



## Article

# Gibberellic Acid Can Improve Seed Germination and Ornamental Quality of Selected *Cyclamen* Species Grown Under Short and Long Days

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**Abstract:** *Cyclamen* species (swinebread spp.) are perennial herbaceous plants which belong to the *Primulaceae* family, widely used as medicinal or ornamental plants. Currently, gibberellic acid (GA<sub>3</sub>), an environmentally friendly bioregulator is widely used to enhance the productivity and phenotypic characteristics of multiple ornamental crops. A dual experiment was conducted to evaluate the influence of gibberellic acid (50, 100 or 150 mg/L), on germination earliness of seeds, along with growth and ornamental quality of selected *Cyclamen* species. Additionally, a greenhouse experiment with long and short sunlight photoperiod was conducted to evaluate its importance in the development of *Cyclamen* plants. Our results indicated that under short sunlight exposure, seeds germinated earlier than those kept in long sunlight photoperiod. Plants treated with foliar applications of GA<sub>3</sub> exhibited higher plant height, leaf area, number of leaves, root length and tuber development. Significant differences were found in germination percentage (GP), mean germination time (MGT) and seedling vigor index (SVI), within different GA<sub>3</sub> supplementation and greenhouse sunlight intensity (short and long days). In conclusion, our study suggests that seedling vigor and ornamental quality can be increased by GA<sub>3</sub> application, an economic strategy for enhancing germination and growth of selected *Cyclamen* species.

**Keywords:** *Cyclamen* species; germination parameters; gibberellic acid; photoperiod

## 1. Introduction

*Cyclamen* species (sowbread or swinebread spp.) are a class/group of popular herbaceous ornamental and medicinal plants distributed in and near the Mediterranean region [1]. Cultivation of *Cyclamen* species in regions such as northern Europe, North America, Japan, Australia and New Zealand underscores their remarkable ability and plasticity to adapt to new ecological environments. However, environmental degradation, displacement and habitat modifications along with tuber over-exploitation have exerted pressure on the native populations of these valuable ornamental species. Therefore, measures of protection were taken by including them in various conservation organizations. *Cyclamen coum* (eastern sowbread), *Cyclamen hederifolium* ‘Alba’ (ivy-leaved cyclamen or sowbread) and *Cyclamen purpurascens* (Alpine, European or purple cyclamen) are included in the IUCN (International Union for Conservation of Nature) Red List of Threatened Species [2]. Although *C. hederifolium* ‘Alba’ is included in CITES Appendix II, artificial propagation of new cultivars is allowed, being also listed as endangered in Switzerland [3]. *C. purpurascens* is considered to be endangered due to the collection

of wild plants for medicinal purposes in Croatia [4]. Permanent loss of habitats resulting from the elimination and degradation of forests is probably a consequence that will lead to shrinking and extirpation of local populations.

Gibberellins (GA) are essential plant regulators for multiple plant development processes, including seed germination, stem elongation, leaf extension, pollen maturation, and flowering induction. Therefore, mutant plants that are deficient in GA have a dwarf phenotype and late flowering; treating these plants with GA restores normal growth. Gibberellic acid (GA<sub>3</sub>) is a natural plant regulator with multiple applications in agriculture and horticulture industry due to its positive impact on plant growth and development. Its positive effects have been demonstrated in multiple crops and ornamental plants by exhibiting enhanced seed germination [5,6], shortening the juvenile phase, leaf expansion and development, stem elongation [7,8], early flowering [9–13] and fruit development; their role in the promotion of flowering in plants was reported in several studies, including *Arabidopsis thaliana* (thale cress) [14], *Brunonia australis* R.Br. (blue pincushion or native cornflower) [15], *Henckelia humboldtianus* (Humboldt's Stone Flower) [16], and *Iris nigricans* Dinsm. (black iris) [17]. Nowadays an increasing interest in patterned foliages and long vase life of *Cyclamen* cut-flowers urged researchers to develop new protocols to fulfill the market requirements. Foliar applications of GA<sub>3</sub> enhanced flower stem length in soil grown cut-flowers [18] and delayed leaf yellowing [19].

It is well known that day length, along with temperature and water supply, is one of the most important traits influencing growth and development of *Cyclamen*. Previous studies have confirmed that GAs contents significantly increased in the long-short day plant *Bryophyllum daigremontianum* (devil's backbone), when transferred from under long-day to short-day condition [20,21]. Huang [22] showed that flowering was induced in *Kalanchoe pinnata* (Goethe plant) and *K. pinnatei* (alligator plant) plants when 5 mg/L GA<sub>3</sub> was applied on them. Therefore, exogenous GAs appears to promote the transition from vegetative growth to reproductive growth [14]. Light intensity is considered to be a stress to multiple plants, especially to *Cyclamen*, but also an important aspect with regards to plant growth and development. As described by the Cyclamen Society, sunlight below 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  causes slow growth and poor quality in ordinary mini cyclamen, requiring supplemental lighting [23]. These conditions usually occur in greenhouses during winter (November to February) and summer rainy season. However, few studies have been conducted to trace the origin of high elongation rate under such conditions [8].

Taking into account the medicinal importance of *Cyclamen*, the aim of the study was to investigate the response of multiple *Cyclamen* species to different concentrations of GA<sub>3</sub> under short and long day exposure. These methods were studied as a potential way to shorten the germination and propagation period of these *Cyclamen* species. Therefore, the main objective of this study was to determine (1) the effects of GA<sub>3</sub> application on seed germination and plant development, and (2) the optimal GA<sub>3</sub> dosage applied to *Cyclamen* accessions as affected by different sunlight intensities.

## 2. Materials and Methods

### 2.1. Plant Materials

A pot experiment was conducted between 2018–2019 (June to May) at the Advanced Horticultural Research Institute of Transylvania (AHRIT), University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, using selected *Cyclamen* species (fall blooming *C. africanum* Boiss & Reut (African cyclamen), *C. cypricum* Schott & Kotschy (Cyprus cyclamen), *C. hederifolium* Aiton 'Alba' (ivy-leaved cyclamen), *C. mirabile* Hildebr (sowbread) and spring blooming *C. pseudibericum* Hildebr (false Iberian cyclamen)). *Cyclamen* seeds (dry, not fresh) were purchased from various botanical gardens in Europe, namely: *C. africanum*, *C. cypricum*, *C. hederifolium* 'Alba' were purchased from Botanic Gardens and Arboretum of Mendel University of Agriculture and Forestry from the Czech Republic, Brno. *C. mirabile* and *C. pseudibericum* were purchased from Friedrich Schiller Universität Jena, Institut für Ökologie und Evolution, Botanischer Garten Jena, Germany.

## 2.2. Experiment Design and GA<sub>3</sub> Application on Selected Cyclamen Species

The first experiment consisted of soaking of the *Cyclamen* seeds in different concentrations of GA<sub>3</sub> (control, 50 mg/L, 100 mg/L, 150 mg/L) in order to evaluate the germination of the selected *Cyclamen* species.

Seeds were surface sterilized (4.0% sodium hypochlorite solution) for 15 min at room temperature followed by triple-rinsing with distilled sterile water. They were placed on cotton pads and covered with two layers of Whatman filter paper no. 1 in 9 cm Petri dishes, with 10 mL of distilled water and gibberellic acid (GA<sub>3</sub>) on 10th June 2018. Petri dishes were kept at 65% humidity and alternating temperatures of 23–25 °C with a photoperiod of 15–16 h light (day) and 8–9 h (night) for the long-day exposure treatment and of 5–6 h light and 17–18 h dark for the short-day exposure treatment. After 5 weeks on 18th July the seeds started to germinate. The seedlings were transplanted into pots (14 × 13 cm in diameter) and watering with and without GA<sub>3</sub> solution was carried out on the basis of plant requirements (4 days interval). The growing substrate (60/20/20 v/v) was a mixture of sowing and propagation soil (pH = 6.0) with a content of N 0.1 m/m%, P<sub>2</sub>O<sub>5</sub> 0.01 m/m%, K<sub>2</sub>O 0.03 m/m%, *Cyclamen* substrate (pH = 6.2) with a content of N 1.0 m/m%, P<sub>2</sub>O<sub>5</sub> 0.1 m/m%, K<sub>2</sub>O 0.3 m/m%, containing 70% organic substances and perlite.

