

**Efficacy of different primers on growth and yield of tulip (*Tulipa gesneriana* L.)****Mariam Atait*, Usman Shoukat Qureshi**

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ABSTRACT

Tulip (*Tulipa gesneriana* L.) is an important and highly valuable flower of the cut flower industry. The most critical step in its cultivation is to break dormancy in order to initiate the growth, especially in tropical and sub-tropical areas of the world. Therefore, the current research was conducted to break bulb dormancy and foster the growth of tulip in Potohar region with the help of different primers. The objective of this study was the selection of best primer at appropriate concentration level to enhance growth, yield and vase life of the flower. Tulip bulbs were treated with different primers: T₀ (distilled water), T₁ (chitosan @ 5 g/L), T₂ (gibberellic acid @ 0.15 g/L), T₃ (humic acid 160 g/L), T₄ (imidacloprid 19 g/L) and T₅ (salicylic acid 0.1 g/L) for 24 hours, respectively. The experiment was laid out using Complete Randomized Design (CRD) with three replications. Statistical results revealed that characteristics including early germination, plant height, number of leaves, stalk length, fresh and dry weight of flower, weight of bulbs, diameter of bulbs and number of daughter bulbs were significantly increased in T₂. Whereas, leaf area, diameter of stem and flower was maximum in T₀. Plants under T₃ showed an increase in chlorophyll content of leaves. While floral characteristics like early formation and opening of flower bud, more number of flowers and vase life were improved in T₁. Thus, statistical results showed that priming can effectively help to improve morpho-physiological attributes of tulip.

Key word: Tulip, primers, dormancy, chitosan, gibberellic acid, humic acid, imidacloprid, salicylic acid.

INTRODUCTION: Tulip (*Tulips gesneriana* L.) is the most popular and lucrative spring blooming bulbous plant of *Liliaceae* family. It is famous for its distinctive flower shape; size and vibrant color range that make it stand out aesthetically among other ornamental flowers. There are about 150 to 160 species of tulip that can be grown in gardens. In addition to this, they are also used as cut flowers. In cut flower industry, it is ranked as 3rd most desirable flower after rose and chrysanthemum (Singh, 2006; Ahmad *et al.*, 2014). This flower holds a significant importance on societal events like Valentine's Day, Easter, New Year and Mother's Day. Along with ornamental uses, its bulb can be used for cooking purposes in place of onions and petals can be used to treat rough skin. As a result of its immense beauty and multiple uses, it is day by day becoming more eminent and favorite among people globally (Buschman, 2004; Jhon and Neelofar, 2006). The demand for cut flowers in Pakistan is also gaining popularity. In Pakistan, where floriculture industry is still struggling to make its way towards development, the annual production of cut flowers is estimated to be 10,000 to 12,000 tons per annum (Younis *et al.*, 2009). Main cut flower crops produced includes: rose, carnation, gerbera, statice, tuberose, narcissus, gladiolus, freesia and lilies (Ahsan *et al.*, 2012). Despite of tulip's high demand, it is not among the few cut flowers that are produced at commercial level in Pakistan. However, some of the wild species of tulip (*Tulipa stellate*) are found in the country, as they wildly grow in West and North West Himalayan region of the world. (Nasir *et al.*, 1987). This perennial plant needs several weeks of low temperature (temperature < 5°C) to break its dormancy for producing beautiful flowers, as a result, its cultivation is restricted to temperate areas (Koksal *et al.*, 2011). It is widely grown in areas with 5-10°C night and 17-20°C day

