

Research Article



Exogenous Application of Calcium Improved the Vegetative Attributes and Corm Production in Gladiolus

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Abstract | Gladiolus is commercially important flowering plant famous for its excellent flowers worldwide. In order to meet the high demand for its quality plants especially cut flowers. Gladiolus is commercially propagated by corms. The quality and health of corm is one of the important factors that affect the production and quality of gladiolus florets and spikes as well as corm production. Keeping in view, the significant role of calcium in plants and its potential effects on vegetative attributes and quality production of gladiolus corms, an experiment was conducted during the years 2016-2017. The influence of foliar calcium application was investigated by spraying the gladiolus plants cv. "White Prosperity" with 0, 100, 200, 300 and 400 mM calcium solutions at different times (3rd, 5th and 7th leaf stages). The findings showed that maximum leaf area (43.21 cm²), leaf chlorophyll content (58.275 SPAD), size and weight of daughter corms (7.10 cm and 61.52 g) and highest calcium content (0.567%) in daughter corms were recorded in gladiolus plants sprayed with 200 mM calcium. Results pertaining to foliar application time showed that maximum leaf area (38.92 cm²), maximum calcium content in leaf and daughter corm (0.971% and 0.442%) were recorded in plants of gladiolus treated with calcium at 5th leaf stage. Whereas application of calcium at 7th leaf stage resulted in maximum size and weight of daughter corm (6.45 cm and 59.17 g). The interaction between calcium concentrations and application time revealed that foliar application of 200 mM calcium at 5th leaf stage resulted in significant increase in leaf area, however application of the same concentration of calcium at 7th leaf stage resulted in maximum size and weight of daughter corm. It is concluded that foliar application of 200 mM calcium at 7th leaf stage increased the size and weight of daughter corm and hence could be recommended for better corm production in Peshawar conditions.

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Introduction

The name "Gladiolus" is derived from Latin word "gladius", which means sword and is probably in reference to the shape of its leaves. The Gladiolus was known as "Xiphium" in ancient time, which is a Greek word "Xiphos", which means sword. Botanically, it is known as *Gladiolus grandiflorus*, which belong to

family Iridiaceae (Bhujbal et al., 2013). Gladiolus is the 2nd most important cut flower in Pakistan after rose with the potential to generate handsome revenue from export. Gladiolus has a diverse range of cultivars and the flowers are available in various colors, shapes and sizes. Besides its cut flowers, gladiolus is also planted in flower beds in landscape gardening and is used as specimen plant in flower shows and exhibitions. The

area under gladiolus cultivation in Pakistan is more than 2000 ha (Saeed et al., 2013; Anonymous, 2015). Gladiolus needs proper cultural practices such as, time of plantation, planting density, irrigation and weed management for quality production of spikes and corms (Seed et al., 2013). It can be planted by keeping corm to corm distance 20 cm and 30 to 40 cm apart in rows.

The vegetative attributes, flower and corm production in gladiolus could be positively influenced by calcium treatment when applied in optimum concentration (Sharma et al., 2013). Good quality corms result in better vegetative growth with healthy foliage and enhanced photosynthetic activity (Sarkar et al., 2014). Leaf chlorophyll content and leaf area are among the important vegetative attributes that determine the photosynthetic efficiency of a plant (Kumar et al., 1988; Dodd et al., 2005). Calcium influence the division and elongation of cells due to its role in regulation of auxin activity (Singla et al., 2006; Quint and Gray, 2006) and it act as secondary messenger and hence retain the amino acids and chlorophyll contents in the foliage of the plant (Poovaiah, 1988; Oloo-Abucheli et al., 2016). Calcium is among the macro-nutrient required for plant growth and development (Marschner, 1995). Calcium plays an important role in growth and development of plant (Harper et al., 2004). Calcium is a multifunctional nutrient and its deficiency can cause death of growing points, resulting in abnormally dark green foliage (Easterwood, 2002).

