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MORPHOLOGICAL DESCRIPTION OF NOVEL PHAIUS PRIMARY HYBRID (ORCHIDACEAE)

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ABSTRACT

Popularly known nun orchid or swamp orchid (*Phaius sps.*) is listed as endangered species under Schedule-I of Indian Wild Life (Protection) Act, 1972. Natural *Phaius* hybrids were not reported and present study is an attempt to develop a new *Phaius* species by using two native species *viz.*, *Phaius tankervilleae* (Banks ex l'Heritier) Bl and *Phaius flavus* (Blume) Lindl. Result of present study suggests crossability and compatibility between these two species which helped in synthesize novel hybrid species. New *Phaius* hybrid produced flowers with dominating yellow-orange colour (RHS 17B/A) sepals and petals are bright crimson colour (RHS 175B) with elongated stripes on lip inside. First flowering of *Phaius* cross progenies (PBX-11-22) was observed in Jan-Mar, 2017 with regular flowering habit in consecutive seasons. Comparison with parents indicated distinct nature in floral attributes. Unlike *Phaius flavus*, the sepals and petals of novel progenies have spreading nature. Flower size varies from 7-8 x 8.5-9 cm with mild fragrance on shiny and warm days. Sequential flowering in acropetal fashion with 8-10 florets observed on moderately strong spike with potted vase life > 40 days.

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1 Introduction

Genus Phaius was established by Jao de Loureiro 1790 in Flora Cochinchinensis and comprises more than 30 species around tropical parts of Old world from Asia, Australia, Africa and various islands of Pacific and Indian oceans (Loureiro, 1790; Lucksom, 2007; Chowdhery & Agrawala, 2013). *Phaius tankervilleae* was introduced in England in 1778 from China by John Fothergill. With course of time, these species were naturalized by introduction in South America, Caribban Islands; Central America (Panama) and was listed in catalogue of plant species at Royal Botanical Gardens, Kew (Desmond, 1995) and The European Garden Flora (Cullen et al., 2011). In India, few species are naturally available in North-Eastern states and legally categorized as endangered; and it was protected under Schedule-I of Indian Wild Life (Protection) Act, 1972 and has serious trade restrictions on international commercial export selling's under Appendix-II of international conventions (Bhuyan, 2010; CITES, 2016). Efforts were also initiated for conservation through long-term seed storage (Hirano et al., 2009) and mass multiplication (Malemnganba et al., 1994). Both *in-situ* and *ex-situ* conservation programmes of *P. tankervilleae* were initiated in India (Kanwal, 2014). Presence of *P. tankervilleae* from other Indian states like Uttarakhand from Corbett National Park, North-Western Himalaya (Naithani et al., 2009) and Southern India (George & Mathew, 2016) indicates spatial distribution in India. *P. flavus* (Blume) Lindl., geographically located in Assam, Eastern Himalayas and *P. mishmensis* (Lindl. & Paxton) Rchb., and *P. nanus* Hook.f., are the other common *Phaius* species available in India (Pearce & Cribb, 2002).

Phaius orchids are considered as highly decorative and have great ornamental value with long inflorescences, bigger size flowers, more florets & different colours with evergreen long broad elliptic pleated leaves suitable indoor cultivation; otherwise often treated as invasive orchids (Ackerman, 2012). Even it has medicinal application in traditional medicine and used in natural dye indigo preparations in Arunachal Pradesh (Kanwal, 2014). The predominant brownish-blue hue of flowers especially when old or damaged may indicate the presence of rich chemicals (Pearce & Cribb, 2002).

