https://doi.org/10.48048/tis.2022.3429

Interspecific and Intergeneric Hybrids of *Aerides* Species with *Rhynchostylis coelestis* Rchb.f. and Germination of Hybrid Seeds *In Vitro*

Nipawan Jitsopakul[1,*], Atcharaporn Chunthaworn[1], Urailuck Pongket[2] and Kanchit Thammasiri[3]

[1]*Department of Plant Science, Textile and Design, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan, Surin Campus, Surin 32000, Thailand*
[2]*Department of Science and Mathematics, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan, Surin Campus, Surin 32000, Thailand*
[3]*Department of Plant Science, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

([*]Corresponding author's e-mail: njitsopakul@hotmail.com)

Received: 24 August 2021, Revised: 7 November 2021, Accepted: 14 November 2021

Abstract

The beautiful Thai orchid species are *Aerides* and *Rhynchostylis coelestis* Rchb.f. that hybrids are valued for potted plants or cut flowers. Interspecific hybridization of *Aerides* species and intergeneric hybridization of *Aerides* species with *Rhynchodtylis coelestis* were investigated to observe their cross ability and the germination of hybrid seeds *in vitro*. The successful crosses were 4 interspecific hybrids including *A. multiflora* × *A. houlletiana*, *A. odoratum* × *A. multiflora*, *A. odoratum* × *A. houlletiana*, *A. falcata* × *A. houlletiana* (40 to 100 % pod formation) and 3 intergeneric hybrids including *A. multiflora* × *R. coelestis*, *A. houlletiana* × *R. coelestis* and *A. falcata* × *R. coelestis* (83 to 100 % pod formation). The age and size of mature pods of interspecific hybrids were 106 to 181 days with length of 1.40 to 2.37 cm and width of 0.70 to 1.23 cm and intergeneric hybrids were 93 to 165 days with length of 1.75 to 2.68 cm and width of 0.81 to 1.18 cm after pollination. Seeds of 4 interspecific and 3 intergeneric hybrids germinated into protocorms after sowing on VW (1949) agar medium for 26 to 84 days and 35 to 50 days, respectively. Hybrid plantlets with well-developed shoots and roots for 150 days after cultured on modified VW (1949) agar medium supplemented with 100 g L^{-1} banana, 150 mL L^{-1} coconut water, 20 g L^{-1} sucrose, 2 g L^{-1} activated charcoal, 7 g L^{-1} agar, and pH 5.2 at 25 ± 2 °C under light condition for 16 h day^{-1}. Hybrid plantlets were transplanted into greenhouse condition after 180 days showed 100 % survival and grew. The successful hybridization of *Aerides* hybrids can be applied for production new varieties of orchids.

Keywords: Orchid, Pollination, Pod, Protocorm, Plantlet

Introduction

The genus *Aerides*, one of the most beautiful Thai orchids consists of approximately 21 species in Southeast Asia [1] that flowers have variation in shapes, sizes and colors (**Figure 1**(**A**) **- 1**(**E**)). *Aerides* spp. or hybrids are valued for potted plants or cut flowers [2]. *Rhynchostylis coelestis* Rchb.f. is native to Thailand that the color of flowers is purple (**Figure 1**(**F**)) used for trades and making hybrids. The genus *Aerides* and *Rhynchostylis* show 2n = 38 chromosomes [3,4]. Hybridization and tissue culture are essential for conservation and propagation of wild orchid parents from deforestation, environmental change and orchid trades. In addition, the advantages of orchid hybrid production are easy to grow, free flowering, beautiful shape, beautiful color and longer shelf life [5]. The successful pollination depends on pollen viability and flower age that pollen plays an important role in hybridization and conservation of genetic resources [6]. The orchid seeds are minute and the absence of endosperm that development of embryo arrested at globular stage [7]. However, they can be germinated *in vitro* [8]. The common culture media used for tissue culture of interspecific and intergeneric hybrid orchids are Vacin and Went (VW) [9] medium [2,8] and Murashige and Skoog (MS) [10] medium [11,12]. There are reported on successful interspecific hybridization of some *Aerides* spp. including, *A. odorata* var. 'Yellow' × *A. quinquevulnera*

var. *calayana*, *A. odorata* × *A. quinquevulnera* var. *calayana*, *A. quinquevulnera* var. *calayana* × *A. odorata* and *A. flabellata* × *A. odorata* without intergeneric hybridization [11]. The purpose of this research is to study the ability to interspecific hybridization of *Aerides* spp. and intergeneric hybridization between *Aerides* spp. with *R. coelestis* for pod formation, seed germination on different media and transplantation of hybrid plants in the greenhouse.

