill the nutritional requirements of plant. Five treatment plan with three replication was designed in this experiment and was laid out under Randomized complete block design (RCBD). Treatments were sprayed twice in the growing cycle of plant once at two leaf stage and second at tight bud stage. All treatments were assigned after randomization with following treatment combinations for foliar application of bacterial strains. The soil characteristics of experimental field were clay loam to silt clay, pH 6.83 and EC 0.38 dS m⁻¹ with adequate drainage and water holding capacity.

Observation

Data was collected from twenty plants randomly selected from each replication. Plant fresh mass was measured on digital balance and their average was calculated. Plant dry mass (g) was measured after chopping the plant into pieces and were packed in paper bags. They were placed in oven for drying (Memmert-110, Schawabach) at 72°C for 72 hr. Leaf area was calculated from fully mature basal leaves with the help of formula devised by Kemp (1960) for monocots: Leaf area = Maximum length × maximum width × 0.68.

Treatment description

T ₀	Control
T ₁	<i>Burkholderia pytofirmans</i> (PsJN)
T ₂	<i>Bacillus</i> sp. (MN-54)
T ₃	<i>Enterobacter</i> sp (MN-17)
T ₄	<i>Caulobacter</i> sp. (FA-13)

Vegetative attributes:

- Plant Fresh mass (g)
- Leaf area(cm²)
- Scape length (cm)
- Dry mass (g)

Flower attributes:

- Flower diameter (mm)
- Bud diameter (mm)
- Scape diameter (mm)
- Tepal diameter (mm)

Postharvest attributes:

- Vase life (days)
- Days to start Tepal Abscission (days)
- Days to start flower senescence (days)

Physiological attributes:

- Leaf Chlorophyll contents (SPAD value)

Bulb attributes:

- Mass of bulbils per clump(g)
- Number of bulbils per plant
- Diameter of bulbils per clump (mm)

Experimental Design and Statistical Analysis

Experimental unit was executed according to RCBD (Randomized complete block design). Data was conducted statistically by using analysis of variance (ANOVA). Difference among the means was compared by applying Tuckey HSD test at level of $\alpha = 0.05$ by using Statistix 8.1 computer software (Steel *et al.*, 1997).

RESULTS

Vegetative attributes

Results indicated significant ($P \leq 0.05$) differences among treatments means regarding plant fresh mass, dry mass, scape length and leaf area. Response of all treatments was found significantly difference. Highest plant fresh mass, dry mass, scape length and leaf area were recorded in T₁ treatment followed by T₃, T₂ and T₄ while least score of these observation was observed in T₀ (control). Maximum dry mass, plant fresh mass and leaf area was seen in T₁ treatment followed by other treatments while minimum value was measured in T₀ control.

Floral attributes

Significant difference was also observed in scape diameter and flower diameter. Mean comparison of treatments presented the dominance of T₁ over all the other treatments by giving maximum scape diameter and flower diameter. In contrast, the minimum scape diameter and flower diameter was recorded in T₀ and placed in lowest order. Results shows that treatments are highly significant with respect to scape diameter and flower diameter of tulip statistically and these attributes increased with the exogenous application of *Burkholderia pytofirmans* (PsJN) that

Table 1 Effects of different Bacterial strains on scape length, fresh mass, and stem diameter of Tulip flower, Clear water cultivar

Scape Length (cm)	Fresh Mass (g)	Scape Diameter (mm)	Flower Diameter (mm)
T ₀	31.017c	30.944c	5.6161b
T ₁	45.178a	41.822a	7.4544a
T ₂	35.893c	37.295b	6.5372a
T ₃	38.358bc	34.030bc	6.1251ab
T ₄	40.376b	33.702bc	6.3975ab

T₀=control, T₁= PsJN, T₂= MN-54, T₃=MN-17, T₄=FA-13**Table 2** Effects of different Bacterial strains on dry mass, flower diameter, and final scape length of Tulip flower, Clear water cultivar