The influence of exposure to short and long day condition (GA<sub>3</sub>) on morphological parameters was evaluated. The plants were germinated at 24 ± 1 °C in approximately 60 days, with two distinct photoperiod regimes: long-day exposure (~14 h light/10 h dark) and short-day exposure (~6 h light/18 h dark). The intensity of the brightness was measured every two-three days using a luxmeter (Testo 440, Turda, Romania). The average of the greenhouse recordings at the end of the experiment were 842.2 μmol m<sup>2</sup> s<sup>-1</sup>, and for the shaded plants 147.6 μmol m<sup>2</sup> s<sup>-1</sup>.

## 2.3. Effect of Exogenous GA<sub>3</sub> on Morphological and Physiological Traits Under Different Light Exposure Treatments

Germinated seeds were counted every three days; a seed was considered as germinated at the moment of radicle protrusion. At the end of the experiments (day 65) multiple germination parameters were calculated; in addition, all germinated and non-germinated seeds were scanned and the ImageJ Programme 1.52a, Wayne Rasband, National Institutes of Health Bethesda, Maryland, USA for image processing was used to measure plant height, radical and petiole lengths (cm), leaf area and tuber diameter (cm<sup>2</sup>). Ten different germination parameters were assessed.

The germination percentage [25] was calculated using the method described by the Equation (1).

$$GP = \frac{\sum Us}{Gs} \times 100\% \quad (1)$$

where *Us* is the number of seeds used and *Gs* in the final number of germinated seeds. To assess germination velocity [24,25], mean germination time (MGT) was calculated using the method described by the Equation (2).

$$MGT = \frac{\sum Dn}{\sum n} \quad (2)$$

where *D* is the days from the beginning of the germination test, and *n* is the number of seeds newly germinated on day *D* [26]. The Seedling Vigour Index (SVI) [24] was calculated using the Equation (3):

$$SVI = GP \times (Mr + Mh) \quad (3)$$

where *GP* is the germination percentage, *Mr* is the mean root length (mm) and *Mh* is the Mean hypocotyl length (mm), as described by Abdul-Baki & Anderson [27]. The following parameters used: mean germination rate (MGR) [24], coefficient of velocity (CV) [24], germination rate index (GRI) [28], germination index (GI) [29], Timson's germination index (TGI) [30], germination energy (GE) [30] and emergence index (EI) [31] did not have significant differences.

## 2.4. Statistical Analysis

For each randomized block (four blocks in total), 5 plants per treatment were used (a total of 20 plants per exposure treatment). The aggregated dataset comprises 5 genotypes of 200 plants (80 control and 120 stress), along with ten germination parameters and five morpho-agronomic traits. The significance of the differences between treatments was tested by applying a one-way analysis of variance (ANOVA), at a confidence level of 95%. When the ANOVA null hypothesis was rejected, Tukey's Post hoc test was carried out to establish the statistically significant differences at  $p < 0.05$ .

## 3. Results

### 3.1. Variation in Germination Parameters with Gibberellic Acid ( $GA_3$ ) Application Under Long-Day Exposure

The morpho-agronomic traits monitored revealed that  $GA_3$  treatments positively influenced plant growth in long day condition. Comparing our results, these traits greatly differ depending on species, the germination power of the seeds and  $GA_3$  administered concentrations (data not shown).

As the usage of multiple germination parameters, such as the MGR, CV, GRI, TGI, GE and EI did not have significant differences (data not shown), we selected the GP, MGT and SVI which provide reliable information regarding seedling development. Compared with the water treatment, the most optimal  $GA_3$  rate increased the germination percentage (GP) by 13.33 and 40.33% with long-day exposure, in *C. africanum*, and *C. hederifolium*, respectively (Figure 1). At the concentration of 150 mg/L, the seeds of *C. africanum*, *C. cyprium*, *C. mirabile*, and *C. pseudibericum* did not germinate (Figure 1A–D). In *C. africanum*, the level of administered  $GA_3$  prolonged the germination period but the plants developed better compared to control (Figure 1A).

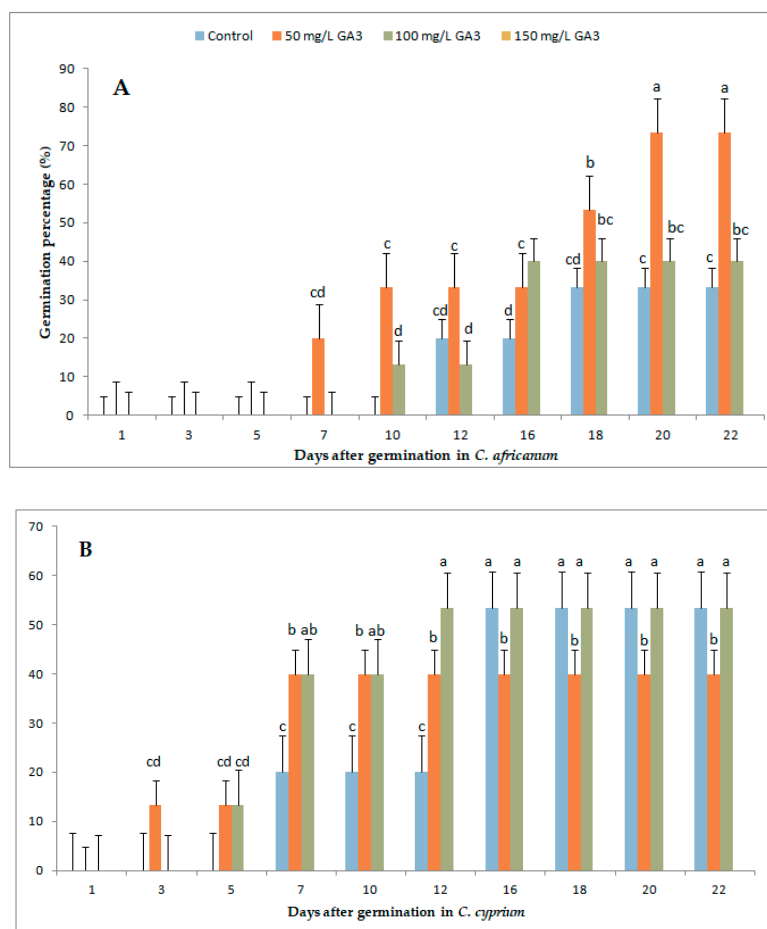
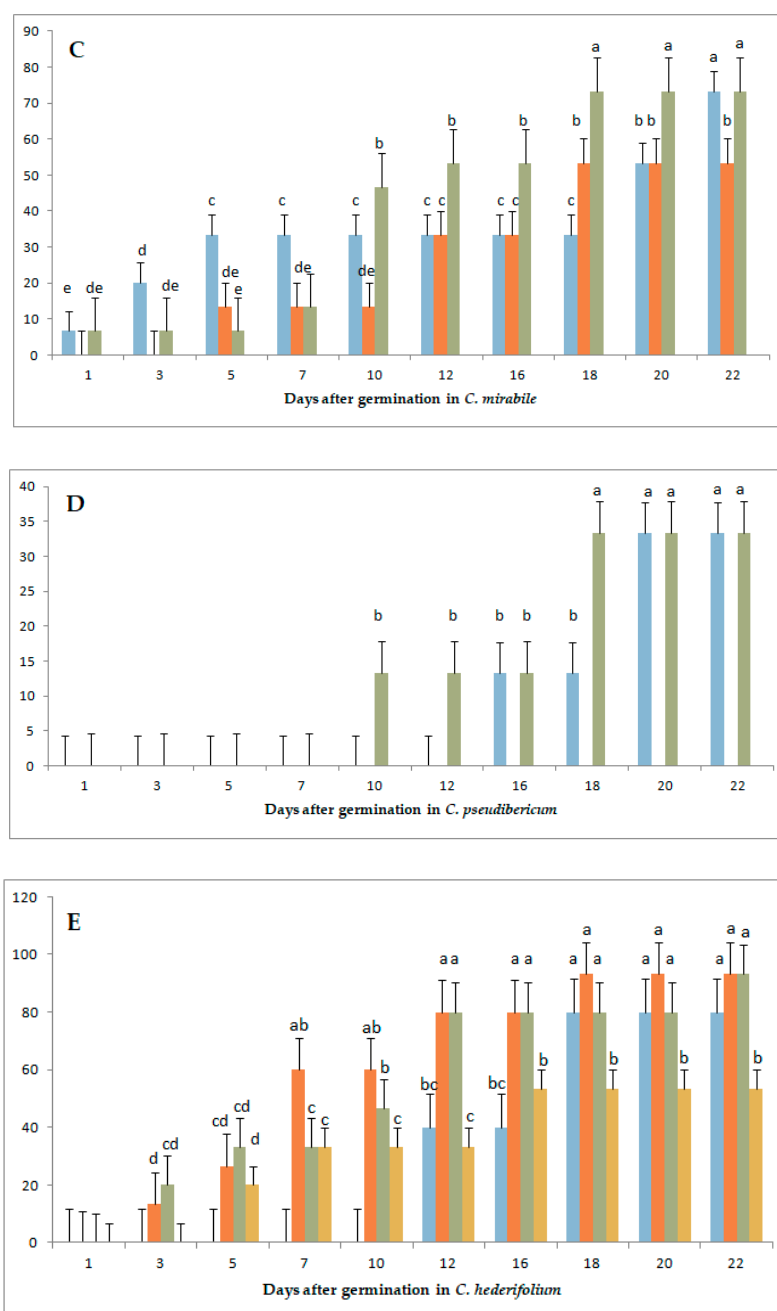


Figure 1. Cont.



