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Impact of 6-benzyl amino purine and spermidine on vegetative growth of gladiolus (*Gladiolus* × *hortulanus*) *cv*. Passos

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Abstract
The present work was conducted to investigate the application of 6-benzyl amino purine and Spermidine for improving vegetative, flowering and chlorophyll contents of Gladiolus (*Gladiolus*× *hortulanus*) cv. Passos". Results revealed that both the plant growth regulators increased plant height, leaf number, leaf area, and chlorophyll content, days to spike emergence, colour break and first floret opening as compared to control. Increasing concentration of both PGRs significantly improved all the parameters whether foliar spray of BAP or sperimidine and/or in their interaction. However, Spermidine significantly increased leaf area followed by BAP, while as BAP significantly improved plant height, leaf number, chlorophyll content (SPAD), spike emergence, colour break and first floret opening followed by sperimidine. Regarding concentrations, 200 mg L^{-1} performed the best for plant height, leaf number, leaf chlorophyll content, days to spike emerergence, colour break and first floret opening followed by 150 mg L^{-1}, 100 mg L^{-1} and control treatment.

Keywords: gladiolus, PGRs, BAP, sperimidine, colour

Introduction
Gladiolus (*Gladiolus hortulanus* L.), commonly known as queen of bulbous flowers, belongs to family Iridaceae and sub-family Ixioideae is native to South Africa, includes 180 species with more than 10,000 cultivars (Sinha and Roy, 2002) [39]. The name gladiolus has been derived from Latin word 'Gladius', meaning 'Sword', because of sword like shape of its foliage. It has gained popularity in many parts of the world owing to its unsurpassed beauty and economic value. It is grown in herbaceous borders, beds, pots and as a commercial crop for cut flower production. Apart from being of superb aesthetic value, gladiolus is known to have medicinal properties and is effective against headache, diarrhoea, rheumatism, and allied pains (Misra and Singh, 1989) [26]. Gladiolus is the second most popular flower in the world, especially from the commercial point of view. Gladiolus has great economic value and wide market in world due to attaractive spikes, big florets, dizzling colours and long vase life (Khan *et al*., 2012 and Farid-Uddin *et al*., 2002)) [18, 15] and occupies 4th place in International cut flower trade after rose, carnation and chrysanthemum (Farhat, 2004) [14]. It has a high demand in global cut flowers trade; which requires development of new, promising, high yield cultivars and their evaluation for their suitability for commercial production (Ahmed *et al*., 2008) [1]. In India, the estimated area under gladiolus cultivation is about 12.67 thousand hectare, with a production 94.89 lakh number cut flowers (Anonymous, 2017) [6].
The gladiolus has the potential not only to fulfil the local requirements but also to earn foreign exchange as the crop is of short duration (110-120 days); wide varietal wealth, better economic returns than conventional crops and wide range of available climatic conditions in the country have contributed to its growth potentials (Sajid *et al*. 2015) [35].
Now a days the use of growth regulators like Cytokinin (BAP), GA_3 and polyamines (spermidine) has revolutionized the flouriculture industry. Many profitable cosequences of different plant growth regulators have been exerted on different flowers and ornamental plants (Sridhar, 2006) [40] including control of growth and flowering in many floral crops to produce high quality produce. It is a common practice for modifying the developmental processes of flowers and ornamental crops. Growth regulating chemical were reported to be effective in manipulating growth and flowering in gladiolus. Growth and development are to be regulated either by single or by interaction of several hormones. They play major role in directing the movement of organic metabolites and in establishing the sink.

Vegetative parameters were playing an important role in all crops, which influence photosynthesis, yield and quality of the particular crop. Green photosynthetic rachis and bracts form a significant role of a gladiolus spike. Cytokinins governs progression linked with plant growth and development, enclosing cell division and leaf senescence (Asil *et al*. 2011) [8]. Cytokinins encaging BAP (6-benzyl amino purine) encourage leaf expension and flower size in some plants (Nishijima *et al*. 2006) [31]. Yellowing, discolouring of the rachis and bracts lowers the commercial value of gladiolus spike. Cytokinins and Spermidine are known to prevent the premature chlorophyll degradation and hence can prevent loss of quality of gladiolus spikes. Further enhancing the chlorophyll content by preventing degradation and increasing duration of crop field life can improve corm yield also (Faraji *et al*. 2011) [13].