Plant Dry mass (g)	Bud diameter (mm)	Final scape length (cm)
T ₀	2.7822c	26.67c
T ₁	4.6144a	33.65a
T ₂	3.6657b	31.02b
T ₃	3.4039bc	32.14ab
T ₄	3.0254bc	30.96b

T₀=control, T₁= PsJN, T₂= MN-54, T₃=MN-17, T₄=FA-13**Table 3** Effects of different Bacterial strains on Days to start Senescence (Days), Tepal Abscission (Days), SPAD Value and Vase life of Tulip flower, Clear water cultivar

Days to flower Senescence	Tepal Abscission (Days)	SPAD Value	Vase life
T ₀	7.24c	9.51c	40.544c
T ₁	13.24a	13.75a	50.252a
T ₂	11.24b	11.63bc	46.961b
T ₃	13.13a	13.41a	48.172ab
T ₄	11.04b	11.33bc	44.161b

T₀=control, T₁= PsJN, T₂=MN-54, T₃=MN-17, T₄=FA-13**Table 4** Effects of different Bacterial strains on Days to floral bud emergence, Length of Tepals (mm) and Width of Tepals (mm) of Tulip flower, Clear water cultivar

Days to flower bud emergence	Leaf Area (cm ²)	Tepals diameter (mm)
T ₀	49.500d	73.026d
T ₁	63.500a	93.396a
T ₂	57.500b	86.597ab
T ₃	54.500bc	80.900cd
T ₄	55.000bc	84.548bc

T₀=control, T₁= PsJN, T₂= MN-54, T₃=MN-17, T₄=FA-13**Table 5** Effects of different Bacterial strains on Bulbils Diameter (mm), No of Bulbils/per plant and Mass of Bulbils, Clear water cultivar

Diameter of Bulbils (mm)	No of Bulbils per plant	Mass of Bulbils per clump (g)
T ₀	12.68d	2.30d
T ₁	25.71a	4.05a
T ₂	20.87b	3.62ab
T ₃	19.50b	3.20bc
T ₄	15.25c	3.15c

T₀=control, T₁= PsJN, T₂= MN-54, T₃=MN-17, T₄=FA-13

advocated the importance of T₁ for floral attributes. Same pattern was also observed in case of flower bud diameter and tepal diameter. Maximum flower bud diameter and tepal diameter were noticed in T₁ followed by T₃, T₂ and T₄ while least value was seen in T₀ control. There was significant difference ($P \leq 0.05$) among treatments pertaining days to flower bud emergence. Maximum score of days to flower bud emergence was seen in T₁ followed by T₂, T₄ and T₃ while the minimum value was measured in T₀ control.

Physiological attributes

Chlorophyll is a critical biomolecule that responsible for the proper food synthesis, growth and development of plant. Results indicated the highest leaf chlorophyll content was achieved in T₁ followed by T₃, T₂ and T₄. Least leaf chlorophyll content was found in T₀ control. There was significantly difference among treatments regarding leaf chlorophyll content (SPAD Value).

Postharvest attributes

Post-harvest response of treatments was significantly different ($P \leq 0.05$) regarding vase life, days to start flower senescence and tepal abscission. Vase life is critical period during in which cut flower retains its appreciable appearance and freshness. Highest vase life, days to start senescence and tepal abscission were noticed in T₁ followed by other treatments while T₀ control exhibited the minimum vase life, days to start senescence and tepal abscission.

Bulb attributes

Bulb attributes are significant to evaluating the potential of underground storage organs that are indicator of ultimate value in geophytes. Data showed significant difference ($P \leq 0.05$) among treatment means. Comparison of mean indicated the highly significant superiority of T₁ in mass of bulbils per clump and bulbils diameter followed by the T₂, T₃, and T₄ while the lowest observation for both measured traits was