**Figure 1.** Effect of gibberellic acid (50, 100, and 150 mg/L GA<sub>3</sub>) and long-day exposure on germination percentage/day  $\pm$  SE of selected *Cyclamen* species: (A) *Cyclamen africanum* (African cyclamen); (B) *Cyclamen cyprium* (Cyprus cyclamen); (C) *Cyclamen mirabile* (sowbread); (D) *Cyclamen pseudibericum* (false Iberian cyclamen); (E) *Cyclamen hederifolium* 'Alba' (ivy-leaved cyclamen). Values (mean  $\pm$  SE) followed by different letters are significantly different based on Tukey's HSD (honestly significant difference) test at  $p < 0.05$ .

In *C. africanum* the level of administered GA<sub>3</sub> prolonged the germination period, but the plant developed better compared to the control. The 50 mg/L concentration administered proved to be favorable for both seed germination and seedling development, with a germination percentage of 80%. At the concentration of 100 mg/L, the plants developed similar to those of the control, the only difference being the early germination of seeds treated with GA<sub>3</sub> (Figure 1A).

Germination occurred faster in *C. cyprium* compared to *C. africanum*. Although the germination percentage was not high (60%), the most beneficial concentration in terms of seedling development was

100 mg/L which was similar to the control. When GA<sub>3</sub> was administered at a concentration of 50 mg/L, the seeds germinated the earliest, but with a weaker development. When GA<sub>3</sub> was administered at a concentration of 50 mg/L, the seeds germinated the earliest, but the petiole and tuber had a weaker development. At the concentration of 150 mg/L the seeds did not germinate, as in the case of *C. africanum* specie.

Regarding *C. pseudibericum*, the germination percentage showed no significant differences. At the concentrations of 50 and 150 mg/L the germination rate was 0%. In contrast, the concentration of 100 mg/L proved to be effective both in terms of germination and plant development, although the germination percentage was quite low (40%). Compared to the control, the concentration of 100 mg/L was similar in germination and plant development.

The highest germination percentage of *C. mirabile* was noticed in the case of untreated seeds, respectively in the case of seeds treated with a concentration of 100 mg/L (80%). The earliest germination was observed in the case of 100 mg/L concentration, although the plant had a weaker development. At the concentration of 50 mg/L, the GP was low with a poor development of the plants.

In *C. hederifolium* 'Alba' the earliest germination was observed was observed with 50 mg/L, which proved to be the most favorable in terms of germination percentage and plant development. In comparison, the concentration of 100 mg/L had similar results, the only difference being a slightly weaker development compared to the concentration of 50 mg/L. At the concentration of 150 mg/L the germination percentage was the lowest, compared to the control, as well as a late development.

### 3.2. Variation in Plant Growth and Morpho-Agronomic Parameters of Species Under Long-Day exposure

Although the percentage of germination was low in most species, the period required for germination showed significant differences depending on the concentration of GA<sub>3</sub> administered (data not shown). Seed of *C. cyprium* showed the greatest germination percentage with control condition (water germination) which were reduced with 50 mg/L GA<sub>3</sub> (Table 1). Comparatively, *C. africanum* and *C. hederifolium* 'Alba'; had a higher germination percentage with 100 mg/L. *C. pseudibericum* had the lowest germination percentage of all the species. In the case of *C. africanum*, the highest percentage of germination was observed at the administered concentration of 50 mg/L. In the *C. cyprium* species, concentrations of 50 and 100 mg/L (36%) gave better results than the lower GA<sub>3</sub> concentration of 50 mg/L. In the case of *C. mirabile* the germination percentage was decreased by higher concentrations of GA<sub>3</sub> (Table 1).

The period of time required for germination under control corresponded approximately to the percentage of germination, with the highest values (lowest MGT) calculated for the species *C. cyprium*, *C. mirabile* and *C. hederifolium* 'Alba' (64–66 days) and higher MGT, between 77 and 81 days, for *C. africanum* and *C. pseudibericum*, respectively. Increased concentration of GA<sub>3</sub> generally led to a significant decrease in MGT (increased germination rate), except for *C. cyprium*, which showed a reduction in MGT (increased germination rate) of 47% at the concentration of 50 mg/L. Comparatively, the lowest MGT value was recorded in *C. hederifolium* 'Alba', 20% at the concentration of 100 mg/L, being the species with the highest germination rate. Good results were also recorded at a concentration of 150 mg/L. With the species *C. mirabile* and *C. hederifolium* 'Alba', the lowest value of MGT was recorded at concentrations of 100 (43%) and 50 mg/L (56%). Compared to control, the plants of *C. pseudibericum* at 100 mg/L GA<sub>3</sub> concentration required a greater period for germination, with a MGT value of 54% (Table 1).

The SVI of the *Cyclamen* accessions was significantly affected by GA<sub>3</sub> application and was enhanced with increasing GA<sub>3</sub> (Table 2). The highest SVI was observed in *C. mirabile* with GA<sub>3</sub> application (100 mg/L) followed by *C. cyprium* and *C. hederifolium*. (50 mg/L and 100 mg/L, respectively) compared to that of control. Moreover, a significant difference was found in the SVI of different accessions, and the SVI of *C. mirabile* at 100 mg/L GA<sub>3</sub> was remarkably higher than that of *C. africanum* and *C. pseudibericum* at the administered GA<sub>3</sub> concentration of 100 mg/L, which had a lower SVI (82.6 and 100.4, respectively). The variation in SVI might be attributed to improved germination which depends



upon the roots and shoots length of the seedlings, as well as stimulation of enzymatic activities. The results are in accordance with the findings of Kumar et al. (2018) [32] who reported significant increase in SVI of coriander (*Coriandrum sativum* L. (Chinese parsley)) with the application of GA<sub>3</sub>.

**Table 1.** Effects of increasing GA<sub>3</sub> concentrations (mg/L) on the indicated seed germination parameters of the five selected *Cyclamen* taxa.

Parameter	Genotype	GA <sub>3</sub> Concentrations (mg/L)			
		0	50	100	150
Germination percentage (GP%)	<i>C. africanum</i> (African cyclamen)	33.33 ± 0.76 c	73.33 ± 0.8 a	40.00 ± 1.0 b	0.0 ± 0.0 d
	<i>C. cyprium</i> (Cyprus cyclamen)	53.33 ± 0.8 a	40.00 ± 0.9 b	53.33 ± 0.7 ac	0.0 ± 0.0 c
	<i>C. hederifolium</i> 'Alba' (ivy-leaved cyclamen)	80.00 ± 0.7 b	93.33 ± 0.8 a	93.33 ± 0.76a	53.33 ± 0.8 c
	<i>C. mirabile</i> (sowbread)	73.33 ± 0.8 a	53.33 ± 0.7 b	73.33 ± 0.8 a	0.00 ± 0.0 c
	<i>C. pseudibericum</i> Hildebr (false Iberian cyclamen)	0.00 ± 0.0 b	33.33 ± 0.76 a	0.00 ± 0.0 b	33.33 ± 0.8 a
Mean germination time–MGT (days)	<i>C. africanum</i> (African cyclamen)	33.62 ± 1.2 c	23.90 ± 0.7 b	42.72 ± 1.5 d	0.00 ± 0.0 a
	<i>C. cyprium</i> (Cyprus cyclamen)	31.22 ± 0.8 c	47.50 ± 1.0 d	32.70 ± 0.9 b	0.00 ± 0.0 a
	<i>C. hederifolium</i> 'Alba' (ivy-leaved cyclamen)	49.22 ± 1.3 d	29.36 ± 0.9 b	19.57 ± 0.4 a	32.07 ± 0.6 c
	<i>C. mirabile</i> (sowbread)	73.59 ± 2.8 b	55.64 ± 1.4 d	43.32 ± 0.4 c	0.00 ± 0.0 a
	<i>C. pseudibericum</i> (false Iberian cyclamen)	37.51 ± 1.9 b	0.00 ± 0.0a	53.9 ± 1.8 c	0.00 ± 0.0 a
Seedling vigor index I (SVI)	<i>C. africanum</i> (African cyclamen)	20.0 ± 2.6 b	185.5 ± 2.7 d	82.6 ± 4.2 c	0.0 ± 0.0 a
	<i>C. cyprium</i> (Cyprus cyclamen)	190.8 ± 3.2 b	223.8 ± 3.2 c	346.4 ± 4.4 d	0.0 ± 0.0 a
	<i>C. hederifolium</i> 'Alba' (ivy-leaved cyclamen)	114.8 ± 2.3 b	328.2 ± 3.2 d	312.4 ± 2.9 c	110.4 ± 2.6 a
	<i>C. mirabile</i> (sowbread)	461.9 ± 3.0 c	187.3 ± 3.7 b	812.4 ± 2.5 d	0.0 ± 0.0 a
	<i>C. pseudibericum</i> (false Iberian cyclamen)	63.6 ± 2.2 b	0.0 ± 0.0 a	100.4 ± 1.9 c	0.0 ± 0.0 a

