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TAXONOMY, KARYOMORPHOLOGY AND POLLEN VIABILITY OF *HYMENOCALLIS LITTORALIS* (JACQ.) SALISB. (AMARYLLIDACEAE)

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Abstract

The present study offers the taxonomy, karyomorphology, and pollen-pistil interactions in the bulbous species *Hymenocallis littoralis* (Jacq.) Salisb. of the family Amaryllidaceae. The genus *Hymenocallis* is closely allied to *Pancratium*, however, differs from the later by filament, number and shape of ovule, and seed characteristics. Detailed descriptions and illustrations of *H. littoralis* are provided, alongside information on its habitat, distribution, examined specimens, and economic significance. A new somatic chromosome number of 2n = 50 is reported for *H. littoralis*, and this count was not found to be consistent with any of the earlier reports, offering additional insights into its chromosomal characteristics. Furthermore, the study reveals a high pollen viability of 95% in *H. littoralis*.

Introduction

The genus *Hymenocallis*, belonging to the Amaryllidaceae family, comprises approximately 70 species (Tapia-Campos *et al.*, 2012), and is valued for both its horticultural appeal and medicinal properties (Ogden, 2007). According to Angiosperm Phylogeny Group (APG IV, 2016), this genus falls into the family Amaryllidaceae, though Cronquist (1981) placed it into the family Liliaceae. Initially, the members of *Hymenocallis* were considered the American representatives of the Old World genus *Pancratium* L. (Sealy, 1954; Meerow *et al.*, 2002). However, Salisbury (1812) established *Hymenocallis* as a distinct genus, on the basis of distinct differences in seed characteristics: *Pancratium* produces black, dry, compressed seeds with a phytomelan layer, while *Hymenocallis* has nearly ovoid, green, fleshy, and often viviparous seeds. Along with the genera *Ismene* Salisb. and *Leptochiton* Sealy, *Hymenocallis* forms the tribe Hymenocallideae (Meerow *et al.*, 2002). Commonly known as 'spider lilies' these plants are distinguished by their unique staminal membrane enveloped by long, slender tepals. In Bangladesh, the genus *Hymenocallis* is represented by a single species, *H. littoralis*, which is rarely found in Mymensingh, Chattogram and Sylhet districts. Apart from horticultural and ornamental value, *Hymenocallis littoralis* has been reported to possess antibacterial and anti-inflammatory properties (Noormi *et al.*, 2012; Karthikeyan *et al*., 2016).

Karyomorphology plays a pivotal role in plant taxonomy, offering insights into evolutionary relationships, genetic diversity and classification. Recent studies underscore the significance of karyomorphological and cytogenetic data in species delimitation, phylogenetic reconstruction, taxonomic revision, conservation, and genomic studies. Karyomorphological studies aids in differentiating closely related species and defining species boundaries based on chromosomal

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characteristics (Martins *et al*., 2020). Karyotype data contribute to taxonomic revisions and the establishment of robust classification systems by providing additional characters for systematic analysis leading to more accurate taxonomic classifications (De Moraes *et al*., 2021). Karyomorphological studies also provide insights into genomic evolution processes such as genome duplication, chromosome rearrangements, and genome size changes (Nkongolo and Mehes-Smith, 2012; Sun *et al.*, 2020).

The pollen-pistil interaction encompasses a series of events that determines whether the gametes are recognized and accepted or rejected (Dumas and Guade, 1981). In cases of compatible pollination, pollen grains attach to the stigma, undergo hydration, germinate, and develop pollen tubes that penetrate the stigma's cell layers. These tubes then extend within the transmitting tissue of style, eventually reaching the ovary for fertilization. In contrast, incompatible pollination can lead to the arrest of pollen tube growth. The tissues of the pistil are believed to provide both chemical and physical support, along with directional guidance, to facilitate pollen tube development (Knox, 1984).

Despite the ecological and economic importance of *Hymenocallis littoralis*, it has not undergone taxonomic revision, nor has it been investigated from cytological and palynological perspectives in Bangladesh. Therefore, this study aims to conduct a comprehensive taxonomic analysis of *H. littoralis*, examine its pollen and pollen-pistil interaction, and investigate cytological parameters of the species for the first time in Bangladesh.

Materials and Methods

Taxonomic identity

The plant specimen collected from Sonargaon, Narayanganj and grown in the Botanical Garden of the University of Dhaka was examined critically. In order to ascertain its identity, floral parts were studied in detail using light microscope, and the relevant literatures were consulted to ascertain its identity (Dassanayake and Clayton, 2000; Karthikeyan *et al.*, 1989). The voucher specimen has been housed at Dhaka University Salar Khan Herbarium (DUSH).

