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Ex-situ propagation of *Anacamptis pyramidalis* (L.) from an agroecological perspective

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ABSTRACT

The pyramidal orchid (*Anacamptis pyramidalis*) in Lebanon is facing random collection and potential extinction risks. To address this conservation concern, we conducted a study to investigate the impact of different agroecological treatments, including substrate type, mycorrhizal application, and mother bulb separation, on the growth and flowering characteristics of the orchid. The primary aim was to propose effective methods for ex-situ propagation of this endangered species. Various substrates were employed, including Own-soil, Pinebark, Pinebark-Peat (1/1 ratio), and Peat-Sand (1/1 ratio). Son bulbs were planted separately (SB) or combined with mother bulbs from the previous season (SB+MB), with or without mycorrhizal application (M: Yes and M: No). The selection of treatments was based on the natural requirements of the Pyramidal orchid in the wild state. The trial was conducted over a two-year period. The control treatment (Own-soil/SB+MB/M: No) yielded the highest plant length (37cm), width of the longest leaf (1.6cm), length of the longest leaf (17cm), width of the shortest leaf (1.4cm), dorsal sepal length (0.9cm), labellum length (0.9cm), petal length (0.9cm), and length of bulbs (3cm). Mother bulb separation resulted in enhanced growth for all indicators except bulb length and width. Mycorrhiza application improved all indicators, except the width of the shortest leaf and lateral sepal length. The combination of mycorrhizal treatment with mother bulb separation yielded the most significant improvements across all indicators compared to the control. Notably, in the Peat+Sand/SB/M: Yes treatment, there was a substantial increase in the width of the longest leaf (by 0.5cm) and the length of the longest leaf (by 3cm) compared to the control. Integrating the three studied factors provided a suitable ex-situ conditioning approach for the orchid, surpassing in-situ conditions. This study provides valuable insights into effective strategies for the conservation and ex-situ propagation of the pyramidal orchid in Lebanon.

1. Introduction

The botanical family Orchidaceae exhibits a diverse array of distinctive anatomical and morphological adaptations, showcasing parallels with only a limited number of other plant families (Hagsater and Dumont, 1996). Broadly speaking, orchids are renowned for their adaptability to various environments, with the exception of cultivated agricultural lands and salt marshes. Nevertheless, the occurrence and abundance of orchids are currently undergoing rapid decline (Tsiftsis et al., 2008).

Anacamptis pyramidalis (L.) or Pyramidal Orchid belongs to the family Orchidaceae, genus Anacamptis (Foley and Clarke, 2005). Louis Claude Marie Richard, the French botanist, established the *Anacamptis* genus in 1817 (Wood and Ramsay, 2004). It consists of 25 taxa and 11 species all under 7 sections (Kretzschmar et al., 2007). This species exhibits a distribution range encompassing North and Central Europe, Russia, North Iran, the Mediterranean area, Caucasia, Crimea, and the Northwest (Sevgi et al., 2012). This species thrives in habitats characterized by chalk, limestone, or other calcium-rich soils that are arid or well-drained, exhibiting an organic matter content ranging from 0.78% to 23.94%. The pH levels of these soils

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typically fall within the range of 4.94 to 7.99, while their phosphorus content ranges from 0.87 to 4.32 mg/100g (Tsiftsis et al., 2008).

Anacamptis pyramidalis is a perennial herb (Ilves et al., 2016) with an underground part consisting of ellipsoidal bulbs and roots (Tohmé and Tohmé, 2014). The aboveground part consists of an erect stem, 20–60cm high, lower leaves in rosette, linear-lanceolate (Ilves et al., 2016). Stems are almost circular in cross-section (del Prete et al., 1991). The inflorescence (8cm tall) consists of small flowers on a pyramidal raceme which is light rose to dark purple and rarely white (Ilves et al., 2016). During flowering, *Anacamptis pyramidalis* takes a small pyramid pink shape (Mehdiyeva et al., 2017).

The pyramidal orchid is being extensively collected from the wild causing a risk of extinction of this species in its natural habitat. In addition, the remaining population of this orchid in the wild state is not enough to meet consumers' demand for salep which induced the need for importing salep powder from Turkey. In addition, although people extensively collect this flower for ornamental purposes due to its highly appreciated beautiful appearance, however, it is not being domesticated and commercially produced for ornamental purposes.

In Lebanon, wild orchids are found in mountains at an altitude of 400 to 2000m and a flowering period that begins early, March to July. Pyramidal orchid is one main richness in the natural Lebanese diversity where it is widely found. It is collected as well for medicinal uses. Although this species is found in a high density, however, its extensive random collection could lead to a population decline in the following years. Lebanese forests are being fragmented by both anthropogenic and natural causes (Jomaa, 2008). Especially due to their high sensitivity to climatic changes (Penuelas et al., 2004). An example of these changes is the reduction in water availability in semi-arid areas of the Mediterranean (Vicente-Serrano et al., 2006). Several studies have pointed out the efficient domestication of endemic wild floral species facing risks of extinction through vegetative propagation methods. Therefore, the domestication and multiplication of pyramidal orchids for further commercial production could be a way to limit its random collection from the wild in Lebanon and to match market demands for salep powder limiting the need for importation. Its propagation could also allow using it as an ornamental plant. In Lebanon, possibilities of propagation were not tested or tried yet. Therefore, the objective of this study was to investigate the possibilities of ex-situ propagation of pyramidal orchids under different growth conditions based on substrates, planting bulb state, and mycorrhizal application. The current study is the first step for the domestication of *Anacamptis pyramidalis* under ex-situ conditions through the study of its performance, growth, and viability. Based on this step, the following phases will be adopted for both environmental and economic purposes.

2. Materials and methods

2.1. Experiment establishment

The experiment was done over two consecutive years 2017 and 2018. The collection of Pyramidal orchids was from the Wedeh el Karem-Mount Lebanon/Lebanon area (33°57'05.8"N 35°45'09.6"E) at an altitude of 1140m. During the flowering period of the studied species, 140 plants were collected in early June in each experimental year (Lind et al., 2007). The collected orchids consist of parts above ground (stem, leaves, and flower) and parts under the ground: The two bulbs and a root system. The two bulbs comprised of a mother bulb (MB) that has previously sprouted and given the flowering of the current season and a new bulb that will sprout in the next season after a dormancy period that was used in this study and referred to as son bulb (SB).

2.2. Treatments

Planting materials with a uniform dimension (bulb diameter and weight) were selected for the trial. In four soil types (Own soil, Pine, Pine+Peat, and Peat+Sand), son bulbs were planted with or without mycorrhizal application (M: Yes or M: No) and with or without the mother bulb ((SB+MB) or (SB)).