Values are means ± SE. Different lowercase letters denote significant differences under different treatments based on genotype at the 0.05 probability level according to the Tukey's HSD test.

**Table 2.** Effects of increasing GA<sub>3</sub> concentrations (mg/L) on the indicated seed germination parameters of the five selected *Cyclamen* taxa.

Parameter	Genotype	GA <sub>3</sub> Concentrations (mg/L)			
		0 GA <sub>3</sub>	50 GA <sub>3</sub>	100 GA <sub>3</sub>	150 GA <sub>3</sub>
Germination percentage (GP%)	<i>C. africanum</i>	33.33 ± 0.75 b	0.00 ± 0.0 c	73.33 ± 0.7 a	33.33 ± 0.8 b
	<i>C. cyprium</i>	33.33 ± 0.8 b	73.33 ± 1.0 a	73.33 ± 0.76 a	73.33 ± 1.0 a
	<i>C. hederifolium</i> 'Alba'	33.33 ± 1.2 d	93.33 ± 0.7 a	73.33 ± 0.8 b	60.00 ± 1.1 c
	<i>C. mirabile</i>	93.33 ± 0.7 a	93.33 ± 0.8 a	53.33 ± 0.76 a	53.33 ± 0.8 a
	<i>C. pseudibericum</i>	53.33 ± 0.75 a	0.00 ± 0.0 b	53.33 ± 0.8 a	0.00 ± 0.0 b
Mean germination time–MGT (days)	<i>C. africanum</i>	64.3 ± 0.4 b	0.0 ± 0.0 d	13.9 ± 0.6 a	37.6 ± 1.9 c
	<i>C. cyprium</i>	34.4 ± 0.7 d	74.4 ± 1.4 a	33.2 ± 0.9 c	47.7 ± 1.2 b
	<i>C. hederifolium</i> 'Alba'	41.3 ± 0.8 a	26.1 ± 0.4 c	30.5 ± 0.7 b	30.7 ± 0.4 b
	<i>C. mirabile</i>	28.3 ± 0.7 c	50.8 ± 0.9 a	38.6 ± 0.4 b	51.7 ± 0.6 a
	<i>C. pseudibericum</i>	35.4 ± 1.9 a	0.0 ± 0.0 c	23.2 ± 1.4 b	23.9 ± 1.4 b
Seedling vigor index I (SVI)	<i>C. africanum</i>	206.4 ± 1.5 a	0.0 ± 0.0 d	178.0 ± 3.4 c	134.4 ± 2.2 b
	<i>C. cyprium</i>	1123.8 ± 0.6 a	417.8 ± 0.5 d	611.6 ± 0.4 b	517.9 ± 0.8 c
	<i>C. hederifolium</i> 'Alba'	143.5 ± 0.8 d	304.8 ± 0.1 a	234.9 ± 0.4 b	198.2 ± 0.3 c
	<i>C. mirabile</i>	640.9 ± 0.3 a	577.0 ± 0.5 b	456.8 ± 0.7 c	245.3 ± 1.2 d
	<i>C. pseudibericum</i>	176.3 ± 0.5 b	0.0 ± 0.0 d	248.4 ± 0.0 a	75.2 ± 1.2 c

Values shown are means ± SE (*n* = 3). For each parameter and accession, different lowercase letters in a row indicate significant differences between treatments according to Tukey's HSD test (*p* < 0.05)

The plants' height was measured from the highest point of the leaf to the beginning of the tuber.

Germination of *C. pseudibericum* was irregular and none of the *Cyclamen* species germinated when 150 mg/L GA<sub>3</sub> was applied (Figure 2). Height and petiole length of *C. cyprium* and *C. mirabile* was highest with 150 mg/L GA<sub>3</sub>. Regarding the plants of *C. africanum*, elongation of the plant and petiole, as well as leaf area, tuber diameter, and radicle length were greatest with 50 mg/L.

### 3.3. Variation in Germination Parameters with Gibberellic Acid (GA<sub>3</sub>) Application and Short-Day Exposure

Compared to long-day exposure, it was observed that the germination percentages differed greatly when species were exposed to GA<sub>3</sub> and short days (data not shown). With short days, higher GA<sub>3</sub> rates increased germination (Figure 3). In *C. africanum*, GA<sub>3</sub> administration delayed germination and 100 mg/L had the greatest germination percentage. Unlike long days, at a concentration of 50 mg/L the seeds did not germinate having 0% germination percentage. The best concentration with regards

to plant development was that of 100 mg/L, being the concentration with the highest germination percentage, i.e., 80%.

In *C. cyprium*, germination occurred faster than in the case of *C. africanum* all GA concentrations delayed germination. The most beneficial concentrations in terms of development were 100 and 150 mg/L, while the concentration of 50 mg/L administered prolonged the period of germination. The germination rate of the seeds was affected by GA<sub>3</sub> but not the germination percentage of 80%.

The germination percentages in *C. pseudibericum* showed no significant differences, only in terms of plant development. At concentrations of 50 mg/L the germination percentage was 0%. In contrast, the concentration of 100 mg/L increased both germination and plant development. Compared to the control, the concentration of 150 mg/L showed similarities regarding germination and plant development.

The highest germination percentage of *C. mirabile* was in the case of seeds treated with GA<sub>3</sub> at a concentration of 50 mg/L, where the germination percentage reached 100%. The earliest germination was observed in the case of 50 and 100 mg/L, compared to control at which the germination took place later.

Regarding the species *C. hederifolium* 'Alba' the earliest germination was observed in the case of 50 and 150 mg/L concentrations. Also, the highest germination percentage was at 50 and 100 mg/L administration doses.