Cytological investigation

Root tips were collected from *H. littoralis*, planted in the Botanical Gardens of the University of Dhaka as well as in the Jagannath University, Dhaka. The roots were pretreated with a 1:1 solution of paradichlorobenzene (PDB) and 0.002 M 8-hydroxyquinoline for 3 h and 30 min at room temperature. Subsequently, they were fixed in 45% acetic acid for 15 min at 4°C. Afterwards, the roots were hydrolyzed in a solution of 1 N HCl and 45% acetic acid (2:1) for 15 min at 60℃. The root tips were then stained and squashed in 1% aceto-orcein solution (Das *et al.*, 2020). Chromosomes were observed under an Optica electron microscope and photographs were captured with a Euromex camera.

Pollen viability and pollen-pistil interaction

Freshly opened flowers were used for controlled pollinations. Suitable flower buds were emasculated one day prior to pollinations in case of both self- and cross-pollinations. Pollinations occurred between 7:30 to 10:00 am, with self-pollinations involving the removal of open-pollinated flowers the day before to ensure fresh buds. Self-pollination involved touching freshly dehisced anthers onto the stigma using fine forceps. In cross-pollination, conventional methods were used, with emasculation before anthesis to prevent contamination. Anthers were carefully removed with pointed forceps and rubbed against the stigma of emasculated flowers. Identification tags were tied around peduncles to track pollinated buds (Ram *et al*., 2006). To investigate pollen-pistil interaction, the pollinated pistils were collected at 12-, 24-, and 48 h intervals post-

pollination, and were fixed in aceto-alcohol solution (1:3 v/v). After washing the pistils with distilled water to remove the fixative, they were treated with 1N NaOH and incubated for 12 min at 55°C to soften them. Following cooling, the pistils were washed with distilled water again to remove any NaOH residues, and then stained with 0.1% decolorized aniline blue for 8-10 min (Patil *et al*., 2013). The stained pistils were mounted in a 50% aqueous glycerol and observed under a Nikon (Optiphot) microscope which is equipped with epi-fluorescence UV illumination using the UV-2A and BV-2A filters. Pollen grains, germinated and non-germinated, were counted from 10 pollinated pistils for each pollination type.

Results and Discussion

Taxonomic account

Hymenocallis littoralis (Jacq.) Salisb., Trans. Hort. Soc. Lond. 1: 338 (1812). *Pancratium littorale* Jacq., Select. Stirp. Amer. Hist.: 99 (1763). *Hymenocallis adnata* Herbert, Amaryll.: 215 (1837). *Hymenocallis tenuiflora* Herbert, Amaryll.: 215 (1837). *Pancratium illyricum* auct. *non* L.: Blanco, Fl. Filip. ed. 3: 316 (1877). *Pancratium maritimum* auct. *non* L.: Blanco, Fl. Filip. ed. 3: 316 (1877). (**Fig. 1**).

Bengali name: *Bok phul*.

Bulbous perennial herb; bulbs about 4-5 cm in diameter, with cylindric neck. Leaves up to 90 cm long and 7 cm across, radical, linear, distichous. Scapes compressed, attain up to 80 cm in length; bracts hyaline, linear or lanceolate. Inflorescence umbellate, 6-12 flowered umbels; flowers white. Perianth 6-lobed, with a tube up to 14 cm long and approximately 0.5 cm across, light green; lobes can reach to 14 cm long, white. Stamens 6, with a white staminal cup, approximately 4 cm in length; filament around 6 cm long; anthers versatile, linear, approximately 2 cm long. Ovary 3-chambered, approximately 1.6 cm long and 0.6 cm across, inferior; ovules 4-5 in every chamber; style around 10 cm long; stigma 3-lobed. Fruit a subglobose capsule, triangular. Seeds angular, black. *Flowering period:* June-August.

Specimens examined: **Dhaka**: Dhaka University Botanical Garden, 20.02.1980, Mahbuba Halim 740 (DACB). **Narayanganj**: Sonargaon, Amgaon, 25.08.2011, Sumona 69; **Bhola**: Char Kukri Mukri, 02.07.2014, Sumona 89 (DUSH).

Habitat: H. littoralis grows in well-drained soils.

Distribution: H. littoralis is native to America. Though cultivated, this species has become naturalized in tropical regions of Africa and Asia, Malaysia and Pacific Islands.

