

Effects of a Gibberellin Inhibitor on Flowering, Vegetative Propagation, and Production of Rapid Generation Cycling Gladiolus for Potted Plant Production

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Abstract. *Gladiolus* (*Gladiolus* × *hybridus*) is an asexually propagated, herbaceous perennial and an economically important cut flower crop. In commercial production, gladioli have tall flower stalks, which limit their use to cut flowers and annual garden plants. The gladiolus breeding program at the University of Minnesota has bred and selected rapid generation cycling (RGC) cycle 1 gladiolus, which can flower in <1 year from seed instead of the norm of 3 to 5 years (which are vegetatively propagated as corms). Gibberellin inhibitors, such as ancymidol, are used as plant growth retardants to control height in potted plants. Higher concentrations can inhibit flowering along with other negative side effects. The aim of this study was to investigate the growth, flowering, and corm/cormel production response of cycle 1 gladiolus to the gibberellin inhibitor, ancymidol (0, 100, and 400 mg·L⁻¹ soak) in comparison with noncycle 1 genotypes and commercial cultivars for potted gladiolus production. Cycle 1 genotypes flowered with all ancymidol concentrations while noncycle 1 genotypes had significantly fewer flowers or were completely nonflowering under higher concentrations. All tested genotypes had increased leaf width as ancymidol concentration increased. Conversely, flower stalk heights were shorter as the ancymidol concentration increased while the number of stalks was nonsignificant. Corms, cormel number, and fresh weights decreased in all genotypes except for one cycle 1 genotype, which had an increase in both corm number and fresh weight when treated with 100 mg·L⁻¹ ancymidol. Cycle 1 gladiolus are more resilient to this gibberellin inhibitor even at high concentrations and can potentially be used for gladiolus potted plant production.

Gladiolus (*Gladiolus* × *hybridus* Rodigas) is a member of the Iridaceae, native to South Africa (Goldblatt and Manning, 1998), and a major cut flower in the floriculture industry (ranked in the top 10 species). The 2018 wholesale farmgate value of cut flower gladiolus is \$20.175M in the United States (U.S. Department of Agriculture, National Agricultural Statistics Service, 2019),

whereas in China, according to Forestry Bureau of China, the value was ≈\$41M in 2011 (Fukai, 2012). It is also commonly grown as an ornamental garden plant (nonhardy in northern latitudes, USDA Z3–4) (Anderson et al., 2012).

Due to its economic importance, the University of Minnesota flower breeding program is developing cold-tolerant gladiolus for USDA Z3–4 (Anderson et al., 2012) with reduced generation cycling (Anderson et al., 2015). Other breeding objectives are dwarf gladiolus for potted plant production, RGC, and seed-propagated F₁ hybrids. These would be new traits for this crop. RGC with the ability to flower from seed in <1 year (as early as 4–6 months) with a reduced juvenility and/or dormancy period is now a possibility (Anderson and Aljaser, 2019) due to 20 years of directed breeding and selection for flowering earliness (Anderson, 2019; Anderson et al., 2015); a U.S. Plant Utility Patent has been filed for breeding and selecting these phenotypes (Anderson and Aljaser,

2019). Such seed-propagated hybrids could also be forced to flower as vegetative clones without a cold treatment (Aljaser, 2020).

All gladiolus species are geophytes with corms (compressed stems) as underground storage organs (De Hertogh and Le Nard, 1993). In commercial production, gladioli are planted as mature (3–5 years old) corms (Dole and Wilkins, 2005). Gladioli are vegetatively propagated through daughter corms and cormels for commercial production. “Daughter corms” are defined as a specialized underground organ consisting of an enlarged stem axis with distinct nodes and internodes and enclosed by dry, scale-like leaves, whereas cormels refer to small corms arising from a mother corm (De Hertogh and Le Nard, 1993). Usually only one daughter corm is produced by the “mother corm” each year, whereas the number of cormels produced per year varies among cultivars (Cohat, 1993). Asexual propagation by any means (division, scooping, scoring, tissue culture) that produces the highest number of propagules (cormels, corms) as fast as possible is critical for meeting market demands for a clonal gladiolus cultivar. The need to increase the number of daughter corms and cormels quickly increases propagule pressure. Tissue culture provides an alternative method to produce cormels (Simonsen and Hildebrandt, 1971). The use of plant growth regulators, such as gibberellins, particularly GA₃ and GA₄₊₇ (Thomas and Hedden, 2006), has also been shown to increase cormel production. Gibberellins can increase the number of gladiolus cormels by means of exogenous applications (Khan et al., 2011).

