Brazilian Journal of Botany (2025) 48:59
https://doi.org/10.1007/s40415-025-01100-8

REPRODUCTIVE BIOLOGY - ORIGINAL ARTICLE

Different reproductive strategies influence in vitro germination in *Chloraea gavilu* and *Chloraea crispa* (Orchidaceae)

G. Pereira[1] · D. Castillo-Novales[2,3] · O. Jeldes-Cajas[4] · J. T. Otero[5] · C. Arriagada[6] · C. Atala[4]

Received: 22 January 2025 / Revised: 10 June 2025 / Accepted: 4 July 2025

Abstract

Orchids are highly evolved plants with complex pollination strategies. Different species may have different reproductive strategies, related to their evolutionary and ecological context. Pollination in Chilean species have almost not been studied, and could have consequences for conservation and propagation programs. Seed germination of the terrestrial orchids *Chloraea crispa* and *C. gavilu*, both endemic to Chile, was evaluated under different reproductive strategies. Plants of the two species were collected in the field and at flowering, plants were subjected to four reproductive strategies: autonogamy, geitonogamy, xenogamy and interspecific crossing. For each reproductive strategy, six flowers per plant were selected, hand-pollinated and covered with fine mesh to avoid natural pollination and the percentage of fertilized capsules was assessed afterward. Four capsules were randomly selected per treatment for the germination experiment. Asymbiotic seed germination was carried out on Malmgren modified terrestrial orchid medium (MM) and Tomato culture medium (MT), without mycorrhizal fungi. Capsule formation and seed production occurred in all treatments but reproductive strategies did significantly impact germination in both species. We found differences in seed development depending on the culture medium used, with *C. crispa* reaching shoot stage and *C. gavilu* the rhizoid stage after 8 weeks. The highest germination percentages were obtained with geitonogamy and MT medium for both species. The two *Chloraea* species achieved seed germination in the interspecific crossing strategy, indicating potential hybridization of the species may occur. These results contribute to understanding the ecology of Chilean orchids and could be essential for managing, propagation, conservation and restoration programs in the future.

Keywords Asymbiotic seed germination · *Chloraea* pollination · Reproductive strategies · Terrestrial orchids

1 Introduction

Most species survive and reproduce only by interacting with other species, and the evolution of biodiversity seems to intimately depend on the evolution of these interactions (Thompson 1987). In fact, both pollination by animals and mycorrhizal symbioses with fungi are considered pivotal drivers in the diversification of flowering plants (Waterman and Bidartondo 2008; Waterman et al. 2011). In nature, pollination can occur by different means and follow different strategies. Some species can reproduce through autogamy (with pollen from the same flower falling on the stigma),

✉ C. Atala
cristian.atala@pucv.cl

[1] Laboratorio Biotecnología de Hongos, Departamento de Ciencias y Tecnología Vegetal, Campus Los Ángeles, Universidad de Concepción, Casilla 234, Los Ángeles, Chile

[2] Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química, Universidad Técnica Federico Santa María, Avenida España 1680, 2390123 Valparaíso, Chile

[3] Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, San Francisco S/N La Palma, 2260000 Quillota, Chile

[4] Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Campus Curauma, Avenida Universidad 330, Valparaíso, Chile

[5] Departamento de Ciencias Básicas, Sede Palmira, Universidad de Colombia, Carrera 32 # 12-00 Palmira, Valle del Cauca, Colombia

[6] Departamento de Ciencias Forestales, Facultad de Ciencias Agropecuarias y Medioambiente, Universidad de La Frontera, Casilla 54-D, Temuco, Chile

Published online: 22 July 2025

geitonogamy (with pollen from different flowers of the same plant), xenogamy (pollen from a flower belonging to a different individual of the same species), and even outcrossing between individuals of different related species that can produce viable hybrid offspring. The Orchidaceae (the orchid family) is one of the most diverse among flowering plants (Christenhusz and Byng 2016). Their pollination is a very complex part of their biology with very distinct flowers and intricate ecological interactions with their pollinators (Barth 2019). Almost all orchids (97%) require animals for effective pollen transfer (Heywood et al. 2007; Darren et al. 2017), typically having exclusive relationships with pollinators (Adam et al. 2014; Phillips et al. 2017). These are usually bees, wasps, and flies, but many orchids also utilize moths, butterflies, fungus gnats, or birds to cross-pollinate their flowers. Orchids are renowned for their enormous diversity of pollination mechanisms and unusually high occurrence of non-rewarding flowers compared to other plant families (Jersáková et al. 2006). Using maximum parsimony analysis of orchid phylogenies, Bateman et al. (2003) suggested that orchids originated from a nectar-less ancestor that offered pollen as a reward.

Of the many hypotheses that has been proposed to explain the evolution and/or the persistence of floral deceive strategies, cross-pollination is the one that has received most theoretical and empirical support (Borrás and Cursach 2021). However, the benefits of cross-pollination, i.e. higher seed quality and a more resource-efficient pollen transport, depend on the abundance of pollinators (Borrás and Cursach 2021). When pollinator visit rate drops under a given threshold in deceiving orchids, low seed output could overcome the benefits mentioned above. In such ecological context, natural selection could favor rewards for pollinators and/or autogamy (Jersáková et al. 2006). Deception has the advantage of reducing inbreeding due to the tendency of pollinator of visiting less flowers per inflorescence of a given individual, resulting in fewer visits and, therefore, less reproductive success (Borrás and Cursach 2021). Nectariferous orchid species, on the other hand, are usually more attractive and receive more visits per individual, risking inbreeding depression (Jersáková et al. 2006).