Uses: H. littoralis is valued for its ornamental and medicinal properties. This species possesses strong anti-inflammatory activities (Zhang *et al*., 2022).

Propagation: By bulbs.

Taxonomic notes: *Hymenocallis* is closely allied to the genus *Pancratium*, however, the former differs from the later by filament, number and shape of ovule and seed characters. In *Hymenocallis*, filament is straight, whereas in many *Pancratium*, free staminal filament is incurved from the corona. Ovule is globose and less than 10 in number per locule in the former but flattened and numerous per locule in the later. In *Hymenocallis*, seeds are hard, while they are fleshy in *Pancratium* (Table 1).

Cytological investigation

Chromosomal characteristics of *Hymenocallis littoralis* including the length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes are summarized in Table 2. In *H. littoralis*, orcein staining revealed homogenously stained interphase nuclei (Fig. 2A), categorized as the “Diffused Type” according to Tanaka (1971). Similarly, the

prophase chromosomes exhibited uniform staining along their length (Fig. 2B), classified as the "Continuous Type" based on classification of Tanaka (1971).

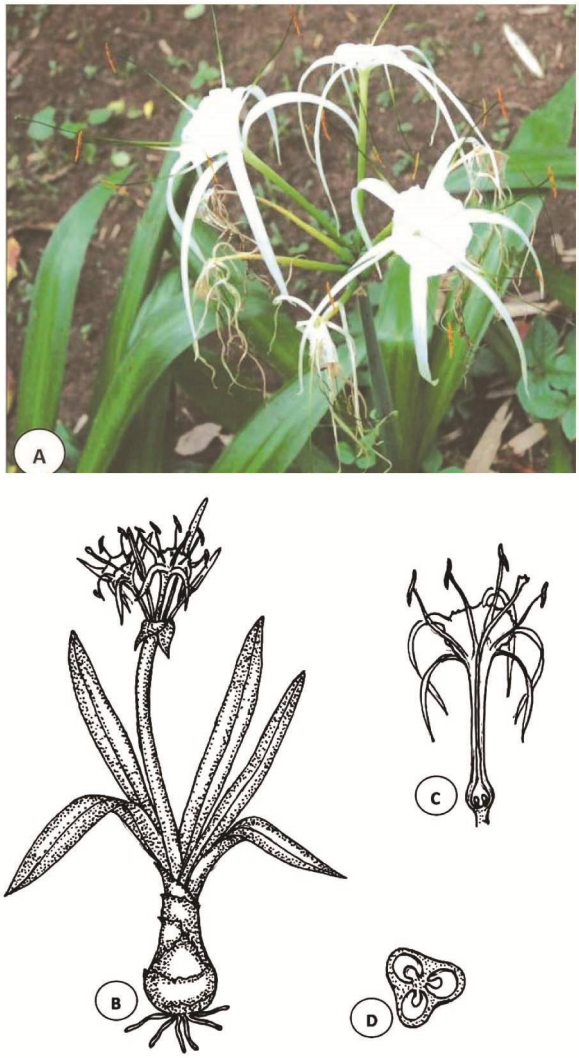

Fig. 1. ***Hymenocallis littoralis***: (A). Habit in nature, (B). Habit sketch (×0.05). (C). L. S. of flower (×0.15), (D) T. S. of ovary (×2).

Table 1. A comparative account of *Hymenocallis* with its closely allied genus *Pancratium*.

Characters	*Hymenocallis* Salisb.	*Pancratium* Dill *ex* Linn.
Filament	Straight	Incurved from the corona
Number of ovules	Less than 10 per locule	More than 15 per locule
Shape of ovules	Globose	Flattened
Seed	Black or brown, hard	Green, fleshy

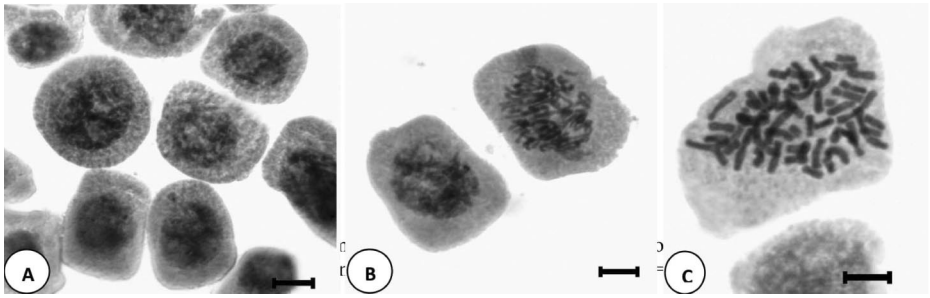

Fig. 2. Different stages of chromosomes of *Hymenocallis littoralis* after orcein staining. A. Interphase nuclei; B. Prophase chromosomes; C. Metaphase chromosomes (Bar=10 μm).

