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# OPTIMIZED PROTOCOL FOR ARTIFICIAL POLLINATION AND IN-VITRO PROPAGATION OF *DENDROBIUM SONIA* (ORCHIDACEAE)

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**Abstract**

Orchids hold significant ornamental and economic value but face challenges in propagation due to low natural pollination rates, seed germination, and survival. Artificial pollination and in-vitro culture techniques provide an alternate approach to overcome these limitations. This study aimed to develop an effective protocol for artificial pollination and large-scale propagation of the orchid *Dendrobium sonia* under semi-controlled greenhouse conditions. Healthy, mature, and fully blooming potted *D. sonia* plants were selected for the artificial pollination experiment. Distinct changes in artificially pollinated flowers were observed, such as wilting of flower petals and a swollen ovary with a color change. Mechanical pollination was calculated as pollination percentage (PP) with 91.72% success across *D. Sonia* plants, demonstrating its efficiency in controlled breeding programs. After maturation, pods were sterilized, and seeds were sown on two types of MS media for asymbiotic germination. High asymbiotic germination efficiency was observed for *D. sonia* seeds on both MS media, with seeds turning from white to cream color after 4 days and green color after 15 days. Maximum germination (95.5%) was observed on full MS medium supplemented with PGRs. Plantlets acclimatized in coconut husk + charcoal reached a height of 13.0 cm, compared to 12.8 cm (coconut husk alone) and 10.7 cm (charcoal alone). These findings highlight the potential of this optimized protocol for efficient, large-scale propagation of *D. sonia* in a semi-controlled environment.

**Key words:** Dendrobium; Induced pollination; Pod formation; Asymbiotic germination; Acclimatization

## Introduction

The family Orchidaceae is among the largest and ecologically significant families of angiosperms, having more than 800 genera and 25,000-35,000 species (Zhang *et al*., 2022; Tiwari *et al*., 2024). Commercially cut flowers of the genus *Dendrobium* have the highest rank because of their longevity, short production cycle, and high number of flowers per inflorescence (Lo *et al*., 2004; Ching *et al.*, 2012). Orchids are either terrestrial or epiphytic (Carl & Hew, 2000). Orchidaceae is an exotic and cosmopolitan family that inhabits tropical and subtropical areas of the world (Lehnebach *et al*., 2004; Tiwari *et al*., 2024). However, their amazing variation in floral forms has long attracted the interest of evolutionary biologists (Jersakova *et al*., 2006).

Cut flowers constitute an important industry that generates about two billion dollars in the world. Orchids are among the highly demanded cut flowers and are produced in different countries like Thailand, the Netherlands, Hawaii, Singapore, and Italy (Chandran *et al*., 2006; Yuan *et al*., 2021; Tiwari *et al*., 2024). Besides their ornamental value, *Dendrobium* species are also used in herbal medicines, cosmetic products, and fragrance industries (Groves & Rutherford, 2017; Teoh, 2019). Pakistan is also trying to contribute to this industry at present. Despite global advances, standardized protocols for induced pollination and propagation of *Dendrobium* in Pakistan remain undeveloped. This study marks the first successful insight from Pakistan on induced pollination in *Dendrobium sonia*, presenting a comprehensive protocol that spans from artificial pollination to seed germination and plantlet acclimatization, thus significantly contributing to the propagation of this commercially valuable orchid species.

Orchids are growing worldwide, accounting for almost 8% of the world's floriculture trade (Rafique *et al*., 2012). However, the *Dendrobium* genus itself contributes 85% of its share to the floriculture industry. This may be attributed to the diverse vegetative and floral characteristics, assorted colors, enduring blooms, and extensive geographic distribution among the members of this genus (Tiwari *et al*., 2024; Pyati, 2022). The demand for high-quality orchid plants is increasing due to the rising popularity of orchid flowers (Kauth *et al*., 2008a).

Orchid flowers have three petals and three sepals, with the third petal forming a specialized structure called the labellum or lip, positioned at the bottom. This labellum serves as a landing platform, guiding pollinators toward the gynostemium. A key characteristic of orchids is the gynostemium, a fusion of the style, stigma, and stamens. The anther cap and pollinia are positioned at the front, while the stigma is located behind the anther on the underside (Thakur & Dongarwar, 2012).