After successful pollination, orchid seeds, which are very small and lack the reserve structures (endosperm) typical of other seeds (Heywood et al. 2007; Ruiz et al. 2008; Takao et al. 2012; Dearnaley et al. 2016), are usually dispersed by the wind. Their germination depends on mycorrhizal fungi present in the soil, as seeds lack reserves for embryo growth and rely on these fungi to obtain essential nutrients and carbon compounds that compensate for the absence of an endosperm (Warcup 1973; Mitchell 1989; Rasmussen 1995; Leake 2004). In laboratory conditions, specific culture media, such as Malmgren modified terrestrial orchid medium (MM), can be used to mimic the nutrients obtained from the fungi and achieve asymbiotic germination of orchid seeds (Pereira et al. 2015, 2017; Quiroz et al. 2017; Romero et al. 2017; Castillo-Novales et al. 2022). In fact, this method can result in high germination rates in some species (Pereira et al. 2015). However, other species have low asymbiotic germination, but this likely due to low seed viability (Castillo-Novales et al. 2022; Jeldes-Cajas et al. 2024). Asymbiotic germination is preferred in laboratory conditions due to some practical advantages to symbiotic methods such as: (i) less contamination, (ii) easier evaluation of seed development, and (iii) it does not require previous isolation and culture of the mycorrhizal fungi (Johnson et al. 2007; Pereira et al. 2017). However, some species may increase their germination inoculating the seeds with specific fungal strains (Herrera et al. 2017; Jeldes-Cajas et al. 2024). Orchid seed development progresses through defined stages, from ungerminated seeds to the emergence of shoots with initial leaves (Miyoshi and Mii 1995; Yamazaki and Miyoshi 2006). These developmental stages provide a standardized framework to compare germination efficiency and growth across methods and media.

Chloraea Lindl. is a genus of terrestrial orchids exclusively found in South America (Correa 1969), encompassing the majority of Chilean species (Novoa et al. 2015; Fuentes 2023). Within this genus, *C. crispa and C. gavilu* stand out for their high ornamental value and potential as cut flowers (Pereira et al. 2017). Several studies have been conducted to understand their propagation methods through symbiotic and asymbiotic germination (Dixon 1991; Steinfort et al. 2010; Fracchia et al. 2014; Pereira et al. 2014; Atala et al. 2015; Castillo-Novales et al. 2022). However, viability and germination in seeds produced by distinct reproductive strategies have not been previously documented. It has been described that the fruiting rate of species of the genus *Chloraea* by natural pollination is usually relatively low (Lehnebach and Riveros 2003; Humaña et al. 2008), as reproductive success is pollen-limited which has been attributed to the poor fidelity (Beardsell et al. 1986) and the habitat preferences of pollinators (Johnson and Bond 1992). By significantly increasing the number of capsules formed per plant through hand pollination, it is reaffirmed that capsule production is limited by pollinators and is not limited by the cost of capsule production and nutrient availability (Janzen et al. 1980; Schemske 1980; Ackerman 1989; Calvo 1990a,b, 1993; Calvo and Horvitz 1990; Berry and Calvo 1991; Christensen 1992; Rodriguez-Robles et al. 1992; Bartareau 1994).

In the present study, we evaluated asymbiotic seed germination of two terrestrial orchid species endemic to Chile, *C. crispa* and *C. gavilu*, subjected to different reproductive strategies, namely: autogamy, geitonogamy, xenogamy, and inter-specific outcrossing. By integrating pollination biology and seed germination analyses, this work aims to contribute to the understanding of orchid ecology and support

conservation, propagation, and restoration efforts for these species.

2 Materials and methods

Sample collection and controlled pollination Individuals of *Chloraea crispa* Lindl. and *C. gavilu* Lidl. were collected from natural populations located in Central-South Chile during spring 2019. *C. crispa* was collected in the Pedregal sector (37°19′50.25″ S, 72°15′9.42″ W) and *C. gavilu* was collected close to Yumbel city (36°59′54.93″ S, 72°34′24.10″ W), both located in the Bio-Bio region. Plants were sampled when the floral stalk was visible, but with barely evident floral buds and were then transferred to 3000 cc pots and put in a greenhouse at Universidad de Concepción, Campus Los Ángeles, Los Ángeles, Chile (37°27′46″ S, 72°21′40″ W). Upon flowering, we randomly assigned plants to one of the following pollination treatments representing different reproductive strategies; autogamy (pollen from the same flower), geitonogamy (pollen from a different flower of the same plant), xenogamy (pollen from a flower of a different individual), and interspecific crossing (pollen from a flower of an individual of the other species). For each species, we used 4 plants per treatment (16 plants in total per species). In each plant, we manipulated and followed 6 flowers that, after manual pollination, were covered with a thin plastic mesh to avoid interaction with natural pollinators. After the capsule started forming, we removed the mesh to avoid affecting capsule development. Pollination was conducted by manually extracting pollinia and then carefully placing them in the stigma of the corresponding flower, depending on the pollination treatment. 28 days after pollination we evaluated the percentage of capsules formed. Four out the total capsules formed in each individual were randomly selected and seed were collected before dehiscence. Capsules were dried in the laboratory at room temperature and their weight was registered in a digital scale (RADWAG AS-220/C/2). Seeds contained in each capsule were then weighted and then kept at 4 °C for later use in the germination experiment (see below).