The properties of soil types are represented in the following Table 1. Own soil substrate represented the soil collected at a depth of 30cm from the site where orchids were found. This substrate was considered as ‘Control’. The pine substrate was formed by pieces of pine bark collected from the same site and cut into small pieces prior to use. Pine+Peat and Peat+Sand substrates were prepared by mixing peat with pine bark pieces and sand respectively in a ratio 1:1 in terms of volume.

Table 1: The composition of used soil types

	Own soil	Pine	Pine+Peat	Peat+Sand
EC	0.832	0.884	0.574	0.174
pH	7.57	5.96	5.77	7.51
Nitrogen (Kjeldahl)(%)	0.6	0.6	0.67	0.326
Organic matter (%)	4.6	76.3	84.3	7.3
K_2O total (digestion) (ppm)	375	1066.35	1531.46	588.61
P_2O_5 total (digestion) (ppm)	13.71	2771.77	424.61	67.09
MgO total (digestion) (%)	1.4	0.7	1	0.3
CaO total (digestion) (%)	9.4	6.1	4.3	1.9

Bulbs were transferred to 15cm diameter plastic pots within two days of their collection. In order to promote growth, vesicular-arbuscular mycorrhiza (VAM) containing Glomus sp. was applied to all soil types, except for those where it naturally occurred. To facilitate the experiment, a specially designed chamber was employed to house the pots.

Throughout the months of November, December, and January, a controlled environment was maintained at a consistent temperature of 5°C. In February, the temperature was incrementally raised to 10°C, and further increased to 15°C during March and April, ultimately reaching a final temperature of 25°C in May. These temperature adjustments were carefully regulated to simulate the optimal growth conditions experienced by the pyramidal orchid in its native wild habitats.

In order to create favorable conditions for the orchid bulbs, continuous watering of the substrates was conducted. The primary objective was to sustain a high level of moisture in the growth medium of the orchid bulbs. For this purpose, sprayers were utilized to dispense 0.25L of water at intervals of 2 days between successive irrigations.

2.3. Measurements

Throughout both experimental years, the study was conducted over a period of 7 months, encompassing the time from the emergence of the plants until the conclusion of the growing cycle, specifically after the stage of flowering. The emergence date was determined by calculating the number of days between the transplantation date, which took place on the first of July, and the appearance of the first shoot tip at the soil level.

The rate of emergence was expressed as the percentage of emerged bulbs out of the total number of bulbs planted. As the plants progressed through their growing cycle after emergence, their length was regularly recorded over a span of 5 months. This measurement was taken from the soil level to the top of the stem, providing a comprehensive indication of plant growth.

After an interval of 3 months from the emergence date, the length, and width of the longest and shortest leaves were carefully assessed. To obtain these measurements, the length of each leaf was determined from the stem level to the top of the leaf, while the width was measured at the midpoint of each leaf.

Flowering parameters were also meticulously recorded. This included the length of the dorsal and lateral sepals, the length of the petals, and the length of the labellum. Each part of the flower was methodically removed from the plant and measured individually to ensure accurate data collection.

Finally, at the conclusion of the experiment and after removing the plants from their pots, bulb dimensions, specifically the length, and width, were thoroughly assessed to gain insights into bulb growth and development.

2.4. Statistical analysis

A fractional factorial design (FFD) was employed within a framework of a complete randomized design (CRD) to investigate the effects of various treatments on the experimental outcomes. The study encompassed 14 treatments, which were formed by combining three factors: Soil type (with three levels, excluding the own soil type), VAM application (with two levels: Yes or No), and mother bulb separation (with two levels: Yes or No). Additionally, two treatments involving the substrate's own soil were included, one with mother bulb separation and one without. In total, each treatment group consisted of 10 replicates, resulting in 10 plants grown under identical experimental conditions.

The FFD was chosen as the experimental design to efficiently explore the effects of the selected factors and their interactions. Multifactorial analysis of variance (ANOVA) was conducted to assess the impact of the various treatments on the measured parameters. STATISTICA Software version 12 was utilized for this purpose. A significance level of $P<0.05$ was adopted to determine statistically meaningful results.

3. Results and discussion

3.1. Date of leaf emergence

The findings presented in Fig. 1 demonstrate that, despite the application of mycorrhizal treatment and the separation of mother bulbs, the date of emergence exhibited significant variation across all soil types. The earliest emergence was observed in the treatment with own soil and no mother bulb separation (131 days after plantation), whereas the latest emergence occurred in the treatment involving Pine+Peat substrate and no mother bulb separation (145 days after plantation).

Moreover, the date of emergence was consistently earlier in substrates that included solely sterilized bulbs (SB) compared to those that included both sterilized and non-sterilized bulbs (SB+MB) despite the presence of mycorrhizal application. For instance, the average date of emergence for treatments with no mother bulb separation was 138 days, 140 days, 145 days, and 142 days, respectively, for Own soil/SB+MB, Pine/SB+MB, Pine+Peat/SB+MB, and Peat+Sand/SB+MB, while it was 131 days, 135 days, 138 days, and 134 days, respectively, for Own soil/SB, Pine/SB, Pine+Peat/SB, and Peat+Sand/SB.

Furthermore, the inclusion of mycorrhiza in the Pine+Peat substrate resulted in earlier emergence for SB (by 6 days) and SB+MB (by 2 days) compared to treatments without mycorrhizal application.

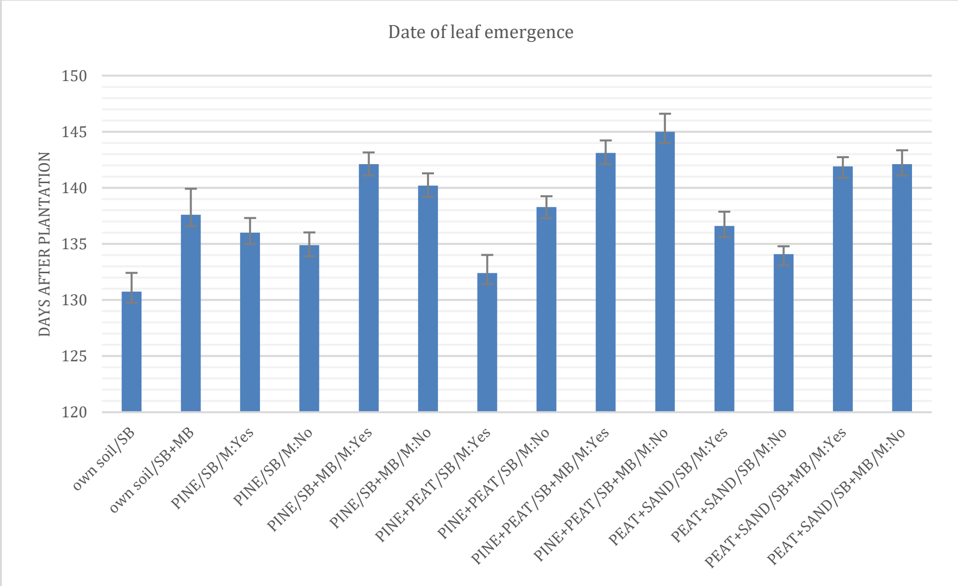

Fig. 1: Variation of the date of leaf emergence in experimental treatments (Means±SD)

3.2. Plant length

The results presented in Fig. 2 indicate a significant increase in plant length across all soil types with the application of mycorrhiza. Specifically, in the Peat+Sand substrate, the plant length for sterilized bulbs (SB) and bulbs with both sterilized and non-sterilized bulbs (SB+MB) increased from 34.8cm and 21.6cm, respectively, to 40.04cm and 39.9cm, respectively, following mycorrhizal treatment.