Typically, specimens showing the "Diffused Type" in interphase nuclei, also display the "Continuous Type" in prophase chromosomes, as observed in *H. littoralis*. This indicates a homogeneous distribution of diffused heterochromatin at the interphase stage, which continues uniformly along the prophase chromosomes. This pattern aligns with the general characteristics of orcein staining in both interphase nuclei and prophase chromosomes. This study reveals that *H. littoralis* has a somatic chromosome number of 2n = 50 (Fig. 2C, Table 2).

The somatic chromosome number of *H. littoralis* has been reported to vary in previous studies, with 2n=44 (Sharma and Bal, 1956) and 2n=46 (Sato, 1938, 1942; Raina and Khoshoo, 1971). Recently, Tanee *et al.* (2018) reported different chromosome numbers of 2n=44, 46, 48, 49 and 68 in *H. littoralis* using conventional staining techniques. In contrast, our study reveals a new chromosome number of 2n=50 for *H. littoralis*, marking the first report of this chromosomal count. This finding differs from all previous studies (Sato, 1942; Sharma and Bal, 1956; Tanee *et al*., 2018), suggesting potential intraspecific chromosomal variation in *H. littoralis*. Such variations could result from numerical chromosomal aberrations, including euploidy and secondary modifications of polyploidy within species of this genus. Alternatively, these variations might arise from distinct cytotypes or the presence of some B-chromosome, indicating that the genus *Hymenocallis* holds significant interest for future cytogenetic studies. The total length of diploid chromosome complement in *H. littoralis* was measured at 291.94 μm (Table 2). This species was found to have 42 metacentric chromosomes, 6 sub-metacentric chromosomes and 2 sub-telocentric chromosomes, as classified by Levan *et al.* (1964) (Figs. 3 & 4; Table 2).

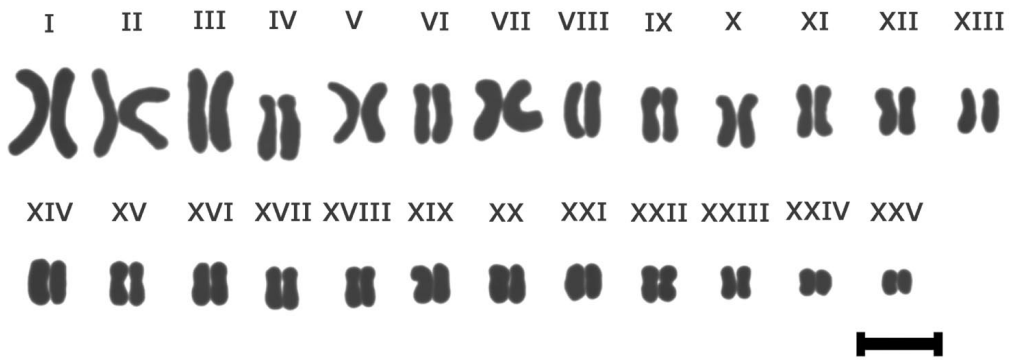

Fig. 3. Karyotypes of *Hymenocallis littoralis* (Bar=10 μm).

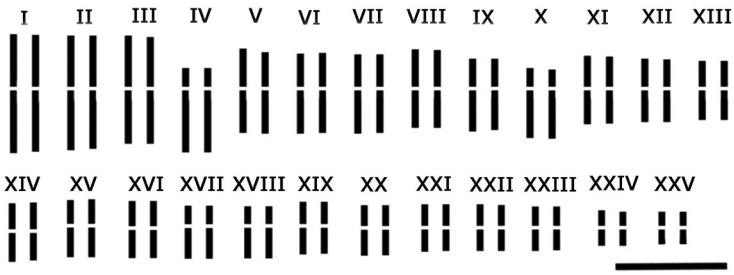

Fig. 4. Ideograms of *Hymenocallis littoralis* (Bar=10 µm).