Flowering in gladiolus is governed by factors such as corm size, vernalization, light intensity, long-day photoperiod, ambient temperature, and gibberellins (Ehrlich, 2013; Kamenetsky et al., 2012). In geophytes such as *Zantedeschia*, storing the tubers resulted in a sharp increase in the endogenous gibberellin levels in the buds (Naor et al., 2008). Likewise, in gladiolus corms, gibberellin levels increase during cold storage while abscisic acid (ABA), an inhibitor, decreases in concentration (Wu et al., 2015). In addition, exogenous applications of gibberellins can hasten flowering (Sudhakar and Kumar, 2012; Tonecki, 1980).

Gibberellin inhibitors consist of a wide range of plant growth retardants. Their primary use in commercial plant production is to reduce plant height through decreased cell elongation and cell division (Rademacher, 1991). Commercial applications are used to restrict plant height and establish a uniform height in ornamental crops using an appropriate concentration of gibberellin inhibitors for bulbous crops, such as *Hippeastrum* (Miller et al., 2012).

The PGR [ancymidol: α-cyclopropyl-α-(p-methoxyphenyl)-5-pyrimidinemethanol] is a pyrimidine analog. Its mode of action is blocking the monooxygenase enzyme that catalyzes *ent*-kaurene oxidation, a necessary step in the pathway between *ent*-kaurene and gibberellins (Rademacher, 1991). Ancymidol

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is applied at low rates ranging between 10 and 200 mg·L⁻¹ for foliar sprays and 0.15 to 0.5 mg per 15.24 cm² container for substrate drenches (Whipker and Evans, 2012). For gladiolus, a drench application rate of 1.5 mg A-Rest per 1.89 L was reported to effectively reduce height (Shaw et al., 1991). Conversely, drench applications of other types of gibberellin inhibitors, such as 0.8% 2-chloroethyltrimethylammonium chloride (CCC), resulted in increases of stem length, the number of florets per spike and slightly later flowering dates (Halevy and Shilo, 1970). These plant growth regulator compounds have not been tested on newly developed winter-hardy and dwarf germplasms in the University of Minnesota gladiolus breeding program, as well as new cycle-1 seed-propagated hybrids that flower in <1 year from sowing (Anderson, 2019; Anderson et al., 2015).

While gladiolus is currently valued as a cut flower and landscape ornamental, its long juvenile period and tall stature have made it unsuitable for potted plant production or as a bedding plant. Thus, there is a need to develop gladiolus plants with shorter juvenile periods and shorter statures both for breeding and genetic improvement of gladiolus as well as for potted plant production (Anderson and Aljaser, 2019). The University of Minnesota Flower Breeding and Genetic gladiolus program has the objective of producing new phenotypes of gladiolus with RGC, whereby as many as three cycles per year (where a cycle is equivalent to 1-year growth, dry down, and vernalization treatments) enables faster breeding and selecting (Anderson, 2019; Anderson et al., 2015; Anderson and Aljaser, 2019). In gladiolus, the period of seed to flower may encompass 3 to 5 years (Anderson, 2019). Anderson et al. (2015) created an RGC program for gladiolus that included enhanced selection of seed-propagated hybrids for early germination, early leaf unfolding, high leaf numbers in RGC 1 to 3, early flowering stalk emergence, and hastened flowering (flower bud initiation and development). All selection was done under standard growing conditions for the species, i.e., seed germination (2–7 weeks) in glasshouse mist systems (21 °C day/night) followed by subsequent growth of transplants for 7 weeks in glasshouses (24/20 °C day/night; inductive long-day photoperiods for flowering, ≥150 μmol·m⁻²·s⁻¹, 0600–2200 HR). Several genotypes were selected that

flowered <1 year from seed in cycle 1, meaning they did not require a vernalization period to mobilize GA and breakdown ABA concentrations. Because these unique hybrids were bred and selected without vernalization treatments, it is unknown whether these genotypes have the same response to GA inhibitors based on published literature on geophytes. The objective of this study was to demonstrate cycle 1 gladiolus genotypic response to a GA inhibitor for flowering, vegetative propagation production (corm and cormels), and their overall use for potted plant production of gladiolus. The null hypothesis was: H₀ = Treatment of ancymidol negatively impact gladiolus plant growth and flowering capability. The alternative hypothesis was: H_A = Ancymidol concentrations positively impact gladiolus plant growth and flowering capability.