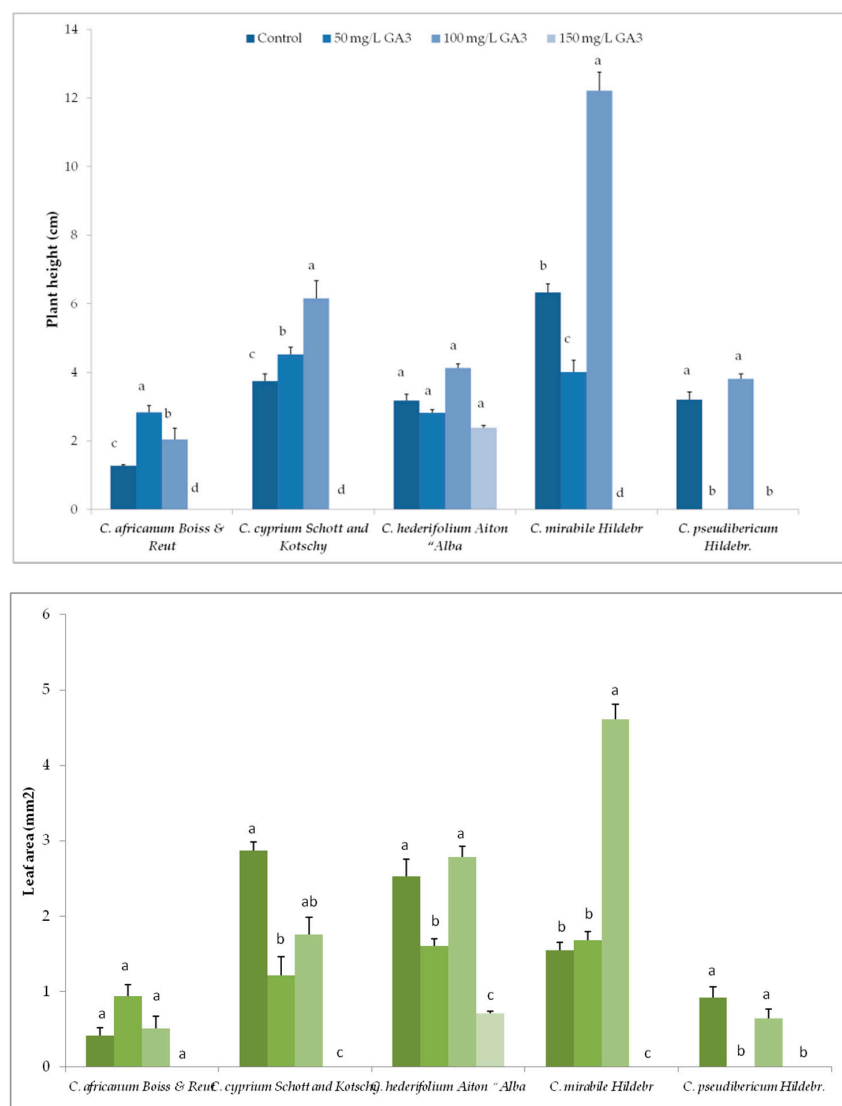
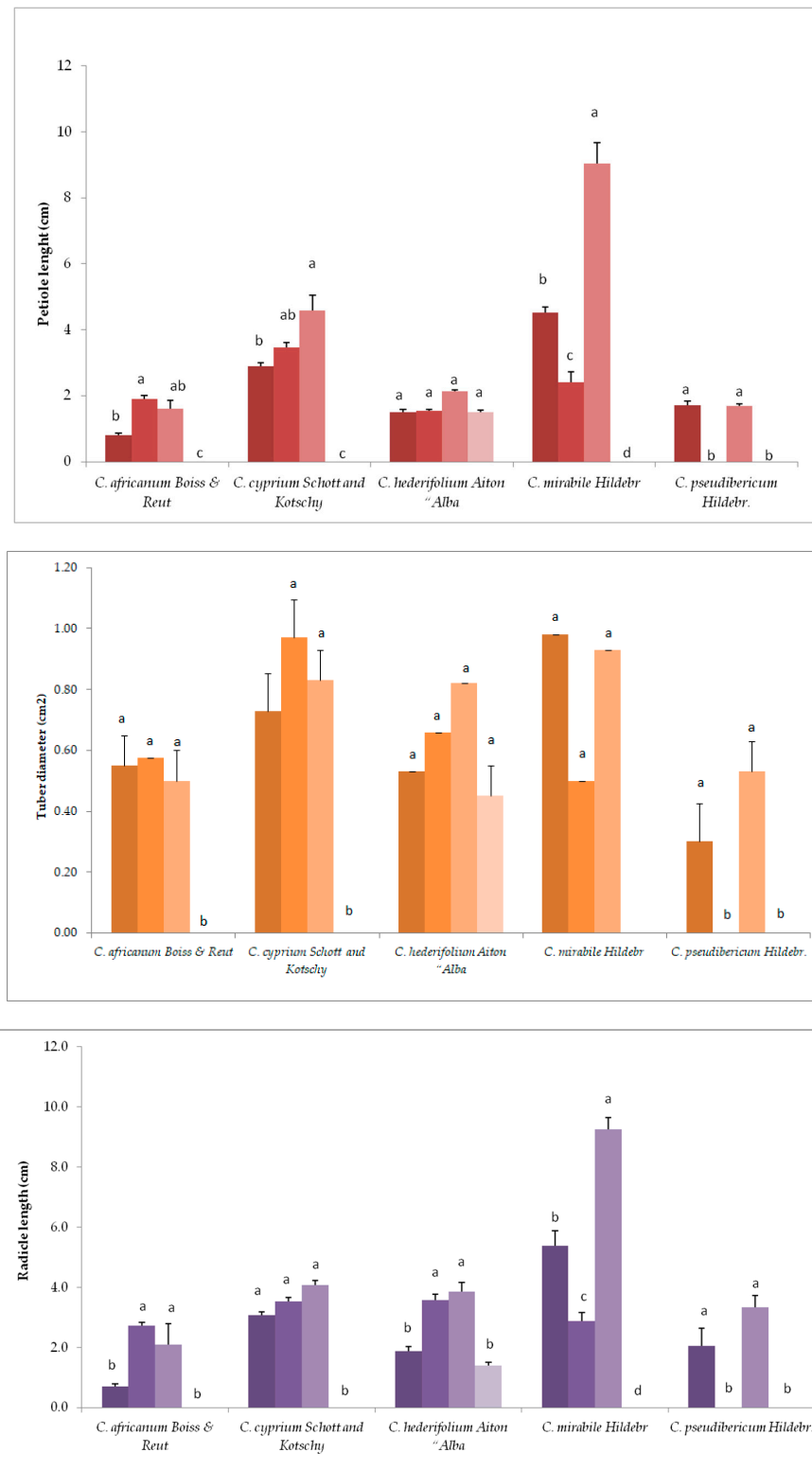


Figure 2. Cont.





**Figure 2.** Variation of morphological parameters in *Cyclamen* species based on different concentration of gibberellic acid (GA<sub>3</sub>). Different lowercase letters above the bars indicate significant differences between treatments, for each genotype, according to the Tukey's HSD test ( $p < 0.05$ ).

### 3.4. Variation in Plant Growth and Morpho-Agronomic Parameters of Short-Day Exposed Cyclamen Species

Seeds from *Cyclamen* species (*C. cyprium* and *C. mirabile*) showed high germination percentage (47.4–55.3%) under control conditions (water germination). The 50 and 150 mg/L GA<sub>3</sub> treatments decreased the percentage of germination by 15.3–31.3% in *C. cyprium* and by 8.1–17.8% in *C. mirabile*. Comparatively, *C. hederifolium* ‘Alba’ treated with 50 mg/L GA<sub>3</sub> had a higher germination percentage than the control. *C. pseudibericum* had the lowest germination percentage of the *Cyclamen* species but was higher with the 100 mg/L GA<sub>3</sub>. In the case of *C. africanum*, the highest percentage of germination was observed in the presence of the concentration of 100 mg/L and with no germination at 50 100 mg/L concentration. In the *C. cyprium* species, concentrations of 100 and 150 mg/L (42%) gave better results than the lower GA<sub>3</sub> concentration of 50 mg/L (Table 2).

Although the percentage of germination was low in most species, the period required for germination showed significant differences depending on the concentration of GA<sub>3</sub> administered (data not shown). The period required for germination under control corresponded approximately to the percentage of germination, with the highest values (lowest MGT) calculated for the species *C. mirabile*, *C. hederifolium* ‘Alba’ and *C. cyprium* (63–68 days) and higher MGT, between 65 and 81 days, for *C. africanum*, and *C. pseudibericum*, respectively (Table 2). Increased concentration of GA<sub>3</sub> generally led to a significant decreased MGT (i.e., earlier germination), except for *C. mirabile* and *C. cyprium*, which showed an increase in MGT (delayed germination) of approx. 22.5–40.0% at the concentration of 50 mg/L. Comparatively, the lowest MGT value was recorded in *C. africanum* Boiss & Reut, 13.9% at the concentration of 100 mg/L. With the species *C. cyprium* and *C. hederifolium* ‘Alba’, the lowest value of MGT was recorded at concentrations of 100 mg/L (30.5–33.2%). For the species *C. pseudibericum*, all concentrations of GA<sub>3</sub> took less time to germinate than the control with a MGT value of 23% at both 100 and 150 mg/L concentrations. The SVI of the *Cyclamen* accessions slightly decreased by GA<sub>3</sub> application in the case of *C. africanum*, *C. cyprium* and *C. mirabile* (data not shown). The highest SVI was observed in *C. cyprium* on GA<sub>3</sub> application (100 mg/L and 150 mg/L, respectively) followed by *C. mirabile*. (50 mg/L and 100 mg/L, respectively) compared to that of control (Table 2). Moreover, a significant difference was found in the SVI of different accessions and the SVI of *C. cyprium* at 100 mg/L GA<sub>3</sub> was remarkably higher than that of *C. africanum*, *C. hederifolium* and *C. pseudibericum* which had a lower SVI (178.0, 234.9 and 148.4, respectively).