temperature throughout the growing season (Singh, 2006). Although, it has high demand worldwide but there are only 15 tulips producing countries in the world. Among few tulip producing countries, Netherlands tops the list due to her favorable climatic conditions. The total production area of tulips in Netherlands is 10,800 hectares that contributes 60% of the world's total production. The reason behind its limited production in the world is the inability of tulip bulbs to break dormancy under unfavorable climatic conditions. Dormancy is a state in which flower bulbs do not show any physical growth due to physical and physiological barriers. Therefore, dormancy breaking is the utmost important step while growing tulips anywhere in the world (De Klerk *et al.*, 1992). Thus, aforementioned restrictions and sensitivity of crop towards its growth requirements has also affected its production in Pakistan. Its cultivation is restricted to Murree, Abbottabad and Swat only. Some other parts of the country, including the Potohar region have great potential to grow tulip by putting in a little effort to cope with the challenge of dormancy breaking due to relatively high temperature. The winter period in Potohar region is from November to March. Moreover, December, January and February are the coldest months with a mean annual temperature between 10°C to 15°C. As a result, the time of planting is very critical for dormancy breaking and fast growth in such areas, as late planting would cause an abortion of flowers due to a raised field temperature at the time of flowering. In order to grow tulips in areas with mild winters different techniques are adopted that includes: pre-chilling, seed priming and protected cultivation method to achieve early growth and high yield of flowers before the temperature rises. Out of all additional efforts, seed priming can be an effective method for growing tulips in open fields because it promotes

early growth and good yield (Anjum *et al.*, 2010; Benschop *et al.*, 2010; Kumar *et al.*, 2013; Ramzan *et al.*, 2014; Sarfaraz *et al.*, 2014; Khan, 2019). Seed priming is a method of soaking seeds in solution with high osmotic potential which provides optimum level of hydration and aggravates the germination process, but don't show the radical emergence by prolonging the lag phase. Lag phase makes the seed metabolically active and helps to convert the stored food reserves into the available form to be used during germination (Taylor *et al.*, 1998; Reid *et al.*, 2011; Nawaz *et al.*, 2013). Application of different chemicals as primer including salts (chitosan), growth regulator (gibberellic acid), plant hormone (salicylic acid), organic compounds (humic acid) and insecticides (imidacloprid) can result in a reduced forcing period, enhanced growth, early flowering and high flower yield. Therefore, careful selection of variety and use of priming as dormancy breaking technique is the essential step of Tulip cultivation in the Potohar area (Horii *et al.*, 2007; Shakarami *et al.*, 2013; Nakasha *et al.*, 2014; Baldotto *et al.*, 2016).

OBJECTIVES: Keeping in view that tulip is an excellent cut flower and its demand is increasing globally, present study was designed to analyze and study the effects of best concentration of different primers on early growth (vegetative and reproductive), yield and vase life of tulip in the Potohar region of Northern Punjab, Pakistan.

MATERIALS AND METHODS: Experimental site and planting material: The experiment on *Tulipa gesneriana* L.

was conducted at the experimental area, Department of Horticulture, PMAS-Arid Agriculture University, Rawalpindi with longitude 73.07° E and latitude 33.6° N, during the year 2017-2018. *Tulipa gesneriana* L. was established through bulbs. Tulip bulbs were purchased from reliable sources and were planted by the end of November in pots after priming treatment in open field conditions.

Maintenance practice: Regular watering and fortnightly fertilizer application of NPK (Grow more (17:17:17) @ 10g/m² was done to maintain plant health.

Priming treatments: Tulip bulbs were treated with different primers including: T₀ (distilled water), T₁ (chitosan @ 5 g/L), T₂ (gibberellic acid @ 0.15 g/L), T₃ (humic acid 160 g/L), T₄ (Imidacloprid 19 g/L) and T₅ (salicylic acid 0.1 g/L) for 24 hours, respectively.

Parameters: Both vegetative and reproductive parameters were analyzed to determine the efficacy of primers including days to sprouting of bulbs (days), plant height (cm), leaf area (cm²), number of leaves, diameter of flower stem (mm), days to flower bud formation (days), days to flower opening stage (days), diameter of flower (mm), number of flowers per plant, stalk length (cm), fresh weight of flower (g), dry weight of flower (g), diameter of bulbs (mm), weight of bulbs (g), number of daughter bulbs per plant and vase life (days).

Statistical analysis: Experiment was laid out randomly using Complete Randomized Design (CRD). The collected data was analyzed through appropriate statistical package i.e. MSTAT-C. Statistical significance was compared with LSD test at 5% level of significance (Steel *et al.*, 1997).