Gladiolus is commercially propagated and grown from its specialized underground structure for production of flowers and corm as well as cormels (Hartman et al., 1990; Adkins and Miller, 2011). Usually one daughter corm (new corm) along a number of cormels is produced (depending upon the size of daughter corm) in one season. The daughter corm produces flowering spike in the same season whereas the cormels needs 2-4 seasons in order to get the reasonable size and produce marketable spikes (Memon et al., 2009). The corms and cormels of gladiolus remain dormant for 4-5 months with slight variations depending upon the cultivars and growing conditions (Priyakumari and Sheela, 2005). Production of both, flowering spikes and corms depends upon the size of mother corm and or cormels (Bose et al., 2003; Chourasia et al., 2015). Calcium being an important structural component of the middle lamella of cell and hence has a key role in development of the plant structure including corms

and cormels (Hepler, 2005).

Calcium treatment before harvest enhances the yield of corms and cormels in gladiolus (Gerasopoulos and Chebli, 1999; Ahmad et al., 2011; Reddy and Sarkar, 2016; Mortazavi et al., 2016).

Foliar application of calcium could be used as an alternative fertilization method to improve the growth and corm production of gladiolus (Chaturvedi et al., 1986). It is environment friendly and gives high yields and superior quality (Weinbaum et al., 2002; Kadir, 2004). Foliar application could be done when there is decreased nutrient availability in soil, when topsoil is dry and when root activity is declined (Wojcik, 2004).

Keeping in view the above facts and importance of calcium to enhance vegetative attributes and production of corms in gladiolus, the present project was designed with the following objectives:

- To investigate the influence of foliar calcium applications on the vegetative attributes and corm production of gladiolus.
- To establish the optimum stage for foliar application of calcium for corm production of gladiolus.
- To evaluate the interactive effects of calcium and application time on vegetative attributes and corm production of gladiolus.

Materials and Methods

The experiment on the influence of foliar applied calcium treatments on the foliage and corm production of *gladiolus grandiflorum* was carried out at ornamental nursery, Department of Horticulture, the University of Agriculture, Peshawar during 2016 and 2017.

The corms of gladiolus cultivar 'White Prosperity' were planted in January 2016 and January 2017. The corms were planted at a distance of 20 cm and 40 cm apart in rows. Nitrogen, phosphorus and potassium were applied to the field at the rate of 100 kg ha⁻¹. Urea, Superphosphate and Potassium sulfate were used as source of nitrogen, phosphorus and potassium (Zubair et al., 2013). Phosphorus and potassium were applied to the field before plantation of corms. Nitrogen was applied in two split doses i.e. half at planting time and another half two weeks after plant emergence. The soil of the experimental plot is

alkaline in nature with a pH of 8.1 having 120 mg.kg⁻¹ of calcium. The texture of the soil silt loam.

All the cultural practices including weeding, hoeing, irrigation etc. were carried out uniformly according to the need of the plants. Calcium chloride was used as source of calcium and was applied as foliar spray. Experiment was laid out as Randomized Complete Block design (RCBD) with split plot arrangement.

Main plot (Time of foliar application)	Sub plot (Ca Concentrations)
T1. 3 rd Leaf stage	C1. 000 mM (Distilled water)
T2. 5 th Leaf stage	C2. 100 mM
T3. 7 th Leaf stage	C3. 200 mM
	C4. 300 mM
	C5. 400 mM

Data were recorded on the leaf area, leaf chlorophyll content, leaf calcium content, size and weight of daughter corm as well as calcium content in daughter corm of gladiolus.

Statistical analysis

The data were subjected to analysis of variance combined over years as per procedure appropriate for randomized complete block design (Jan et al., 2009). The least significant difference test was used for significant means comparison.