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The mode of pollination and interactions with pollinators play a significant role in plant evolution. Orchid flowers exhibit structural adaptations that promote cross-pollination, primarily facilitated by insects. Orchid flowers have a column (*gynostemium*), which could facilitate self-fertilization, but this occurs in only about 3% of species (Stort & Galdino, 1984). *Dendrobiums* normally reproduce asexually by the division of offshoots, but the multiplication ratio is low. Sexual reproduction and seed germination are difficult due to the complicated structure of flowers, poorly developed embryos and seeds, which are very minute and non-endospermic, and specific mycorrhizal requirements (Hossain *et al*., 2012).

Orchids rely on mycorrhizal associations for germination, as these fungi supply essential nutrients and water. Finding compatible mycorrhizae is a challenge, limiting natural germination to less than 5% of seeds in the wild, which threatens species survival (Pyati, 2022). Early attempts to germinate orchid seeds collected from the wild faced high seedling mortality rates. However, the advent of in-vitro techniques in the early 1900s improved reliability. These methods have since facilitated the successful germination and propagation of many orchid species by addressing crucial factors like photoperiod, temperature, and mineral nutrition (Kauth *et al*., 2008b). Therefore, plant tissue culture is an alternative and most robust strategy to conserve and propagate these unique plants on a large scale (Pyati, 2022).

Orchids are fascinating due to their unique flower structures. Most species store pollen in compact structures called pollinia. Many orchids attract pollinators by offering nectar, oils, resins, or aromatic compounds (Singer, 2003). Some use deception, mimicking territorial cues, mating signals, brood sites, or food sources to lure pollinators (Lammi *et al*., 2003; Jersakova *et al*., 2006).

After pollination, the ovary forms a seed capsule containing thousands of tiny seeds, with the *Dendrobium* capsule holding around ten thousand seeds (Xu *et al*., 2013). Seed development demands additional energy and resources, so pollination is best suited for healthy, well-established plants. To support seeded capsule growth, the plant extends the lifespan of the capsule-bearing shoot (Camiel, 2002). The expansion of orchid production is driven by advancements in breeding and greenhouse techniques, boosting the industry significantly (Zhang *et al*., 2022).

Propagation techniques have become crucial for the orchid industry, as the commercial demand for ornamental orchids far exceeds their natural regeneration. While asymbiotic seed germination is commonly used, it poses challenges for some terrestrial orchids, like *Dendrobium*, and is less ideal for large-scale production due to the long juvenile period and genetic variability (Park *et al*., 2018). To produce genetically stable and uniform orchids, micropropagation through tissue culture is widely used for mass production and regeneration (Yam & Arditti, 2018). Rooting media for the growth of the orchid were optimized by Kazmi *et al*., (2024). Rapid in-vitro flowering offers commercial benefits, particularly for species with long juvenile phases. Along with shortening the reproduction cycle, it also aims to enhance the production of valuable secondary metabolites (Zhang *et al*., 2022).

This study aimed to optimize a protocol for successful artificial pollination and large-scale propagation of *D. sonia*. We hypothesized that mechanical pollination, followed by in-vitro seed germination and substrate-based acclimatization, would lead to successful plantlet development.

To our knowledge, this is the first documented study from Pakistan presenting a complete protocol for induced pollination, asymbiotic seed germination, and acclimatization of *D. sonia*, contributing to both conservation and commercial orchid propagation.

## Material and Methods

**Plant selection and growth conditions:** Healthy, well-established *D. sonia* plants with purple flowers were chosen for induced pollination. These plants were potted in clay pots with side holes for aeration, filled with wooden charcoal as a growth substrate. Greenhouse conditions were maintained with a 60/40% light intensity ratio, 60% humidity, and temperatures between 28-32°C using fine mist irrigation.