Material and Methods

Present work was carried out under open field conditions at Urban Technology Park, Habak, Srinagar (Kashmir) during the Kharif season 2018. Habak is situated at 34º.9´ N latitude and 74º.50´ E longitude at an elevation of 1606 meters above mean sea level. The average annual precipitation is 944.6 mm (average over past thirty years) and more than 80 per cent of precipitation is received from western disturbances. It can be observed from the obtained meteorological data that mean maximum temperature ranged from 9.37 to 30.66 ºC and minimum from -4.56 to 17.16 ºC during the growing season. The relative humidity ranged between 70.15 per cent in May to 93.68 per cent in December. The total rainfall received in month of August was 140.10 mm.

The soil for planting corms was thoroughly prepared and plots were laid out according to randomized complete block design (RCBD) with factorial arrangement. The experimental area was divided into three main blocks which were further divided into sixteen plots which received different treatment combinations as per plan of layout. There were two plant growth regulators (BAP and Sperimidine) with control, four concentrations (0, 100, 150 and 200 mgL^{-1}) and three replications. The different treatment combinations are presented in Table 1.The calendar of the operations during the same year are represented in Table 2. The first application of growth regulators was applied at five leaf stage and second after twenty days of first spray. Control plants were sprayed with normal water. Uniform cultural practices were followed throughout the growing period. Irrigation, weeding cum hoeing, plant protection measures were carried out as when required. Staking was provided to every clump after slipping stage and the details about the different cultural operations adopted during growing period.

Treatment details

Code	Symbol	Details
T_1	B_0S_0	Control
T_2	B_0S_1	Spermidine (100 ppm)
T_3	B_0S_2	Spermidine (150 ppm)
T_4	B_0S_3	Spermidine (200 ppm)
T_5	B_1S_0	BAP (100 ppm)
T_6	B_1S_1	BAP (100ppm) + Spermidine (100pmm)
T_7	B_1S_2	BAP (100ppm) + Spermidine (150ppm)
T_8	B_1S_3	BAP (100ppm) + Spermidine (200ppm)
T_9	B_2S_0	BAP (150ppm)
T_{10}	B_2S_1	BAP (150ppm) + Spermidine (100ppm)
T_{11}	B_2S_2	BAP (150ppm) + Spermidine (150ppm)
T_{12}	B_2S_3	BAP (150)ppm + Spermidine (200ppm)
T_{13}	B_3S_0	BAP (200ppm)
T_{14}	B_3S_1	BAP (200ppm) + Spermidine (100ppm)
T_{15}	B_3S_2	BAP (200ppm) + Spermidine (150ppm)
T_{16}	B_3S_3	BAP (200ppm) + Spermidine (200ppm)

Calendar of operations during the year 2018

	Operation	Date
1.	Land preparation and layout	May 15
2.	Farm yard manure application	May 16
3.	Corm planting	June 3
4.	Fertilizer application	
	✓ N fertilizer	
	✓ 1st split dose	June 3
	✓ 2nd split dose	July 4
	✓ 3rd split dose	August 5
	✓ P and K fertilizers	June 3
5.	Hoeing-cum-weeding	
	1st	June 29
6.	Irrigation	
	✓ 1st	July 5
	✓ 2nd	July 21
	✓ 3rd	July 27
	✓ 4th	August 8
7.	Staking	August 16
8.	Plant protection measures	
	Fungicidal drenches (Bavistin @ 0.1%)	
	✓ 1st	July 12
	✓ 2nd	August 16
	Insecticide spray (Methyl parathion @ 0.04%)	August 6
9.	Harvesting of corms and cormels	December 4

Preparation of Spermidine stock solution

A stock solution of 1000 ppm was prepared by dissolving 1000 mg of putrescine in 1 litre of distilled water and further dilutions were made as follows:

$$\text{Volume to be used} = \frac{\text{Concentration required}}{\text{Concentration given}} \text{ X Volume to be prepared}$$

Therefore, for preparing 1000 ml of 100 ppm = 100 × 1000/1000 = 100 ml of stock Solution + 900 ml of distilled

water. Similarly, 150 and 200 ppm Spermidine solutions were prepared.