Furthermore, mycorrhiza appeared to mitigate the impact of bulb separation on this parameter, as no significant difference in plant length was detected between SB and SB+MB in all soil types when mycorrhizal treatment was applied. However, in the absence of mycorrhizal treatment, the plant height was higher for SB and SB+MB in all soil types, except in the Pine substrate, where it was higher compared to other treatments.

Interestingly, the soil type Pine+Peat exhibited the lowest plant length when mycorrhizal treatment was applied. This trend was consistent in the second year of the trial, where the combination of mycorrhizal application and mother bulb separation had a more pronounced effect on plant length.

In conclusion, mycorrhizal application played a significant role in increasing plant length across various soil types, and its presence seemed to attenuate the differences between SB and SB+MB in terms of plant height. However, the interplay between mycorrhizal treatment and mother bulb separation had a notable impact on plant length, particularly in the Pine+Peat substrate. These findings contribute to our understanding of the complex interactions that influence plant growth in diverse environmental conditions.

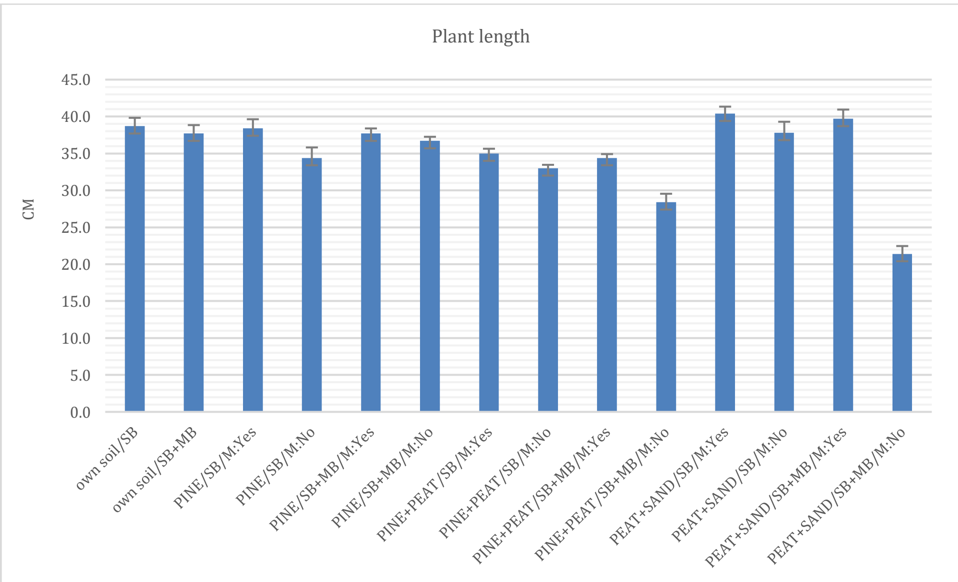

Fig. 2: Variation of plant length in experimental treatments (Means±SD)

3.3. Length of longest leaf

The findings from Fig. 3 reveal a significant increase in the length of the longest leaf across all soil types with the application of mycorrhizal treatment. Specifically, in the Peat+Sand substrate, the length of the longest leaf for sterilized bulbs (SB) and bulbs with both sterilized and non-sterilized bulbs (SB+MB) increased from 15.2cm and 6.7cm, respectively, to 20.3cm and 18.8cm, respectively, following mycorrhizal application.

Moreover, the separation of mother bulbs (SB treatments) also led to a significant increase in the length of the longest leaf in all soil types, with the exception of the Pine substrate. In the Peat+Sand substrate, the length of the longest leaf increased from 6cm in SB+MB to 15cm in SB, despite the presence of mycorrhizal application. Notably, the highest length of the longest leaf was observed in the treatment with Peat+Sand, SB, and mycorrhizal application (M:Yes), measuring 20cm, while the lowest length was found in the treatment with Peat+Sand, SB+MB, and no mycorrhizal application (M:No), measuring 6cm.

The same patterns were observed in the second year of the trial, where the treatment involving Peat+Sand, SB, and mycorrhizal application (M:Yes) exhibited the highest length of the longest leaf, measuring 21cm.

These results underscore the positive impact of mycorrhizal application on the elongation of the longest leaf in diverse soil types. Furthermore, the separation of mother bulbs also played a significant role in promoting leaf length, with the exception of the Pine substrate. These findings contribute valuable insights to our understanding of the factors influencing leaf growth and development in different environmental conditions.

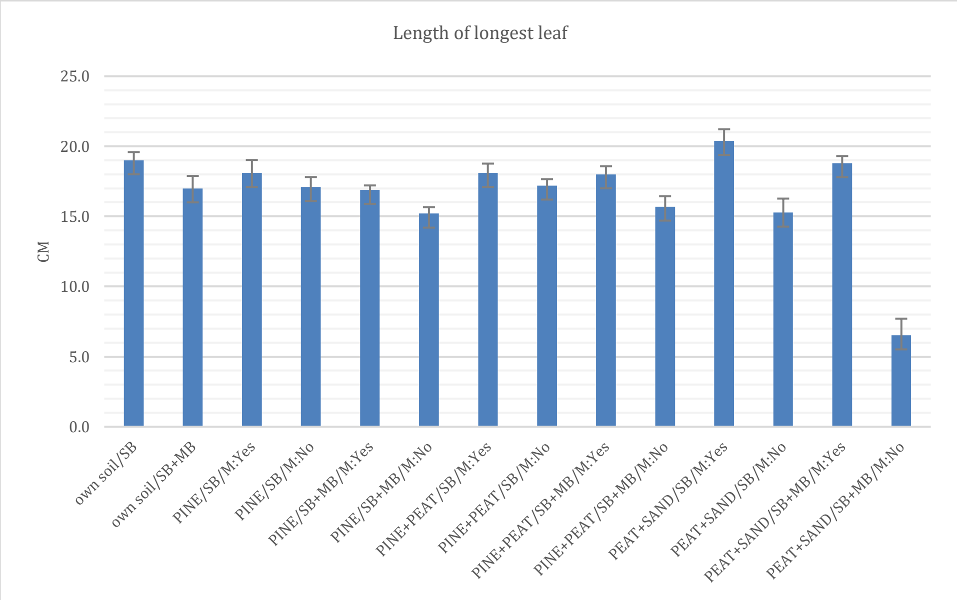

Fig. 3: Variation of length of longest leaf in experimental treatments (Means±SD)