Asymbiotic germination Two culture media were used for the asymbiotic germination of orchid seeds; Malmgren Modified Terrestrial Orchid Medium (MM, Malmgren 1996) and Culture Medium Tomato (MT, Barbery and Molares 2014) (Table 1). The seeds used were obtained from the different pollination treatments described in the previous paragraph. Both culture media were sterilized at 121 °C and 1 atm for 20 min in an autoclave and pH was set to 5.8. Four Petri dishes were used per treatment. In each Petri dish, we placed approximately 200 seeds resulting from each pollination treatments, which were previously superficially sterilized following previously described protocols (Batty and Brundett 2001; Pereira et al. 2015, 2017). After sterilization, seeds were then put at 24 ± 1 °C in the dark for two weeks and later put in 16/8 h light/dark cycles for six more weeks. Germination percentage was determined by counting the number of germinated seeds relative to the total number of viable seeds. The criteria for seed germination followed Yamazaki and Miyoshi (2006), and corresponded to the time at which the embryo emerged from the seed coat.

Table 1 Comparative composition of culture media used for orchid seed germination: Malmgren's modified terrestrial orchid medium (MM) and modified tomato culture medium (MT) (modified from Jeldes-Cajas et al. 2024)

	MM	MT
Macronutrients (mg/L)		
KH_2PO_4	75	
$Ca_3(PO_4)_2$	75	
$MgSO_4$	97.69	
Na_2 EDTA—2 H_2O	75	
Micronutrients (mg/L)		
$MnSO_4$—H_2O	1.54	
$FeSO_4$—7 H_2O	27.8	
Others (mg/L)		
Ripe tomato (pulp)		150,000
Yeast extract	1000	
Pineapple powder	20,000	
D-Biotin	0.05	
Casein, enzymatic hydrolyzate	400	
Folic acid	0.5	
Glycine (free base)	2	
Pyridoxine	5	
Myo-Inositol	100	
Nicotinic acid (free acid)	5	
Thiamine	10	1200
Sucrose	10,000	15,000
Sulfate streptomycin		
Agar	7000	10,000
Activated charcoal (plant based)	1000	
Deionized water (mL)	1000	1000

Data analysis The software Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA) was used for the statistical analyses. The Shapiro–Wilk test was used to evaluate the normality of the data distribution. The percentage of formed capsuled was analyzed using a proportion (z) test. A one-way ANOVA with pollination treatment as a fixed factor was used to determine differences in capsule and seed weight between treatments, followed by an a posteriori Tukey Test. For capsule weight, data was normalized using square root. Germination was analysis likewise using a two-way ANOVA and Tukey test. In this case, pollination treatment and culture media

were used as factors. We did not compare between orchid species and they were analyzed separately.

3 Results

In all pollination treatments, plants of both species formed capsules (Fig. 1). Autogamy, geitonogamy, and interspecific crosses resulted in 100% of flowers maturing into capsules. In contrast, xenogamy led to slightly lower capsule formation; however, this difference was not statistically significant compared to the other treatments ($p > 0.05$). All comparisons were performed within each species, and no statistical comparisons were made between *C. crispa* and *C. gavilu*.

There were significant differences between pollination treatments in capsule and seed mass for both species ($F(7, 24) = 17.58$, $p < 0.0001$ in all cases). Tukey's post hoc test shows that the xenogamy and autogamy treatments resulted in a higher capsule mass compared to geitonogamy and interspecific crossing for *C. crispa* (Table 2, $p < 0.05$). In this species, seed mass per capsule was significantly higher in xenogamy than in the other treatments, which did not statistically differ between them (Table 2, $p > 0.05$). In *C. gavilu* xenogamy and autogamy seed mass did not differ between them (Table 2, $p < 0.05$), but was statistically above to geitonogamy, which was also similar to Interspecific crossing (Table 2, $p > 0.05$).

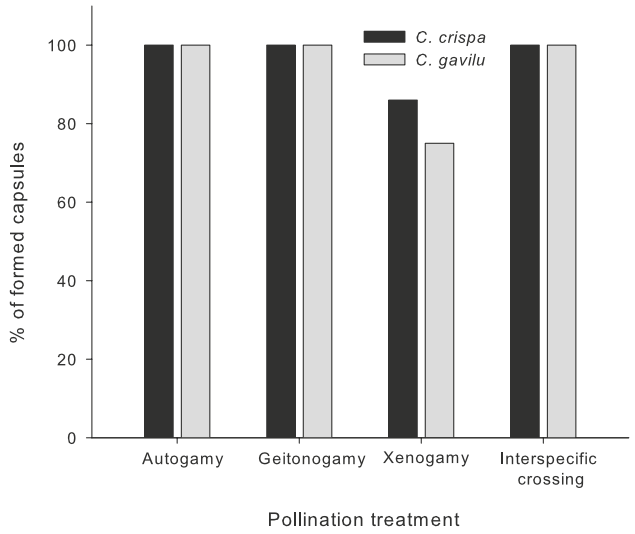

Fig. 1 Formed capsules (% respect to total flowers) in *Chloraea crispa* and *C. gavilu* subjected to four different pollination treatments ($n = 6$ plants per treatment)

In vitro germination percentages was significantly affected by pollination treatments in both orchid species (Fig. 2, $F(3, 24) = 23.26$, $p < 0.0001$). Additionally, there were differences in germination between the two used culture media, only in *C. crispa* (Fig. 2, $F(1, 24) = 50.19$, $p < 0.0001$). Overall, germination surpassed 50% in most cases for *C. crispa*, especially using MT, and were close to 10–20% in *C. gavilu* (Fig. 2). In *C. crispa*, the highest germination (close to 80%) was reached in geitonogamy using MT, and the lowest in interspecific crossing with MM (Fig. 2). On the other hand, for *C. gavilu*, regardless of the culture medium, the highest germination percentages, close to 20%, were observed in geitonogamy and interspecific crosses, while autogamy resulted in the lowest germination (below 10%) and was significantly different than the other treatments (Fig. 2, $p < 0.05$).