Preparation of 6- benzyl amino purine (6- BAP) stock solution

A stock solution of 1000 ppm was prepared by dissolving 1000 mg of BAP in 1 litre of distilled water and further dilutions were made as follows:

$$\text{Volume to be used} = \frac{\text{Concentration required}}{\text{Concentration given}} \times \text{Volume to be prepared}$$

Therefore, for preparing 1000 ml of 100 ppm = 100 x 1000/1000 = 100 ml of stock Solution + 900 ml of distilled water. Similarly, 150, 200 ppm Benzyl amino purine (BAP) solutions were prepared.

Results and Discussion

Plant height

Statistical analysis of the data for plant height, (Table 1 and Fig 1) revealed significant differences among PGR treatments, their concentrations and the interaction between these two factors. Both 6-BAP and Spermidine significantly increased the plant height as compared to the control. Among the concentrations used, 200 ppm of each 6-BAP and spermidine were most effective treatment in increasing the plant height followed by 150 ppm each 6-BAP and Spermidine treatments. The control treatment registered the lowest plant height. Among the interaction means, 6-BAP 200 ppm + spermidine 200 ppm performed best (120.40 cm) followed by 118.53 cm with 6-BAP @ 200 ppm + spermidine @ 150 ppm.The control resulted in the lowest plant height. As cytokinin including 6-BAP are involved in many physiological processes related with plant growth and development while polyamines are known for stem elongation. Therefore both the PGRs resulted in enhanced plant height as compared to the control plants. Increase in plant height has already been reported by Khan *et al.*, 2011 [19] in gladiolus by treating corms with BAP before planting, possibly due to its role in increasing cell division and shoot formation. Similar results were also found by Sajid *et al.* (2015) [35] in gladiolus. The positive effect of polyamines (PAs.) on growth through enhancing cell division and expansion have been reported by cohen 1998 [10]. According to some researchers the augmentation effect of polyamine on growth rate is because they help in the uptake of minerals like N, P and K from soil, as N promotes the vegetative growth and also helps in protein synthesis there by increases the growth and development of crop.P_2O_5 helps in better root system that results in better uptake and inturn increases growth or height of crop and K provides tolerance / resistance to crop (Shawky, 2003) [38].

Table 1: Effect of Foliar Application of 6- benzyl amino purine and Spermidine on Plant height (cm) in Gladiolus cv. Passos

Growth regulators		Spermidine				
		S_0 (water)	**S_1 (100 ppm)**	**S_2 (150 ppm)**	**S_3 (200 ppm)**	**Mean**
Benzyl amino purine (BAP)	B_0 (water)	97.46	104.50	112.46	112.90	106.83
	B_1(100 ppm)	100.40	113.80	114.30	115.46	110.99
	B_2(150 ppm)	103.66	115.66	116.30	117.00	113.15
	B_3(200 ppm)	109.80	118.20	118.53	120.40	116.73
	Mean	102.83	113.04	115.40	116.44	
	C.D ($p \leq 0.05$)					
		6- Benzyl amino purine (B)	:	0.66		
		Spermidine (S)	:	0.66		
		B × S	:	1.33		