3.4. Width of longest leaf

The analysis of the width of the longest leaf (Fig. 4) revealed significant variations across all soil types following the application of mycorrhiza. In the Pine+Peat substrate, the width of the longest leaf increased from 1.2cm and 1.2cm in the treatments with sterilized bulbs (SB) and bulbs with both sterilized and non-sterilized bulbs (SB+MB), respectively, to 1.5cm and 1.4cm, respectively, after mycorrhizal treatment.

Interestingly, mycorrhiza seemed to moderate the effect of mother bulb separation, as no significant difference was observed between the width of the longest leaf in SB and SB+MB treatments in all soil types, with the exception of Pine+Sand substrate.

Furthermore, the width of the longest leaf exhibited significant variations across all soil types. Notably, the highest width was observed in the Peat+Sand substrate, followed by the Own soil substrate.

These experimental results were consistent with those obtained in the second experiment, wherein the effects of mycorrhizal application, mother bulb separation, and soil types on the width of the longest leaf remained consistent. Specifically, the treatment involving Peat+Sand, SB, and mycorrhizal application (M:Yes) displayed the highest width.

In conclusion, mycorrhizal application had a significant impact on the width of the longest leaf across various soil types, and its presence appeared to moderate the differences between SB and SB+MB treatments. Additionally, the substrate type played a critical role, with Peat+Sand substrate showing the highest width. These findings contribute to our understanding of the complex interactions influencing leaf width under different experimental conditions.

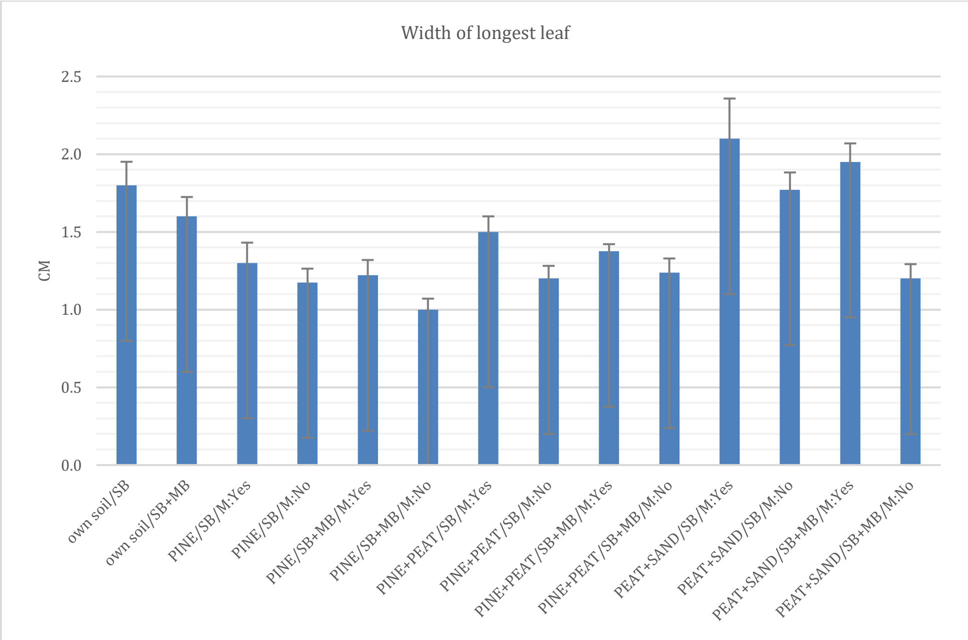

Fig. 4: Variation of width of longest leaf in experimental treatments (Means ±SD)

3.5. Length of shortest leaf

The results presented in Fig. 5 demonstrate that mycorrhizal application led to a significant increase in the length of the shortest leaf across all substrates. However, it appeared to moderate the effect of bulb separation, except for the Peat+Sand substrate, where the treatment with sterilized bulbs (SB) exhibited a higher length of the shortest leaf compared to the treatment with both sterilized and non-sterilized bulbs (SB+MB). For instance, in the Peat+Sand substrate, the length of the shortest leaf in SB and SB+MB treatments increased from 12.1cm and 6.1cm, respectively, to 15.8cm and 14.4cm, respectively, following mycorrhizal application.

Moreover, the separation of mother bulbs significantly increased the length of the shortest leaf in all substrate types, regardless of mycorrhizal application. The highest length was observed in the treatment with own soil, sterilized bulbs, and no mother bulb separation (M:No), measuring 16.8cm, while the lowest length was recorded in the treatment with Peat+Sand, both sterilized and non-sterilized bulbs, and no mycorrhizal application (M:No), measuring 6.1cm.

In summary, mycorrhizal application positively influenced the length of the shortest leaf in all substrates, and its effect on bulb separation was more pronounced in the Peat+Sand substrate. Furthermore, the separation of mother bulbs had a significant impact on this parameter across all substrates, irrespective of mycorrhizal application. These findings contribute valuable insights into the intricate interplay between mycorrhizal treatment, bulb separation, and substrate type on the length of the shortest leaf in experimental conditions.

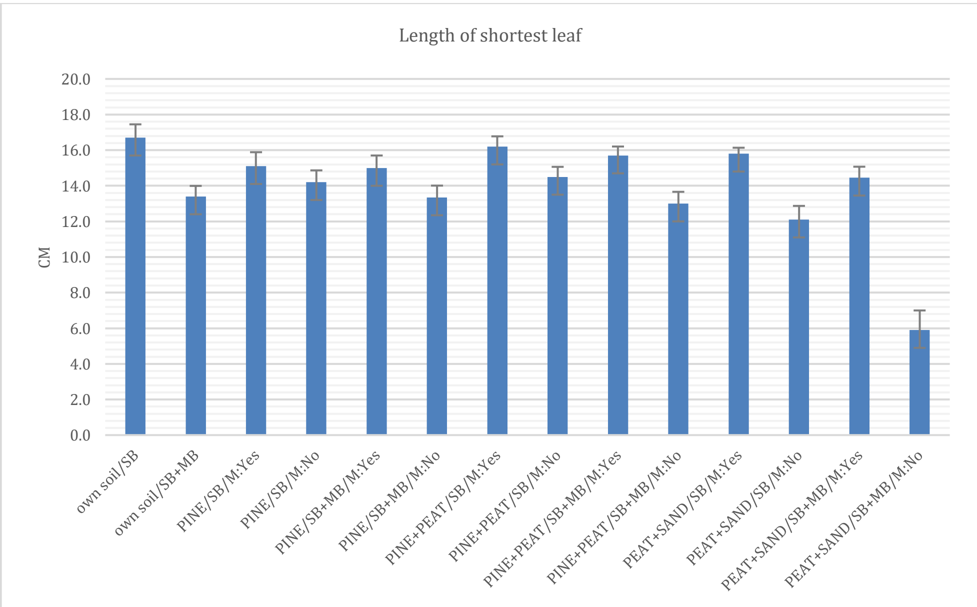

Fig. 5: Variation of length of shortest leaf in experimental treatments (Means±SD)