Material and Methods

Plant material. Seven gladiolus genotypes (vegetative clones) were used in this experiment. Two genotypes were commercial cultivars, ‘Amsterdam’ and ‘Bananarama’, which served as controls (comparisons). Flowering size corms of ‘Amsterdam’ and ‘Bananarama’ were obtained from Noweta Gardens (Table 1) during 2017 and had been produced in the same field conditions by the producer. ‘Amsterdam’ is a white-colored gladiolus that is a hybrid cultivar derived from unknown parents bred by J. and P. Snoek and Sons, Ltd. (Flevoland, The Netherlands) in 1992 (North America Gladiolus Counsel, 1999). ‘Bananarama’ is a new yellow-colored cultivar bred by Coöperatieve Kwekersvereniging “For Ever” U.A. and released in 2013 (Saint Maarten, The Netherlands; KAVB, 2014). On receipt of mature commercial corms with flowering capacity, they were cooled at 2 °C in darkness (Widmer, 1958) for >1000 h before experimentation. Five University of Minnesota RGC gladiolus breeding lines were also tested: RGC-Genotype 1, RGC-Genotype 2, RGC-Genotype 3, RGC-Genotype 4, and RGC-Genotype 5 (Table 1). All RGC hybrid cormels were produced in the same greenhouse selection environment (Anderson et al., 2015) and were capable of flowering. Thus, RGC cormels are physiologically equivalent to the mature commercial corms for flowering capacity. As many RGC hybrid cormels as possible

were obtained and vernalized along with the commercial cultivars for the same duration before the commencement of this experiment.

Treatments. Three treatments (0, 100, and 400 mg·L⁻¹) of the gibberellin inhibitor ancymidol (A-Rest 0.0264% a.i.; SePRO Corp., Carmel, IN) were used (Table 1). All corms were soaked in the solutions for a period of 24 h before planting. There were n = 4 to 8 replications/genotype, depending on the availability of cormels from the RGC genotypes (Table 1). Thus, this experiment was an unbalanced design.

Greenhouse growing conditions. After 24 h of treatment, corms were planted into 1679.8 cm² square, deep pots (Belden Plastics, St. Paul, MN) in week 23 (2017) and grown for 18 weeks. Containers were filled with a peatmoss based on soilless substrate (SunGrow SS#8-F2-RSi; Sun Gro Horticulture, Agawam, MA). The corms were grown in a long-day photoperiod (0800–1600 HR supplied by 400-W high-pressure sodium lamps + 2200 to 0200 HR night interruption, >150 μmol·m⁻²·s⁻¹) at a minimum setpoint of 18 °C (day/night), 70% to 80% relative humidity, with irrigation accomplished using constant liquid feed of 125 mg·L⁻¹ N from water-soluble 20N–4.4P–16.6K (Peters Professional 20–10–20 peat-lite; ICL Fertilizers, Tel-Aviv, Israel) and deionized water on weekends. Standard fungicide drenches and insecticides were applied either monthly or as needed, respectively.

Data collected. Foliage height (cm) was recorded at the peak of highest growth (measured from soil line to the tip of the uppermost leaf), flower stalk height (cm; measured as length from the uppermost leaf to the tip of the uppermost floral bud), flowering (+/–), and leaf width (cm) for widest leaf on each replicate. At the termination of the experiment, the number of corms and cormels were counted for production purposes and their respective fresh weights (g) recorded.

Statistical analysis. Replicates were arranged in a complete random design, and all quantitative data were analyzed as unbalanced analysis of variance and Tukey’s honestly significant difference mean separations at $P \leq 0.05$ using JMP 13 statistical software (Campus Drive, Cary, NC). Chi-squares (χ^2) were calculated using a 1:1:1 χ^2 test ratio for equal distribution of flowering response among the three different ancymidol treatments (0, 100, and 400 mg·L⁻¹).

Table 1. Number of replicates per treatment (0, 100, and 400 mg·L⁻¹ of ancymidol) and commercial or breeding source of the tested *Gladiolus* genotypes.