The embryos development showed similar results in *C. crispa* using both culture media (MT and MM). In these media seeds reached stage 2 (germination) after 2 weeks in all the pollination treatments (Table 3). After 8 weeks, shoot stage was reached only in autogamy, xenogamy and interspecific crossing (Table 3). In *C. gavilu*, we obtained different results when using MT or MM. After 4 weeks, stage 2 was reached using MT in geitonogamy, xenogamy and interspecific crossing, and only in xenogamy and interspecific crossing using MM (Table 3), in autogamy only reached stage 2 after 6 weeks on both media. Depending on the culture media, rhizoid stage we reached in embryos after 6–8 weeks in xenogamy and interspecific crossing (Table 3).

4 Discussion

Orchids are renowned for their remarkable ecological diversity, a result of various adaptations aimed at achieving optimal vegetative and reproductive strategies in a given environment. However, survival can be constrained by the normal functioning of ecological interactions, such as

Table 2 Capsule and seed mass (g) in *Chloraea crispa* and *C. gavilu* subjected to four different pollination treatments ($n = 4$ per treatment)

		Autogamy	Geitonogamy	Xenogamy	Interspecific crossing
C. crispa	Capsule mass (g)	0.807 ± 0.995^{a}	0.378 ± 0.661^{b}	0.920 ± 0.150^{a}	0.506 ± 0.612^{b}
	Seed mass (g)	0.313 ± 0.032^{b}	0.248 ± 0.017^{b}	0.416 ± 0.054^{a}	0.282 ± 0.023^{b}
C. gavilu	Capsule mass (g)	0.046 ± 0.005^{b}	0.057 ± 0.004^{ab}	0.065 ± 0.005^{a}	0.061 ± 0.009^{a}
	Seed mass (g)	0.030 ± 0.002^{a}	0.012 ± 0.004^{b}	0.031 ± 0.003^{a}	0.019 ± 0.004^{b}

Different letters indicate statistically significant differences between pollination treatments (Tukey test $p < 0.05$)

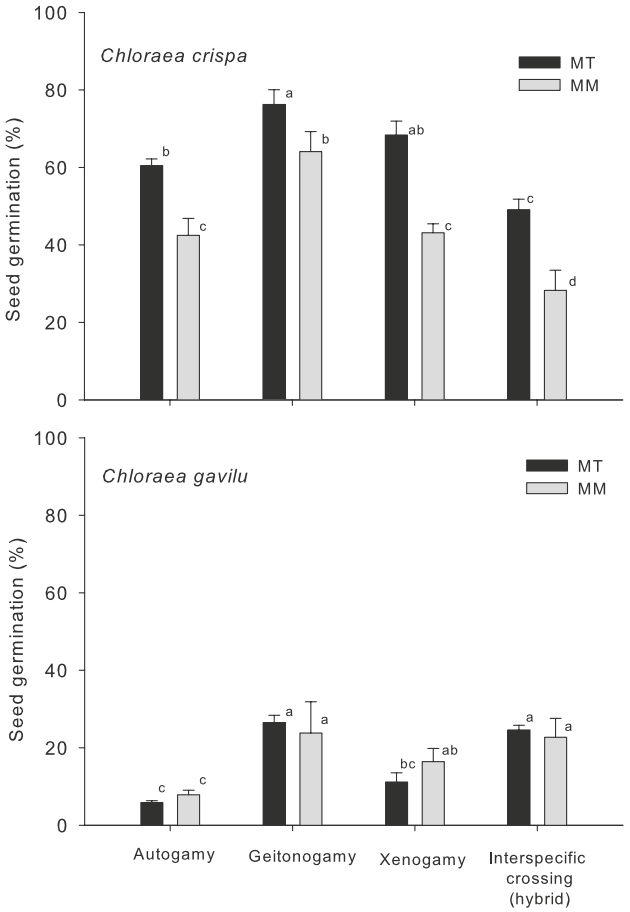

Fig. 2 In vitro seed germination in *C. crispa* (A) and *C. gavilu* (B) after 8 weeks. $n = 4$ Petri dishes per treatment

plant-pollinator and plant-mycorrhiza relationships. These interactions, typically species-specific, render orchids highly vulnerable to disturbances in the ecosystem (Wells 1981).

This study assessed reproductive capability through different strategies: autogamy, geitonogamy, xenogamy, and interspecific crossing. It was observed that both species formed capsules in all reproductive treatments. For both species, three of the four reproductive strategies tested (autogamy, geitonogamy, and interspecific crossing) obtained a percentage of 100% fruiting, suggesting that capsule formation does not seem to be limited by pollination treatment in these species. This contrasts with the results obtained by Humaña et al. (2008) in which, when evaluating different pollination treatments, they found that four species of the genus *Chloraea*, including *C. crispa*, did not form capsules by autogamy. Although the frequency of autogamy as a reproductive strategy within floras usually increases toward geographic extremes, as the spectrum of potential pollinators declines (Hagerup 1952; Kevan 1972; Strathdee and Bale 1998), autogamy has been reported for certain orchid species (Catling 1990), but it is usually morphologically prevented by the herkogamous structure of flowers (Proctor et al. 1996), so it only seems to be possible through hand-pollination.