Results and Discussion

Leaf area (cm²)

Two years' average data pertaining to leaf area in response to calcium concentrations and application time at various leaf stages of gladiolus are presented in Table 1. Statistical analysis showed that calcium concentrations, time of application and their interaction significantly affected leaf area of gladiolus. Means for different years showed non-significant effect on leaf area. Two years averaged data concerning various concentrations of calcium showed that the maximum leaf area (43.21cm²) was observed in plants of gladiolus that were applied with 200 mM calcium as foliar spray, followed by (40.56 cm²) with the foliar application of calcium at the rate of 300 mM. The least leaf area (31.67 cm²) was recorded in control plots. The mean data across two years regarding time of application revealed that highest leaf area (38.92 cm²) was recorded in plants sprayed at 5th leaf stage, followed by leaf area (37.91cm²) at 7th leaf stage

which was statistically similar to leaf area (37.64 cm²) observed in plants treated with calcium at 3rd leaf stage. The interaction between calcium concentrations and application time significantly affected the leaf area of gladiolus (Figure 1). The leaf area of gladiolus was the least (31.4 cm²) in control plants (treated with distilled water), which increased to the maximum (43.6 cm²) with increasing calcium concentration to 200 mM, applied at 5th leaf stage that was followed by the leaf area of 43.6 cm² and 42.9 cm² in plants treated with 200 mM calcium at 3rd and 7th leaf stages respectively.

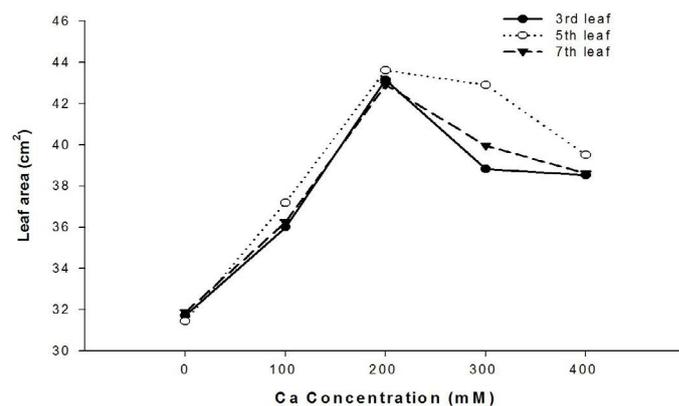


Figure 1: The influence of calcium concentrations and application time on the leaf area of gladiolus.

Leaf area is one of the main factors that influence plant growth and development. Plants having more leaf area will offer more space for efficient photosynthesis by receiving more photosynthetically active radiations (PAR) and hence more leaf area results in more photosynthates that enhance growth and production (Dodd et al., 2005). Exogenous application of 200 mM calcium significantly enhanced leaf area when sprayed at 5th leaf stage. Calcium arbitrates the action of auxin (Singla et al., 2006), which is involved in cell division and elongation (Quint and Gray, 2006; Cleland et al., 1999). The calcium is also involved in increasing the antioxidant activity (Sairam et al., 2011) by decreasing the level of ROS (Agarwal et al., 2005). Hence the application of 200 mM calcium at 5th leaf stage resulted in larger leaf area. The influence of auxin might have been mediated through its modulation of auxin activity resulting in increased cell division and expansion in leaf tissue (Quint and Gray, 2006; Cleland et al., 1999) as compared to low levels of auxin which are responsible for decreased leaf size (Romheld and Marchner, 1991). The reduced auxin activity in plants is also correlated with high oxidative stress and generation of reactive oxygen species (ROS) (Marshner, 1995). Hence calcium effects

Table 1: Leaf area (LA), chlorophyll content (CHL), Leaf calcium content (LCa), Floret calcium content (FCa), Size of daughter corm (SDC), Weight of daughter corm (WDC) and Calcium content in daughter corm (DCCa) as affected by calcium concentrations and time of application during 2016 and 2017.

Ca concentrations (C) mM	LA (cm ²)	CHL (SPAD)	LCa (%)	SDC (cm)	WDC (g)	DCCa (%)
000	31.67 e	43.425d	0.155e	5.06d	50.62d	0.121d
100	36.47 d	56.196b	0.470d	6.38c	60.68c	0.360c
200	43.21a	58.275a	1.258c	7.10a	61.52a	0.567a
300	40.56 b	54.349c	1.418a	6.72b	60.99b	0.546b
400	38.87 c	54.180c	1.398b	6.28c	60.80bc	0.544b
LSD0.05	0.96	0.596	0.010	0.32	0.22	0.004
Time of application (T)						
3 rd leaf	37.64 b	50.315b	0.938b	6.21b	58.78b	0.427
5 th leaf	38.92 a	55.041a	0.971a	6.26ab	58.82b	0.442
7 th leaf	37.91 b	54.498a	0.911c	6.45a	59.17a	0.413
LSD0.05	0.80	0.596	0.012	0.32	0.22	NS
Year						
Year 1	38.16	52.76	0.935	6.29	58.91	0.434
Year 2	38.15	53.81	0.945	6.33	58.94	0.421
Significance	NS	*	NS	NS	NS	**
Interaction C x T	* Fig. 1	** Fig. 2	* Fig. 3	* Fig. 4	* Fig. 5	NS