Genotype	No. of replicates / treatment of ancymidol concn (mg·L ⁻¹)			Commercial or Breeding Source
	0 (Control)	100	400	
‘Amsterdam’	8	8	8	Noweta Gardens, Inc., Three Rivers, MI
‘Bananarama’	8	8	8	Noweta Gardens, Inc., Three Rivers, MI
RGC-1	4	4	4	University of Minnesota, St. Paul, MN
RGC-2	4	4	4	University of Minnesota, St. Paul, MN
RGC-3	7	7	7	University of Minnesota, St. Paul, MN
RGC-4	4	4	4	University of Minnesota, St. Paul, MN
RGC-5	5	5	5	University of Minnesota, St. Paul, MN

Results

Ancymidol treatments resulted in variation in flowering between genotypes (Table 2). 'Amsterdam' and 'Bananarama' did not flower even after 18 weeks from corm planting in pots in comparison with control, which flowered in 10 and 11 weeks, respectively (Table 3). Genotype RGC-1 did not flower in all treatments, whereas RGC-2, RGC-3, RGC-4, and RGC-5 all flowered even at the highest ancymidol concentration, yet with variation in number of flowering plants in each treatment (Table 2). All flowering genotypes were not significantly different in 1:1:1 χ^2 except for 'Bananarama' (Table 2). RGC-2, RGC-3, RGC-4, and RGC-5 genotypes, which flowered at high ancymidol concentration and were delayed in flowering by 1 to 2 weeks from the control (Table 3).

Plant height ranged from 19 to 108 cm (Table 4). Ancymidol significantly decreased plant height in the higher concentrations in all

tested genotypes in exception of RGC-4 in which 100 mg·L⁻¹ (105.3 cm in height) was not statistically different from 0 mg·L⁻¹ treatment (106.0 cm in height) (Table 4). Both the number of stalks and leaf width were significantly different in the genotype, while in the treatment, leaf width was significantly increased in width with the increase of ancymidol concentration, ranging from 1.5 to 5.6 cm (Table 4). Corms and cormels were harvested after all plants senesced. They ranged in number from 1.0 to 8.0 with mean fresh weights of 3.7 to 40.8 g (Table 4).

Discussion

Gibberellin inhibitors such as ancymidol and paclobutrazol are reported to delay flowering in *Tulipa* (McDaniel, 1990), *Lilium* (Bailey and Miller, 1989), and *Gladiolus* (Ahmad et al., 2014). However, high concentrations of ancymidol resulted in completely inhibiting flowering in 'Bananarama' (Fig. 1), as it was the only genotype that flowered in the

0 mg·L⁻¹ (control) treatment with complete lack of flowering in the other two treatments (100 and 400 mg·L⁻¹ ancymidol). This was also reported in different *Watsonia* species, as dipping the corms in 0.5, 1, and 2 mg of paclobutrazol produced nonflowering plants. However, post-planting applications of 5, 10, and 25 mg of paclobutrazol resulted in flowering *Watsonia* as marketable potted plants (Ascough et al., 2006). Treatments of exogenous gibberellins after pretreatment with paclobutrazol in *Tulipa* reversed the influence of paclobutrazol and promoted flowering (Rebers et al., 1994). This demonstrates the importance of gibberellins in the geophytic flowering pathway (Naor et al., 2008). Furthermore, RGC-2, RGC-3, RGC-4, and RGC-5 genotypes, which flowered at high ancymidol concentration were delayed in flowering by 1 to 2 weeks from the control (Table 3). This has been demonstrated using paclobutrazol on gladiolus, which resulted in delayed flowering as the concentration increased (Milandri et al., 2008). However, the RGC-3, RGC-4, and

Table 2. Influence of ancymidol concentrations (0, 100, and 400 mg·L⁻¹) on the number (n) of flowering plants (frequency of flowering) and 1:1:1 χ^2 test ratios of the tested *Gladiolus* genotypes.

Genotype	No. of flowering plants in ancymidol (mg·L ⁻¹)			1:1:1 χ^2	Significance
	n	0 (control)	100	400	
'Amsterdam'	24	4	0	0	5.20 NS ^z
'Bananarama'	24	5	0	0	9.98 **
RGC-1	12	0	0	0	— ^y
RGC-2	12	2	2	1	0.40 NS
RGC-3	21	6	1	5	3.50 NS
RGC-4	12	4	4	2	0.80 NS
RGC-5	15	5	1	3	2.67 NS
Pooled	120	26	9	11	11.26 **
Noncycle 1	72	11	3	1	11.20 **
Cycle 1	48	15	6	10	3.94 NS

^zNS, **Nonsignificant or significant at $P = 0.01$, respectively.