**Induced pollination:** Pollination was performed on fully bloomed flowers using sterile toothpicks for pollinia transfer. Ten uniformly grown, healthy plants per repeat (three repeats) were selected for mechanical pollination over a year. Pollinia from mature flowers were carefully picked with a sterilized toothpick and deposited onto the stigma of the same flower. Pollinated flowers were tagged, and the total number of flowers per plant, along with pollination success, was recorded. Seed capsule formation confirmed successful pollination. Pollination percentage (PP) was calculated according to Thakur & Dongarwar (2012) using the formula:

$$\mathrm{PP} = \left(\frac{\text{No. of capsules developed}}{\text{No. of pollinated flowers}}\right) \times 100$$

Plants were maintained in the greenhouse for 19 weeks until pods ripened, turning from dark green to light green or yellow-green, indicating readiness for seed germination (Fig. 1f).

**Capsule sterilization:** Greenish-yellow capsules were collected after 15 weeks and washed with tap water, followed by sterile distilled water. Pods were then dipped in 70% ethanol for 3-5 minutes (Fig. 1d) and briefly flamed 3-4 times in a laminar flow cabinet (Fig. 1e). After sterilization, pods were cut longitudinally using a sterile surgical blade for seed extraction.

**Media selection and asymbiotic germination:** The asymbiotic germination experiment was conducted using two media types: i) ½ MS medium (Murashige & Skoog, 1962) without plant growth regulators (PGRs) as a control; ii) Full-strength MS medium supplemented with 0.1 mg/L BAP (6-Benzylaminopurine) and 0.5 mg/L NAA (Naphthalene Acetic Acid).

A single capsule of *D. sonia* can produce approximately 10,000 seeds, which are non-endospermic and lack stored nutrients. Consequently, their germination and development in nature depend on a symbiotic association with fungi. In this study, the fine white seeds were sown on MS media, and the jars were placed in a growth room at 25°C for germination (Fig. 1f).


Fig. 1. Experimental highlights from mechanical pollination to in-vitro seedbed preparation; (a) Pollinated flowers, (b) Pod formation, (c) Mature pods in flower stems, (d) Pod in 70% alcohol, (e) The pod fired on flame, (f) Shading seeds on MS Media.