NS: Non-significant; *: Significant at $P \leq 0.05$; **: Significant at $P \leq 0.01$; Means followed by different letters in respective columns are significantly different from each other at $P \leq 0.05$.

might be due to lowered oxidative stress when applied at the optimum concentration (Agarwal et al., 2005; Abdolmaleki et al., 2015). It is clear from the results that 5th leaf stage is the best for calcium application. It is possible that the 3rd leaf stage exposes small area for calcium absorption to cause a significant effect, by contrast the 7th leaf stage is about their terminal stage of vegetative growth in gladiolus and hence calcium application may not contribute to the leaf area of the plant.

Chlorophyll content (SPAD)

The mean data across two years regarding the chlorophyll content as affected by foliar application of calcium and application time are presented in Table 1. Statistical analysis revealed significant differences in leaf chlorophyll content in response to calcium concentration and application time; the interaction of both factors was also significant. The mean data for two years indicated that the chlorophyll content was the least (43.425 SPAD), recorded in control plants increased to the maximum (58.275 SPAD) with 200 mM calcium treatment which was significantly higher than 56.196, 54.349 and 54.180 SPAD reading recorded with application of 100, 300 and 400 mM calcium concentrations respectively.

The two years averaged data among application times revealed that the chlorophyll content of the leaf was the least (50.315 SPAD) in plants of gladiolus sprayed at 3rd leaf stage, which increased significantly to 55.04 SPAD when calcium was applied at 5th leaf stage but declined to 54.498 SPAD with application of calcium at the 7th leaf stage. The difference in 5th and 7th leaf stages was, however, non-significant. Average data between years showed that chlorophyll content was higher in 2017 as compared to 2016. The interaction (Figure 2) between calcium concentrations and application time revealed that the chlorophyll content increased with increasing calcium concentration up to 200 mM calcium. But further increase in calcium concentration to 300 and 400 mM resulted in decline of chlorophyll content, irrespective of the application time. However, the highest chlorophyll content (60.725 SPAD) was recorded in plants sprayed with 200 mM calcium at 7th leaf stage, followed by (59.662 SPAD) with treatment of 200 mM calcium at 5th leaf stage, while the least chlorophyll content (40.74 SPAD) was found in plants sprayed with distilled water at 3rd leaf stage (Figure 2).

Chlorophyll is very important pigmented compound in autotrophic plants, required for the process of photosynthesis by capturing photosynthetically active

radiations (PAR) for plant growth and development (Kumar et al., 1988; Saeed et al., 2013). Calcium is an important intracellular messenger in plants and maintains protein and chlorophyll contents in their leaves; hence exogenous foliar application of calcium with various concentrations resulted in significant differences in leaf chlorophyll contents of rose cultivars (Poovaiah, 1988; Poovaiah, 1992; Oloo-Abucheli et al., 2016). Our results are also in agreement with Kumar et al. (2010) and Chang et al. (2012) who found that calcium treatment increased the chlorophyll and protein content in mungbean and oriental lily.

calcium whereas least calcium content was observed in plants treated sprayed with distilled water (no calcium). Interaction data pertaining leaf calcium content revealed the highly significant increase in leaf calcium content was recorded with treatment of 200 mM calcium at 5th leaf stage (Figure 3). Calcium is an essential nutrient required by plants for strengthening of cell wall and membrane (Marschner, 1995). As secondary messenger, it plays key role in regulating plant growth and development (Poovaiah, 1988; Hepler, 2004). Changes in calcium concentrations at cellular level have significant effect on physiological processes in plants (Poovaiah, 1988). The highest uptake of calcium at 5th leaf stage also increased the leaf area, leaf chlorophyll content (Table 1). The foliar application of calcium increased the concentration of calcium in tissues and leaves of tomatoes and roses (Eraslan et al., 2007; Abdolmaleki et al., 2015). Hence, it has been commonly used to satisfy the calcium demand of the plants or ameliorate calcium deficiency in the tissue (White and Broadley, 2003).