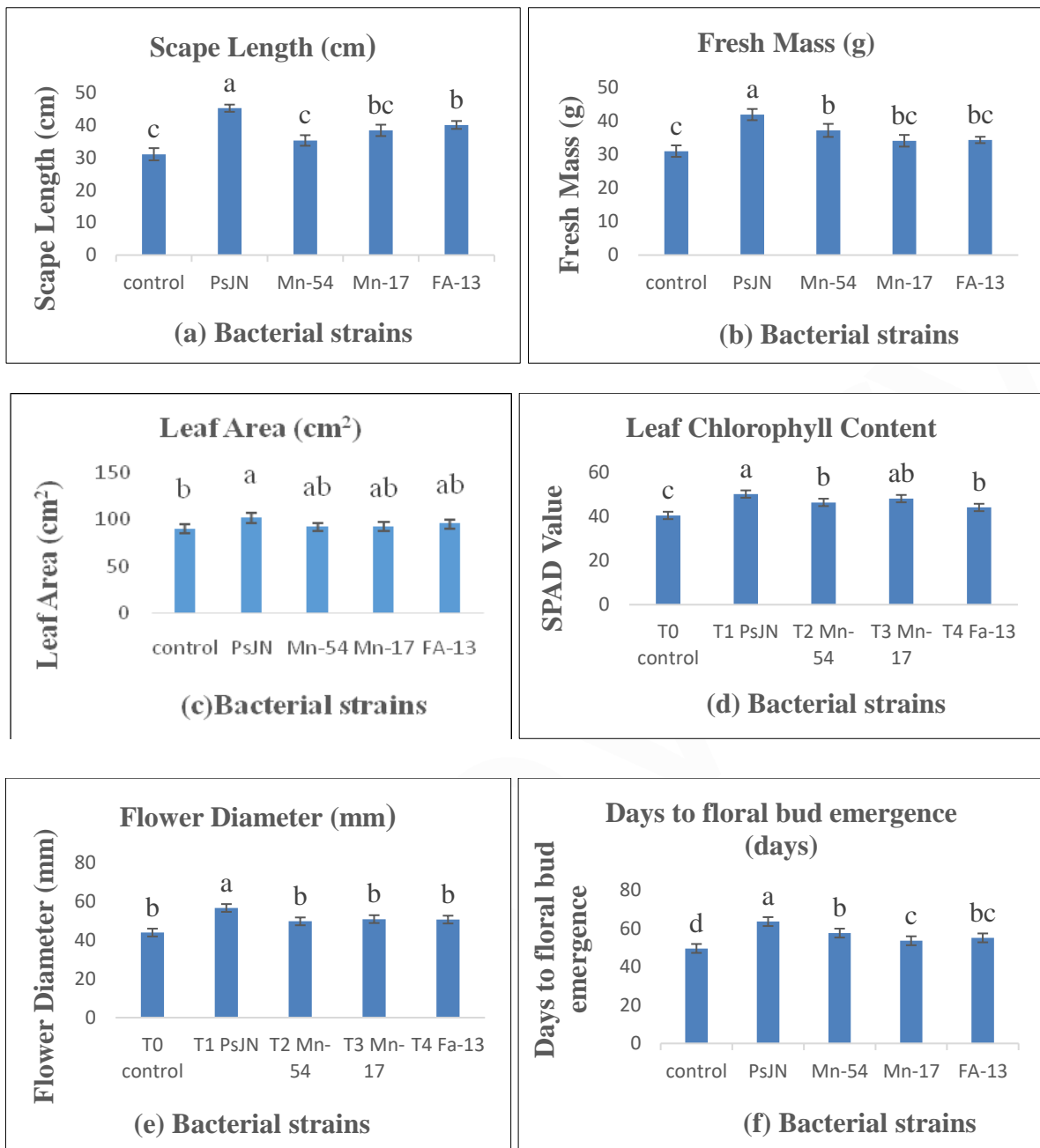
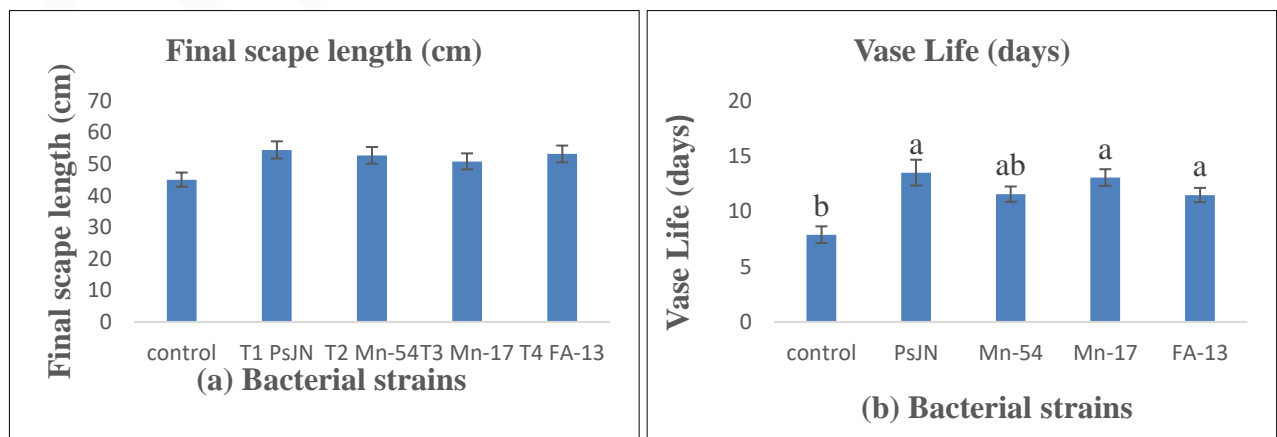