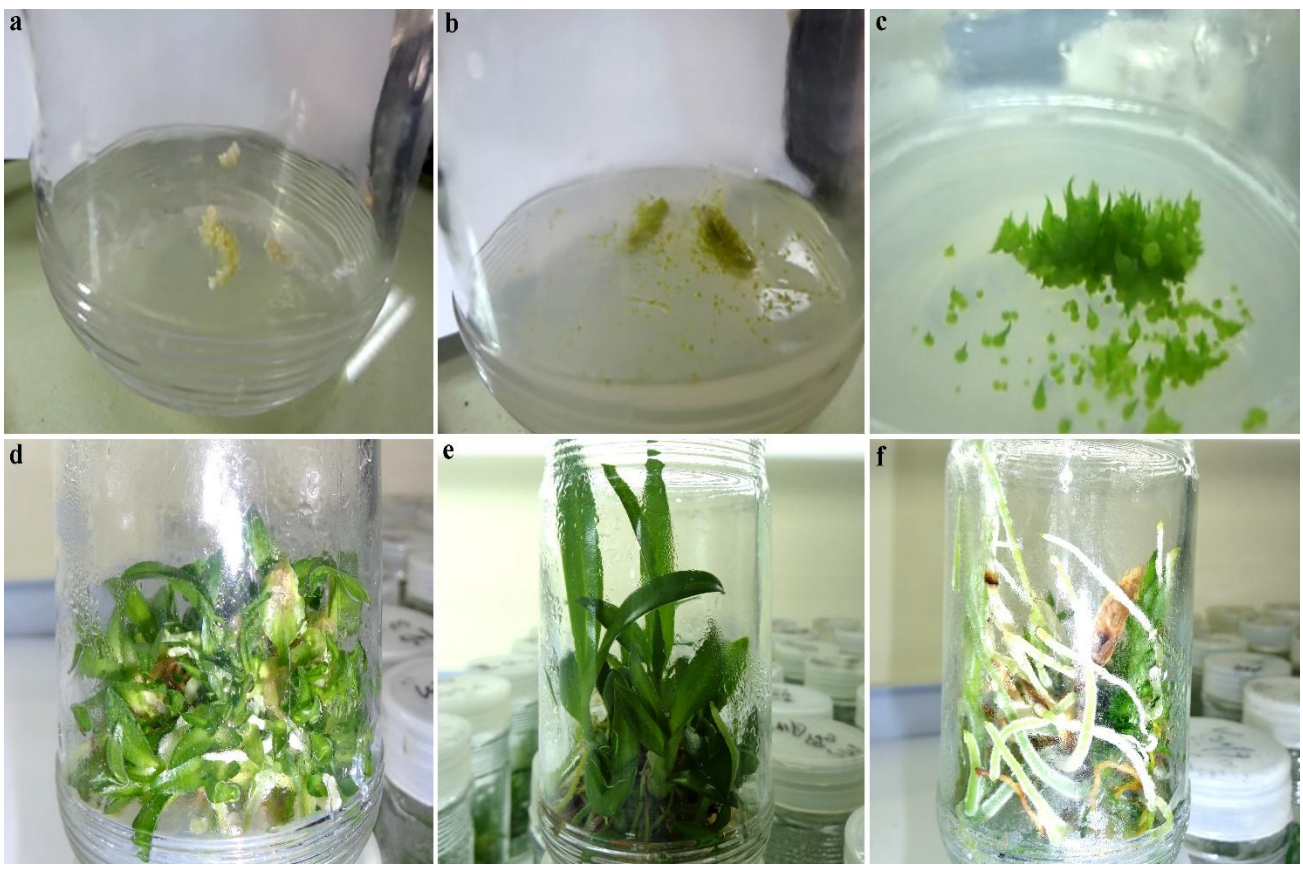


Fig. 2. Experimental highlights of asymbiotic seed germination and seedling growth on various MS media; (a) White seeds turning to cream color, (b) Seeds turning to yellow-green color, (c) Sprouting of seeds, (d) Multiplication of orchid on media, (e) Shoot developed in plantlets, (f) Root developed in plantlets.

**Table 1. Pollination percentage in the *Dendrobium sonia* orchid.**

| No. of plants | No. of flowers | No. of flowers pollinated | No. of pods developed | Pollination percentage % |
|---|---|---|---|---|
| 1 | 14 | 11 | 10 | 90.91% |
| 2 | 19 | 18 | 17 | 94.44% |
| 3 | 18 | 16 | 14 | 87.50% |
| 4 | 21 | 19 | 17 | 89.47% |
| 5 | 22 | 20 | 18 | 90% |
| 6 | 17 | 14 | 13 | 92.86% |
| 7 | 18 | 16 | 15 | 93.75% |
| 8 | 20 | 20 | 18 | 90% |
| 9 | 16 | 14 | 14 | 100% |
| 10 | 23 | 21 | 19 | 90.48% |
| **Total** | **196** | **167** | **157** | **91.72** |

**Table 2. ANOVA table for the effect of two MS media on germination percentage of *Dendrobium sonia* seeds. The analysis depicts significant variations among treatments (*F*-value = 275, *P*-value = 0.0001).**

| Source of variations | DF | SS | MS | *F*-value | *P*-value |
|---|---|---|---|---|---|
| MS media | 1 | 111.802 | 111.802 | 275 | 0.0001 |
| Error | 4 | 1.627 | 0.407 | | |
| **Total** | **5** | **113.428** | | | |

Note: DF = Degrees of freedom, SS = Sum of squares, MS = Mean squares

**Table 3. ANOVA for the Plant height of *Dendrobium sonia* plantlets grown in different potting media. The analysis shows that the variation due to potting media is highly significant (*F*-value = 424, *P*-value = 0.0001), indicating that the choice of potting media significantly influences the growth of *Dendrobium sonia* plantlets.**

| Source of variations | DF | SS | MS | *F*-value | *P*-value |
|---|---|---|---|---|---|
| Potting media | 2 | 15.5293 | 7.76467 | 424 | 0.0001 |
| Error | 12 | 0.2200 | 0.01833 | | |
| Total | 14 | 15.7493 | | | |

Note: DF = Degrees of freedom, SS = Sum of squares, MS = Mean squares

Fifty, fine, and healthy orchid seeds were dusted onto the surface of both media in sterilized jars using forceps. Each pod's seeds were distributed across 5 jars per treatment. The media pH was adjusted to 5.75-5.78 using 1% NaOH before autoclaving at 121°C for 20 minutes. Seed-containing jars were stored in a growth room under controlled conditions. Each treatment was performed in triplicate to evaluate the media's effectiveness in seed germination and seedling development.