The plants’ height was measured from the highest point of the leaf to the beginning of the tuber. According to Figure 4, the values show the existence of statistically significant differences. The concentration of GA<sub>3</sub> to which the *Cyclamen* species had the most significant response in terms of elongation and plant development was 100 mg/L.

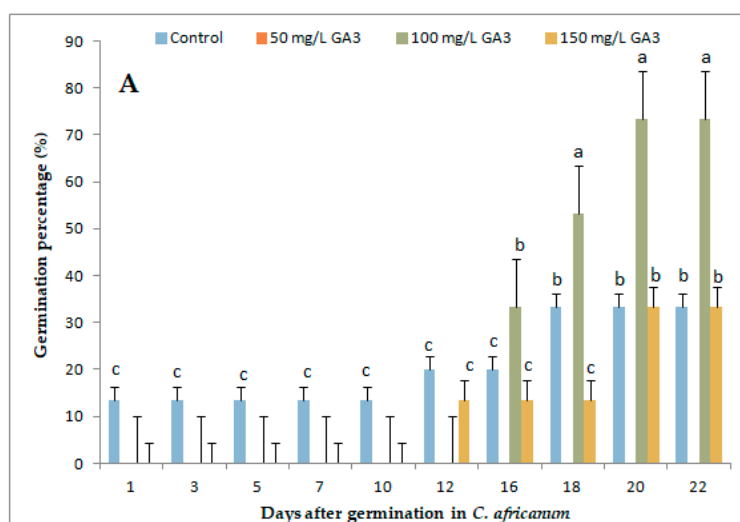


Figure 3. Cont.

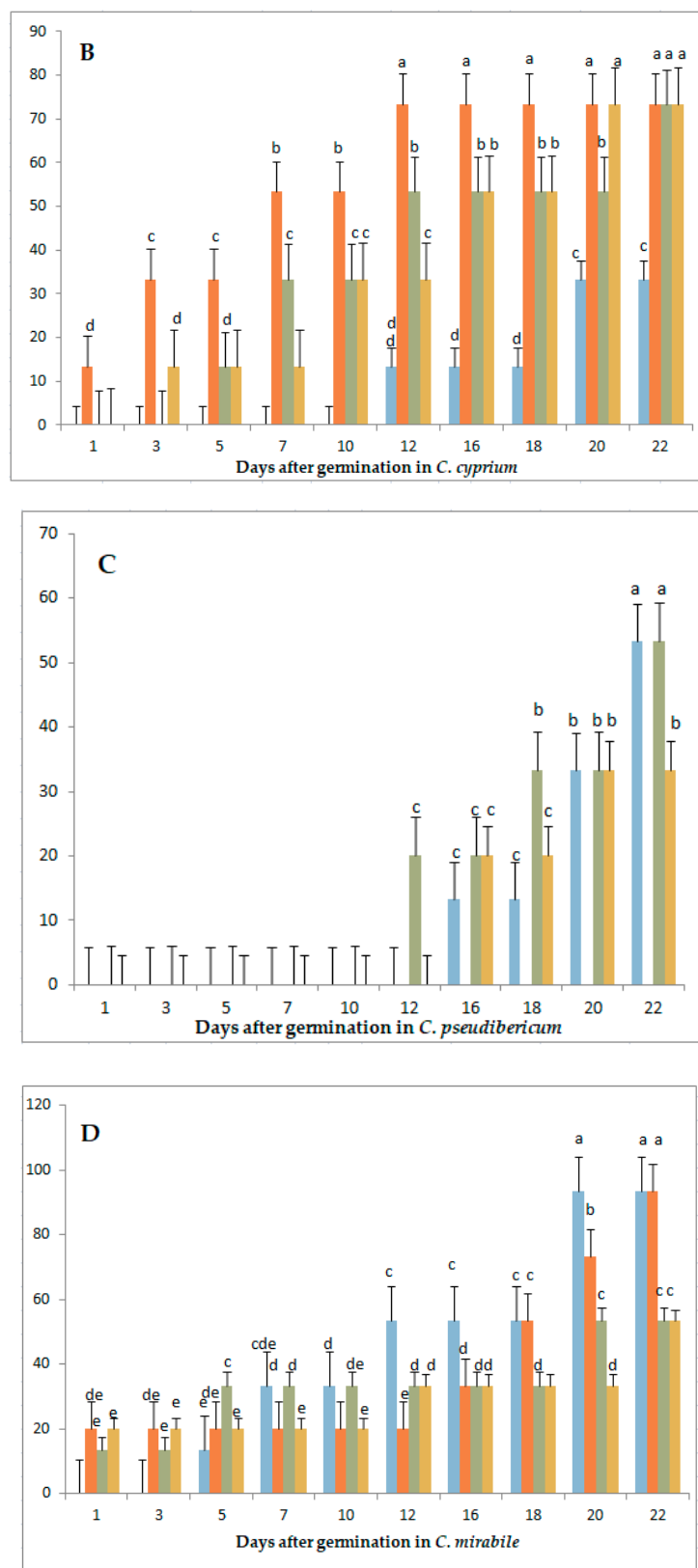
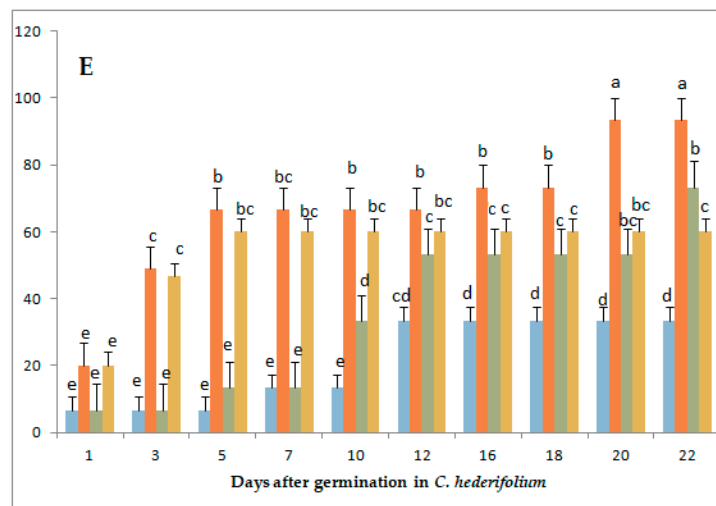
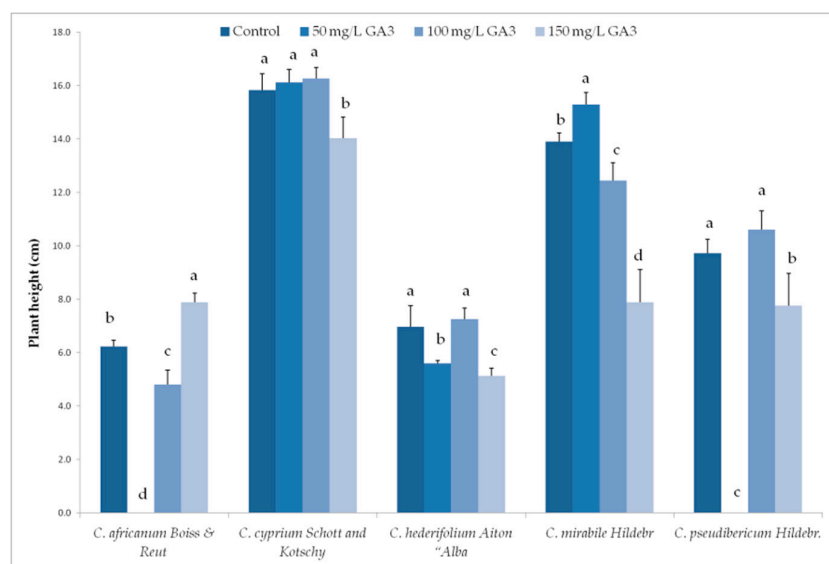


Figure 3. Cont.