^yNot estimable due to no flowering in all replicates.

Table 3. Influence of ancymidol concentrations (0, 100, and 400 mg·L⁻¹) on the number (n) of flowering plants (frequency of flowering) and mean number of weeks reached to flowering in each genotypes of the tested *Gladiolus* genotypes.

Genotype	n	Treatments	No. of flowering plants	Mean no. of weeks reached to flowering
'Amsterdam'	8	0	4	10
	8	100	1	12
	8	400	0	— ^z
'Bananarama'	8	0	5	11
	8	100	0	—
	8	400	0	—
RGC-1	4	0	0	—
	4	100	0	—
	4	400	0	—
RGC-2	4	0	2	11
	4	100	2	12
	4	400	1	13
RGC-3	7	0	6	10
	7	100	1	11
	7	400	5	12
RGC-4	4	0	4	11
	4	100	4	12
	4	400	2	13
RGC-5	5	0	5	10
	5	100	1	11
	5	400	3	12
Treatment				— ^y
Genotype				*** ^x
Treatment × genotype				***

^zNot estimable due to no flowering in all replicates.

^yNot estimable due to the number of nonflowering genotypes.

^xAnalysis of variance for NS or *** (nonsignificant or significant at $P = 0.001$, respectively).

Table 4. Mean plant height (cm), flower stalk height (cm), number of stalks, leaf width (cm), no. of corms, fresh weight (FW) of corms (g), number of cormels, and FW of cormels (g) for seven gladiolus genotype corms treated with different concentrations of ancymidol (0, 100, and 400 mg·L⁻¹).

Genotype	Ancymidol treatments	Plant ht (cm)	Flower stalk ht (cm)	No. of stalks	Leaf width (cm)	No. of corms	FW of corms (g)	No. of cormels	FW of cormels (g)
'Amsterdam'	0	102.8 ab ^z	101.3 abc	1.7 bc	4.4 bcde	1.8 c	22.9 bcde	0.0 b	— ^x
	100	78.0 cde	80.0 abc	2.0 bc	5.6 a	1.9 c	18.5 de	0.0 b	— ^x
	400	52.4 fg	— ^y	1.6 bc	4.9 abcd	2.0 c	8.4 e	0.0 b	— ^x
'Bananarama'	0	106.3 a	107.8 ab	2.1 bc	3.5 efgh	2.1 c	28.1 abcd	0.0 b	— ^x
	100	62.9 ef	— ^y	2.0 bc	5.2 ab	2.0 c	19.3 cde	0.0 b	— ^x
	400	40.7 g	— ^y	1.7 bc	5.1 abc	1.8 c	17.1 de	0.0 b	— ^x
RGC-1	0	101.5 abc	— ^y	3.0 abc	2.2 hi	3.0 bc	29.2 abcd	2.5 b	1.4
	100	53.3 efg	— ^y	2.0 bc	2.3 hi	2.3 c	14.2 de	0.8 b	0.2
	400	19.0 g	— ^y	1.0 c	1.5 hi	1.0 c	3.7 e	0.0 b	— ^x
RGC-2	0	83.3 abcde	56.0 c	4.0 abc	2.6 fghi	3.3 bc	32.6 abcd	2.7 b	0.3
	100	56.3 efg	55.5 c	2.8 bc	2.6 ghi	3.8 bc	17.2 de	0.0 b	— ^x
	400	33.5 g	56.0 c	1.3 bc	2.2 hi	2.3 c	8.5 e	0.0 b	— ^x
RGC-3	0	108.0 a	121.2 a	1.3 bc	3.7 defg	1.3 c	26.1 abcd	24.0 a	4.1
	100	75.7 cdef	107.0 abc	1.8 bc	4.4 bcde	2.0 c	26.1 abcd	7.5 b	1.6
	400	67.0 def	65.0 c	1.0 c	4.1 cdef	1.5 c	21.2 bcde	2.8 b	0.9
RGC-4	0	106.0 a	113.0 ab	4.0 ab	3.7 defgh	3.5 bc	37.6 abc	1.3 b	0.2
	100	105.3 a	98.5 abc	6.5 a	3.8 defg	8.0 a	40.8 a	0.8 b	0.1
	400	89.0 abcd	82.5 abc	6.3 a	4.1 bcdef	6.5 ab	37.9 ab	0.0 b	— ^x
RGC-5	0	98.8 abc	85.6 abc	3.2 abc	3.0 fghi	3.3 bc	20.9 bcde	0.6 b	1.2
	100	77.0 cdef	72.0 abc	3.3 abc	3.3 efghi	4.3 abc	8.9 e	0.0 b	— ^x
	400	66.2 def	61.3 c	2.4 bc	3.1 fghi	2.0 bc	7.7 e	0.2 b	0.1
Treatment		***	— ^w	NS	***	*	***	**	— ^w
Genotype		***	***	***	***	***	***	***	— ^w
Treatment × genotype		***	NS	NS	***	NS	NS	***	— ^w