3.6. Width of shortest leaf

The analysis of the width of the shortest leaf (Fig. 6) revealed significant variations among all soil types. Notably, the widest width was observed in the treatment with own soil, sterilized bulbs, and no mycorrhizal application (M:No), measuring 1.54cm. Conversely, in the Peat+Sand substrate with sterilized bulbs and mycorrhizal application (M:Yes), the width reached 1.84cm, which was significantly higher compared to the other treatments.

The separation of mother bulbs had a significant impact on the width of the shortest leaf in all soil types, except for the Pine+Peat substrate. For example, in the Pine substrate, the width increased from 0.82cm to 1.08cm after the mother bulb separation.

Consistent results were obtained in the second year of the experiment, indicating that the effects of mother bulb separation and substrate types on the width of the shortest leaf remained stable over time.

In conclusion, the width of the shortest leaf exhibited significant variations across different soil types. Moreover, mother bulb separation had a notable influence on this parameter in most soil types. These findings contribute to a deeper understanding of the factors influencing the width of the shortest leaf in diverse experimental conditions.

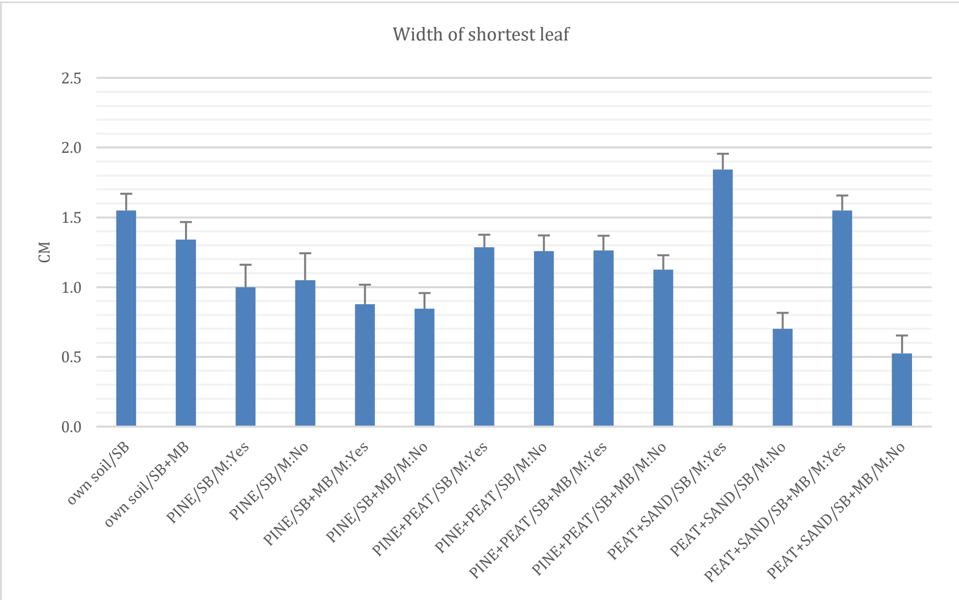

Fig. 6: Variation of width of shortest leaf in experimental treatments (Means±SD)

3.7. Dorsal and lateral sepal length

The analysis of dorsal sepal length (Fig. 7) revealed significant variations among all soil types. The highest length was observed in the treatment with own soil, sterilized bulbs, and no mycorrhizal application (M:No), measuring 0.9cm.

Furthermore, mycorrhizal application had a significant impact on sepal length in all soil types, leading to an increase in the length of the dorsal sepal. Interestingly, mycorrhiza seemed to moderate the effect of mother bulb separation on this parameter, as no significant difference was observed between the treatments with sterilized bulbs (SB) and bulbs with both sterilized and non-sterilized bulbs (SB+MB) in all soil types.

When mycorrhizal treatment was not applied, mother bulb separation only affected the Peat+Sand substrate, where dorsal sepal length increased from 0.6cm to 0.8cm. Consistent results were obtained in the second year of the experiment, indicating that the effects of mycorrhizal application, mother bulb separation, and soil types on dorsal sepal length remained stable over time. In conclusion, dorsal sepal length exhibited significant variations across different soil types, with mycorrhizal application playing a significant role in enhancing sepal length. Additionally, mother bulb separation had a limited effect on this parameter, and its influence was more pronounced in the Peat+Sand substrate in the absence of mycorrhizal treatment. These findings contribute valuable insights to our understanding of the factors influencing dorsal sepal length in various experimental conditions.Lateral sepal length (Fig. 8) varied between all soil types. It was significantly the highest in own soil/SB/M:No (1cm) and the lowest in peat+sand/SB+MB/M:No (0.4cm). Mycorrhizal application increased significantly this parameter except in Pine+Peat/SB+MB. On the other hand, mother bulbs separation increased significantly the length of the lateral sepal when no mycorrhiza was applied for all soil types except for pine. The same results were obtained in the second experimental year however mother bulb separation significantly increased the length of lateral sepal for all soil types.

3.8. Labellum length

The longest labellum length (Fig. 9) was observed in the treatment with Own soil, sterilized bulbs, and no mycorrhizal application (M:No), measuring 1.1cm. Across all substrates, mycorrhizal application had a positive impact on labellum length. For example, in the Peat+Sand substrate with sterilized bulbs and mycorrhizal application (M:Yes), the labellum length was 1cm, whereas it was 0.6cm in the treatment without mycorrhizal application (M:No).