Generally, *P. tankervilleae* is very commonly cultivated than other species (Schuiteman & de Vogel, 2000). However, the commercial varieties of *Phaius* are unavailable for domestic cultivation and not registered under 'Protection of Plant Varieties and Farmers Rights Act, 2002' for Plant Breeder's rights in India. At international level, various experiments were conducted at large scale to develop novel Grex/hybrids (https://www.orchids.org/grexes/phaius-magniflorus); but only few hybrids are available within closed circuits by amateur growers, which are not yet legally protected nor notified by Plant Variety Protection (PVP) of TRIPS agreement or similar governing laws enacted under Patent Cooperation Treaty (http://www.aos.org/orchids/orchids-a-to-z/letter-p/phaius.aspx).

Further, the occurrence of natural hybrids involving wild species was not reported so far in genus *Phaius*. Nototribic mode of pollination assisted by insects was found in *Phaius tankervilleae* (Banks ex L'Herit) Bl (Buragohain et al., 2016). However, auto self pollination was absent due to obstructive floral structures, although genetic barriers were never reported (Bhuyan, 2010). Royal Horticultural Society (RHS) data base reveals only one hybrid 'Ashworthianus' obtained from *P. flavus* (seed parent) and *P. wallichii* (male parent) in 1893 by Sanders (St Albans). So far, no hybrid registered with *P. flavus* and *P. tankervilleae* as parents for both direct and reciprocal crosses (http://apps.rhs.org.uk/horticulturaldatabase/orchidregister/orchidregister.asp). Development of hybrids using rare and endangered species for commercial sales will reduce the threatening pressure on their wild species (Kishor & Sharma, 2009). At gobal level, there are no trade restrictions on hybrids derived from Orchid species for import and exports as per *'Convention on International Trade in Endangered of Wild Flora and Fauna'* (CITES, 2004; Devadas et al., 2010). Hence, in present study an attempt has been made to develop new interspecific hybrid using native indigenous species available and novel progeny generated characterized at flowering stage to assess the taxonomic and breeding value for further genetic improvement.

2 Materials and methods

2.1 Mother plants

Phaius species viz., *P. tankervilleae* (Banks ex l'Heritier) Bl., and *P. flavus* (Bl.) Lindl. were used as parent material in the present studies, these were catalogued species accessions from the 'National Active Germplasm Site' (NAGS) collections at ICAR-NRC on Orchids, Pakyong, Sikkim.

2.2 Hybridization programme

A series of crossing programme was carried out with *Phaius* species and other orchid species from 2005 to 2014 to assess the cross compatibility nature, with long term objective to generate novel inter-specific and inter-generic hybrids for Orchid genetic improvement. Based on flowering synchrony, *Phaius* species were crossed with other species and hybrids from other genera for several years viz., *Paplionanthe, Calanthe, Dendrobium, Dienia, Coelogyne, Phalaenopsis, Lycaste, Thunia, Cymbidium, Arundina, Paphiopedilum, Vanda, Coelogyne and Vanda* group (Table 1).

Table 1 List of crosses attempted in Phaius with other Orchids and pod set status