However, seed mass and capsule mass does seem to be limited by pollination treatment. The most noteworthy results were observed in xenogamous pollination, for both *C. crispa* and *C. gavilu*, where the highest capsule weight and seed weight were recorded (Table 2), for this reproductive strategy a lower percentage of capsules were formed but they were of higher mass compared to the rest of capsules. Consequently, producing a few capsules containing only several thousand seeds may be considered an effective strategy to conserve plant resources (Gregg 1991). On the contrary, the rest of the treatments produced lower mass capsules and lower seed mass. These findings align with Lehnebach and Riveros. (2003) observations, where manual pollination in *C. lamellata* increased the percentage of formed capsules, although the number of viable seeds produced was low, suggesting that the reproductive success of orchid does not only depend on the supply of pollen, but is related to the amount of resources that can be made available for fruit production.

Table 3 Highest developmental stage of seeds/embryos observed in *C. crispa* and *C. gavilu* subjected to four pollination treatments and using two culture media

Species	Treatments	2 weeks		4 weeks		6 weeks		8 weeks	
		MT	MM	MT	MM	MT	MM	MT	MM
C. crispa	Autogamy	2	2	3	3	4	4	4	5
	Geitonogamy	2	2	3	3	4	4	4	4
	Xenogamy	2	2	3	3	4	4	5	5
	Interspecific crossing	2	2	3	3	4	4	5	5
C. gavilu	Autogamy	1	1	1	1	2	3	3	3
	Geitonogamy	1	1	2	1	3	2	3	3
	Xenogamy	2	2	3	3	3	4	3	4
	Interspecific crossing	2	2	3	3	4	4	4	4

Developmental stages: *1* pre germination stage, *2* Germination stage. Embryo emerges from the seed coat. *3* Protocorm stage, *4* Rhizoid stage, *5* shoot stage. Developmental stages modified form Yamazaki and Miyoshi (2006)

This is crucial in terrestrial orchids, as artificially increasing fruit set can significantly impact individual fecundity and future growth (Snow and Whigham 1989; Ackerman and Montalvo 1990).

The culture medium significantly impacts the in vitro germination of terrestrial orchids. Specific media formulations play a crucial role in promoting successful germination and growth, and subsequent acclimatization of terrestrial orchids (Diantina et al. 2020). There was no major difference between the culture media for *C. gavilu*. While, for *C. crispa* there were differences between growing media, although MT achieved a higher germination percentage, probably due to its higher concentrations of readily assimilable carbohydrates (sucrose), vitamins (thiamine), and organic nitrogen sources (tomato pulp) (Table 1), both media allowed similar levels of development, meaning that both met the nutritional requirements necessary for seedling development. An important factor is the form and availability of nitrogen in the culture media. Different media vary in their ability to stimulate protocorm development because of differences in nitrogen source and bioavailability (Johnson et al. 2007). Media supplying primarily inorganic nitrogen may limit germination, since young protocorms exhibit low nitrate reductase activity during early development (Van Waes and Debergh 1986). By contrast, organic nitrogen—provided as free amino acids or complex organic matter—can be taken up directly, bypassing energy-intensive assimilation steps and thus supporting faster protocorm growth (Malmgren 1992, 1996). Moreover, because the physiology and nutritional needs of Chilean orchids remain largely uncharacterized, it is challenging to determine whether these species require specific nutrients or precise ratios for optimal development, underscoring the need for detailed investigations into nitrogen forms, carbon sources, and micronutrient assimilation.

Although both species have been previously asymbiotically germinated in vitro (Pereira et al. 2017; Quiroz et al. 2017; Romero et al. 2017), this was done only using seeds produced by random cross-pollination. The germination percentage for *C. gavilu* is significantly lower in comparison to *C. crispa*, however, it is in agreement with previous studies (Pereira et al. 2017). After 16 weeks from the start of the assay, germination percentages should reach similar values to those previously described for this species, which suggests that *C. gavilu* has slower development times under asymbiotic conditions, i.e., in the absence of its mycorrhizal fungus. Significant differences were noted in germination percentage for both the pollination strategy and the culture medium used for both species. According to our results, the geitonogamy pollination strategy yielded the best seed germination outcomes for both species across different growth media. This strategy has been extensively documented in terrestrial orchids from temperate regions, regarded as an adaptation to the limited presence of insects and/or low rates of insect visitation (Lehnebach and Riveros 2003). It is also considered the initial step towards mechanical autopolination (Sipes and Tepedino 1995; Galetto et al. 1997; Lehnebach 2003; Lehnebach and Riveros 2003). However, from an evolutionary perspective, geitonogamy and autogamy are considered undesirable due to the low genetic variability it generates (Pérez-Martínez and Pacheco-Salamanca 2005; Scopece et al. 2014; Duarte et al. 2021; Zhang et al. 2021). A small error in evolutionary chance could lead to the complete elimination of the species population due to an evolutionary bottleneck in a scenario of increasing inbreeding and reduced genetic variability (Yun et al. 2020; Lee et al. 2022). In contrast, with xenogamous pollination or interspecific crossing, genetic variability is ensured, allowing for the development of individuals resistant to novel disturbances such as specific pests or diseases (Pérez-Martínez and Pacheco-Salamanca 2005; Duarte et al. 2021). However, for *C. cripa*, in autogamy, xenogamy, and interspecific crossing treatments, the "shoot" stage was achieved, while geitonogamy only reached the "rhizoid" stage in both culture media. Meanwhile, for *C. gavilu*, the "rhizoid" stage was reached in both culture media in xenogamy and interspecific crossing. In autogamy and geitonogamy treatments, only the "protocorm" stage was achieved. Overall, although geitonogamy had the highest germination percentage for both species, it was not the reproductive strategy that allowed the greatest degree of development, indicating slower growth in both cultures.