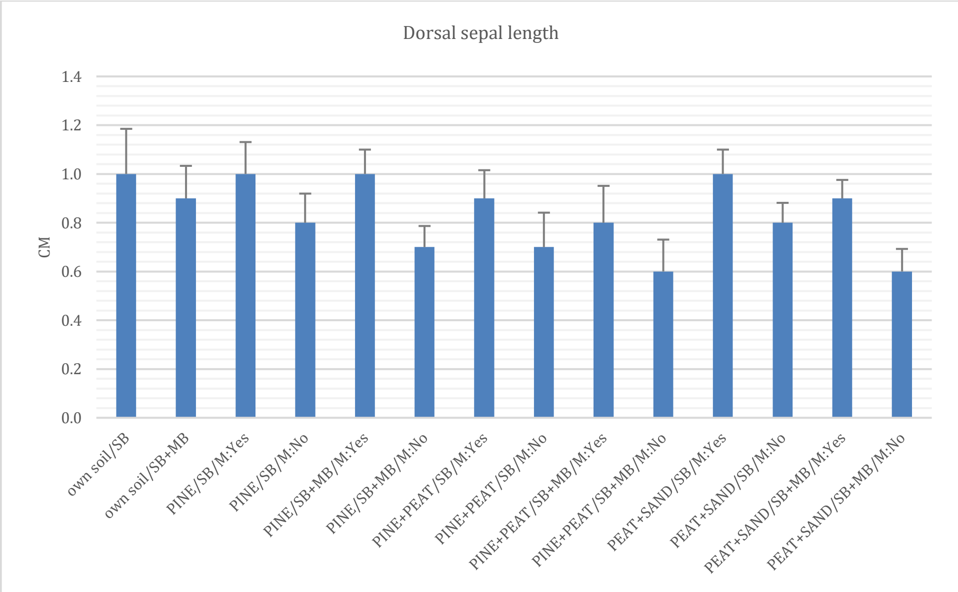

Fig. 7: Variation of length of dorsal sepal in experimental treatments (Means±SD)

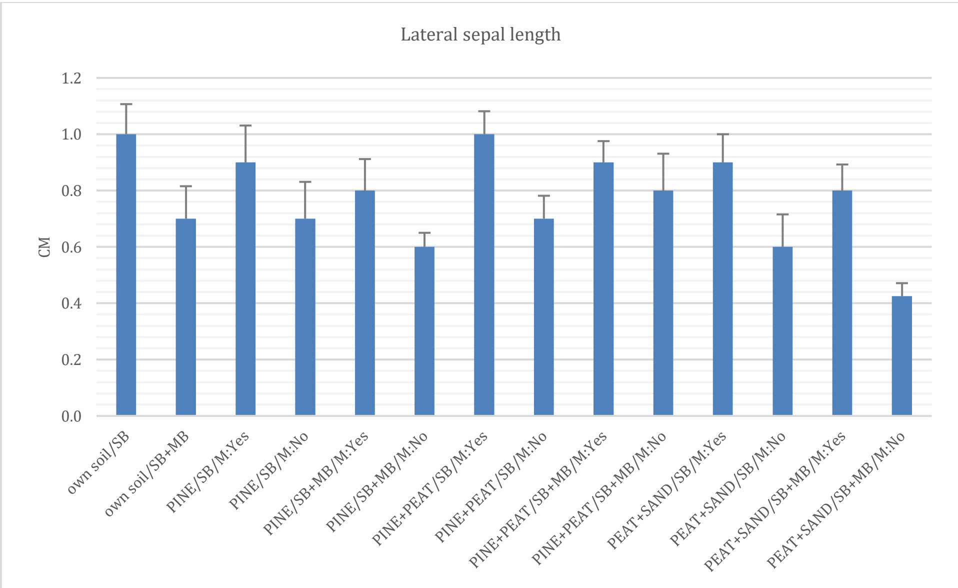

Fig. 8: Variation of length of lateral sepal in experimental treatments (Means±SD)

Furthermore, mother bulb separation (SB) also had a significant enhancing effect on this parameter, regardless of mycorrhizal application. This effect was particularly evident in the Own soil substrate, where labellum length increased from 0.9cm in the treatment with both sterilized and non-sterilized bulbs (SB+MB) to 1.1cm in the treatment with sterilized bulbs only (SB).

Consistent results were obtained in the second year of the experiment, indicating that all treatments similarly affected labellum length. Notably, the combination of mycorrhizal application and mother bulb separation in the Peat+Sand substrate provided the longest labellum length, measuring 1cm.

In conclusion, labellum length showed variations across different treatments, with mycorrhizal application and mother bulb separation positively influencing this parameter. Additionally, the combined treatment of mycorrhizal application and mother bulb separation in the Peat+Sand substrate resulted in the longest labellum length. These findings contribute to our understanding of the factors affecting labellum length in diverse experimental conditions.

3.9. Petal length

Petal length, as depicted in Fig. 10, exhibited significant variations among all soil types, with the highest length observed in the own soil substrate, measuring 0.92cm. The results revealed that mycorrhizal application had a substantial impact on petal length in all soil types. For instance, in the Peat+Sand substrate, petal length increased from

0.6cm and 0.4cm in the treatments with sterilized bulbs (SB) and bulbs with both sterilized and non-sterilized bulbs (SB+MB), respectively, to 0.9cm and 0.7cm, respectively, following mycorrhizal application.

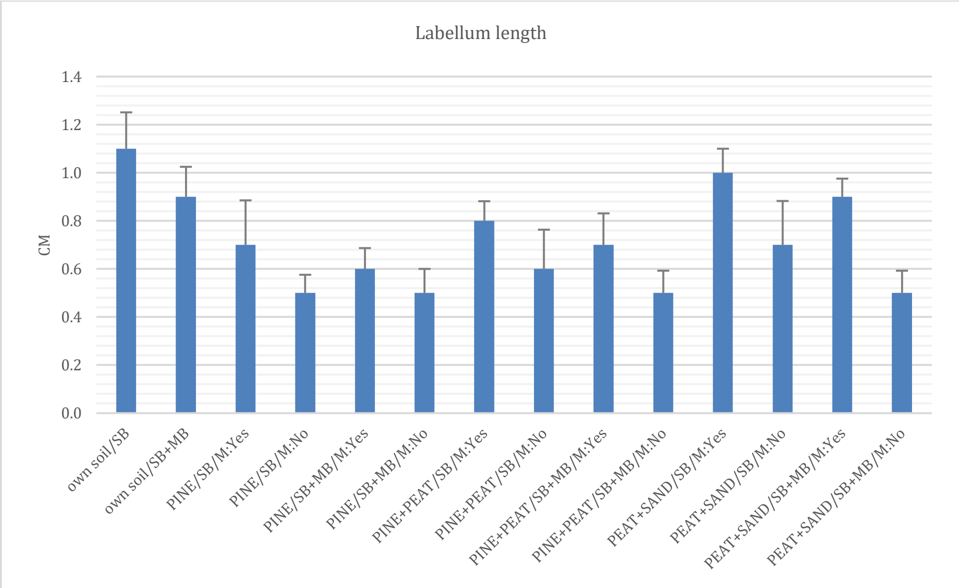

Fig. 9: Variation of averages of the length of labellum in experimental treatments (Means±SD)

Moreover, mother bulb separation significantly increased petal length in the Peat+Sand substrate, regardless of mycorrhizal application. In the second year of the experiment, all treatments had similar effects on petal length, except for the treatment involving Own soil and no mycorrhizal application (M:No), where petal length decreased significantly after bulb separation.