S. no	*Cross code*	*Female parent*	*Male parent*	*Pod set*
1	PBS-05-271, 272	*P. tankervilleae*	(selfing)	√
2	PBS-05-273, 274	*Paplionanthe teres*	*P. tankervilleae*	x
3	PBX-05-279, 280	*P. tankervilleae*	*Calanthe masuca*	x
4	PBX-05-289	*P. tankervilleae*	*D. moschatum*	x
5	PBX-05-290	*Calanthe masuca*	*P. tankervilleae*	√/x
6	PBX-05-328	*P. tankervilleae*	*Dienia ophrydis*	x
7	PBX-05-1009 to 1011	*D. aphyllum*	*P. flavus*	x
8	PBX-05-1012 to 1014	*P. flavus*	*D. aphyllum*	x
9	PBX-05-1015 to 1016	*D. nobile*	*P. flavus*	x
10	PBX-05-1017 to 1018	*P. flavus*	*D. nobile*	x
11	PBX-05-1019	*P. flavus*	(selfing)	x
12	PBX-05-1024 to 1025	*Coelogyne flaccida*	*P. flavus*	x
13	PBX-06-20 to 21	*P. flavus*	*Phalaenopsis lobbii*	x
14	PBX-06-22 to 23	*Phalaenopsis lobbii*	*P. flavus*	x
15	PBX-06-24	*P. flavus*	*D. nobile*	x
16	PBX-06-25	*D. nobile*	*P. flavus*	x
17	PBX-06-59	*P. flavus*	*Lycaste crueata*	x
18	PBX-06-60	*Lycaste crueata*	*P. flavus*	x
19	PBX-06-78	*Lycaste crueata*	*P. tankervilleae*	√/x
20	PBX-06-79	*P. tankervilleae*	*Lycaste crueata*	x
21	PBX-06-84	*P. flavus*	*Lycaste crueata*	x
22	PBX-06-85	*Lycaste crueata*	*P. flavus*	x
23	PBS-06-129 to 132	*P. tankervilleae*	(selfing)	√
24	PBX-06-143	*C.* 'Sheragreen'	*P. tankervilleae*	x
25	PBX-06-144	*P. tankervilleae*	*C.* 'Sheragreen'	x
26	PBX-06-307	*Calanthe triplicata*	*P. tankervilleae*	x
27	PBX-06-308	*P. tankervilleae*	*Calanthe triplicata*	√/x
28	PBX-06-339	*P. tankervilleae*	*Thunia marshalliana*	x
29	PBX-06-340	*Thunia marshalliana*	*P. tankervilleae*	x
30	PBX-06-376	*C. aloifolium*	*P. tankervilleae*	x
31	PBX-06-377	*P. tankervilleae*	*C. aloifolium*	x
32	PBX-06-411 to 412	*P. tankervilleae*	(selfing)	√
33	PBX-06-465 to 466	*P. tankervilleae*	*Arundina graminifolia*	x
34	PBS-06-473	*P. tankervilleae*	(selfing)	√
35	PBX-07-21	*Lycaste crueata*	*P. tankervilleae*	X
36	PBX-07-22	*P. tankervilleae*	*Lycaste crueata*	x
37	PBX-09-01	*P. tankervilleae*	*Lycaste crueata*	X
38	PBX-09-02	*Lycaste crueata*	*P. tankervilleae*	x

Table 1 List of crosses attempted in Phaius with other Orchids and pod set status

S. no	*Cross code*	*Female parent*	*Male parent*	*Pod set*
39	PBX-09-03	*P. tankervilleae*	*P. teres*	√/x
40	PBX-09-04	*P. teres*	*P. tankervilleae*	x
41	PBX-11-21, 22, 23	*P. tankervilleae*	*P. flavus*	√
42	PBX-11-24, 25, 26	*P. flavus*	*P. tankervilleae*	√
43	PBX-13-144	*Lycaste crueata*	*P. tankervilleae*	x
44	PBX-13-145	*P. tankervilleae*	*Lycaste crueata*	x
45	PBSx-13-146, PBSx-14-208	*P. tankervilleae*	(natural selfing)	√
46	PBX-14-255	*C.* 'Soul Hunt-I'	*P. tankervilleae*	x
47	PBX-14-256	*P. tankervilleae*	*C.* 'Soul Hunt-I'	x
48	PBX-14-257	*C.* 'PCMV'	*P. tankervilleae*	x
49	PBX-14-258	*P. tankervilleae*	*C.* 'PCMV'	x
50	PBX-14-259	*C.* Unknown hybrid	*P. tankervilleae*	x
51	PBX-14-260	*P. tankervilleae*	*C.* Unknown hybrid	x
52	PBX-14-261	*C.* 'Fire Storm Blaze'	*P. tankervilleae*	x
53	PBX-14-262	*P. tankervilleae*	*C.* 'Fire Storm Blaze'	x
54	PBX-14-263	*C.* 'Caripepper Peach Keen'	*P. tankervilleae*	x
55	PBX-14-264	*P. tankervilleae*	*C.* 'Caripepper Peach Keen'	x
56	PBX-14-265	*P. villosum* (#2410)	*P. tankervilleae*	√/x
57	PBX-14-266	*P. tankervilleae*	*P. villosum* (#2410)	x
58	PBX-14-267	*V.* 'Berniece Miller'	*P. tankervilleae*	x
59	PBX-14-268	*P. tankervilleae*	*V.* 'Berniece Miller'	x
60	PBX-14-269	*D.* 'Emma White'	*P. tankervilleae*	x
61	PBX-14-270	*P. tankervilleae*	*D.* 'Emma White'	-
62	PBX-14-271	*V.* 'Berniece Miller'	*P. tankervilleae*	x
63	PBX-14-272	*P. tankervilleae*	*V.* 'Berniece Miller'	x
64	PBX-14-273	*C. flaccida* var *cristata*	*P. tankervilleae*	x
65	PBX-14-274	*P. tankervilleae*	*C. flaccida* var *cristata*	√/x
66	PBX-14-275	*P. vandarum*	*P. tankervilleae*	x
67	PBX-14-276	*P. tankervilleae*	*P. vandarum*	x
68	PBX-14-277	*E. pubescens*	*P. tankervilleae*	x
69	PBX-14-278	*P. tankervilleae*	*E. pubescens*	x
	Total number of self pollinations (12 no.)			91.67%
	Total number of cross pollinations (79 no.)			13.18%
	Total number of crosses germinated (13 no.)			14.28%
	Total number of crosses germinated excluding selfing (6 no.)			6.59%
	Total number of pedigree germinated excluding selfing (2 no.)			2.19%