^zMeans within a column not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference means comparison.

^yNo production of cormels.

^xNonflowering.

^wNot estimable due to the number of nonflowering genotypes.



Fig. 1. 'Bananarama' gladiolus treated with ancymidol concentrations of 400, 100, and 0 mg·L⁻¹, left to right respectively. Photo credit: Jaser Aljaser.

RGC-5 genotypes are different in response because they are bred to be cycle 1 gladiolus with flowering in less than 1 year from sowing, all of which show short dormancy (Aljaser, 2020). These are genotypically different in response to commercial gladioli with deep dormancy (Kumar and Raju, 2007) overcome only with a cooling period. Pooled data for all genotypes were significantly different in flowering response (did not fit the 1:1:1 χ^2) as well for pooled noncycle 1 genotypes ('Amsterdam', 'Bananarama', RGC-1, and RGC-2) (Table 2). However, no differences were found with three of four cycle 1

genotypes (RGC-3, RGC-4, and RGC-5), indicating a complete lack of effect from the ancymidol treatment. This difference among RGC genotypes is most likely genetic in nature, although further testing would be required to determine the exact causal gene(s). Nonetheless, the parents and RGC3 to RGC5 are novel genotypes in gladiolus and will be used to enhance development of dwarf, seed-propagated cycle 1 selections that flower in <1 year from sowing. The reduction of plant height (Table 4) matches the reports of gladiolus using flurprimidol (Ahmad et al., 2014). Because gibberellin inhibitors are used to control the plant height by reducing gibberellin biosynthesis, this leads to limiting cell expansion and, thus, affecting plant height (Cosgrove and Sovonick-Dunford, 1989). The application of ancymidol, paclobutrazol and uniconazole decreased lily plant height even with a 1-minute dip at different concentrations (Ranwala et al., 2002). In addition to plant height, the flower stalk height was also decreased by ancymidol in 'Amsterdam', RGC-3, RGC-4, and RGC-5, whereas the flower stalk height of RGC-4 was not influenced by ancymidol treatments and the height was almost identical in all treatments. In RGC-3 and RGC-5, the 400 mg·L⁻¹ ancymidol concentration resulted in more marketable gladiolus for potted production in terms of plant height and flowering capability (Fig. 2). In freesia, *Freesia ×hybrida*, dipping corms in 200 mg·L⁻¹ of ancymidol was also reported to reduce flower stalk height (Berghoef and Zevenbergen, 1989). In genotype RGC-1 and RGC-2, the number of stalks

decreased significantly as ancymidol concentration increased, whereas only RGC-4 had an increase in the number of stalks. However, low concentrations (10 and 20 mg·L⁻¹) of paclobutrazol on *Ornithogalum thyrsoides* (a bulbous geophyte) increased the number of spikes per plant (Banswal, 2012). This indicates that increased levels of gibberellin inhibitors may influence the number of emerging stalks by genotype and species level.

Leaf width was significantly increased with ancymidol treatment, ranging from 1.5 to 5.6 cm (Table 4). Ancymidol at 100 mg·L⁻¹ resulted in the leaves with widest length that were wider than both the control and 400 mg·L⁻¹ treatments in all genotypes with the exception of RGC-2 (Table 4). This increase in leaf width does not necessarily mean a corollary increase in leaf area. As reported by Bailey and Miller (1989), gibberellin inhibitors tend to reduce whole leaf area with increased applied concentration in *Lilium longiflorum* 'Nellie White' as well as *Freesia ×hybrida* (De Hertogh and Milks, 1989).