RESULTS AND DISCUSSION: Effect of priming on vegetative growth attributes: Results were exhibiting significant difference among vegetative growth attributes of the treated plants (table 1). The 0.15 g/L of gibberellic acid treated

plants showed early sprouting (25 days) and maximum increase in plant height (33cm), number of leaves (6), stalk length (29.05cm) and diameter of flower stem (9.66mm), followed by 5 g/L of chitosan, 160g/L of humic acid and 19 g/L of Imidacloprid, respectively.

Parameters	Treatments					
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
Days to sprouting of bulbs	27.55 ab	26.3 bc	25.44 c	26.4 bc	30.22 a	31.33 a
Plant height (cm)	26.4 b	31.7 ab	33.5 a	30.4 ab	28.9 ab	15.6 c
Number of leaves	5.44 a	5.77 a	6.33 a	4.88 ab	4.77 ab	4 ab
Leaf area (cm ²)	39.07 a	26.49 c	32.55 b	35.17 b	23.58 c	24.26 c
Chlorophyll content of leaves	57.25 c	60.46 b	59.49 c	62 a	58.64 d	59.77 c
Diameter of flower stem (mm)	8.90 a	9.54 a	9.66 a	9.44 a	8.78 ab	6.04 b
Stalk length (cm)	26.88 a	27.99 a	29.05 a	26.79 a	25.05 a	12.33 b
Diameter of bulbs (mm)	36.92 b	39 ab	41 a	37.5 b	37.7 ab	39.3 ab
Weight of bulbs (g)	21.66 b	23.77 ab	25.99 a	22.33 ab	23.45 b	24.66 ab
Number of daughter bulbs per plant	3.66 ab	4.22 a	4.22 a	3.66 ab	3.11 b	3.88 ab

Table 1: Effect of different primers on vegetative growth of Tulip (*Tulipa gesneriana* L.).

Minimum plant height (15.6cm), number of leaves (4), stalk length (12.33cm) diameter of flower stem (6.04mm) and delayed bulb sprouting (31 days) was observed in 0.1 g/L of salicylic acid. Improvement in vegetative characteristics shown by T₂ plants revealed that gibberellic acid helped in dormancy breaking, cell division and elongation in actively growing plant parts (Kumar *et al.*, 2013). As further result confirmed that the maximum leaf area (39.07cm²) was observed in control plants and treated plants didn't show significant increase in leaf area, because of the use of energy in increasing plant height and number of leaves. Previous studies also showed that plants with more number of leaves had a less leaf area and color of the leaves was also lighter (Khangoli, 2001; Janowska and Jerzy, 2004). Moreover, the maximum amount of chlorophyll content (62) was observed in 160 g/L of humic acid followed by 5 g/L of chitosan, 0.15 g/L of gibberellic acid and 19 g/L of Imidacloprid, respectively. Whereas, minimum amount of chlorophyll content was observed in 0.1 g/L of salicylic acid (58). Tulip bulbs treated with Humic acid effectively increased photosynthetic activity of the plant which in result increased the chlorophyll content of the leaves and produced more plant food. Leaf area of the humic acid treated plants was also increased as compared to other treatments that also caused an increase in the chlorophyll content of the leaves (Chanprasert *et al.*, 2012; Salachna and Zawadzińska, 2014). Furthermore, bulb characteristics were also improved under the influence of priming. Maximum diameter (41mm) and weight of bulbs (26g) was observed in 0.15g/L of Gibberellic acid followed by 0.1g/L of salicylic acid, 5 g/L of chitosan, 19 g/L of Imidacloprid and 160 g/L of humic acid respectively. Whereas, minimum diameter (36mm) and weight (21g) of bulbs was observed in control treatment which proved the efficacy of primers in

enhancing characteristics of tulip bulbs. Bulb diameter and weight was increased because of the presence of good amount of food in the bulb which helped in its growth (Arteca, 2013). Furthermore the number of daughter bulbs were maximum in 0.15 g/L of gibberellic acid (4.22) and 5 g/L of Chitosan (4.22) followed by 0.1 g/L of salicylic acid and 160 g/L of Humic acid respectively. Minimum number of bulb-lets was observed in 19 g/l of Imidacloprid (3.11). Increased rate of cell division and multiplication, plus availability of good nutrition in bulbs helped to increase the number of daughter bulbs in the treated plants (Shakarami *et al.*, 2013). Thus, results confirmed that 0.15 g/L of gibberellic acid effectively improved both plant (figure 1) and bulb (figure 2) characteristics.