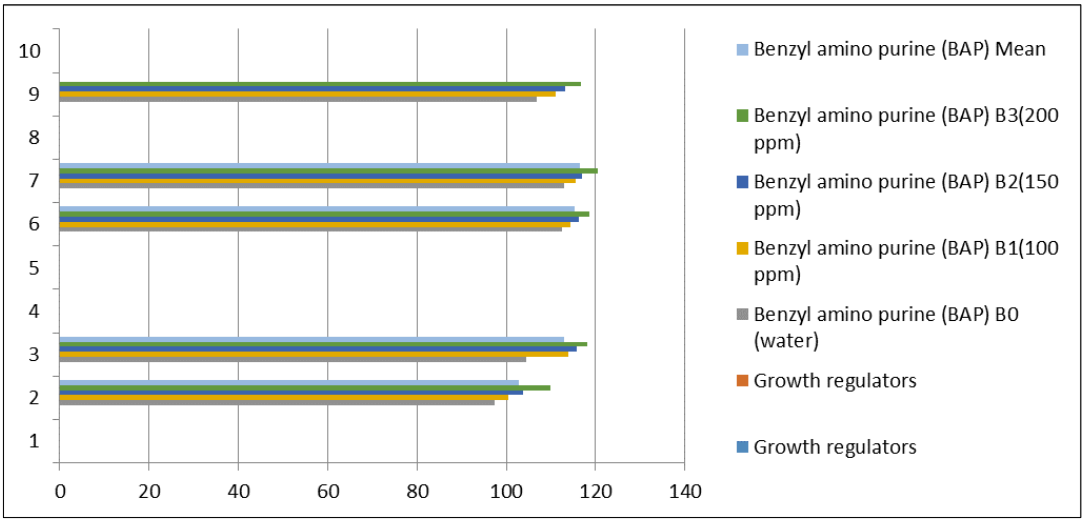

Fig 1: Graphical representation of Plant height (cm) in Gladiolus *cv.* Passos

Number of leaves plant^{-1}

The data related to number of leaves plant^{-1} (table 2 and fig 2) indicated significant differences in mean leaf number plant^{-1} due to differential PGR treatments (6-BAP and Spermidine), their concentrations and their interaction. Both the PGRs (6-BAP and Spermidine) significantly increased the number of leaves plant^{-1} as compared to control treatment. Among the concentrations used, 200 mg L^{-1} was most effective in

increasing the number of leaves plant^{-1} followed by 150 mg L^{-1} in both 6-BAP and Spermidine. But incase of spermidine 200 mg L^{-1} was at par with 150 mg L^{-1}. Among the interaction means, 6-BAP 200 mg L^{-1} + spermidine 200 mg L^{-1} performed best and resulted maximum number of leaves plant^{-1} followed by 6-BAP at 200 mg L^{-1} + spermidine at 150 mg L^{-1} and lowest number of leaves were recorded in control treatments. The increased number of leaves with application of 6-BAP and Sperimidine may be due to increased cell division, cell enlargement and elongation that might have produced more nodes and internodes leading to the production of more number of leaves. The present findings are in line with the reports of Baskaran *et al.* (2014) [9] in gladiolus. The positive effect of polyamines on growth has also been earlier attributed to enhanced cell division and expansion (Cohen, 1998) [10]. These findings are in accordance with Youssef *et al.* (2004) [42]. Increase in the concentration of 6-BAP increases the number of leaves plant^{-1}. This might be due to increased cell division and number of suckers per plant resulting in more number of leaves and increased leaf area as compared to untreated (control). These results are in accordance with the findings of Padmalatha *et al.* (2012) [32]. Similar results have been reported by Sharma *et al.* (2004) [37], Kumar *et al.* (2008) [20], Rana *et al.* (2005) [34] and in gladiolus.

Table 2: Effect of Foliar Application of 6- benzyl amino purine and Spermidine on number of leaves plant^{-1} in Gladiolus cv. Passos

Growth regulators		Spermidine				
		S$_0$ (water)	**S$_1$ (100 ppm)**	**S$_2$ (150 ppm)**	**S$_3$ (200 ppm)**	**Mean**
Benzyl amino purine (BAP)	B$_0$ (water)	7.43	8.33	8.80	9.13	8.42
	B$_1$(100 ppm)	7.76	9.33	9.56	9.83	9.12
	B$_2$(150 ppm)	7.96	9.93	10.13	10.23	9.56
	B$_3$(200 ppm)	8.36	10.83	11.53	11.96	10.66
	Mean	7.88	9.608	10.00	10.29	
	C.D ($p \leq 0.05$)					
		6- Benzyl amino purine (B)	:	0.29		
		Spermidine (S)	:	0.29		
		B × S	:	0.59		