**Figure 3.** Effect of gibberellic acid (50, 100 and 150 mg/L  $GA_3$ ) and short –day exposure on germination percentage/day  $\pm$  SE of selected *Cyclamen* species (A) *Cyclamen africanum* (African cyclamen); (B) *Cyclamen cyprium* (Cyprus cyclamen); (C) *Cyclamen mirabile* (sowbread); (D) *Cyclamen pseudibericum* (false Iberian Cyclamen); (E) *Cyclamen hederifolium* ‘Alba’ (ivy-leaved cyclamen). Values (mean  $\pm$  SE) followed by different letters are significantly different based on Tukey’s HSD test at  $p < 0.05$ .

Except for *C. africanum*, in general,  $GA_3$  applied at 150 mg/L reduced the plant height, petiole length and tuber diameter, but increased the leaf area and radicle length. The 100 mg/L  $GA_3$  increased the height, petiole length, and tuber diameter but decreased the leaf area and radicle length. Regarding the species *C. africanum* a major increase in height of the plant and elongation of the petiole as well as a slight increase in leaf area was observed at the concentration of 150 mg/L. The tuber developed best at the concentration of 150 mg/L and the radicle at control. At the concentration of 50 mg/L the seeds did not germinate. Similar results regarding leaf area and plant height were recorded in *Gazania rigens* L (treasure flower) at 100 mg/L  $GA_3$  concentrations [33].



**Figure 4.** Cont.

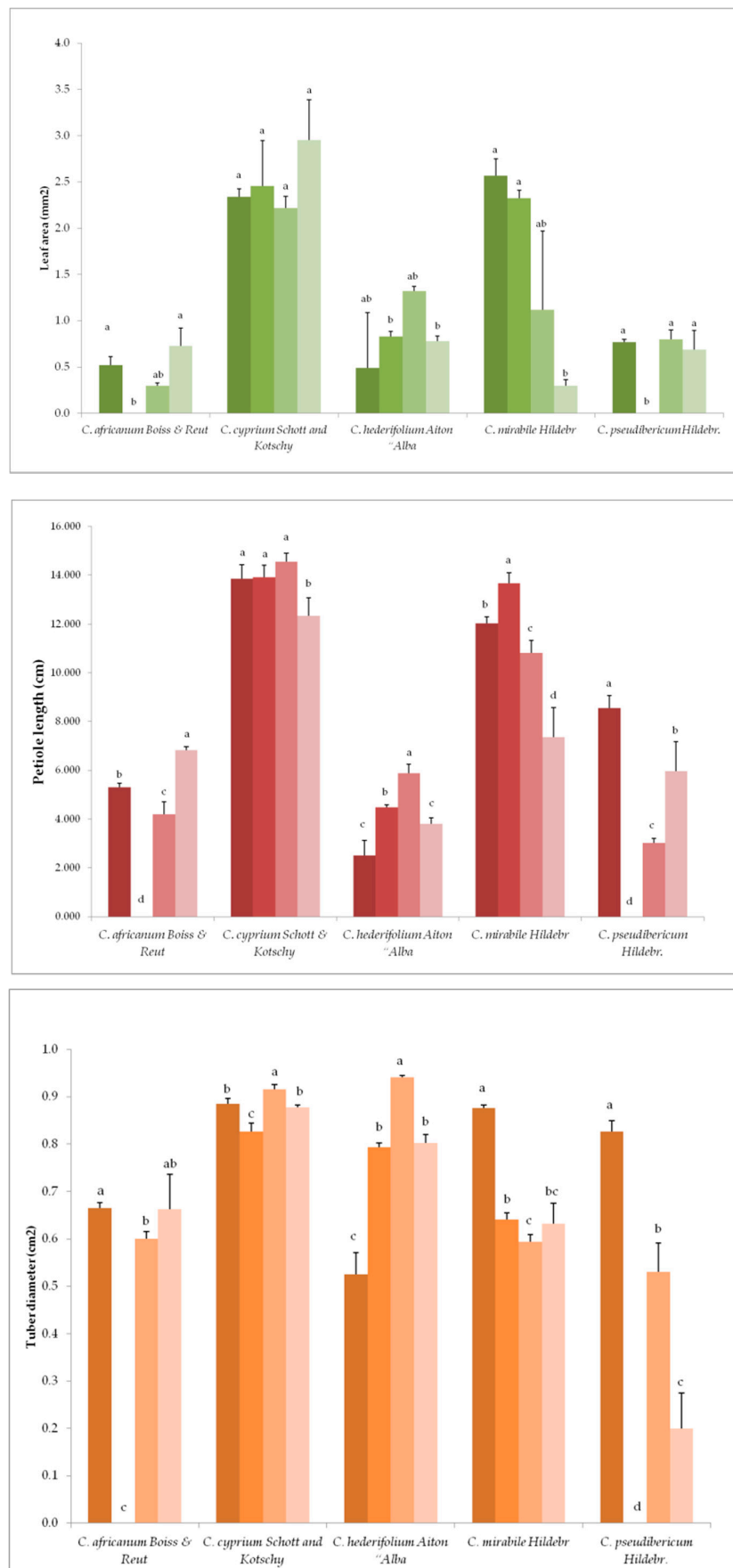
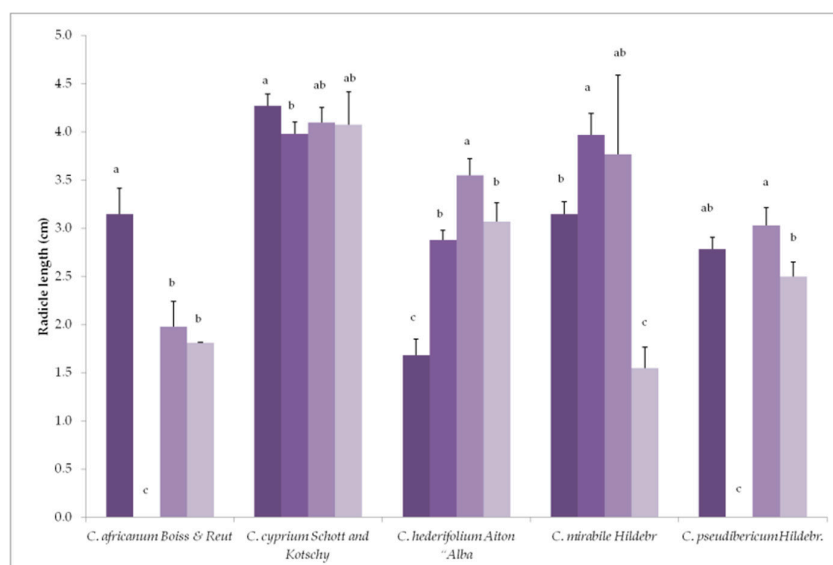


Figure 4. Cont.



**Figure 4.** Variation of morphological parameters in *Cyclamen* species based on different concentration of gibberellic acid ( $GA_3$ ). Different lowercase letters above the bars indicate significant differences between treatments, for each genotype, according to the Tukey's HSD test ( $p < 0.05$ ).

#### 4. Discussion

The results of this experiment showed that proper concentration of  $GA_3$  could significantly support seed germination and seedling growth of some *Cyclamen* species under different day length exposure. GA are extensively used in agriculture and horticulture as tools for boosting productivity, cost reduction and enhancing ornamental value of multiple plants.  $GA_3$  plays a role in seed germination, stem elongation, response to abiotic stress, flowering and fruit development. The effect of GA treatments depends on species, concentrations used and time of applications. Hanyan et al. 2006 [34], found that treatment with multiple concentrations of  $GA_3$  (20–400 mg/L enhanced seed germination, but with significant differences among the varieties of *Cyclamen persicum* used. Furthermore, the juvenile phase may last from only a few days in annual plants to several years in perennials [35], and depends on seed harvesting, especially in *Cyclamen* species, the age of the plant, number of leaves, or size of meristem.

The activity promoted by exogenous GA has been frequently reported in multiple studies. GAs led to the extension of the plant of peas [36], the increase of the internode to *Ilex paraguariensis* A.St.-Hil (yerba mate) [37], the elongation of the petiole to *Thlaspi arvense* L. (field pennycress) [38] and the elongation of *Triticum aestivum* L. (common wheat) leaves, respectively [39]. Recent advances highlight the importance of internal and external factors (amount of GA, short or long day exposure, developmental stage) which regulate the expression of FLOWERING LOCUS T gene in leaves. Furthermore, in *Arabidopsis thaliana* (L.) Heynh. long day conditions accelerate flowering through the expression of this gene [40,41].