Figure 1: Effect of gibberellic acid treatment (T₂) on vegetative growth of tulip (*Tulipa gesneriana* L.)



Figure 2: Effect of gibberellic acid treatment (T₂) on bulb characteristics of tulip (*Tulipa gesneriana* L.).

Effect of priming on reproductive growth attributes: Results showed significant differences in plants for reproductive growth attributes in response to priming (table 2). Minimum days of bud formation (122 days) and flower opening stage (124 days) were showed by 5 g/L of chitosan followed by 0.15 g/L of gibberellic acid, 160g/L of humic acid and 19 g/L of Imidacloprid, respectively. Whereas, 0.15 g/L of salicylic acid took maximum days in the formation (127 days) and opening (129 days) of flower buds. Chitosan helps plant in maintaining its vegetative and reproductive growth under stress conditions like drought and high temperature. As a result, the plant maintains its growth under stress conditions and give early flowers, because its reproductive growth attributes remain unaffected under any abiotic stress, as previously studied in orchid as well (Saniewska, 2001; Uthairatanakij *et al.*, 2007). Moreover, the number of flowers per plant were maximum in 5 g/L of Chitosan (3.33) and 0.15 g/L of gibberellic acid (3.33), followed by 160 g/L of humic acid.

Parameters	Treatments					
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
Days to flower bud formation	124.7 8 b	122.55 c	122.78 c	123.22 bc	123.44 bc	127 a
Days to flower opening stage	126.7 8 b	124.44 c	124.78 c	125.22 bc	125.44 bc	129 a
Diameter of flower (mm)	40.73 a	39.23 a	37.37 a	36.5 a	36.81 a	11.55 b
Fresh weight of flower (g)	22.7 ab	28.8 ab	33.8 a	24.4 b	20.5 bc	12.2 c
Dry weight of flower (g)	1.23 a	1.87 a	2 ab	1.57 ab	1.54 a	0.98 c
Number of flowers per plant	2.66 a	3.33 a	3.33 a	2.66 a	2.33 ab	1.44 b

Table 2: Effect of different primers on reproductive growth of Tulip (*Tulipa gesneriana* L.).

Whereas, 19 g/L of Imidacloprid (2.33) and 0.1 g/L of Salicylic acid (1.44) gave minimum flower yield. Along with enhancing the defense mechanism, chitosan also helped in increasing flower number in Freesia and other flowers, previously (Salachna and Zawadzińska, 2014). Furthermore, maximum fresh weight (33g) and dry weight of flower (2g) were observed in 0.15 g/L, of gibberellic acid followed by 5 g/L of chitosan, 160 g/L of humic acid and 19 g/L of imidacloprid, respectively. Minimum fresh weight of flower (12 g) and dry weight of flower (0.98 g) was observed in 0.1 g/L of salicylic acid. Gibberellic acid effectively increases plant height and diameter of stem that caused an increase in fresh and dry weight of flower due to presence of more plant nutrients and maintenance of turgidity. Diameter of the flower was maximum (40mm) in control plants, this showed priming of tulip bulbs didn't have any effect on increasing flower size due to increase in flower number (Rashad *et al.*, 2009; Hashemabadi, 2010). Thus, the aforementioned results confirmed that 5 g/L chitosan was most effective in improving floral attributes (figure 3) of tulip.



Figure 3: Effect of chitosan treatment (T₁) on flower growth of tulip (*Tulipa gesneriana* L.)

Effect of priming on vase life: According to results (table 3), 5 g/L of Chitosan and 0.1 g/L of salicylic acid showed maximum vase life (8 days) followed by 19 g/L of imidacloprid, 0.15 g/L of gibberellic acid and 160 g/L of humic acid, respectively. Flowers under control treatment showed minimum vase life (6) as compared to treated plants.

Parameters	Treatments					
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
Vase life (days)	6.66 b	8.66 a	7.33 ab	6.88 b	7.66 ab	8 ab

Table 3: Effect of different primers on vase life of Tulip (*Tulipa gesneriana* L.).