**Germination percentage (GP):** The seed germination percentage (GP) was calculated using the formula provided by Yari *et al*., (2010).

$$\mathrm{GP} = \left(\frac{\text{No. of germinated seeds}}{\text{Total no. of seeds sown}}\right) \times 100$$

**Plant multiplication and growth:** Orchid seed germination occurred in a growth room with a 16/8 hours photoperiod (light and dark period) at 25±2°C. The data on seed germination were recorded at different time intervals. Seed cultures were regularly monitored for development. The plantlet multiplication was achieved on media, as reported by Khan *et al*. (2010). Following complete root and shoot development, the plantlets were transferred to a green net house with 60% humidity for acclimatization/hardening into different substrates, including coconut husk, wooden charcoal, and a combination of coconut husk + wooden charcoal (Fig. 3abc). The experiment followed a completely randomized design (CRD) with 5 replications, and 10 healthy plantlets were selected and transferred to each potting medium.

**Statistical analysis**

A one-way ANOVA was performed to test the effects of different growth media and potting media on germination and growth parameters, respectively. Data were analyzed using Statistix (version 8.1). Tukey's HSD test was used to identify which treatment means differed significantly. The significance was evaluated at $p \leq 0.05$.

**Results**

**Induced pollination:** Successful pollination in *D. sonia* was evident after three days, as indicated by swollen ovaries on the flower stalk or the formation of pods (Fig. 1ab). The pollinated flowers progressed to seed capsule development within three months (Fig. 1c). The results of this experiment demonstrated a high degree of success among the ten *Dendrobium sonia* plants (Table 1). On average, each plant had approximately 19.6 flowers, with the number of successfully pollinated flowers ranging from 10 to 20 per plant. This resulted in an average PP of 91.72%. However, the highest pollination rate observed was 100%, while the lowest was 87.50% (Table 1).


Fig. 3. (a) and (b) depict the acclimatization of *Dendrobium sonia* plantlets into different media. While Figure c depicts a green net house view.

**Asymbiotic seed germination:** The seeds first imbibed and turned from white to cream color (Fig. 2a), then turned green (Fig. 2b), and started propagating within three weeks (Fig. 2cd). The percent germination was significantly different ($p \leq 0.0001$) on the two MS media with $F$-value of 275 (Table 2). The maximum germination percentage (95.5%) of seeds was found in full MS media with PGRs (Fig 4). While the percent germination (86.9%) also increased on ½ MS media. Despite this, the growth of seedlings on ½ MS media was low. To enhance the growth, a precise concentration of PGRs, i.e., BAP (0.1 mg/L) and NAA (0.5 mg/L), was tested. Seedlings cultured on full-strength MS media supplemented with PGRs exhibited faster growth compared to those on ½ MS media. After three weeks, root and shoot development were observed in seedlings grown on full-strength MS media containing 0.1 mg/L BAP and 0.5 mg/L NAA (Fig. 2ef).

**Acclimatization:** The growth of *D. sonia* plantlets was significantly influenced by the type of potting media used during acclimatization ($p \leq 0.0001$, Table 3; Fig. 5). The average plant height in coconut husk was 12.8 cm, while plantlets grown in coconut husk + wooden charcoal reached an average height of 13.0 cm (Table 4). In contrast, plantlets grown in wooden charcoal alone had a lower average height of 10.7 cm (Table 4).

**Acclimatization sensitivity:** During the acclimatization or hardening stage, plantlets transitioning from a controlled environment exhibit heightened sensitivity and require adequate moisture levels. Charcoal lacks water retention capacity, making it unsuitable for the moisture-dependent acclimatization of plantlets. The greenhouse setup maintained a 60/40 light intensity ratio; however, an increase in sunlight intensity beyond 40% could lead to a higher mortality rate among the plantlets. Plantlets potted into coconut husk and coconut husk + charcoal showed excellent growth with a bright green color, but slow growth and reddish green leaves were observed in charcoal-potted plantlets (Table 4).