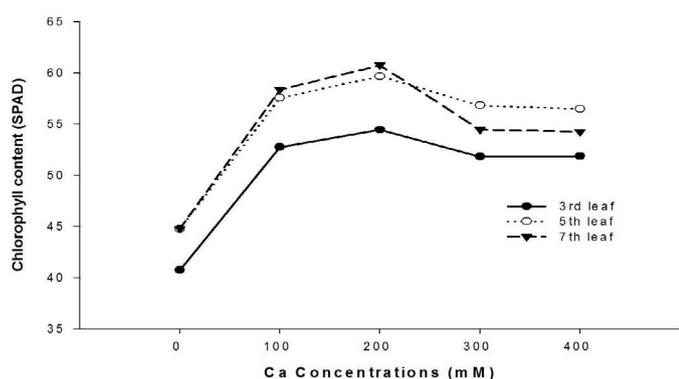


Figure 2: The influence of calcium concentrations and application time on the leaf chlorophyll content of gladiolus.

Leaf calcium content (%)

Data regarding calcium content in leaves are given in Table 1. Statistical analysis shows that calcium content in leaves was significantly influenced by calcium concentration, time of application and their interaction. Among various concentration of calcium, foliar application of 300 mM resulted in the highest leaf calcium content (1.418%) that was statistically at par with calcium content (1.398%) with treatment of 400 mM calcium concentration. The mean data across two years for foliar calcium application time showed that the highest calcium content (0.971%) were recorded in plants when foliar application of calcium was done at 5th leaf stage, followed by 0.938% with 3rd leaf stage. The leaf calcium content was the least (0.911%) with the foliar application of calcium at 7th leaf stage. Average data between years pertaining calcium content in leaf showed no-significant variations (Table 1). The interaction between calcium concentration and time of application (Figure 3) revealed that calcium contents of leaves were increased to the maximum (1.48%) with 300 mM of calcium at 5th leaf stage, followed by calcium content (1.44%) recorded with foliar application of 400 mM

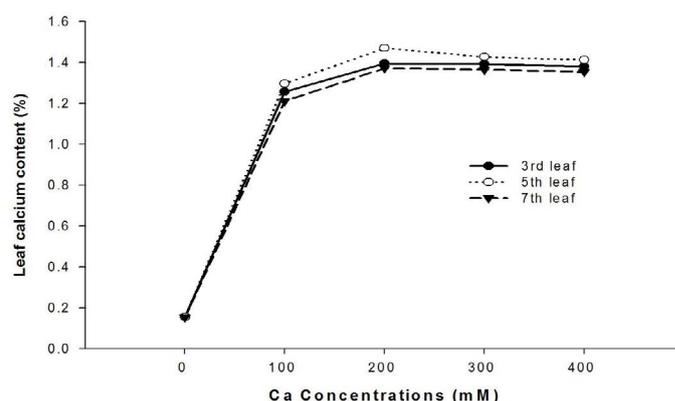


Figure 3: The influence of calcium concentrations and application time on leaf calcium content of gladiolus.