In summary, petal length displayed notable variations across different soil types, with mycorrhizal application positively influencing this parameter in all substrates. Additionally, mother bulb separation had a significant enhancing effect on petal length in the Peat+Sand substrate, independent of mycorrhizal application. However, in the second year, the effect of bulb separation on petal length in the treatment with Own soil and no mycorrhizal application differed from other treatments. These findings contribute valuable insights into the factors influencing petal length in diverse experimental conditions.

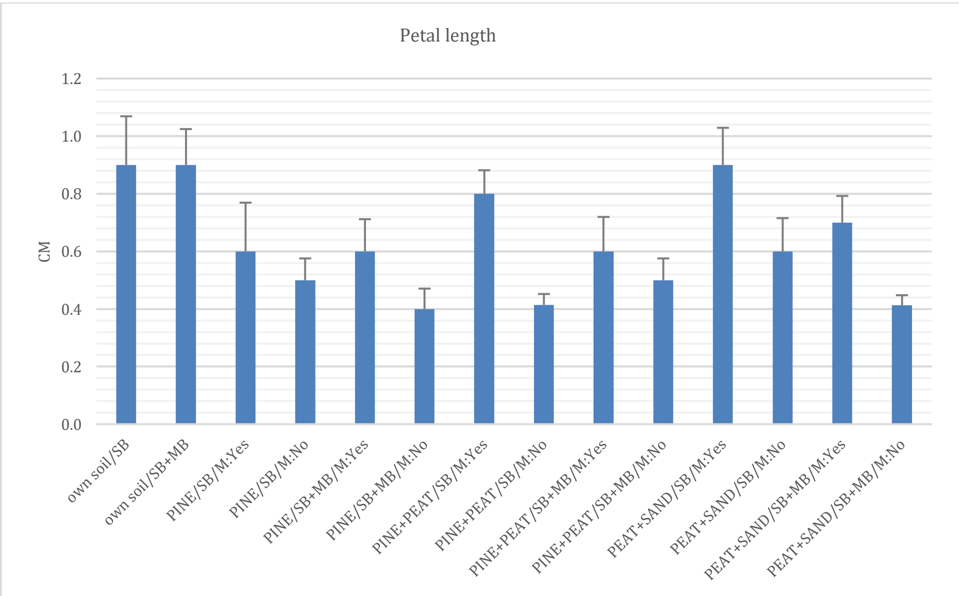

Fig. 10: Variation of petal length in experimental treatments (Means±SD)

3.10. Length and width of bulbs

Mother bulbs separation had a negative effect on bulb dimensions (Fig. 11) in all soil types except Peat+Sand. On the other, mycorrhizal application increased bulb length in the substrate Peat+Sand in SB+MB (by 0.2cm) and in SB (by 1.2cm). The same results were obtained in the second experimental year for all studied factors.

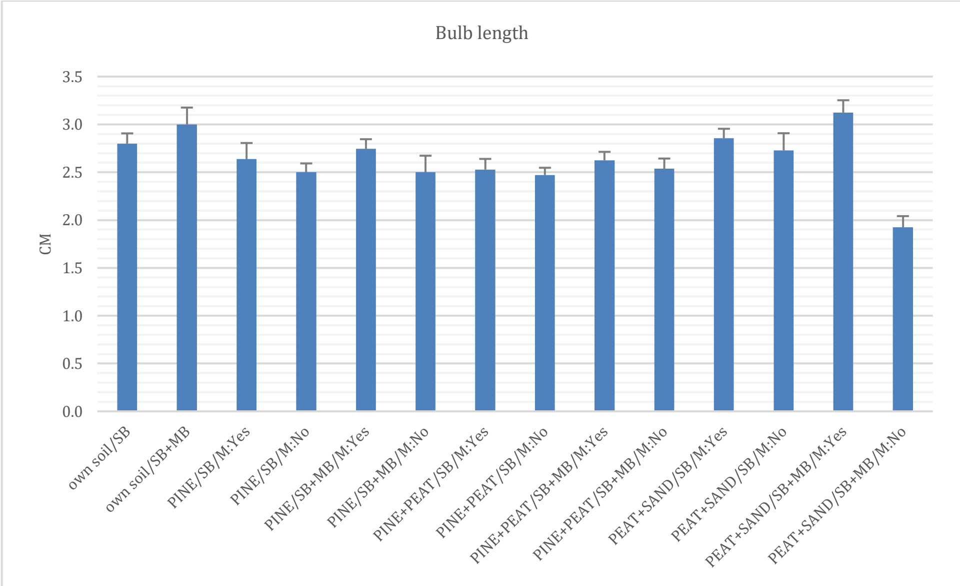

Fig. 11: Variation of bulb length in experimental treatments (Means±SD)

Bulb width (Fig. 12) was similarly negatively affected by mother bulb separation. For instance, in own soil, bulb width scored 1.12cm in (SB+MB), while it decreased following mother bulb separation to reach 0.74cm in (SB). In addition, mycorrhiza significantly increased bulb width in substrate pine for SB and SB+MB. In the second experimental year, all treatments had the same effect on all studied factors.

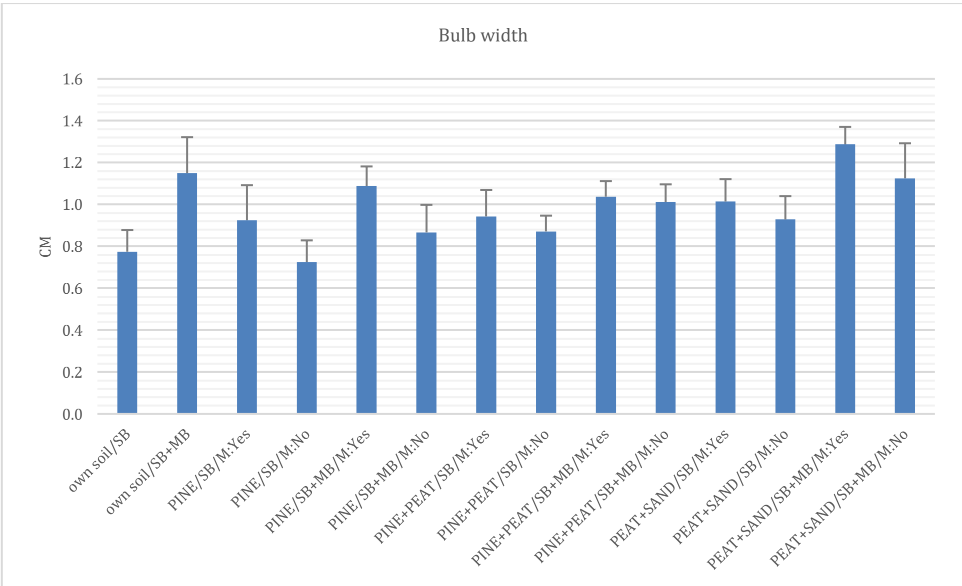

Fig. 12: Variation of bulb width in experimental treatments (Means±SD)

The separation of the mother bulb resulted in an earlier emergence of leaves across all soil types. This early emergence of leaves in son bulbs (SB) compared to mother bulbs (SB+MB) may be attributed to differences in dormancy stage and bulb maturity at the time of plantation. Mother bulb separation appears to trigger an accelerated breakage of dormancy in son bulbs, leading to their earlier emergence.