Different studies have shown that exogenous GAs and GA biosynthesis inhibitors have affected petiole elongation. From these results it is assumed that endogenous  $GA_1$  (or  $GA_3$ ) and  $GA_4$  may be involved in petiole elongation of *C. persicum* Mill. In our experiments, we evaluated the effects of  $GA_3$  treatments on seed germination parameters and morpho-agronomic features as well quantitatively comparing the germination parameters used for seed germination studies (Table 2 and 6). Our results showed that by increasing the  $GA_3$  concentration, the plants significantly developed, especially at the administration of 100 mg/L  $GA_3$  in long-day exposed species and 50 and 100 mg/L  $GA_3$  in short-day exposed species.

Under long-day condition, *C. africanum* exhibited a major elongation of the plant and petiole, as well as root length and shoot formation when 50 mg/L  $GA_3$  was applied. Comparatively, under



short-day condition, a larger number of leaves and shoot formations were observed with 100 mg/L GA<sub>3</sub>, whereas under 150 mg/L GA<sub>3</sub> larger leaf areas and tubers were observed.

In *C. cyprium*, under long-day condition, fewer numbers of roots, but an increased length were observed at the concentration of 50 mg/L GA<sub>3</sub>, whereas an increased tuber diameter and shoot formations were observed at the concentration of 100 mg/L GA<sub>3</sub>. Under short-day condition, at the concentration of 100 mg/L GA<sub>3</sub> the tuber diameter increased and at 150 mg/L GA<sub>3</sub> the leaf area increased, while the roots length decreased a better shoot formation was observed.

*C. hederifolium* exhibited a larger number of roots and an increased root length when 50 mg/L GA<sub>3</sub> was applied. The 100 mg/L GA<sub>3</sub> increased the petiole length and the shoot formation, under long-day exposure treatment. Comparatively, under short-day condition an increase in leaf area, tuber diameter and a decrease in root length but a better shoot formation were recorded when 100 mg/L GA<sub>3</sub> was applied. At the concentration of 150 mg/L GA<sub>3</sub> a larger number of leaves were noticed and a decrease in tuber diameter.

Under long-day exposure treatment, *C. mirabile* exhibited an increased number of leaves under control and at 100 mg/L GA<sub>3</sub> an increase in petiole length and leaf area were recorded. Under short-day condition an increase in petiole, root length and tuber diameter were noticed when 50 mg/L GA<sub>3</sub> was applied.

In the case of *C. pseudibericum* the germination was irregular and none of the *Cyclamen* species germinated when 150 mg/L GA<sub>3</sub> was applied, under long-day exposure treatment. Compared to control, the concentration of 100 mg/L GA<sub>3</sub> exhibited an increased leaf area and tuber formation. Furthermore, increased root length and shoot formation were recorded. Under short-day condition, a larger number of leaves and an increase in tuber diameter were noticed at control. At the concentration of 100 mg/L GA<sub>3</sub> an increase in leaf area and root length were recorded.

Similar findings had been described by Oh and Kim [7], where the administration of GA<sub>3</sub> at a concentration of 100 mg/L increased petiole elongation and plant height of *Cyclamen persicum* 'Metis Scarlet Red'. Alshakhaly and Qrunfleh [9] reported that foliar applications of GA<sub>3</sub> with concentrations ranging from 25 to 100 mg/L on *Cyclamen persicum* specie effectively increased petiole elongation and flower development. Consequently, increased active GAs promoted petiole elongation and the positive effect of total contents of active GAs was greater under low rather than high irradiance [8]. This result might be caused by different GA<sub>3</sub> concentrations and time of application. Positive effects have been obtained with regards to plant growth and development by GA<sub>3</sub> applications in *Antirrhinum majus* L. cv. Rocket Pink (Rocket Pink snapdragon) and *Polianthes tuberosa* L. (tuberose) [42,43]. The latter had similar results to *Cyclamen* in petiole and leaf area with GA<sub>3</sub> at 100 mg/L. Alcalá-Rico et al. [44] reported that gibberellic acid increased the desirable characteristics of physiological potential of the germination of *Capsicum annuum* var. *glabriusculum* (Indian pepper) genotypes. Their results showed that dormancy of *Capsicum* seeds is generally influenced by physiological aspects. Furthermore, GA<sub>3</sub> slightly promoted stem elongation in *Matthiola incana* (L.) R.Br (hoary stock) [11].

Fagge and Manga (2011) [45] studied the effect of GA<sub>3</sub> application on the growth and seedling establishment of *Bougainvillea glabra* (paperflower), *Ixora coccinea* (jungle geranium) and *Rosa chinensis* (Chinese rose). Their results showed that the concentration of 100 mg/L GA<sub>3</sub> gave better results in terms of root establishment, growth and development in all studied species. Punetha et al., 2018 [46], studied the effect of GA<sub>3</sub> application (100 mg/L) on *Hydrangea* (hortensia) cuttings with better results in terms of shoot number and length, number of leaves and survival percent and lower mortality under shade conditions, compared to open conditions.

Four concentrations of GA<sub>3</sub>, (0.05, 0.5, 5.0, or 50mg/L) were applied to the root systems of seven *Pelargonium* (geranium) cultivars. With increased GA<sub>3</sub> concentration the relative growth rate of all cultivars tested increased with GA<sub>3</sub> treatments. In conjunction with the increase in growth rate, each of the cultivars showed a reduction in the root: shoot ratio [47].

As mentioned above, irradiance is also an important factor in the development of *Cyclamen* species which are mostly found at the base of *Abies-Fagus* forests. Ravnjak et al. [48] found that populations of

*Cyclamen* species are capable of surviving in forest gaps due to increased anthocyanin and quercetin content, which protect plants against strong solar radiation. Furthermore, Osterc et al. [49] found that *Cyclamen* rosettes of long-day exposed plants were larger compared to those of short-day exposed. In *Arabidopsis* high-light exposure had a negative effect on plant development, compared to low-light exposure. [50].

In previous reports, Oh et al. [51,52] found that *Cyclamen persium* cv. 'Metis Scarlet Red' plants kept in short day conditions induced leaf expansion and petiole elongation, whereas those kept in long day conditions mainly expressed longer petioles. Similar results were found in petiole elongation at *C. persicum* at different developmental stages [7]. Furthermore, Cheon et al. [53] reported that most of the growth characteristics, including plant height, number of leaves and flowers, leaf area, tuber size and roots length were enhanced under long-day conditions. On the other hand, higher concentrations of GA<sub>3</sub> (200 mg/L, 300 mg/L and 400 mg/L) increased the vegetative growth of *Chrysanthemum morifolium* cv. Thai Chen Queen (florist's daisy) under short day conditions [54].

GA<sub>3</sub> treatment was shown to be specie-specific and dose-dependent.

Due to the diversity of the genotypes and their great adaptability to different environments, these species are important phylogenetic resources for *Cyclamen* breeding programs [55]. Differences in genotypes' genetic diversity can present high phenotypic plasticity and variation of morphological traits [56]. Therefore, variations of the germination capacity among and within *Cyclamen* specie are an adaptation to the particular conditions of local populations and habitats [57]. Within the same context, Curuk et al. [58,59] found that measurements related to morphological characteristics had great variation indicating differences between the studied species, confirmed also by the use of PCA which showed grouping of characters into species-specific clusters.

The data provided here reveal the response of long and short day exposed *Cyclamen* species under different GA<sub>3</sub> concentrations, which furthers our knowledge of how these plants respond to high and low light intensity stress, as well as their germination capacity induced by GA<sub>3</sub> application. The relationships between the used germination parameters are important aspects that will allow us to predict certain traits in future work. Furthermore, these findings suggest the need to deepen the research on the dose and methods of application of GA<sub>3</sub>, as well as the usage of multiple germination parameters in order to provide reliable germination data.

## 5. Conclusions

To our knowledge, this study presents the first report on the investigation of several germination parameters, seedling growth performance and photosynthetic capacity in five *Cyclamen* species. There were distinct morphological differences and germination parameters among all studied species which allowed them to be clearly distinguished based on germination traits. In the long-day exposure treatment, in terms of plant development the best GA<sub>3</sub> concentration was 150 mg/L in *C. cyprium* and *C. mirabile*. In the short-day exposure treatment, increased plant elongation was observed in *C. cyprium* and *C. mirabile*, followed by *C. pseudibericum* at the concentration of 50 and 100 mg/L, respectively. The use of wild genetic resources to expand the *Cyclamen* database and in vitro preservation remains a relatively unexplored subject in *Cyclamen* biology. As future opportunities for research, the expression of related genes in lowering the effect of light intensity stress should be evaluated.

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