Materials and methods

Plant materials

Hybridization of *Aerides* spp. was carried out in the greenhouses covered with 70 % shade cloth under natural environmental conditions with an average temperature of 26 - 33 °C at Rajamangala University of Technology Isan, Surin Campus, Surin province, Thailand in May, 2018 to December, 2018. About 5 species of *Aerides* spp. including *A. multiflora* Roxb. (**Figure 1**(**A**)), *A. houlletiana* Rchb.f. (**Figure 1**(**B**)), *A. odoratum* Lour. (**Figure 1**(**C**)), *A. falcata* Lindl. & Paxton (**Figure 1**(**D**)) and *A. crassifolia* C.S.P. Parish ex Burb. (**Figure 1**(**E**)) and 1 species of *Rhynchostylis coelestis* Rchb.f. (**Figure 1**(**F**)) were used to produce intraspecific, interspecific and intergeneric hybridization.

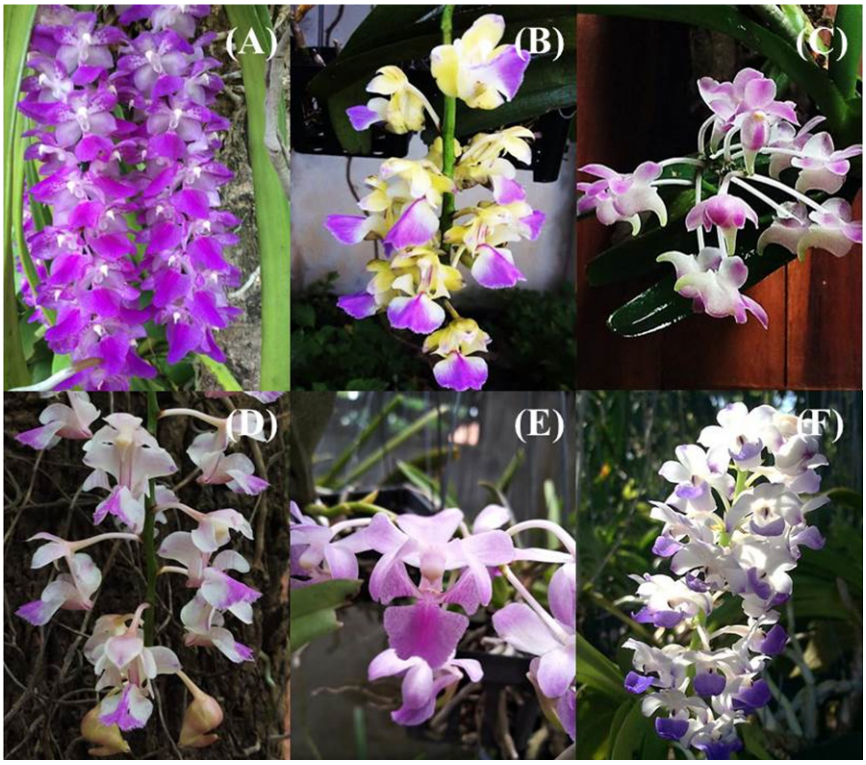

Figure 1 Flowers of parents for hybridization. *A. multiflora* Roxb. (A), *A. houlletiana* Rchb.f. (B), *A. odoratum* Lour. (C), *A. falcata* Lindl. & Paxton (D), *A. crassifolia* C.S.P. Parish ex Burb. (E) and *Rhynchostylis coelestis* Rchb.f (F).

Cross combination to produce interspecific and intergeneric hybrids

Flowering times of *Aerides* spp. and *R. coelestis* were observed in May, 2018. Hybridization was done at 8.00 - 11.00 am by hand-pollination to produce intraspecific, interspecific and intergeneric hybrids of *Aerides* spp. After 2 days of flower blooming, pollinia were removed from male flowers using toothpicks and pollinia from female parents were also removed from the blooms to prevent self-pollination and to be used for intraspecific hybridization (self-fertilization) and reciprocal crosses. Pollinia from male flowers were deposited on the stigma of female parents and then the pollinated flowers were labelled with white tags giving the name of female parent and male parent and date of pollination.

The total crosses to produce intraspecific hybrids were 6 including 5 species of *Aerides* spp. (*A. multiflora*, *A. houlletiana*, *A. odoratum*, *A. falcata* and *A. crassifolia*) and 1 species of *R coelestis*)

(**Figure 1**). Five flowers were used for self-pollination in each treatment. The total crosses to produce interspecific hybrids were 10 including *A. multiflora* × *A. houlletiana*, *A. multiflora* × *A. odoratum*, *A. houlletiana* × *A. odoratum*, *A. houlletiana* × *A. falcate*, their reciprocals, *A. falcata* × *A. multiflora* and *A. houlletiana* × *A. crassifolia*. The number of flowers were used for hand-pollination about 2 - 6 flowers in each pollination treatment (**Table 1**). The total crosses to produce intergeneric hybrids were 3 including 3 species of *Aerides* spp. such as *A. multiflora*, *A. houlletiana* and *A. falcata* as female parents and *R. coelestis* as male parent. Six, 9 and 15 flowers of *A. falcata*, *A. houlletiana* and *A. multiflora* were pollinated with *R. coelestis*, respectively.