Moreover, when bulbs were planted in the Own-Soil substrate, the presence of specific microorganisms crucial for natural bulb emergence, which might be lacking in other substrates, could have contributed to the shorter emergence time. Orchid reproduction is promoted through interactions with specific pollinators and symbiotic fungi essential for soil exploitation (Selosse, 2014). The orchid mycorrhizal fungi play a vital role in seed germination, seedling establishment, reproduction, and the overall survival of orchid species (Li et al., 2021).

In both trial years, mycorrhizal application led to increased plant length, as well as the length and width of the longest leaf across all substrates, with the exception of the Own-Soil substrate, where mycorrhizal treatment was not applied. Additionally, mother bulb separation increased these parameters in all soil types, except in Pine for plant length and length of the longest leaf, and in Pine+Peat for the width of the longest leaf.

The adaptation of orchid nutrient metabolism to mycorrhizal application is attributed to a reduction in nitrogen metabolism, which can be considered an adaptation to the parasitic habit during orchid development.

In conclusion, mother bulb separation and mycorrhizal application had significant impacts on various growth parameters, leading to earlier emergence, increased plant length, and improved leaf dimensions. These findings provide valuable insights into the intricate interactions between bulb separation, mycorrhizal application, and orchid development in different soil types (Press et al., 1986). Moreover, phosphate and nitrogen fluxes are completely influenced in later life (Alexander and Hadley, 1984; Alexander et al., 1984). As a result, the effects of nutrients on the growth of orchids may occur through the symbiotic association and arise by affecting symbiotic fungi growth, or by influencing the symbiotic reaction between fungi and orchids (Dijk and Eck, 1995). Consequently, the incorporation of mycorrhizal application and the separation of mother bulbs have shown to positively enhance plant length and the length and width of the longest leaf, particularly in the Peat+Sand substrate. This substrate was found to be comparatively lower in inorganic nutrients such as nitrogen, phosphorus, and potassium in contrast to the pine and Pine+Peat substrates. This observation aligns with the well-documented beneficial effect of mycorrhiza, which is associated with a reduction in the availability of inorganic nutrients (Dhillion and Friese, 1994).

Mother bulb separation increased the length and width of the shortest leaf for all soil types except for Peat+Sand. The substrate (Own-Soil) seemed to increase shortest leaf elongation in comparison to other soil types even if they were richer in organic matter. This may be explained since own soil has a higher pH (7.57) compared with the one of (Pine) (5.96) and (Pine-Peat) (5.77). In fact, *Anacamptis pyramidalis* likes more soils with a pH of 7.01 (Tsiftsis et al., 2008) and is mainly found in alkaline soil.

Mycorrhizal application had a positive impact on the length of all flowering parameters, including dorsal sepal, lateral sepal, petal, and labellum, with the exception of lateral sepal length for SB+MB in the Pine+Peat substrate. Additionally, the greatest length for all flowering parameters was observed in the Own soil (control) when mycorrhiza was absent. This is likely due to the fact that the Own Soil represents the natural substrate of the Pyramidal orchid, enabling the best growth as the bulb is naturally adapted to this type of soil.

The observations on the length and width of bulbs may be attributed to the substrate texture. The length of bulbs was significantly higher in the Own soil in SB+MB when mycorrhiza was absent. This reaffirms the notion that the own Soil, being the natural substrate of the Pyramidal orchid, fosters the most favorable growth conditions due to the bulb's adaptation to this substrate in its wild state. However, the separation of mother bulbs negatively affected both bulb dimensions (length and width), except for the Peat+Sand substrate where the length of bulbs increased with mother bulb separation. Moreover, mycorrhizal application only increased the length and width of bulbs in the Peat+Sand substrate for SB+MB and the width in the Pine substrate for SB and SB+MB.

For example, the physical stress and pressure exerted by the Pine substrate may have contributed to the lower bulb dimensions, whereas the addition of peat to pine bark in the Pine+Peat substrate allowed for better bulb growth due to the lighter texture of this substrate, which resulted in less physical pressure on the bulbs. Notably, mycorrhizal addition did not significantly influence bulb dimensions (length and width), suggesting that it may have had a more pronounced effect on the development of the root system of the Pyramidal orchid than on its bulbs. According to Dhillion and Friese (1994), the mycorrhizal fungi create a supplementary root system through their hyphal network, leading to an increase in water volume and available nutrients in the soil. This finding is consistent with the results reported by Dubova et al. (2019), where mycorrhizal application enhanced the root system of faba bean. Additionally, Heydarian et al. (2018) observed a stimulatory effect of VAM application on wheat under nickel stress. These studies collectively support the notion that mycorrhizal application can positively influence root development and nutrient uptake in various plant species.

Furthermore, the beneficial effects of mycorrhizal fungi were found to be influenced by soil and climatic conditions, including temperature and humidity. The positive impact of VAM on plant growth and stress tolerance is contingent upon the prevailing environmental factors in a particular region.

In conclusion, research by several authors has demonstrated the positive influence of mycorrhizal application on root systems, water uptake, and nutrient availability in plants. However, the specific effects of VAM may vary depending on soil and climatic conditions. These studies shed light on the importance of considering environmental factors when assessing the efficacy of mycorrhizal application in enhancing plant growth and stress resistance (Loit et al., 2018).

4. Conclusion

Anacamptis pyramidalis exhibited optimal growth when subjected to conditions closely resembling its natural habitat in the wild. Nevertheless, the integration of mother bulb separation, utilization of specific substrates, particularly Peat+Sand, and mycorrhiza application resulted in a more favorable growth environment, surpassing that observed in its natural habitat. These combined treatments demonstrated superior growth conditions for *Anacamptis pyramidalis* compared to its wild counterparts.

Compliance with ethical standards

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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