(√ - success of pod set, x - failure of pod set, √/x – success of pod set/no germination

The hybridization was done by emasculation of female flowers by removing anther cap and pollinia. Fresh pollinia were collected from male parent and attached to stigma surface of the column for pollination. Both direct and reciprocal crosses were made using *P. tankervilleae* (Banks ex l'Heritier) Bl., and *P. flavus* (Bl.) Lindl. at ICAR-NRC Orchids, Sikkim. One to two flowers were crossed for each combination due to limitation of flowering plants availability. Generally the stigmatic surface of female plants was highly receptive and sticky; however the pollen bags were used to cover the inflorescence to avoid cross pollination with insects. Once the pollination succeeded, flower colour changed in to dark and lip dries after 3-4 days and success of pod set was presented in Table 1. Few crosses were made by selfing using pollen of the same flower on stigma to understand self compatibility among *Phaius* species.

2.3 *Progeny development*

Mature ellipsoid capsules were harvested after 10-11 months from pod formation after crossing and seedlings were raised using *in-vitro* techniques. The response of *in-vitro* studies will be discussed in separate paper. Among the capsules obtained from crosses, seedlings were obtained from selfed progenies and crossed progenies from PBX-11-21 (direct cross) and PBX-11-25 (reciprocal cross) series. Progenies of PBX-11-21 and PBX-11-25 flowered in early 2017 and mid 2018 after four and five years respectively after shifting seedlings for growing conditions under normal greenhouses. All the standard package practices for cultivation of *Phaius* progenies for growth were followed using pots (Devadas et al., 2008). Morphological characters are recorded at the full bloom stages and colour of the flowers was recorded with the help of 'Royal Horticultural Society' (RHS) colour chart (Simpson, 2011). The general descriptions for the morphological characters were based on quantitative data and expressivity of qualitative characters like flower colour at Pakyong, Sikkim (Altitude 1,300 MSL) location are recorded.