Chitosan improved the quality of flower by maintaining its size, color and freshness, but most importantly, it provided protection against many pathogenic fungi that can attack tulip and cause senescence of the flower. As a result of fungal protection and resistance against abiotic stresses, Tulip flower showed increased post-harvest quality and vase life (Saniewska, 2001). In Liliium flower it helped to decrease the production of ethylene and respiration rate and helped in increasing its vase life (Kim *et al.*, 2004).

CONCLUSION: Present research proved that treatment of tulip bulbs with different primers at their best selected concentration levels was an effective method of enhancing early growth and yield in an area with relatively high temperature as compared to temperate region. The Tulip plants showed improvement in sprouting, plant height, number of leaves, chlorophyll content of leaf, leaf area, early flowering, flower size, number of flowers, stalk length, stem diameter, bulbs weight and diameter, number of bulb-lets and vase life. Thus, this method can be used in future for the production of Tulips under tropical and sub-tropical areas.

CONFLICT OF INTEREST: Authors have no conflict of interest.

REFERENCES Ahmad, A., H. Rashid, R. Sajjad, S. Mubeen, B. Ajmal and M. Khan, 2014. Enhancing the vase life of tulip (*Tulipa gesneriana* L.) using various pulsing solutions of humic acid and npk. International journal of plant, animal environmental sciences, 4(2): 193-200.

Ahsan, M., S. Rehman, A. Younis, A. Riaz, U. Tariq and R. Waqas, 2012. Different strategies to create earliness and enhance quality of tuberose (*Polianthes tuberosa* L.) cv. Single. Asian journal of pharmaceutical biological research, 2(1): 84-88.

Anjum, S. A., L. Wang, J. Salhab, I. Khan and M. Saleem, 2010. An assessment of drought extent and impacts in agriculture sector in Pakistan. Journal of food, agriculture environment, 8(3/4 part 2): 1359-1363.

Arteca, R. N., 2013. Plant growth substances: Principles and applications. Springer Science & Business Media.

Baldotto, M. A., J. E. da Rocha, F. D. P. Andrade, M. P. Del Giúdice and L. E. B. Baldotto, 2016. The plant stimulant humic acid extracted from organic waste recycled by composting combined with liming and fertilization. Semina: Ciências Agrárias, 37(6): 3955-3963.

Benschop, M., R. Kamenetsky, M. Le Nard, H. Okubo and A. De Hertogh, 2010. The global flower bulb industry: Production, utilization, research. Horticultural reviews, 36(1): 1-115.

Buschman, J., 2004. Globalisation-flower-flower bulbs-bulb flowers. In: IX International Symposium on Flower Bulbs 673. pp: 27-33.

Chanprasert, W., T. Myint, S. Srikul and O. Wongsri, 2012. Effect of thiamethoxam and imidacloprid treatment on germination and seedling vigour of dry-heated seed of oil palm (*Elaeis guineensis* jacq). African journal of agricultural research, 7(48): 6408-6412.

De Klerk, G.-J., I. Delvallée and A. Paffen, 1992. Dormancy

release of micropropagated bulblets of *lilium speciosum* after long culture in soil. HortScience, 27(2): 147-148.

Hashemabadi, D., 2010. Yield and quality management of rose (*Rosa hybrida* cv. Poison) with plant growth regulators. Plant omics, 3(6): 167.

Horii, A., P. McCue and K. Shetty, 2007. Enhancement of seed vigour following insecticide and phenolic elicitor treatment. Bioresource technology, 98(3): 623-632.

Janowska, B. and M. Jerzy, 2004. Effect of gibberellic acid on the post-harvest flower longevity of *Zantedeschia elliottiana* (w. Wats) engl. Hortorum cultus, 3(1): 3-9.

Jhon, A. and Neelofar, 2006. Tulip in: Bulbous ornamental and aquatic plants, advances in ornamental horticulture. Advances in ornamental horticulture, 3: 1-72.

Khan, S., 2019. Climate classification of pakistan. International journal of economic environmental geology, 10(2): 60-71.