In vitro germination of *C. crispa* and *C. gavilu* seeds reveals the significant influence of different reproductive strategies in terrestrial orchids of the genus *Chloraea*. The different reproductive strategies, although not significant in the percentage of capsules produced, impact the quality of the capsules and seed germination. This study highlights the complexity of reproductive processes and the need to consider multiple variables when designing propagation and conservation strategies for species with complex interactions. Understanding these interactions is crucial for the conservation, restoration, and cultivation of orchid species in the future. Further research is needed to further investigate these mechanisms and their practical application in the management and conservation of these plants with high ornamental and ecological value.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by GP and DC-N. The first draft of the manuscript was written by GP, OJ-C and CA, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability Data not available in repositories but can be requested to the corresponding author.

Declarations

Conflict of interest The authors did not receive support from any organization for the submitted work. The authors have no competing interests to declare that are relevant to the content of this article.

References

Ackerman JD (1989) Limitations to sexual reproduction in Encyclia krugii (Orchidaceae). Syst Bot 14:101–109

Ackerman JD, Montalvo AM (1990) Short- and long-term limitations to fruit production in a tropical orchid. Ecology 71:263–272

Adam D, Chupp L, Battaglia E, Schauber M, Sedonia D, Sipes E (2014) Orchid-pollinator interactions and potential vulnerability to biological invasion. Aob Plants 7:plv099

Atala C, Pereira G, Romero C, Muñoz-Tapia L, Vargas R (2015) Orchidiod fungi of the form-genus rhizoctonia associated with the roots of *Chloraea cuneata* lindl. from Araucanía, Chile. Gayana Bot 72:145–148

Barbery R, Molares I (2014) Manual para el Cultivo in vitro de la orquídea Cattleya nobilior "flor símbolo de Concepción". Editorial el País, CEPAD, Santa Cruz, Bolivia

Bartareau T (1994) The reproductive ecology of *Dendrobium toressae* (Bailey) Dockr. A geographically restricted species in northeast Queensland. The Orchadian 11:106–112

Barth FG (2019) One of the most fascinating stories in biology. J Comp Physiol A 205:281–284

Bateman RM, Hollingsworth PM, Preston J, Yi-Bo L, Pridgeon AM, Chase MW (2003) Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). Bot J Linn Soc 142:1–40

Batty M, Brundett M (2001) Orchid conservation techniques manual. Canberra. Plant Science, Kings Park and Botanic Garden. First International Orchid Conservation Congress—Training Course, 20 September 2001, Perth, Western Australia

Beardsell DV, Clements MA, Hutchinson JF, Williams EG (1986) Pollination of *Diuris maculata* R.Br. (Orchidaceae) by floral mimicry of the native legumes *Daviesia spp.* and *Pultenaea scabra* R.Br. Aust J Bot 34:165–174

Berry PE, Calvo RN (1991) Pollinator limitation and position dependent fruit set in the high Andean orchid *Myrosmodes cochileare* (Orchidaceae). Plant Syst Evol 174:93–101

Borràs J, Cursach J (2021) Female and male fitness of a sexually deceptive orchid with a narrow distribution area: from phenotypic traits to spatial distribution patterns. Plant Biol 23(1):130–139. https://doi.org/10.1111/plb.13184

Calvo RN (1990a) Inflorescence size and fruit distribution among individuals in three orchid species. Am J Bot 7:1378–1381

Calvo RN (1990b) Four-year growth and reproduction of *Cyclopogon cranichoides* (Orchidaceae) in south Florida. Am J Bot 77:736–741

Calvo RN (1993) Evolutionary demography of orchids: intensity and frequency of pollination and the cost of fruiting. Ecology 74:1033–1042

Calvo RN, Horvitz CC (1990) Pollinator limitation, cost of reproduction, and fitness in plants: a transition-matrix demographic approach. Am Nat 136:499–516

Castillo-Novales D, Pereira G, Cid H, Elórtegui S, Atala C (2022) Asymbiotic germination of *Chloraea disoides* Lindl. (Orchidaceae), a critically endangered orchid endemic to Chile. Gayana Bot 79:44–50

Catling PM (1990) Auto-pollination in the Orchidaceae. In: J. Arditti (ed) Orchid Biology: Reviews and Perspectives, V, Portland, OR, Timber Press. pp 121–158

Christenhusz MJM, Byng JW (2016) The number of known plants species in the world and its annual increase. Phytotaxa 261:201–217

Christensen DE (1992) Notes on the reproductive biology of *Stelis argentata* Lindl. (Orchidaceae: Pleurothallidinae) in eastem Ecuador. Lindleyana 7:28–33

Correa M (1969) *Chloraea* género sudamericano de Orchidaceae. Darwiniana 15:374–500