After hybridization, the flowers had withered, pod formation was observed to confirm fertilization. Crossing potential was determined by percentage of pod formation at 30 days after hand-pollination. Mature pods of *Aerides* hybrids were observed when the pod color of the female plants changed from green to yellow. Mature pods were collected from the female plants, age of mature pods and pod size were recorded. Seeds were removed from mature pods and then were incubated in 0.6 % TTC solution for 24 h in the dark at 29 ± 2 °C and then embryo formation was monitored under a compound light source microscope at 40× magnification.

Table 1 Pod formation, age and size of mature pods of intraspecific hybrids, interspecific hybrids of *Aerides* spp. and intergeneric hybrids of *Aerides* spp. with *Rhynchostylis coelestis*.

No.	Female	Male	No. of flowers pollinated	Pod formation (%)	Age of mature pods (day)	Size of mature pods (cm) (length × width)
Intraspecific hybrids (Self-pollination)						
1	*A. multiflora*	*A. multiflora*	5	100^{a}	115 ± 1.4^{h}	$1.40 \pm 0.07^{l} \times 0.73 \pm 0.04^{g}$
2	*A. houlletiana*	*A. houlletiana*	5	100^{a}	112 ± 1.1^{i}	$2.30 \pm 0.19^{e} \times 0.93 \pm 0.33^{de}$
3	*A. odoratum*	*A. odoratum*	5	100^{a}	122 ± 4.8^{g}	$1.90 \pm 0.37^{fg} \times 1.00 \pm 0.12^{d}$
4	*A. falcata*	*A. falcata*	5	100^{a}	126 ± 25.3^{f}	$3.20 \pm 0.12^{a} \times 1.13 \pm 0.04^{b}$
5	*A. crassifolia*	*A. crassifolia*	5	100^{a}	151 ± 1.5^{d}	$3.00 \pm 0.42^{b} \times 1.20 \pm 0.07^{ab}$
6	*R. coelestis*	*R. coelestis*	5	100^{a}	71.3 ± 8.4^{l}	$3.23 \pm 0.11^{a} \times 1.03 \pm 0.08^{cd}$
Interspecific hybrids						
7	*A. multiflora*	*A. houlletiana*	5	60^{e}	181 ± 0.0^{b}	$1.87 \pm 0.05^{g} \times 0.70 \pm 0.00^{g}$
8	*A. houlletiana*	*A. multiflora*	4	100^{a}	121 ± 80^{g}	$1.55 \pm 0.09^{k} \times 1.00 \pm 0.19^{d}$
9	*A. multiflora*	*A. odoratum*	5	80^{c}	128 ± 53^{f}	$1.87 \pm 0.12^{g} \times 0.80 \pm 0.00^{f}$
10	*A. odoratum*	*A. multiflora*	2	100^{a}	122 ± 0.0^{g}	$1.40 \pm 0.10^{l} \times 0.85 \pm 0.05^{e}$
11	*A. houlletiana*	*A. odoratum*	2	100^{a}	201 ± 0.0^{a}	$1.50 \pm 0.00^{k} \times 1.15 \pm 0.05^{b}$
12	*A. odoratum*	*A. houlletiana*	5	40^{f}	106 ± 8.2j	$1.74 \pm 0.33^{j} \times 0.98 \pm 0.04d^{e}$
13	*A. houlletiana*	*A. falcata*	3	100^{a}	41 ± 0.0^{m}	$1.80 \pm 0.22^{h} \times 0.80 \pm 0.08^{f}$
14	*A. falcata*	*A. houlletiana*	3	100^{a}	127 ± 30^{f}	$2.37 \pm 0.26^{d} \times 1.23 \pm 0.17^{a}$
15	*A. falcata*	*A. multiflora*	6	100^{a}	113 ± 47.2^{i}	$1.93 \pm 0.21^{f} \times 1.05 \pm 0.14^{c}$
16	*A. houlletiana*	*A. crassifolia*	3	0^{g}	0^{n}	0^{m}
Intergeneric hybrids						
17	*A. multiflora*	*R. coelestis*	15	93.33^{b}	165 ± 16.5^{c}	$1.80 \pm 0.13^{h} \times 0.81 \pm 0.10^{e}$
18	*A. houlletiana*	*R. coelestis*	9	100^{a}	93 ± 37.3^{k}	$1.75 \pm 0.34^{hi} \times 0.91 \pm 0.26^{de}$
19	*A. falcata*	*R. coelestis*	6	83.33^{c}	132 ± 26.4^{e}	$2.68 \pm 0.39^{c} \times 1.18 \pm 0.15^{ab}$

Note: Mature pods were collected from the female plants when the pod color changed from green to yellow. Values represent means ± SD. In each column, similar letters mean no significant difference at $p < 0.05$ by DMRT test.