Figure 1 Graphical presentation regarding application of bacterial endophytes on (a) scape length (cm), (b) plant fresh mass (g), (c) leaf area (cm²), (d) leaf chlorophyll content (SPAD), (e) Flower diameter (mm) and (f) Days to flower bud emergence (days).



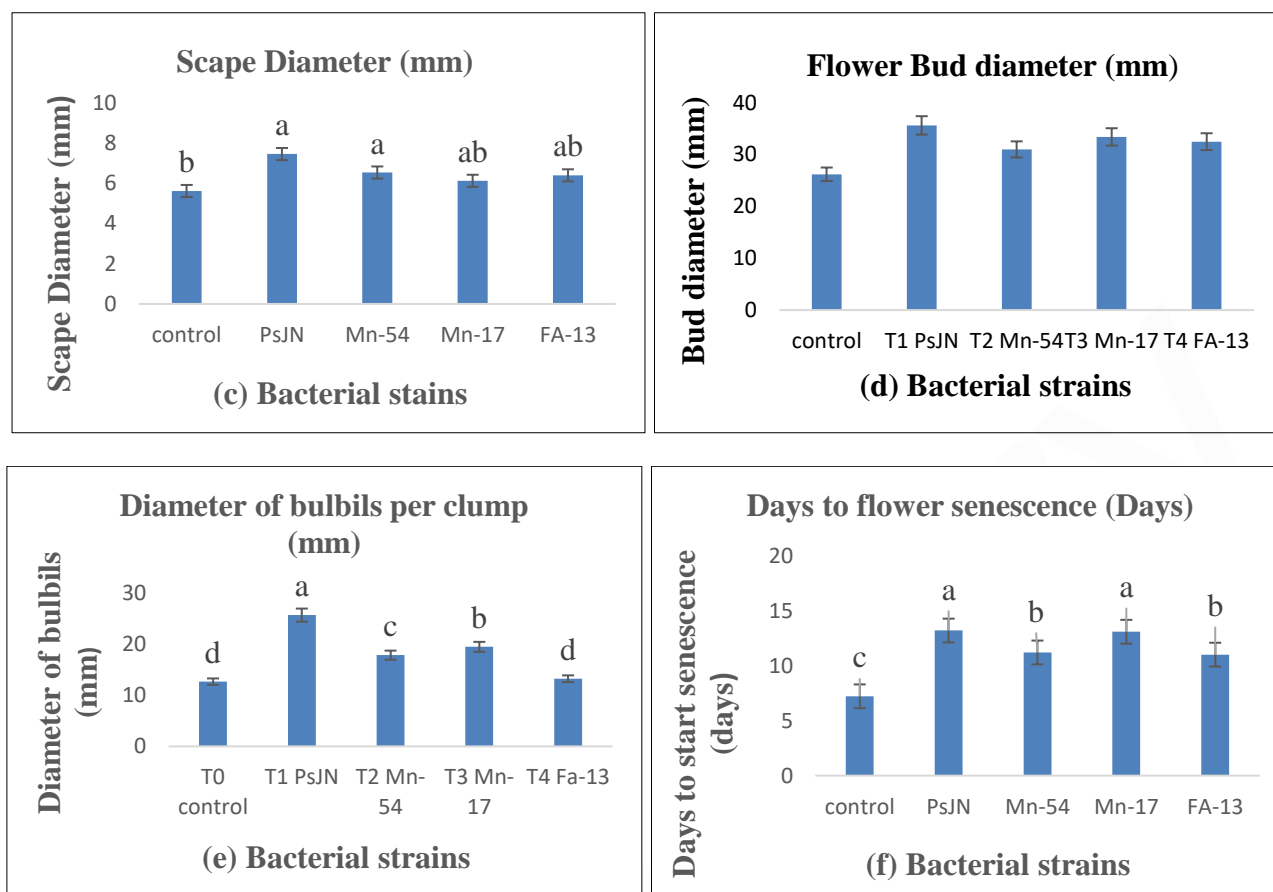


Figure 2 Graphical presentation regarding application of bacterial endophytes on (a) Final scape length (cm), (b) Vase life (days), (c) scape diameter (mm), (d) Bud diameter (mm), (e) Diameter of bulbils (mm) and (f) Days to start senescence (days)

observed in T₀ control. Significant variation was also examined in number of bulbils per clump case and findings clearly confirmed the dominance of T₁ over other treatments followed by T₃, T₂ and T₄ while minimum number of bulbils was recorded in T₀ control.

DISCUSSION

Present study exhibited the significant variation among treatment means in different morphological, floral and bulbous attributes. In global floriculture trade scape length and scape diameter are the most imperious criteria indicating the quality of cut flower. Experimental evidences indicate the appreciable increase in scape length advocated the significance of different bacterial endophytic strains. Foliar application of bacterial strains accelerates the plant height by stimulating the synthesis of various plant growth regulators (PGPRs). They promoted cell division and cell expansion that depicts the scape elongation. Endophytes are prominent to regulate plant growth and development through enhancing nutrients availability, physiological