Khangoli, S., 2001. Potential of growth regulators on control of size and flowering of ornamental plants. In: Proc. First applied Sciences seminar on flowering and ornamental plants. Mahallat, Iran. pp: 75-76.

Kim, J.-H., A.-K. Lee and J.-K. Suh, 2004. Effect of certain pre-treatment substances on vase life and physiological character in *Lilium* spp. In: IX international symposium on flower bulbs 673. pp: 307-314.

Koksal, N., H. Gulen and A. Eris, 2011. Dormancy in tulip (*tulipa gesneriana* l.) bulbs and freesia (*Freesia refracta* Klatt.) corms: Changes in soluble proteins and apx activity. Journal of food, agriculture environment, 9(2): 535-539.

Kumar, R., N. Ahmed, D. B. Singh, O. C. Sharma, S. Lal and M. M. Salmani, 2013. Enhancing blooming period and propagation coefficient of tulip (*Tulipa gesneriana* L.) using growth regulators. African journal of biotechnology, 12(2).

Nakasha, J. J., U. R. Sinniah, A. Puteh and S. A. Hassan, 2014. Potential regulatory role of gibberellic and humic acids in sprouting of *Chlorophytum borivilianum* tubers. The scientific world journal, 1: 1-9.

Nasir, E., Y. J. Nasir and R. Akhter, 1987. Wild flowers of rawalpindi-islamabd districts, national herbarium, PARC, garden graphics Ltd. Karachi, Pakistan.

Nawaz, J., M. Hussain, A. Jabbar, G. A. Nadeem, M. Sajid, M. U. Subtain and I. Shabbir, 2013. International journal of agriculture crop sciences. 6(20): 1373.

Ramzan, F., A. Younis, A. Riaz, S. Ali, M. I. Siddique and K.-B. Lim, 2014. Pre-planting exogenous application of gibberellic acid influences sprouting, vegetative growth, flowering, and subsequent bulb characteristics of 'ad-rem'tulip. Horticulture, environment, biotechnology, 55(6): 479-488.

Rashad, E.-S. M., M. S. A. Abd El-Wahed and A. A. Amin, 2009. Effect of-sitosterol and gibberellic acid on leaf angle, growth, flowering and biochemical constituents of marigold (*Calendula officinalis* L.). Medicinal aromatic plant science biotechnology, 3(1): 21-27.

Reid, J. B., S. E. Davidson and J. J. Ross, 2011. Auxin acts independently of della proteins in regulating gibberellin levels. Plant signaling behavior, 6(3): 406-408.

Salachna, P. and A. Zawadzińska, 2014. Effect of chitosan on plant growth, flowering and corms yield of potted freesia. Journal of ecological engineering, 15(3): 97-102.

Saniewska, A., 2001. The effect of chitosan on limitation of growth and development of some pathogenic fungi for

ornamental plants. *Acta agrobotanica*, 54(1): 17-29.

Sarfaraz, S., M. H. Arsalan and H. Fatima, 2014. Regionalizing the climate of Pakistan using köppen classification system. *Pakistan geographical review*, 69: 111-132.

Shakarami, K., R. Naderi, M. Babalar and Z. Hamzehei, 2013. The effect of different concentrations of gibberellic acid on quantitative and qualitative characteristics of three cultivars lacourtine, yokohama and red favourite tulip (*Tulipa gesneriana* L.). *Journal of ornamental plants*, 3(4): 251-257.

Singh, A., 2006. *Flower crops: Cultivation and management*. new India publishing.

Steel, R. G., J. H. Torrie and D. A. Dickey, 1997. *Principles and*

procedures of statistics: A biological approach. McGraw-Hill.

Taylor, A., P. Allen, M. Bennett, K. Bradford, J. Burris and M. Misra, 1998. Seed enhancements. *Seed science research*, 8(2): 245-256.

Uthairatanakij, A., J. Teixeira da Silva and K. Obsuwan, 2007. Chitosan for improving orchid production and quality. *Orchid science biotechnology*, 1(1): 1-5.

Younis, A., A. Riaz, S. Saleem and M. Hameed, 2009. Potential use of wild flowers in urban landscape. In: *II International Conference on Landscape and Urban Horticulture* 881. pp: 229-233.



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