***In vitro* germination of *Aerides* hybrid seeds and seedling growth**

Mature pods of interspecific and intergeneric hybrids were collected from the female plants and then were cleaned by getting rid of dead tissues and washed with detergent followed by running tap water for 5 min. Mature pods were soaked in 95 % ethanol for 1 min inside a laminar air- flow cabinet and then flamed with a lamp until the flame stop. Mature pods were dissected longitudinal with a sterile surgical blade and then seeds were removed. The seeds were sown on MS agar medium [8] and modified VW agar

medium [9] supplemented with varying concentration of gibberellin at 0, 10, 20 and 30 mg L^{-1}, 150 mL L^{-1} coconut water, 20 g L^{-1} sucrose at pH 5.2. They were cultured at 25 ± 2 °C with illumination (c. 37 μmol m^{-2} s^{-1}) provided by fluorescent tubes for 16 h day^{-1}. Day of hybrid seeds germinated into protocorms at stage 3 by the green pointed shoot-like structure appearance of promeristem (stage 3) [13] was observed and media for hybrid seed germination were recorded. Protocorms at stage 3 were transferred and then cultured on modified VW agar medium [9] supplemented with 100 g L^{-1} banana, 150 mL L^{-1} coconut water, 20 g L^{-1} sucrose, 2 g L^{-1} activated charcoal, 7 g L^{-1} agar and pH 5.2 at 25 ± 2 °C with illumination (c. 37 μmol m^{-2} s^{-1}) provided by fluorescent tubes for 16 h day^{-1} to develop into plantlets.

Morphological characteristic of *Aerides* hybrid plantlets

The *Aerides* hybrid plantlets with well-developed shoots and roots cultured on modified VW agar medium [9] for 150 days were removed from bottles and then washed with water to remove the culture medium. Twenty plantlets were transplanted into 3-inch plastic pots that carried out in the greenhouse condition covered with 70 % shade cloth under natural environmental conditions with an average temperature of 26 - 33 °C. A total of hundred plantlets per hybrid were used for each treatment. After 180 days, survival of hybrid plantlets were recorded and morphological characteristics of *Aerides* hybrid plantlets including leaf size, leaf numbers, and plantlet height were examined.

Statistical analysis

Data of pod formation, age and size of mature pod, day of hybrid seed germination into protocorms at stage 3 and morphological characteristics of *Aerides* hybrid plantlets were analyzed using ANOVA and significant differences were evaluated by Duncan's Multiple Range Test (DMRT).

Results and discussion

Cross combination to produce interspecific and intergeneric hybrids of *Aerides* spp.

The total of 6 cross intraspecific hybrids including 5 species of *Aerides* spp. (*A. multiflora*, *A. houlletiana*, *A. odoratum*, *A. falcata* and *A. crassifolia*) and 1 species of *R. coelestis* and 10 crosses to produce interspecific hybrids of *Aerides* spp. including *A. multiflora* × *A. houlletiana*, *A. multiflora* × *A. odoratum*, *A. houlletiana* × *A. odoratum*, *A. houlletiana* × *A. falcata*, their reciprocals, *A. falcata* × *A. multiflora* and *A. houlletiana* × *A. crassifolia* by hand-pollination was studied and examined (**Table 1**). Results showed that no significant differences in pod formation of intraspecific pollination (100 %) were observed at 30 days after hand-pollination. Pod formation of intraspecific and interspecific pollination was significantly different. The successful pollination depended on pollinia size and flower age [11] and pod formation after hand-pollination provides a measure of success reproduction in orchid breeding [14]. Pod formation of interspecific pollination (40 to 100 %) was observed for 9 crosses except the crosses between *A. houlletiana* × *A. crassifolia* without pod formation after pollination. The highest pod formation (100 %) was obtained in interspecific pollination between *A. houlletiana* × *A. odoratum*, *A. houlletiana* × *A. multiflora* and *A. odoratum* × *A. multiflora*, while their reciprocal cross produced pod formation about 40, 60 and 80 %, respectively. The lower percentage of pod formation after pollination may be due to intergeneric incompatibilities, experimental mishandling and selective abortion [15], flower age of male and female parents and unhealthy state of the off-season flowers [2].