modifications and phyto-hormone regulations (Mei and Flinn, 2010). Bacterial strains improve the metabolic processes and enhance the availability and supply of NP and K nutrients. T₁*Burkholderia pytofirmans* PsJN gave the maximum value of scape length while T₀ control gave the minimum value as compared to other treatments.

Plant fresh mass describes the actual mass of living matter along with absorbed water contents that are physically bound with biomass. Quantity and significant standard of quality of living matter is depicted by plant fresh mass. Exogenous application of bacterial strains exhibited positive response on plant fresh mass. Application of endophytic

bacterial strains promotes the carbohydrates synthesis that leads to the vigorous biomass of plant. More the protoplasmic development more will be the fresh mass of plant, hence improve the quality. Endophytes stimulate the production of phytohormones (Auxins, gibberellins, ethylene, cytokinins and Abscisic acid) that are chemical messengers which improve the plant growth and development by regulating the process of organogenesis, cell division, expansion and differentiation (Ryu and Patten, 2008).

Plant growth can be analyzed in terms of an increase in total plant dry mass and its allocation among organs involved in acquisition of above or below ground resources. Presence of constitutive organic molecules like proteins, fats and carbohydrates of a living is a dry mass. Plant beneficial bacteria are efficient to employ advantageous impact on the plant growth and biomass (Nelson, 2004).