An ANOVA was conducted to statistically validate these observations. The findings revealed a highly significant ($p \leq 0.0001$) effect of potting material on plant height, with $F$-value of 424 and a $P$-value of 0.0001 (Table 3).

**Table 4. Effect of different potting media on plant height and leaf color of *Dendrobium sonia* plantlets. Plantlets grown in coconut husk and a mix of coconut husk + wooden charcoal exhibited bright green leaves and greater heights, while those grown in wooden charcoal alone had shorter heights and reddish green leaves.**

| Potting media | Plant height (cm) | Leaf color |
|---|---|---|
| Coconut husk | 12.8 | Bright green |
| Wooden charcoal | 10.7 | Reddish green |
| Coconut husk + Wooden charcoal | 13 | Bright green |

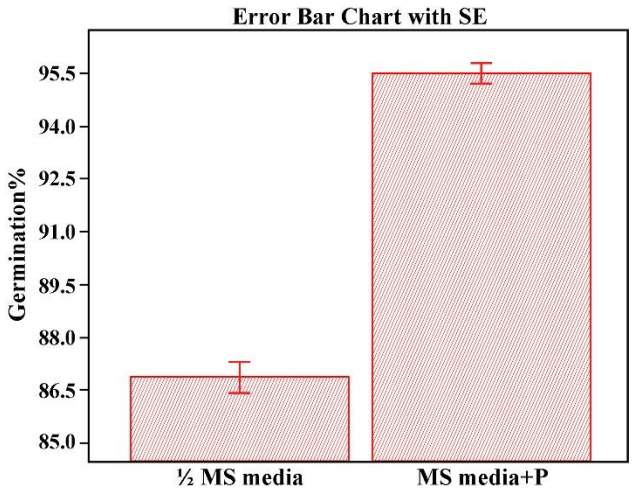


Fig. 4. Germination % of *Dendrobium sonia* on ½ MS media (without PGRs) and Full MS media (with PGRs). The error bars represent the standard error (*SE*) of the mean for Germination% at both media. The small alphabetic letters indicate a significant difference between the treatments as determined by the Tukey HSD test.

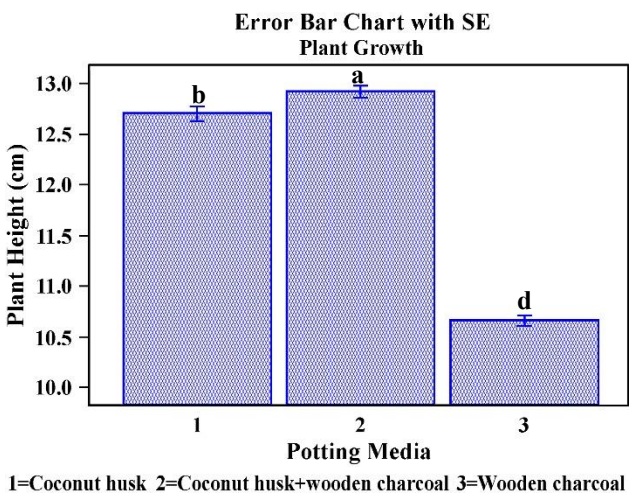


Fig. 5. Plant height (cm) of *Dendrobium sonia* grown in different potting media. The error bars represent the standard error (*SE*) of the mean plant height within each potting media. The small alphabetic letters indicate a significant difference between the treatments as determined by the Tukey HSD test.

## Discussion

Orchids are highly valued in the international market due to their beautiful, complex flower structures and long shelf life. Most orchid flowers remain fresh for an extended period when not pollinated, but wither quickly after pollination (Attri & Nayyer, 2011). In nature, *Dendrobium* **species** reproduce through seeds; however, their germination rate is very low because they require specific fungal symbionts for successful growth. To overcome this limitation, tissue culture techniques have been widely used to propagate orchids efficiently under controlled conditions (Akhtar *et al*., 2008). Natural fruit setting in orchids is not possible in the absence of a specific pollinator. Vegetative multiplication through the splitting of pseudo-bulbs is easy but slow and time-consuming (Saleem *et al.*, 2015).