The duration time of pod developed into mature pods depended on the crosses (**Table 1**). Average age and size of mature pods from different pollination types was significantly different. Results showed that the age and the size of mature pods of *Aerides* spp. were 112 to 151 days after pollination with length of 1.40 to 3.20 cm and width of 0.73 to 1.20 cm. The age and size of *Aerides* hybrid mature pods were varied with female parent plants. The age and the size of mature pods of 9 *Aerides* hybrids were 41 to 201 days with length of 1.40 to 2.37 cm and width of 0.70 to 1.23 cm. Using *A. houlletiana* as female parent for interspecific pollination with *A. odoratum* and *A. falcata* found 100 % pod formation but without seeds formation and pods of *A. houlletiana* × *A. multiflora* produced seeds but seeds did not germinated in all media *in vitro*. The success of pod formation is not truly in the compatibility after hybridization, but getting viable progeny development is important [16]. The failure of hand-pollination has been explained as a female sterility barrier [17] that caused by disrupted ovule development and also can be due to pollen sterility [18] that causes of low fertility may be associated with abnormal meiosis in orchid hybrids. In addition, it is possible that male sterility or low fertility is related to the malfunction of anther tissue [19].

The successful crosses for producing intergeneric hybrids after hand-pollination were observed on pod formation in 3 hybrids about 93 to 100 %. Pod formation of crosses between *A. houlletiana* × *R.*

coelestis was 100 %, followed by *A. multiflora* × *R. coelestis*and and *A. falcata* × *R. coelestis* at 93 and 83 %, respectively. Age and size of mature pods of intergeneric hybrids about 93 to 165 days with length of 1.75 to 2.68 cm and width of 0.81 to 1.18 cm. Hybrid pods produced seeds and observed viable of embryos under a light microscope at 40× magnification after incubation with 0.6 % TTC solution (**Figure 2**). The selection of female parent could be an important factor for success in intergeneric crosses [20]. Using *A. falcata* as female parent for interspecific and intergeneric hybridization gave the largest mature pods that produced a large number of seeds within the pod.

***In vitro* germination of *Aerides* hybrid seeds and seedling growth**

Mature pods of *Aerides* hybrids were collected from the female plants when the pod color changed from green to yellow for seed germination *in vitro* (**Table 1** and **Figure 2**). Mature seeds were removed from mature pods and then sown on MS agar medium [10] and modified VW agar medium [9] supplemented with varying concentration of gibberellin at 0, 10, 20 and 30 mg L^{-1}, 150 mL L^{-1} coconut water, 20 g L^{-1} sucrose at pH 5.2. They were cultured at 25 ± 2 °C with illumination (c. 37 μmol m^{-2} s^{-1}) provided by fluorescent tubes for 16 h day^{-1}. Both media of MS [10] and VW [9] have different concentrations of macronutrient and micronutrients, vitamins, minerals, as well as sucrose concentrations. Composition of VW medium has lower amounts of ingredients than MS medium. Mature seeds of *A. multiflora* × *A. houlletiana*, *A. odoratum* × *A. multiflora*, *A. odoratum* × *A. houlletiana*, *A. falcata* × *A. houlletiana*, *A. multiflora* × *R. coelestis*, *A. houlletiana* × *R. coelestis* and *A. falcata* × *R. coelestis* germinated into stage 3 of protocorms by the green pointed shoot-like structure appearance of promeristem in different media, except seeds of *A. houlletiana* × *A. multiflora*, *A. multiflora* × *A. odoratum* and *A. falcata* × *A. multiflora* did not germinate in all media. VW medium [9] supplemented with 150 mL L^{-1} coconut water can stimulate seed germination of *Aerides* mature hybrid seeds faster than MS medium [10] because VW medium contains coconut water that it beneficially affected germination of orchid seeds and development of plantlets [2,21-24] and phosphate-rich VW medium has been linked to higher seed germination of *Aerides* hybrid seeds than MS medium. Seeds of *A. multiflora* × *A. houlletiana* were the fastest germination into protocorms at stage 3 by the green pointed shoot-like structure appearance of promeristem after 26 days of sowing on VW agar medium [9]. However, MS agar medium [10] can stimulate seed germination of *A. falcata* × *A. houlletiana* and *A. falcate* × *R. coelestis* as fast as VW medium [9]. Germination of some hybrid seeds on VW medium without plant growth regulators might be sufficient endogenous growth hormones required for the initial stages of seed germination [2] that an endogenous or exogenous supply of growth regulators is essential for lipid mobilization during germination of orchid seeds [25]. However, seeds of *A. odoratum* × *A. houlletiana* and *A. multiflora* × *R. coelestis* germinated when sowed on modified VW agar medium [9] supplemented with gibberellin at 10, 30 and 40 mg L^{-1}. Gibberellin is an important plant growth regulator added to the medium to improve the rate of seed germination of orchids [22] because orchid seeds lack endosperm thus seed germination and seedling development have been found to be affected by internal and external factor such as seed maturity, cultivation conditions and media composition [26].