Leaf area is a dimensionless variable and depends upon the sufficient nutrient supply, availability and photosynthetic rate. Highest value was observed in Leaf area, flower bud diameter and flower diameter by the application of T₁ as compared to other treatments. Bacterial strains enhance the photosynthetic rate in plants (Ladwal et al., 2012). They stimulate auxins and gibberellins that enhance the leaf expansion.

Flower size and tepal diameter are important factor that decides the value of cut flower. The calyx and corolla together make up the perianth which is termed as tepal in tulips (Hovarh, 1991). Flower induction is a complex systemic process that regulated by numerous plant promoters and inhibitors that are triggered by various endophytes e.g *Burkholderia* species. Value of flower size depends upon the genetic variability of a

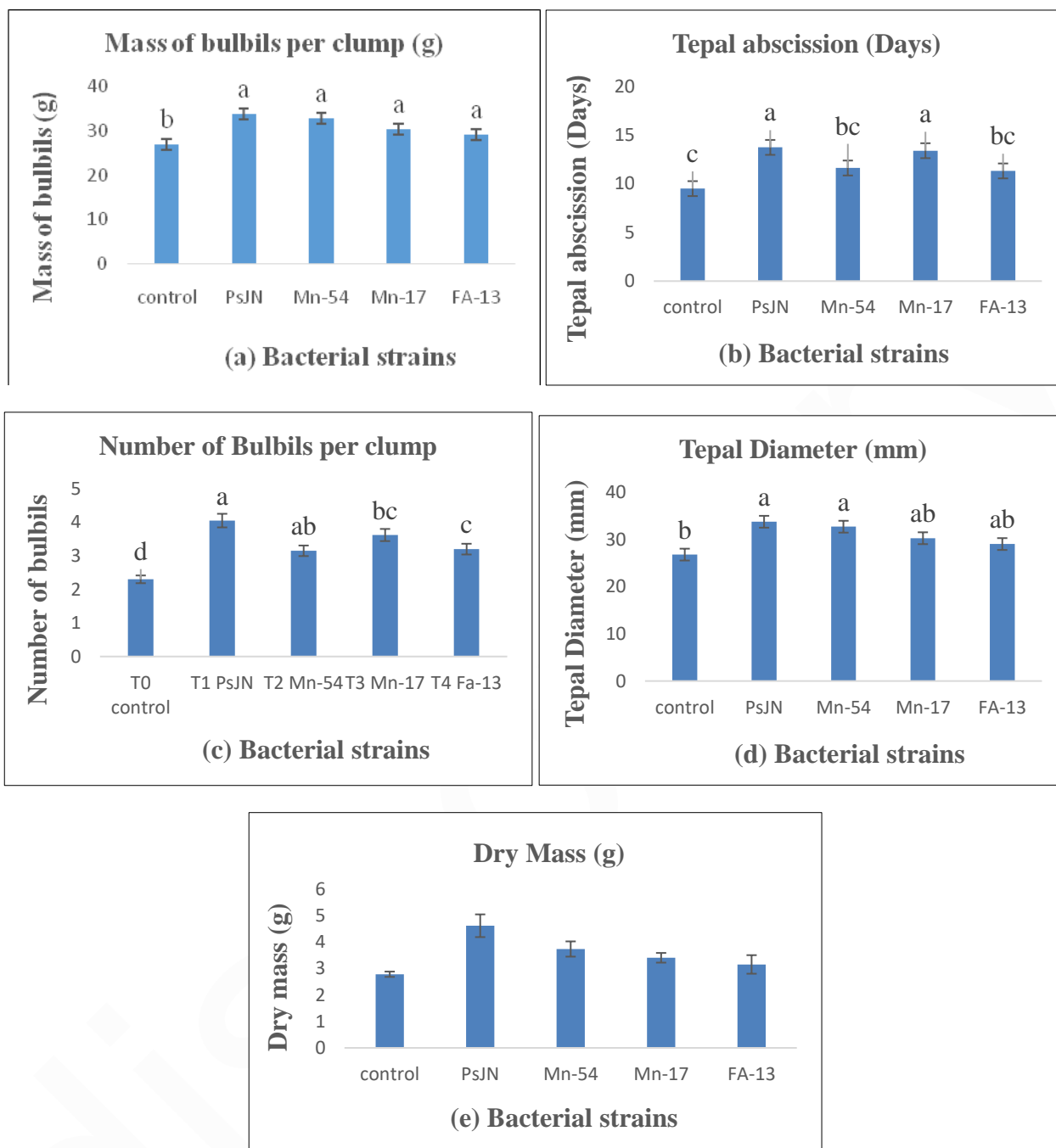


Figure 3 Graphical presentation regarding application of bacterial endophytes on (a) Mass of bulbils per clump (g), (b) Days to start tepal abscission (days), (c) Number of bulbils per clump, (d) Tepal diameter (mm) and (e) Dry mass (g)

plant and market demand. Plants growth promoting bacteria stimulate the various metabolic process include nitrogen fixation, phosphorus solubilization, minimizing the ethylene concentration through enzymatic activity and production of plant hormone in leaves (Kloepper et al. 1989).

Chlorophyll pigment is one of the responsible for photosynthetic activity of plant tissues and maintenance of chemical reactions that necessary for maximum photosynthetic capacity of plants (Wright and Nageswara, 1994). T₁ treatment gave maximum leaf chlorophyll content (SPAD value) by accelerating the process of photosynthesis, and carbohydrates level in plant leaves as compared to the rest of treatments.

Phosphorus solubilizing bacteria (PSB), *azotobacter*, *Rhizobium* and *Azospirillum* exert advantageous effect on life cycle and flowering of *Gladiolus* (*Gladiolus grandiflorus*) Ahmad et al. (2013).

Cut flowers usually carry enough energy in stem to develop completely and therefore, they do not depend on the food produced in the vegetative part of the plant (Bryan, 1989). Physiological and other metabolic processes still continue even after cutting, apical meristem continues to grow to develop flowers. Biochemical modification is at the apex, particularly those caused by plant hormones that are regulated by various beneficial microorganisms. Endophytic strains regulate the