The number of corms showed variation with respect to treatment with 100 mg·L⁻¹ significantly increased the number of corms only in RGC-4 and RGC-5. However, the corollary corm weight did not increase, as the control treatment resulted in the highest fresh weight of all genotypes (Table 4). Similar results were reported for gladiolus (Ahmad et al., 2014). On the other hand, the number of cormels and fresh weight did not increase in number and weight. Likewise, the number of cormels decreased in those genotypes producing cormels. The decrease in number of



Fig. 2. Rapid Generation Cycling (RGC) RGC-3 gladiolus treated with ancymidol concentrations of 400, 100, and 0 mg·L⁻¹, left to right respectively. Photo credit: Jaser Aljaser.



Fig. 3. Gladiolus treated with ancymidol concentration of 400 mg·L⁻¹. RGC-2 reached to flowering (left), whereas 'Bananarama' remained in vegetative state (right). Photo credit: Jaser Aljaser.

harvested cormels and fresh weight was similar to previous reports of gibberellin inhibitors in gladiolus, where concentrations as low as 10 mg·L⁻¹ paclobutrazol decreased cormel formation at the expense of increasing corm swelling in tissue culture (Steinitz and Lilien-Kipnis, 1989). This could mean the corm-cormel relationship is quantitative vs. qualitative, as gibberellin inhibitors influence a reduction in the number of cormels and corms. However, a recent study on corm and cormel formation was linked to the *GhAGPL1* gene, indicating the role of ADP-glucose pyrophosphorylase (AGPase) in starch accumulation, as silencing *GhAGPL1* resulted in a reduction of corms and cormels (Seng and Wu et al., 2017). Therefore, the role of ancymidol as a gibberellin inhibitor could be hypothesized to interrupt the starch accumulation in corms and cormels, thus resulting in a reduction in weight. Future studies will be directed to answer this question.

In conclusion, gibberellin inhibitors such as ancymidol should be applied at precise concentrations for each genotype, as higher concentrations could result in failure to

flower and reduce the fresh weight of both corms and cormels, which are essential for gladiolus floral production in the market. The desirable ideotype in producing potted gladiolus includes the plant height matching the aesthetic ratio of 1.5× to 2× the container diameter or height (whichever is greater) with ≥5 leaves, possessing ≥7 florets that open fully into a decorative floret size (8.9–11.4 cm) (Anderson and Aljaser, 2019; Okubo and Sochacki, 2012). The tested cycle 1 gladioli have an increased tolerance of higher concentrations of gibberellin inhibitors, such as ancymidol, to reduce plant height. Such RGC gladioli are still able to flower, unlike noncycle 1 gladiolus genotypes, which exhibit reduced height and significantly less flowering capability at higher gibberellin inhibitor concentration (Fig. 3). Therefore, the recommended ancymidol concentration for non-cycle 1 gladiolus should not exceed 100 mg·L⁻¹, whereas cycle 1 gladiolus may tolerate as high as 100 mg·L⁻¹ for potted gladiolus production. The use of gladiolus RGC genotypes with the addition of gibberellin inhibitors can achieve the desirable objective for plant height matching the aesthetic ratio for gladiolus potted plant production. Although flowering was eliminated in the commercial, non-RGC gladiolus cultivars tested at either 100 mg·L⁻¹ or 400 mg·L⁻¹ ancymidol (Table 4), a reduction in plant height was realized with flowering (Table 4; Fig. 1). Thus, additional cultivars could be tested with gibberellin inhibitors to potentially find genotypes that will flower with reduced plant height. However, the greatest potential resides in the RGC gladioli to achieve the target height for gladiolus potted plant production; RGC-2 would represent the ideal height gladiolus potted plant, foliage display, and flowering stalk height (Fig. 2; Table 4).

Further research will be conducted at lower concentrations to determine the recommended concentration to achieve the appropriate concentration for gladiolus potted production. Also, the research should include measuring the rachis distance between florets, number of florets, and whole leaf area. In addition, histological cross sectioning of floral differentiation at the three-leaf stage to determine if ancymidol treatments inhibited floral differentiation growth in nonflowering genotypes as gladiolus is reported to have a visible floral spike at the three leaves stage (Schwab et al., 2015). The applied concentrations are relatively high (100 and 400 mg·L⁻¹); thus, lower concentrations would be required to study the influence of ancymidol.

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