Protocorms by the green pointed shoot-like structure appearance of promeristem were transferred to modified VW agar medium [9] supplemented with 100 g L^{-1} banana, 150 mL L^{-1} coconut water, 20 g L^{-1} sucrose, 2 g L^{-1}activated charcoal, 7 g L^{-1} agar and pH 5.2 at 25 ± 2 °C with illumination (c. 37 μmol m^{-2} s^{-1}) provided by fluorescent tubes for 16 h day^{-1} after 150 days developed shoots and roots. VW medium was added activated charcoal for adsorption all toxic compounds released by explants [27] and induced of a high number of buds and roots in seedlings of the orchid hybrid [28]. VW medium for sowing of hybrid seeds without activated charcoal and banana because they inhibited during seed germination but benefited only for further development of seedlings [29].

Morphological characteristic of *Aerides* hybrid plantlets

Plantlets of 4 interspecific and 3 intergeneric hybrids of *Aerides* with well-developed shoots and roots cultured on modified VW agar medium [9] for 150 days were removed from bottles and then washed with water to remove the culture medium. They were transplanted into plastic pots in the greenhouse condition showed 100 % survival after 180 days (**Figure 2**). The high survival of hybrid plantlets in normal condition was observed because of the acclimatization of a high numbers of plantlets in 3-inch pot rather than on plantlet individual in each pot. There were significant difference in leaf length, leaf width and plant height of interspecific and intergeneric hybrid plantlets (**Table 3**). The interspecific hybrid plantlets gave the highest leaf length about 7.7 cm was *A. multiflora* × *A. houlletiana*, followed by *A. odoratum* × *A. multiflora* and *A. falcata* × *A. houlletiana*, respectively. The intergeneric hybrid plantlets gave the highest leaf length was *A. multiflora* × *R. coelestis*, followed by *A. falcata* × *R.*

coelestis and *A. houlletiana* × *R. coelestis*, respectively (**Table 3**). The widest leaf of hybrid plantlets was *A. odoratum* × *A. houlletiana*, followed by *A. odoratum* × *A. multiflora* and *A. houlletiana* × *R. coelestis*, respectively. The highest of hybrid plantlets was *A. odoratum* × *A. houlletiana*, followed *A. odoratum* × *A. multiflora* and *A. falcata* × *A. houlletiana*, respectively. The morphological features of plants derived from reciprocal cross are similar to direct cross [30]. Plantlets of intergeneric hybrids were intermediated to the parents in quantitative characteristics, such as leaf size and width. Plantlets of interspecific hybrids, their reciprocal crosses and intergeneric hybrids were evenly matched to their parental species [31,32].

Figure 2 Mature pods, seeds and plantlets of interspecific and intergeneric hybrids of *Aerides* spp.: *A. multiflora* × *A. houlletiana* (A), *A.odoratum* × *A. multiflora* (B), *A. odoratum* × *A. houlletiana* (C), *A. falcata* × *A. houlletiana* (D), *A. multiflora* × *R. coelestis* (E), *A. houlletiana* × *R. coelestis* (F) and *A. falcata* × *R. coelestis* (G).

Table 2 Effect of media on *Aerides* hybrid seed germination *in vitro* at 25 ± 2 °C under light condition for 16 h day^{-1}.

No.	Female	Male	Seed germination into protocorms (d)	Media for hybrid seed germination
1	*A. multiflora*	*A. houlletiana*	26^{f}	VW
2	*A. houlletiana*	*A. multiflora*	0^{g}	-
3	*A. multiflora*	*A. odoratum*	0^{g}	-
4	*A. odoratum*	*A. multiflora*	81^{b}	VW
5	*A. houlletiana*	*A. odoratum*	0^{g}	-
6	*A. odoratum*	*A. houlletiana*	84^{a}	VW + 30 and 40 mg L^{-1} GA_3
7	*A. houlletiana*	*A. falcata*	0^{g}	-
8	*A. falcata*	*A. houlletiana*	34^{e}	MS, VW and VW+10 mg L^{-1} GA_3
9	*A. falcata*	*A. multiflora*	0^{g}	-
10	*A. multiflora*	*R. coelestis*	50^{c}	VW + 10 and 30 mg L^{-1} GA_3
11	*A. houlletiana*	*R. coelestis*	38^{d}	VW
12	*A. falcata*	*R. coelestis*	35^{e}	MS and VW

Note: Seed germination was observed at stage 3 of protocorms by the green pointed shoot-like structure appearance of promeristem. Values represent means ± SD. In each column, similar letters mean no significant difference at $p < 0.05$ by DMRT test.

Table 3 Morphological characteristics of *Aerides* hybrid plantlets after growing in the greenhouse for 180 days.