Size of daughter corm (cm)

Statistical analysis of data (Table 1) revealed that calcium concentration and timing of application as well as their interaction had significant influence on the size of daughter corm in gladiolus. The mean data (Table 1) for two years regarding various concentration of calcium showed that the maximum corms size (7.10 cm) was attained with foliar application of gladiolus plants with 200 mM calcium, followed by corm size (6.72 cm) with 300 mM calcium. The least corm size (5.06 cm) was recorded in control plots. The corm size also varied significantly in relation to the calcium application time. It is evident from mean data across two years pertaining time of application that foliar application of calcium at 7th leaf stage resulted in the maximum corms size (6.45 cm), followed by 6.26 cm

corm size in plants sprayed at 5th leaf stage. However, the difference in corm size with calcium application at 5th leaf stage was statistically non-significant with the size of daughter corm (6.21) with calcium treatment at 3rd leaf stage. The interaction between calcium concentration and foliar application time (Figure 4) revealed that there was an increasing trend in corm size with increasing concentration of calcium from 100 to 200 mM, but the corm size was decreased with further increase in calcium concentration to 300 and 400 mM at all leaf stages. The highest corm size (7.283 cm) was produced by plants sprayed with 200 mM calcium at 7th leaf stage while least size (5.033 cm) of daughter corm was observed in plants where only distilled water (control) was applied at 3rd leaf stage (Figure 4). Year's effect was statically non-significant on size of daughter corms (Table 1).

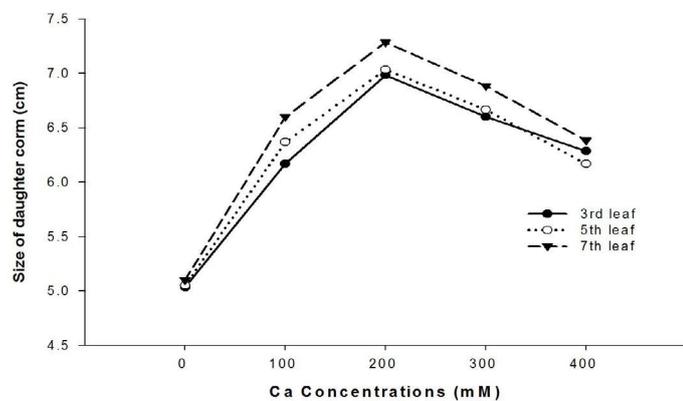


Figure 4: The influence of calcium concentrations and application time on size of daughter corm in gladiolus.

The size of daughter corm was significantly affected by the foliar application of calcium. Since, the calcium is not mobile in the plants (Jones and Jacobsen, 2005); it may have little direct influence on cell division and expansion in daughter corm (Ghoname et al., 2007). Thus, the influence of calcium application may be attributed to the increased leaf area as well as the chlorophyll content, and hence increased photosynthesis. It is likely to assume greater allocation of photosynthates to the daughter corms (Sharma et al., 2013), that resulted in corms with larger size (Reddy and Sarkar, 2016).

Weight of daughter corm (g)

The data related to weight of daughter corm as influenced by foliar application of calcium are presented in Table 1. According to the statistical analysis of data, calcium concentration and application time as well as their interaction significantly affected the weight of daughter corm (Table 1). The mean

data regarding various concentration of calcium showed that the corm weight was the least (50.62 g) in control plants, which increased significantly to 60.68 and 61.52 g with 100 and 200 mM calcium application, but thereafter, declined to 60.99 and 60.80 g with foliar application of 300 and 400 mM calcium. The two years mean data pertaining to the calcium application time revealed that foliar calcium application to the plants at 7th leaf stage produced the maximum corms weight (59.17 g), followed by 58.82 g in plants sprayed at 5th leaf stage that was statistically at par with calcium application at 3rd leaf stage. Two years averaged data pertaining interaction between calcium concentration and timing of foliar application are presented in Figure 5. The data indicated that the weight of daughter corm increased with increasing calcium concentration from 100 to 200 mM followed by a steady decrease with further increase in calcium concentration to 300 and 400 mM, at all the three leaf stages. However, plants that received 200 mM calcium at 7th leaf stage produced corms with highest weight (61.9 g). Average data between years showed that there was no significant variation in weight of daughter corm (Table 1).