Orchid species exhibit diverse breeding systems, ranging from self-pollination to obligate cross-pollination. Seed production by self-pollinated species is lower than that of cross-pollinated species (Pansarin *et al*., 2016; Li *et al*., 2021). Pollination is a crucial process for the sexual reproduction of orchids, enabling genetic diversity and adaptation. Orchids rely on specific pollinators to transfer pollinia (pollen packets) from the anther to the stigma, facilitating fertilization and subsequent seed development. This biological interaction ensures the survival and evolution of orchid species by promoting genetic variation, which is essential for adapting to environmental changes and resisting diseases (Jersakova *et al*., 2006; Cardoso *et al*., 2023).

Self-fertilization in orchids is challenging due to its complex flower structure, which is designed to promote cross-pollination. The intricate arrangement of the gynostemium and the need for precise pollinator interactions make it difficult for these orchids to self-pollinate. Orchid species have co-evolved with their pollinators. Therefore, it is difficult for orchids to find a specific pollinator. Consequently, many orchid species are on the verge of extinction due to the unavailability of their pollinators (Thakur & Dongarwar, 2019).

Additionally, the small, non-endospermic seeds lack the necessary nutrients for independent development, further complicating self-fertilization efforts (Thakur & Dongarwar, 2012; Pyati, 2022). Hence, artificial pollination and propagation through tissue culture offer promising solutions for overcoming the limitations of natural reproduction in *Dendrobium sonia*. By manually transferring pollinia, researchers can ensure successful fertilization and pod formation. The results of our study demonstrated that mechanical pollination achieved a high average success rate of 91.72%, with the highest being 100% (Table 1), indicating its reliability and effectiveness in controlled orchid breeding programs.

Our results are also in line with Thakur & Dongarwar (2012), who also reported improved results of artificial pollination in the *Spathoglottis plicata* orchid. They further revealed that mechanical pollination increased the fruit set and seed production in orchids.

The successful pollination of *D. sonia* orchid plants was evident within three days, as indicated by the swelling of the ovaries on the flower stalks (Fig. 1a). This initial success allowed the flowers to proceed through fruit and seed development stages, culminating in mature capsules after three months (Fig. 1b-f). Each capsule produced approximately 10,000 seeds, relying on symbiotic associations with fungi for germination in nature (Hossain *et al*., 2012).

For sterilization, the capsules were dipped in 70% ethanol for 3-5 minutes and then flamed (Fig. 1de). The seeds were subsequently cultured on full-strength MS media in a growth room maintained at 25 ℃. Within three weeks, the seeds transitioned from cream to green, indicating successful germination and initial propagation (Fig. 2ab). The development of roots and shoots followed, allowing for culturing on MS media supplemented with 0.1 mg/L BAP and 0.5 mg/L NAA to enhance growth. Tissue culture techniques, such as using MS media supplemented with growth regulators, enable the efficient germination and development of orchid seeds in controlled environments (Martin & Madassery, 2006).

This approach enhances the propagation rate and supports the large-scale production of high-quality orchids, catering to the increasing market demand (Kauth *et al*., 2008b; Saengjanchay *et al*., 2020). The composition of growth media plays a pivotal role in the asymbiotic germination and micropropagation of explants (Shukla & Sharma, 2017). MS media improved the in-vitro germination of orchids (Knudson, 1946). The use of MS media supplemented with PGRs in this study paralleled findings by Thakur & Dongarwar (2012) and Martin & Madassery (2006), who unveiled the significant response of full MS medium with BAP and NAA to germination and seedling parameters in *Spathoglottis plicata* and *D. sonia*, respectively.

Several reports claimed the same effects of PGRs, mainly auxins and cytokinins, enhance the seed germination, growth, and development of different orchid species in an in-vitro environment (Gow *et al*., 2008; Lindley *et al*., 2017). This would be due to the addition of cytokinins (BAP) in MS media. The PGRs trigger cell division, cytokinesis, cell enlargement, and shoot and root proliferation. Cytokinins also play a vital role at the cellular level. They are involved in gene expression, RNA, and protein synthesis (Bewley & Black, 1994). Artificially applied cytokinins also promote lipid mobilization in orchid embryos (de Pauw *et al*., 1995).