No.	Female	Male	Leaf length (cm)	Leaf width (cm)	Plant height (cm)
Interspecific hybrids					
1	*A. multiflora*	*A. houlletiana*	7.70 ± 0.07^{c}	0.33 ± 0.04^{e}	1.13 ± 0.04^{d}
2	*A. odoratum*	*A. multiflora*	6.50 ± 0.42^{d}	1.00 ± 0.19^{b}	1.50 ± 0.12^{b}
3	*A. odoratum*	*A. houlletiana*	5.47 ± 0.18^{e}	1.20 ± 0.26^{a}	2.20 ± 0.26^{a}
4	*A. falcata*	*A. houlletiana*	5.87 ± 0.33^{e}	0.77 ± 0.04^{d}	1.30 ± 0.19^{c}
Intergeneric hybrids					
5	*A. multiflora*	*R. coelestis*	11.3 ± 0.26^{a}	0.33 ± 0.04^{e}	0.67 ± 0.04^{f}
6	*A. houlletiana*	*R. coelestis*	4.50 ± 0.07^{g}	0.80 ± 0.07^{c}	0.90 ± 0.12^{e}
7	*A. falcata*	*R. coelestis*	8.00 ± 0.25^{b}	0.77 ± 0.08^{d}	1.10 ± 0.12^{d}

Note: Values represent means ± SD. In each column, similar letters mean no significant difference at $p < 0.05$ by DMRT test.

Conclusions

In this study, the ability to interspecific hybridization of *Aerides* spp. and intergeneric hybridization between *Aerides* spp. with *R. coelestis* were successful for pod formation, seed germination and developed into plantlets. Development of an efficient method can be applied for hybridization and tissue culture of hybrid seeds to produce new outstanding cultivars and for conservation of Thai orchids.

Acknowledgements

We gratefully acknowledge Rajamangala University of Technology Isan Surin Campus, Thailand for supporting and providing fund to carry out the study.

References

[1] A Kocyan, EF Vogel, E Conti and B Gravendeel. Molecular phylogeny of *Aerides* (Orchidaceae) based on one nuclear and two plastid markers: A step forward in understanding the evolution of the Aeridinae. *Mol. Phylogenet. Evol*. 2008; **48**, 422-43.

[2] R Kishor and GJ Sharma. Intergeneric hybrid in two rare and endangered orchids, *Renanthera imschootiana* Rolfe and *Vanda coerulea* Griff. ex Linn. (Orchidaceae): Synthesis and characterization. *Euphytica* 2008; **165**, 247-56.

[3] H Sau and AK Sharma. Chromosome evolution and affinity of certain genera of Orchidaceae. *Cytologia* 1983; **48**, 363-72.

[4] WE Jones, AR Kuehnle and K Arumuganathan. Nuclear DNA content of 26 orchid (Orchidaceae) genera with emphasis on *Dendrobium*. *Ann. Bot*. 1998; **82**, 189-94.

[5] R Kishor, PSSV Khan and GJ Sharma. Hybridization and *in vitro* culture of *Ascocenda* 'Kangla'. *Sci. Hortic*. 2006; **108**, 66-73.

[6] EJ Sacks and DAS Clair. Cryogenic storage of tomato pollen: Effect on fecundity. *Hortscience* 1996; **31**, 447-8.

[7] J Arditii. Factors affecting the germination of orchid seeds. *Bot. Rev*. 1967; **33**, 1-97.

[8] L Knudson. Non-symbiotic germination of orchid seeds. *Bot. Gaz*. 1922; **73**, 1-25.

[9] EF Vacin and FW Went. Some pH changes in nutrient solution. *Bot. Gaz*. 1949; **110**, 605-13.

[10] T Murashige and F Skoog. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*. 1962; **15**, 473-97.

[11] C Sivanaswari, LA Thohirah, AA Fadelah and NAP Abdullah. Hybridization of several *Aerides* species and *in vitro* germination of its hybrid. *Afr. J. Biotechnol*. 2011; **10**, 10864-70.

[12] U Thakur and N Dongarwar. Artificial pollination and *in vitro* asymbiotic seed germination in garden orchid *Spathoglottis plicata* Blume (Orchidaceae). *Recent Res. Sci. Technol*. 2012; **4**, 13-8.

[13] MC Pereira, DI Rocha, T Gomes, OL Pereira, DMT Francino, RMSA Meira and MCM Kasuya. Characterization of seed germination and protocorm development of *Cyrtopodium glutiniferum* (Orchidaceae) promoted by mycorrhizal fungi *Epulorhiza* spp. *Acta Bot. Bras*. 2015; **29**, 567-74.

[14] HC Proctor. Effect of pollen age on fruit set, fruit weight and seed set in three orchid species. *Can. J. Bot*. 1998; **76**, 420-7.