Darren C, Wong J, Pichersky E, Peakall R (2017) The biosynthesis of unusual floral volatiles and blends involved in orchid pollination by deception: current progress and future prospects. Front Plant Sci 8:1955

Dearnaley J, Perotto S, Selosse MA (2016) Structure and development of orchid mycorrhizas. In: Martin F (ed) Molecular mycorrhizal symbiosis. Wiley-Blackwell, Hoboken, pp 63–86

Diantina S, Kartikaningrum S, McCormick AC (2020) Comparative in vitro seed germination and seedling development in tropical and temperate epiphytic and temperate terrestrial orchids. Plant Cell Tissue Organ Cult 143:619–633

Dixon KW (1991) Seeder/clonal concepts in Western Australian orchids. In: Wells TCE, Willems JH (eds) Population ecology of terrestrial orchids. SPB Academic Publishing, The Hague, pp 111–123

Duarte E, Rubenich R, Thalmayr P, Ortiz L, RochaS NF, Küppers G (2021) Efecto del tipo de polinización en la formación de frutos y capacidad germinativa de las semillas de *Cyrtopodium hatschbachii* pabst. Yvyraretá 43:3–5

Fracchia S, Aranda-Rickert A, Flachsland E, Terada G, Sede S (2014) Mycorrhizal compatibility and symbiotic reproduction of *Gavilea australis*, an endangered terrestrial orchid from south patagonia. Mycorrhiza 24:627–663

Fuentes O (2023) Orquídeas de Chile. Primera Edición, 183pp

Galetto L, Bernardello G, Rivera GL (1997) Nectar, nectaries, flower visitors, and breeding system in five terrestrials Orchidaceae from central Argentina. J Plant Res 110:393–403

Gregg K (1991) Reproductive strategy of *Cleistes divaricata* (orchidaceae). Am J Bot 78:350–360

Hagerup O (1952) Bud autogamy in some northern orchids. Phytomorphology 2:51–60

Herrera H, Valadares R, Contreras D, Bashan Y, Arriagada C (2017) Mycorrhizal compatibility and symbiotic seed germination of orchids from the Coastal Range and Andes in south central Chile. Mycorrhiza 27:175–188

Heywood VH, Brummitt RK, Culham A, Selberg O (2007) Flowering plant families of the world. Royal Botanic Gardens Kew, UK

Humaña AM, Cisternas MA, Valdivia CE (2008) Breeding system and pollination of selected orchids of the genus *Chloraea* (Orchidaceae) from central Chile. Flora 203:469–473

Janzen DH, DeVries P, Gladstone DE, Higgins ML, Lewinsohn TM (1980) Self- and cross-pollination of *Encyclia cordigera* (Orchidaceae) in Santa Rosa National Park, Costa Rica. Biotropica 12:72–74

Jeldes-Cajas O, Pereira G, Molina-Montenegro M, Atala C (2024) Symbiotic and asymbiotic germination *in vitro* of *Chloraea longipetala* (Orchidaceae), an endemic orchid from Chile. Gayana Bot 81:66–76

Jersáková J, Johnson SD, Kindlmann P (2006) Mechanisms and evolution of deceptive pollination in orchids. Biol Rev 81:219–235

Johnson SD, Bond WH (1992) Habitat dependent pollination success in a Cape Orchid. Oecologia 91:455–456

Johnson T, Stewart S, Dutr D, Kane M, Richardson L (2007) Asymbiotic and symbiotic seed germination of *Eulophia alta* (Orchidaceae)—preliminary evidence for the symbiotic culture advantage. Plant Cell Tissue Organ Cult 90:313–323

Johnson T, Stewart S, Kauth P, Kane M, Philman R (2009) Confronting assumptions about spontaneous autogamy in populations of *Eulophia alta* (Orchidaceae) in South Florida: assessing the effect