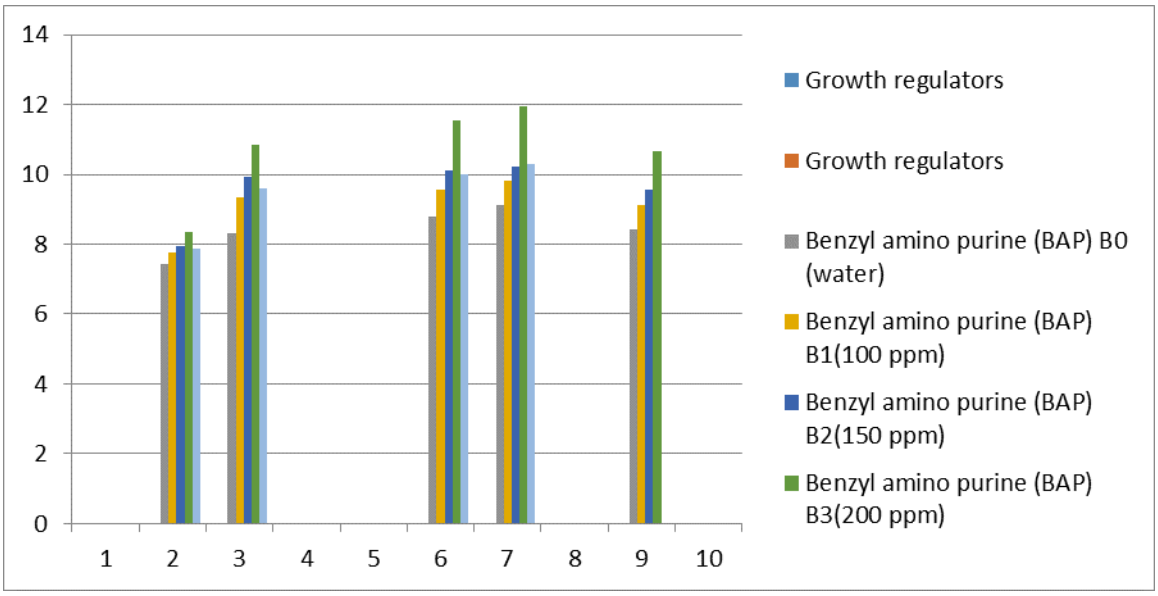

Fig 2: Graphical representation number of leaves plant^{-1} in Gladiolus cv. Passos

Leaf area (cm^2)

Analysis of variance of the data regarding leaf area (Table 3) depicted significant differences among PGRs, their concentrations and their interaction. Among the PGR treatments, application of Spermidine resulted in significant increase in Leaf area, followed by 6-BAP. The control treatment gave the minimum leaf area. Concerning the PGR concentrations, Spermidine at 200 mgL^{-1} performed the best (836.85 cm^2), followed by 789.88 cm^2 with 6-BAP 200 mgL^{-1}. However, the control (no Spermidine) treatment resulted in minimum leaf area (703.30 cm^2) and 748.50 cm^2 with 0 ppm 6-BAP. The interaction between 6-benzyl amino purine and spermidine had also significantly affected the leaf area. Maximum leaf area (852.73 cm^2) was recorded with 6-BAP at 200 mgL^{-1}+ spermidine at 200 mgL^{-1} followed by (823.88 cm^2) with 6-BAP @ 200 ppm + spermidine @ 150 ppm. However minimum leaf area (673.03 cm^2) was recorded with 6-BAP @ 0 ppm + spermidine 0 ppm. Leaf area enlargement may be due to increased cell division and cell enlargement caused by spermidine and BAP application. Both these physiological processes increases the source to sink relation either by increasing the cell number and cell expansion which inturn increases the area of leaf Ahmed *et al.* (2013) [2] also attributed leaf area enlargement in tulip with PGR application due to increased cell division and cell enlargement. Kumar and Singh (2002) found higher leaf width in growth regulator treated plants due to growth enhancing capability. The present results find support from Joshi *et al.* (2011) [17], Neetu *et al.* (2013a) [30] and Aier *et al.* (2015) [3] in gladiolus.

Table 3: Effect of Foliar Application of 6- benzyl amino purine and Spermidine on leaf area (cm^2) of Gladiolus cv. Passos

Growth regulators		Spermidine				
		S_0 (water)	**S_1 (100 ppm)**	**S_2 (150 ppm)**	**S_3 (200 ppm)**	**Mean**
Benzyl amino purine (BAP)	B_0 (water)	673.03	729.25	769.26	822.47	748.50
	B_1(100 ppm)	708.80	728.13	785.68	824.22	761.71
	B_2(150 ppm)	715.70	745.08	814.92	848.01	780.93
	B_3(200 ppm)	715.68	767.23	823.88	852.73	789.88
	Mean	703.30	742.42	798.43	836.85	
	C.D ($p \leq 0.05$)					
		6- Benzyl amino purine (B)	:	3.87		
		Spermidine (S)	:	3.87		
		B × S	:	7.74		

Conclusion

The PGRs applied at the early stage of growth, not only influenced vegetative growth and flowering in gladiolus but also affected chlorophyll contents. From the results it can be concluded that gladiolus can successfully be grown under Kashmir conditions by foliar application of BAP and sperimidine, as the increase in concentration of both all the parameters mentioned increases and vice-versa.

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