3 Results & Discussion

3.1 *Success of hybridization*

A total of 91 handmade pollinations were made that includes self pollinations to study both cross compatibility and self incompatibility nature in *Phaius* species. All 12 crosses attempted for selfing in both species resulted 91.67% pod setting. The status of pod setting with other species and genera are presented in Table 1. Success of crossing at species and genera level was recorded 13.18% pod formation, with success rate of *in-vitro* seed germination at 14.28%. Crosses viz., PBX-05-290, PBX-06-78, PBX-06-308, PBX-09-03, PBX-14-265, PBX-14-274 resulted pod set, when *P. tankervilleae* was used as one of the parent in hybridization programme; however failed to achieve germination of seedlings. Number of pedigree crosses resulted viable seedlings, excluding selfed crosses is limited to 2.19% among crosses performed. Results indicate narrow genetic behaviour of *Phaius* with limited scope for response to genetic improvement through conventional hybridization programmes.

3.2 *Incompatibility behaviour of Phaius*

Sympodial orchids like *Calanthe masuca*, *Lycaste crueata*, *Paphiopedilum villosum* showed only capsule development, when used as female parents in the present study. Similarly, capsule development was recorded in *P. tankervilleae*, when *Calanthe triplicata*, *Paplionanthe teres* and *Coelogyne flaccida* var. *cristata* were used as male parents. It indicates the possibility of pollen influence due to presence of hormones on flower colour change as an indication of pollination with capsule formation, without producing viable seeds (Burg & Dijkman, 1967; Arditti et al., 1971). High level of incompatibility was observed *Phaius* with other Orchid genera, like sympodial orchid viz., Calanthe, Coelogyne, Phalaenopsis, Lycaste, Dienia, Cymbidium, Thunia, Paphiopedilum, Coelogyne, Eria and monopodial orchids viz., Paplionanthe, Dendrobium, Arundina and Vanda (Table 1). Both the pre and post-zygotic barriers could be the reasons with *Phaius* for reproductive isolation between species and the genera for unsuccessful hybridizations (Edmands, 2002). The long journey of pollen grains that usually stimulate ovule formation after pollination, which is distinct in orchid reproductive system (Arditti, 1992) and hurdles in pollen tube elongation after pollen germination might operate among other incompatible crosses.

3.3 *Self and cross compatibility in Phaius*

In the present study the crosses viz., PBX-11-21 to 23 using *P. tankervilleae* as female parent and *P. flavus* as male parent (direct crosses) along with their reciprocal crosses (PBX-11-24 to 26) yielded capsule/ pod formation with viable progeny development. Similarly, except *P. flavus* the attempted self pollinations in *P. tankervilleae* formed capsule development along with successful progeny development. It is indicates the cross compatibility nature of species among *Phaius* and self compatible nature within species. The true hybridity of crosses was confirmed with F1 progeny testing and selfed progenies resulted female type of plants only.

3.4 *Description of novel of Phaius cross (PBX-11-22)*

The data was collected on mature flowering plants (F1) of *Phaius* cross (PBX-11-22) based on 'Common Descriptors of Orchids' essentially required by Plant breeders (**Table 2**). The comparative descriptions of hybrid with parental species for demarcation of floral traits are shown in **Table 3**. The new hybrid