of pollination treatments on seed formation, seed germination and seedling development. Bot J Linn Soc 161:78–88
Kevan PG (1972) Insect pollination of high arctic floras. J Ecology 60:831–847
Leake JR (2004) Myco-heterotroph/epiparasitic plant interaction with ectomycorrhizal and arbuscular mycorrhizal fungi. Curr Opin Plant Biol 7:422–428
Lee S-R, Choi T-Y, Jung S-Y (2022) Genetic diversity on a rare terrestrial orchid, *Habenaria linearifolia* in South Korea: Implications for conservation offered by genome-wide single nucleotide polymorphisms. Front Plant Sci 13:772621
Lehnebach CA (2003) Preliminary checklist of the orchids of Chile. Bot J Linn Soc 143:449–451
Lehnebach CA, Riveros M (2003) Pollination biology of the Chilean endemic orchid *Chloraea lamellata*. Biodivers Conserv 12:1741–1751
Malmgren S (1992) Large-scale asymbiotic propagation of *Cypripedium calceolus*, plant physiology from a surgeon's point of view. Bot Gardens Micropropag News 1:59–63
Malmgren S (1996) Orchid propagation: theory and practice. In: Allen C (ed) American native terrestrial orchids: propagation and production. North American Native Terrestrial Orchid Conference, Maryland, pp 63–71
Mitchell R (1989) Growing hardy orchids from seeds at Kew. Plantsman 11:152–169
Miyoshi K, Mii M (1995) Enhancement of seed germination and protocorm formation in *Calanthe discolor* (Orchidaceae) by NaOCl and polyphenol absorbent treatments. Plant Tissue Cult Lett 12:267–272
Novoa P, Espejo J, Alarcón D, Cisternas M, Domínguez E (2015) Guía de campo de las orquídeas Chilenas, segunda ed. ampliada. Ediciones corporación chilena de la madera, concepción
Pereira G, Romero C, Suz LM, Atala C (2014) Essential mycorrhizal partners of the endemic Chilean orchids *Chloraea collicensis* and *C. gavilu*. Flora 209:95–99
Pereira G, Albornoz V, Muñoz-Tapia L, Romero C, Atala C (2015) Asymbiotic germination of *Bipinnula fimbriata* (Orchidaceae) seeds in different culture media. Seed Sci Technol 43:367–377
Pereira G, Albornoz V, Romero C, Lara S, Sánchez-Olate M, Ríos D, Atala C (2017) Germinación asimbiótica en tres especies de *Chloraea* (orchidaceae) de chile. Gayana Bot 74:131–139
Pérez-Martínez BA, Pacheco-Salamanca RA (2005) Germinación in vitro de la orquídea *Masdevallia ignea* rchb. fa partir del cultivo de semillas provenientes de diferentes tipos de polinización. Pérez-Arbelaezia 16:45–55
Phillips RD, Brown GR, Dixon KW, Hayes C, Linde CC, Peakall R (2017) Evolutionary relationships among pollinators and repeated pollinator sharing in sexually deceptive orchids. J Evol Biol 30:1674–1691. https://doi.org/10.1111/jeb.13125
Proctor M, Yeo P, Lack A (1996) The natural history of pollination. Timber Press, Portland
Quiroz K, Saavedra J, Vogel H, Verdugo G, Caligari PDS, García-Gonzáles R (2017) In vitro asymbiotic germination for micropropagation of the recalcitrant terrestrial orchid *Chloraea crispa* (Orchidaceae). Appl Plant Sci 5:1600142
Rasmussen HN (1995) Terrestrial orchids: from seed to mycotrophic plant. Cambridge University Press, Cambridge
Rodriguez-Robles JA, Melendez EJ, Ackerman JD (1992) Effects of display size, flowering phenology, and nectar availability on effective visitation frequency in *Comparettia falcata* (Orchidaceae). Am J Bot 79:1009–1017
Romero C, Cuba-Díaz M, Silva R (2017) In vitro culture of *Chloraea gavilu* Lindl., an endemic terrestrial orchid from Chile. Plant Biosyst 152:612–620
Ruiz BC, Laguna CA, Iglesias AL, Damon A, Marín HT, Azpíroz RH, Moreno MJ (2008) Germinación in vitro de semillas de *Encydia adenocaula* (La Llave & Lex.) Schltr (Orchidaceae). Oytón 77:203–215
Schemske DW (1980) Evolution of floral display in the orchid *Brassavola nodosa*. Evolution 34:489–493
Scopece G, Musacchio A, Widmer A, Cozzolino S (2014) Patterns of reproductive isolation in Mediterranean deceptive orchids. Evolution 68:596–606
Sipes S, Tepedino VJ (1995) Reproductive biology of the rare orchid, *Spiranthes diluvialis*: breeding system, pollination, and implications for conservation. Conserv Biol 9:929–938
Snow AA, Whigham DF (1989) Cost of flower and fruit production in *Tipularia discolor* (orchidaceae). Ecology 70:1286–1293
Steinfort U, Verdugo G, Besoain X, Cisterna M (2010) Mycorrhizal association and symbiotic germination of the terrestrial orchid *Bipinnula fimbriata* (poepp.) johnst (Orchidaceae). Flora 205:811–817
Strathdee AT, Bale JS (1998) Life on the edge: insect ecology in Arctic environments. Annu Rev Entomol 43:85–106
Takao S, Castilho C, Seaton P, Marks T, Machado-Neto N (2012) Improved assessment of viability and germination of *Cattleya* (Orchidaceae) seeds following storage. In Vitro Cell Dev Biol Plant 48:127–136
Thompson JN (1987) Symbiont-Induced speciation. Biol J Linn Soc 32:385–393
Van Waes JM, Bebergh PC (1986) Adaptation of the tetrazolium method for testing the seed viability, and scanning electron microscopy study of some Western European orchids. Physiol Plant 66:435–442
Warcup JH (1973) Symbiotic germination of some Australian terrestrial orchids. New Phytol 72:387–392
Waterman RJ, Bidartondo MI (2008) Deception above, deception below: linking pollination and mycorrhizal biology of orchids. J Exp Bot 59:1085–1096
Waterman RJ, Bidartondo MI, Stofberg J, Combs J, Gebauer G, Savolainen V, Barraclough T, Pauw A (2011) The effects of above- and belowground mutualisms on orchid speciation and coexistence. Am Nat 177:54–68
Wells TCE (1981) Population ecology of terrestrial orchids. In: Synge H (ed) The biological aspects of rare plant conservation. Wiley, Chichester
Yamazaki J, Miyoshi K (2006) In vitro asymbiotic germination of immature seed and formation of protocorm by *Cephalanthera falcata* (Orchidaceae). Ann Bot 98:1197–1206
Yun SA, Son HD, Im HT, Kim SC (2020) Genetic diversity and population structure of the endangered orchid *Pelatantheria scolopendrifolia* (Orchidaceae) in Korea. PLoS ONE 15:0237546
Zhang Y, Zhang D, Barrett SCH (2021) Reproductive assurance through autonomous self-pollination and its effects on the mating system in a food-deceptive orchid. Ann Bot 127:673–684