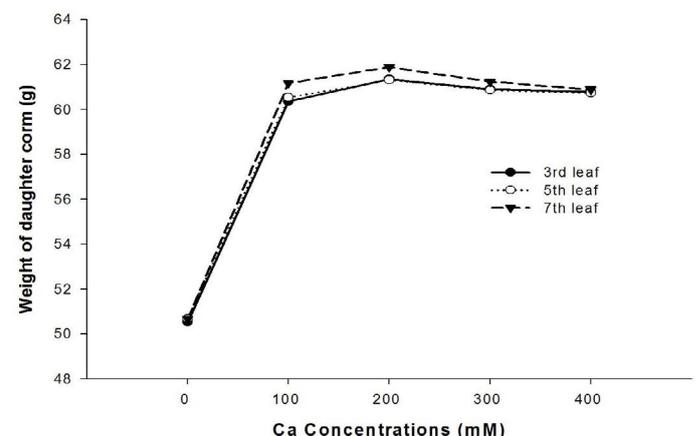


Figure 5: The influence of calcium concentrations and application time on weight of daughter corm in gladiolus.

The weight of daughter corms was affected in similar fashion to the size of daughter corm. The maximum weight of daughter corm (Figure 5) was recorded with foliar application of calcium at 7th leaf stage. Since, the calcium application resulted in increased leaf area, and increased the chlorophyll content of the leaf (Table 1), hence the plant may have had greater reserve carbohydrates available for the growth of the daughter corm, which resulted in increased weight of daughter corm (Reddy and Sarkar, 2016). Weight of daughter corm was higher with foliar application of calcium at later stage (7th leaf stage) as compared to

3rd and 5th leaf stages. It might be because of the fact that at early stage, the carbohydrates were allocated to both spikes and corms. But at 7th leaf stage, translocation of photosynthates was probably more towards daughter corms and hence plants produced corms with maximum size and weight (Reddy and Sarkar, 2016).

Calcium content (%) in daughter corms

Data pertaining to calcium content (%) as affected by calcium concentrations and time of foliar application in gladiolus are presented in Table 1. Statistical analysis (Table 1) showed that calcium concentrations had significant influence on endogenous calcium in daughter corms of gladiolus; however time of application and interaction between calcium concentration and application time was non-significant. Two years averaged data regarding various calcium concentration revealed that foliar application of 200 mM calcium resulted in the maximum calcium contents (0.567%), followed by (0.546%) with the foliar application of calcium at 300 mM that was statistically similar to the effect of 400 mM calcium. The least calcium content (0.121%) was recorded in daughter corms of control plots that were sprayed with distilled water.

The calcium content of daughter corms in gladiolus plants increased with foliar application of calcium. In a comparable study, Collier et al. (1978) observed higher calcium content in tubers of potato with treatment of calcium. The application of calcium has also been known to increase the concentrations of phosphorus, potassium and calcium and reduces the incidence of black spot in potato tubers (Clough, 1994; Karlsson et al., 2006). Thus, it could be the greater allocation of phosphorus and potassium due to the addition of calcium that contributed to increased corm size and weight (Memon et al., 2009).

Conclusions and Recommendations

- Plants of gladiolus treated with 200 mM calcium resulted in largest leaf area, highest leaf chlorophyll content, size and weight of daughter corms with highest calcium content in daughter corms. Foliar application of 300 mM resulted in the maximum calcium contents in leaves.
- Among timing of foliar applied calcium, plants that received calcium at 5th leaf stage produced maximum leaf area, chlorophyll content as well as

maximum calcium content in leaf and daughter corm of gladiolus. Foliar application of calcium at 7th leaf stage resulted in maximum size and weight of daughter corm.

- Interaction between calcium concentrations and application times revealed that foliar spray of 200 mM calcium at 5th leaf stage enhanced leaf area to the maximum, whereas application of the same concentration of calcium at 7th leaf stage resulted in maximum size and weight of daughter corm.
- Application of 200 mM calcium at 7th leaf stage could be recommended for better size and weight of daughter corm in gladiolus.

Author's Contribution

The article is a part of the Ph.D. dissertation and both the authors have contributed: Masood Ahmad (As PhD Scholar) conducted experiments, data collection, data analysis and writing of the manuscript. Prof. Dr. Abdur Rab supervised the whole research and provided assistance in technical writing of the Dissertation and this manuscript.

Novelty Statement

The research findings revealed the potential role of calcium for the quality production of corms in the gladiolus "One of the leading ornamental bulbous plants in Pakistan".

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