Table 2 Description of Phaius F1 progeny of PBX-11-22

Character	Description of trait
Plant	sympodial nature, 76--80 cm height, spreading between 53 – 70 cm.
Pseudobulbs	9 x 3.5 cm, 3 to 5 number, conical to avoid shape, sheathed by leaf bases, 3 – 4 annual cicatrices in old bulbs.
Leaves	5 – 6, longest 80 x 8 cm, medium green colour (RHS 143C), spreading above pseudostem, narrow elliptic-lanceolate, acute, plicate, petiole long, pseudostem with sheath base 20 cm long.
Inflorescence	1 – 2, emerge from base, erect, > 65 cm long, with 8 pedicellate flowers; peduncle stout, long, > 14 cm internodal length, yellow-green colour (RHS 144B), smooth, 4 cm long, tubular, deep veined, sheathing bracts in medium yellow-green colour, caducous;
Flowers	8 – 10, 7.5 – 9 cm, acropetal flower opening, colour dominated by Yellow-orange (RHS 17B/A) sepals & petals with faint brownish shade, crimson colour on lip, moderately scented during shiny ward days with potted vase life of > 40 days.
Sepals	sub-equal, fleshy spreading; dorsal sepal 5 x 1.8 cm, narrow oblong, acute tip, 7 veins deep visible in same colour, yellow colour (RHS 13B) on ventral side; lateral sepals elliptic oblong, inside margin slightly curved forward, median vein touchy on ventral side.
Petals	size 5.2 x 2 cm, oblanceolate, acute, seven nerved, sub equal, colour same as sepals, shiny yellow on ventral side, margin slightly reflexed.
Lip	5.5 x 2 cm, oblong, semi-concave, spreading, open type, mentum in yellow colour apprx. 1.2 cm, side lobes erect, yellow-orange colour (RHS 17B/A); anterior lobe deflexed in middle, conchiform, corrugated; 3 obscure lamellae/calli become prominent at disc, attractive with multiple crimson colour (RHS 175B) thick lines inside from base to tip of apical lobe, crimson rich coloured at margin.
Column	2.5 x 0.52 cm, erect, incurved, white.
Anthers	dome shaped, pure white colour, 4 chambered and deeply seated in column, pollinia yellow colour and laterally compressed in groups.

Table 3 Comparison of floral attributes of Phaius hybrid (PBX-11-22) with parents

Character	Phaius hybrid (PBX-11-22)	*P. tankervilleae* (Banks ex l'Heritier) Bl. (♀ parent)	*P. flavus* (Bl.) Lindl. (♂ parent)
Inflorescence length	long	long	medium
Flower colour	yellow-orange	brown-purple	yellow
Flower nature	spreading & erect	spreading & erect	erecto-petal
Sepal colour	yellow-orange	brown-purple	yellow
Petal colour	yellow-orange	brown-purple	yellow
Dorsal sepal shape	oblanceolate	elliptic-oblong	oblong-elliptic
Lateral sepals shape	elliptic-lanceolate	elliptic-lanceolate	oblong-elliptic
Sepal tip	acute	acute	obtuse tip
Petal shape	oblanceolate	oblanceolate	oblong-oblanceolate
Petal tip	acute	acute	obtuse tip
Lip shape	broad oblong	ovate	oblong
Lip nature	Semi-open	tubular	concave
Lateral lobes	erect	overlapping	erect
Median calli	present	present	present
Lip colour	Crimson	pink	Reddish-brown

PBX-11-22 indicates pedigree code of Plant Breeding crossing register with year 2011 & cross 22

Figure 1. Phaius primary hybrid (PBX-11-22/02)

Figure 2. Rachis of Phaius primary hybrid (on 23.02.2017)

Phaius hybrid F1 flower (PBX-11-22)

Phaius tankervilleae (Banks ex l'Heritier) Bl. (♀ parent)

Phaius flavus (Bl.) Lindl.(♂ parent)

Figure 3. Comparison of hybrid (PBX-11-22) with female and male parents

plant (**Figure 1**), inflorescence (**Figure 2**) and as well as flower configuration along with lip differences was depicted in **Figure 3** for identification of specific characters.

The floral configuration of novel *Phaius* hybrid progeny (PBX-11-22) is compared on both front and ventral view to assess the exact shape, size and colour of parts (**Figure 4**). The floral parts of *P. tankervilleae*, which was used as one of the parent depicted separately for trait comparison (**Figure 5**). Other parent, *P. flavus* (#Old NOAC 659) was lost during course of time from the active collections, due to disease infection and limited population; but morphological characters were compared from previous year's data. The reciprocal combination of same cross, where *P. flavus* and *P. tankervilleae* were used as female and male parents (PBX-11-25/01) flowered during July-Aug, 2018. The morphological features of plants derived from reciprocal cross are similar to direct cross, except smaller size of flowers and light tinge of pale green colour on tips of sepals and petals (**Figure 6**).