Progress of flower and bulb of Tulip



water balance, delaying the process of senescence and blocking harmful microbial agents.

As the petals of flowers mainly to determine the freshness, the petal senescence are the basic floral organs of the body is necessary to study the biochemical and genetic processes (Gandin et al., 2011). Plant growth promoting bacteria (PGPB) contain an enzyme 1-aminocyclopropane-1- carboxylic acid (ACC) deaminase, that diminish the ethylene concentration by hydrolysing ACC into α -ketobutyrate and ammonia NH_3 (Glick et al., 1998). Endophytes decelerate the process of senescence in petal of carnation flower (Nayani et al. 1998).

This is due to improvement of plant growth in terms of scape elongation by hormonal activity like stimulation by phyto-hormones which improved tissue water status which are the essential for normal metabolism, growth and development.

CONCLUSION

It is concluded that bacterial endophytes (treatment) are excellent growth promoting substances which have significant role in pre-harvest as well as postharvest significance for cut flowers. This study declares that bacterial strains played a significant role in plant metabolic process and stimulate the plant growth promoters (PGPR). Application of *Burkholderia pytofirmans* (PsJN) T₁ treatment improved the postharvest life of cut tulip and other quality parameters like scape length, fresh mass, stem diameter, flower longevity and bulb attributes as compared to control.

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Article Keywords

Tulipa gesneriana, exogenously sprayed, *Burkholderia pytofirmans*, *Bacillus* sp. *Enterobacter* sp, Colony Forming Unit (CFU), *Caulobacter* sp. Bacterial strains, endophytes.

Article History

Received: 02 December 2018

Accepted: 15 January 2019

Published: 1 February 2019

Citation

Mohsin Bashir, Muhammad Asif, Muhammad Naveed, Rashad Waseem Khan Qadri, Nazar Faried, Allah Baksh. Pre-harvest exogenous application of bacterial strains to assess the flower and bulb quality of cut Tulip (*Tulipa gesneriana* L.) Cv. Clear Water. *Discovery*, 2019, 55(278), 73-80

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