[15] YJ Shiau, AP Sagare, UC Chen, SR Yang and HS Tsay. Conservation of *Anoectochilus formosanus* Hayata by artificial cross pollination and *in vitro* culture of seeds. *Bot. Bull. Acad. Sin.* 2002; **43**, 123-30.

[16] GJ Wilfret and H Kamemoto. Genome and karyotype relationships in the genus *Dendrobium* (Orchidaceae) II. Karotype relationships. *Cytologia* 1969; **36**, 604-13.

[17] MNS Stort. *Sterility barriers in some artificial F1 orchid hybrids: Female sterility*. Revista Brasileira de Genetica. 1987, p. 109.

[18] MNS Stort. Sterility barriers of some artificial F1 orchid hybrids: Male sterility. 1. Microsporogenesis and pollen germination. *Amer. J. Bot*. 1984; **71**, 309-18.

[19] CJ Hu, N Lee and YI Lee. Meiotic defects and premature tapetal degeneration are involved in the low fertility of *Oncidesa* Gower Ramsey, an important cut-flower orchid. *Hortscience* 2018; **53**, 1283-7.

[20] DG Kim, KK Kim and CG Been. Development of Intergeneric hybrids between Wind orchids (*Sedirea japonica* and *Neofinetia falcata*) and Moth orchids (*Phalaenopsis alliances*). *Hort. Environ. Biotechnol*. 2015; **56**, 67-78.

[21] ESW Utami and S Hariyanto. The effect of organic nutrient and growth regulators on seed germination, embryo and shoots development of *Dendrobium antennatum* Lindl. orchid by *in vitro*. *Biosaintifika* 2016; **8**, 165-71.

[22] M Zahara, A Datta, P Boonkorkaew and A Mishra. The effects of different media, sucrose concentrations and natural additives on plantlet growth of *Phalaenopsis* hybrid 'Pink'. *Braz. Arch. Biol. Technol*. 2017; **60**, e17160149.

[23] H Kang, KW Kang, DH Kim and I Sivanesan. *In vitro* propagation of *Gastrochilus matsuran* (Makino) Schltr., an Endangered Epiphytic Orchid. *Plants* 2020; **9**, 524.

[24] B Kunakhonnuruk, P Inthima and A Kongbangkerd. *In vitro* propagation of *Epipactis flava* Seidenf. an endangered rheophytic orchid: A first study on factors affecting asymbiotic seed germination, seedling development and greenhouse acclimatization. *Plant Cell Tissue Organ Cult*. 2018; **135**, 419-32.

[25] SF Lo, SM Nalawade, CL Kuo, CL Chen and HS Tsay. Asymbiotic germination of immature seeds, plantlet development and *ex vitro* establishment of plants of *Dendrobium tosaense* Makino: A medicinally important orchid. *In Vitro Cell. Dev. Biol. Plant* 2004; **40**, 528-35.

[26] MM Hossain, M Sharma and P Pathak. *In vitro* propagation of *Dendrobium aphyllum* (Orchidaceae)-seed germination to flowering. *J. Plant Biochem. Biotechnol*. 2013; **22**, 157-67.

[27] DH Kim, KW Kang, G Enkhtaivan, U Jan and I Sivanesan. Impact of activated charcoal, culture medium strength and thidiazuron on non-symbiotic *in vitro* seed germination of *Pecteilis radiata* (Thunb.) Raf. *S. Afr. J. Bot*. 2019; **124**, 144-50.

[28] EC Prizao, LDM Goncalves, MAM Gutierre, CA Mangolin and MDFPDS Machado. Activated charcoal and graphite for the micropropagation of *Cattleya bicolor* Lindl. and a orchid double hybrid 'BLC Pastoral Innocence'. *Acta Sci. Agron*. 2012; **34**, 157-61.

[29] RLM Pierik, PA Sprenkels, BVD Harst and QGVD Meys. Seed germination and further development of *Paphiopedilum ciliolare* Pfitz. *in vitro*. *Sci. Hortic.* 1988; **34**, 139-53.

[30] P Sinumporn, T Narumi-Kawasaki and S Fukai. Development of interspecific hybrids between *Habenaria radiata* and *Habenaria rhodocheila* complex. *Adv. Hortic. Sci*. 2020; **34**, 3-10.

[31] T Punjansing, M Nakkuntod, S Homchan, P Inthima and A Kongbangkerd. Production and molecular identification of interspecific hybrids between *Phaius mishmensis* (Lindl. and Paxton) Rchb. f. and *Phaius tankervilliae* (Banks) Blume. *Agriculture* 2021; **11**, 306.

[32] R Devadas, RK Pamarthi, AL Meitei, SL Pattanayak and R Sherpa. Morphological description of novel *Phaius* primary hybrid (Orchidaceae). *J. Exp. Biol. Agric. Sci*. 2019; **7**, 138-47.