Figure 4. Frontal (R) and ventral (L) view of floral part of Phaius primary hybrid (PBX-11-22)

Figure 5. Frontal (R) and ventral (L) view of floral part of *Phaius tankervilleae*

Figure 6. Phaius reciprocal cross (PBX-11-25/01) plant, flower & floral parts

Phaius flowers produced from both direct (PBX-11-22) and reciprocal (PBX-11-25) crosses are found to be similar in flower traits and colour. The characterization of progenies at F1 stage, indicate the novel features with combined features of floral characters from both parents. The flower colour found to be more attractive due to rich yellow-orange colour, than the yellow coloured *P. flavus*. The size of sepals and petals were found be intermediate; but shape was similar to *P. tankervilleae* with acute tip. Characters of lip found to be grand with distinct and dark crimson colour with thick stripes starting adjacent to three obscure calli extending up to tip of terminal lobe and spreading all over anterior lobe on yellow-orange back ground. Lateral lobes of lip were erect and semi-open covering above column, similar to *P. flavus* and lip colour sometimes extended towards the lower margin of lateral lobes. In case of *P. tankervilleae* the lateral lobes were overlapped, which was not found in any Phaius F1 progenies. A similar cross was noticed as 'Joan Hart' by G. Coram from web reference (http://orchids.wikia.com/wiki/Phaius_Joan_Hart; Orchidwiz, 2013) with similar features, but morphological description of plant was not available. In several other countries, *Phaius* species ranked as endangered species (Harrison et al., 2004; Simmons et al., 2017) and efforts were lacking to develop hybrids through systematic breeding approaches. Hence, the present novel *Phaius* breeding lines developed can serve as model to develop new varieties.

Conclusion

This scientific study could be useful for botanists and taxonomist to reassess genetic diversity among species of Phaius and associated gene flow. High incompatibility with other orchid genera indicates narrow genetic base, but found to be compatible within genera and also self compatible. The new primary hybrid from indigenous species developed can serve as an ideal reference material for taxonomical studies and can useful as genetic stock for cultivation as terrestrial plants with good adaptability in sub-tropical conditions. Population development at F_2 generation may unravel the genetic control of various traits and assist in construction of classical genetic map in *Phaius*. Further improvement can be done through markers, mutation breeding and hybridization with its near relatives like Calanthe *etc*.

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Conflict of interest

Authors hereby declare that there is no conflict of interest that could possibly arise.

References

Ackerman JD (2012) Orchids gone wild: Discovering Naturalized Orchids in Hawaii. (Orchids gone wild: Discovering Naturalized Orchids in Hawaii.). Bulletin of the American Orchid Society 81 : 88-93.

Arditti J (1992) Fundamental of Orchid Biology. Wiley, New York.

Arditti J, Jeffrey DC, Flick BH (1971) Post-pollination phenomena in Orchid flowers. III. Effects and interactions of auxin, kinetin or gibberelin. The New Physiologist 70 : 1125-1141.

Bhuyan J (2010) Floral biology of *Phaius tankervilleae* an endangered terrestrial orchid. Advances in Plant Sciences 23: 611-612

Buragohain B, Chaturvedi SK, Puro N (2016) Pollination biology of *Phaius tankervilleae* (Banks ex L'Herit) Bl. (Orchidaceae). The International Journal of Plant Reproductive Biology 8: 75-81.

Burg SP, Dijkman J (1967) Ethylene and auxin participation in pollen induced fading of Vanda orchid blossoms. Plant Physiology 42: 1648-1650.

Chowdhery HJ, Agrawala D K (2013) A Century of West Himalayan Orchids. Bishen Singh Mahendra Pal Singh, Dehra dun – 248001, India. pg 318.

CITES (2004) Orchid hybrids exempted from CITES. Available on https://www.cites.org/sites/default/files/common/cop/13/E13-P41Annex.pdf access on 25th December, 2018.