However, during the natural or symbiotic germination of orchids in the wild, cytokinins are supplemented by mycorrhizae (Crafts & Miller, 1974). In our study, the addition of a precise concentration of cytokinins (BAP) increased the asymbiotic seed germination when combined with the synthetic auxin (NAA). Synthetic cytokinins (BAP) and auxins (NAA) improved the seedling characteristics and their micropropagation in *Dendrobium aphyllum* (Hossain *et al*., 2012). Our results were also in agreement with the findings of Seeja *et al*., (2018), who found some interesting results for seed germination on BAP and NAA supplemented with MS media. However, supplementation of $N^6$-benzyladenine with ½ MS media shortens the growth phase and triggers early flowering in *Dendrobium sonia* (Tee *et al*., 2008).

Saengjanchay *et al*., (2020) explored the effects of different nitrogen-to-phosphorus ratios and cytokinins on the growth of *D. sonia* Earsakul. The better performance of lower PGR concentrations in our study may be attributed to their ability to maintain a physiological balance between auxin and cytokinin activity, thereby supporting normal morphogenesis. In contrast, higher concentrations of cytokinins, as reported by Saengjanchay *et al*., (2020), often disrupt hormonal balance, leading to abnormal shoot proliferation, dwarf shoot growth, or distorted leaves. Such abnormalities are due to excessive cytokinin-induced cell division without coordinated cell elongation and differentiation. This highlights the importance of optimizing growth regulator concentrations to strike a balance between inducing shoot proliferation and maintaining normal plant morphology. Moreover, the success of our study of using lower concentrations of BAP combined with NAA suggests a potential approach to improve the efficiency and quality of *Dendrobium* propagation without the abnormalities associated with higher concentrations.

The significant increase in orchid propagation observed in our study aligns with the findings of Pyati (2022), who demonstrated efficient in-vitro regeneration of *Dendrobium ovatum* through somatic embryogenesis. In their study, the use of specific PGRs such as NAA and BAP in combination with other PGRs was found to be crucial in optimizing embryogenic callus and somatic embryos. However, the precise concentration of said PGRs also played a crucial role in successful orchid propagation (Pyati, 2022).

The acclimatization phase involved transferring the fully developed plantlets to a greenhouse with 60% humidity. Different substrates, including coconut husk, wooden charcoal, and a mix of coconut husk and charcoal, were tested. Plantlets in coconut husk and the coconut husk + charcoal mixture demonstrated superior acclimatization compared to those in charcoal alone (Fig. 3ba). The higher moisture retention of the coconut husk substrates likely supported better growth, as plantlets from controlled environments are sensitive to moisture levels during acclimatization. The 60/40 light intensity ratio in the greenhouse was optimal, as higher sunlight intensity increased mortality rates (Kauth *et al*., 2008b; Larkin, 2024). The better acclimatization results were also observed by Seeja *et al*., (2018) with different potting mixtures.

Overall, plantlets potted in coconut husk and the coconut husk + charcoal mixture exhibited excellent growth and vibrant green coloration. In contrast, those in charcoal alone showed slower growth and reddish-green leaves, indicating suboptimal conditions. This study confirms that using suitable substrates and maintaining appropriate environmental conditions are crucial for the successful acclimatization and growth of *Dendrobium sonia* (Yuan *et al*., 2021).

This study demonstrated a successful mechanical pollination strategy and tissue culture practice that will provide a novel avenue for the conservation of endangered orchid species. These findings not only establish an efficient protocol for *Dendrobium sonia* propagation but also highlight the potential for commercial-scale orchid micropropagation in Pakistan, thereby contributing to the advancement of national horticultural and floricultural industries.

## Conclusion

This study successfully developed and validated a reliable protocol for the artificial pollination, asymbiotic germination, and acclimatization of *Dendrobium sonia*. Full strength of MS medium with BAP and NAA significantly enhanced germination percentage and seedling vigor. Coconut husk + wooden charcoal-based potting media promoted vigorous growth of plantlets with chlorophyll-rich foliage under semi-controlled conditions. As the first successful report from Pakistan, this work holds significant promise for advancing orchid propagation and supporting local floriculture industries, as well as a potential application in orchid conservation and commercial propagation programs.

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**Data Availability** The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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