CITES (2016) Checklist of CITES Species. Appendix II. UNEP-WCMC. Available on http://checklist.cites.org/#/en access on 25th December, 2018.

Cullen J, Knees S, Cubey HS (2011) The European garden flora, flowering plants: A manual for the identification of plants cultivated in Europe, both out-of-doors and under glass, Second edition. Cambridge, UK: Cambridge University Press.

Desmond R (1995) Kew: The History of Botanic Gardens, UK. The Harvill Press and Royal Botanic Gardens Kew.

Devadas R, Das J, Naik SK, Medhi RP (2008) Influence of potting mixture on acclimatization and growth of *in-vitro* developed plantlets of *Zygopetalum intermedium*. Journal of Ornamental Horticulture 11 : 241-247.

Devadas R, Medhi RP, Das SP (2010) Interspecific hybrid in Epidendrum Orchid: *E. radicans* Pav. ex. Lindl. x *E. xanthinum* Lindl. Journal of Horticultural Sciences 5 : 144-147.

Edmands S (2002) Does parental divergence predict reproductive compatibility? Trends in Ecology & Evolution 17: 520-527.

George KV, Mathew J (2016) *Phaius tankervilleae* (Orchidaceae) – A new record for Southern India. Indian Forester 142 : 300-301.

Harrison DK, Kwan H, Johnston ME, Harris WK (2004) Molecular taxonomy of the Australian swamp orchids (*Phaius* spp.). *Acta Horticulturae* [Proceedings of the international symposium on harnessing the potential of horticulture in the Asian-Pacific Region, Coolum, Queensland, Australia, 1-3 September, 2004], No.694: 121-124.

Hirano T, Godo T, Miyoshi K, Ishikawa K, Ishikawa M, Mii M (2009) Cryopreservation and low-temperature storage of seeds of *Phaius tankervilleae*. Plant Biotechnology Reports3 : 103-109

Kanwal KS (2014) Conservation of *Phauis tankervilleae* a valuable orchid of Arunachal Pradesh, India. Indian Forester 140 : 1263-1264.

Kishor R, Sharma G J (2009) Intergeneric hybrid of two rare and endangered orchids, *Renanthera imschootiana* Rolfe and *Vanda coerulea* Griff. Ex (Orchidaceae): Synthesis and characterization. Euphytica 165 : 247-256. DOI 10.1007/s10681-008-9755-9.

Loureiro J (1790) *Phaius grandifolius*. Flora Cochinchinensis 2 : 529-530

Lucksom S Z (2007) The Orchids of Sikkim and North East Hilalaya. Concept, Siliguri 734 001, Pp. 607.

Malemnganba H, Bhattacharya S, Deka P C (1994) A new cost effective embryo culture medium for orchids. Journal of the Orchid Society of India (1/2): 67-71

Naithani HB, Chandola S, Chandran M (2009) On the wild occurrence of orchid *Phaius tankervilliae* in North India. Indian Forester 135: 578-579.

Orchidwiz (2013). Orchidwiz Encyclopedia. Version 9.03. Louisville, USA.

Pearce N R, Cribb P J (2002) The Orchids of Bhutan. Flora of Bhutan including a record of Plants from Sikkim and Darjeeling, Vol 3 (Part 3), Royal Botanic Garden Edinburgh and Royal Government of Bhutan, Pp 643

Schuiteman A, de Vogel EF (2000) Orchid genera of Thailand, Laos, Cambodia and Vietnam. Thai-English edition, Universiteit Leiden, National Herbarium Nederland, The Netherlands. (ISBN 90-71236-44-7) Pp. 118

Simmons L, Mathieson M, Lamont R W, Shapcott A (2017) Genetic diversity of endangered orchid *Phaius australis* across a fragmented Australian landscape. Conservation Genetics 19: 41-465. DOI: 10.1007/s10592-017-1022-y.

Simpson N (2011) Colour and contemporary digital botanical illustration. Optics & Laser Technology 43 : 330–336