The relative length of each individual chromosome varied from 0.02-0.07, while the length of individual chromosome ranged from 2.64-10.80 µm (Table 2). The presence of metacentric, sub-metacentric and sub-telocentric chromosomes indicate that *H. littoralis* has asymmetric karyotypes. According to Stebbins (1971), asymmetric karyotypes are considered as advanced character. Thus, *H. littoralis* can be considered as evolutionarily advanced based on its chromosomal architecture.

Pollen-pistil interaction

The study revealed *Hymenocallis littoralis* exhibited 95% pollen viability; however, despite this high viability, no fruit set was observed throughout the investigation. Pollen grains failed to germinate, and there was an absence of pollen tubes in all types of pollination experiments, including self-, cross-, and open pollination experiments (Fig. 5).

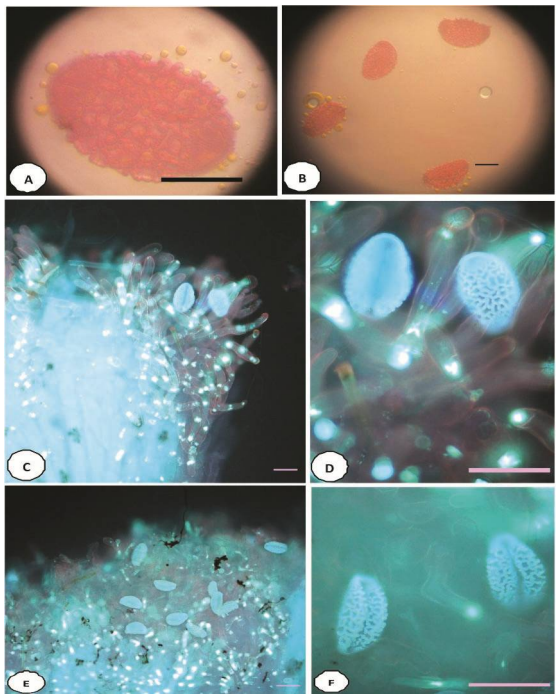

Fig. 5. Pollen grains and pollen-pistil interaction of *H. littoralis*; A&B. Pollen grains; C&D. Self-pollination; E&F. Cross-pollination. Bar=100 µm.

Table 2. Chromosomal characteristics of *Hymenocallis littoralis*.

Chromosome Pair	Long arm (µm)	Short arm (µm)	Total length	Arm ratio	Relative length	Centromeric index	Centromeric type
I	5.87	4.93	10.80	1.19	0.07	45.65	m
	5.77	4.88	10.65	1.18	0.07	45.82	m
II	5.63	4.81	10.44	1.17	0.07	46.07	m
	5.47	4.79	10.26	1.14	0.07	46.69	m
III	4.98	4.81	9.79	1.04	0.07	49.13	m
	5.00	4.64	9.64	1.08	0.07	48.13	m
IV	5.81	1.76	7.57	3.30	0.05	23.25	st
	5.81	1.76	7.57	3.30	0.05	23.25	st
V	3.99	3.57	7.56	1.12	0.05	47.22	m
	4.06	3.26	7.32	1.25	0.05	44.54	m
VI	4.08	3.14	7.22	1.30	0.05	43.49	m
	3.97	3.19	7.16	1.24	0.05	44.55	m
VII	4.08	3.05	7.13	1.34	0.05	42.78	m
	3.99	3.05	7.04	1.31	0.05	43.32	m
VIII	3.52	3.5	7.02	1.01	0.05	49.86	m
	3.52	3.43	6.95	1.03	0.05	49.35	m
IX	3.83	2.67	6.5	1.43	0.04	41.08	m
	3.75	2.65	6.40	1.42	0.04	41.41	m
X	4.44	1.73	6.17	2.57	0.04	28.04	sm
	4.51	1.64	6.15	2.75	0.04	26.67	sm
XI	3.19	2.89	6.08	1.10	0.04	47.53	m
	3.14	2.87	6.01	1.09	0.04	47.75	m
XII	2.95	2.63	5.58	1.12	0.04	47.13	m
	2.95	2.58	5.53	1.14	0.04	46.65	m
XIII	2.76	2.42	5.18	1.14	0.04	46.72	m
	2.75	2.42	5.17	1.14	0.04	46.81	m
XIV	2.65	2.35	5.00	1.13	0.03	47.00	m
	2.53	2.42	4.95	1.05	0.03	48.89	m
XV	2.72	2.18	4.90	1.25	0.03	44.49	m
	2.70	2.20	4.90	1.23	0.03	44.90	m
XVI	2.56	2.3	4.86	1.11	0.03	47.33	m
	2.58	2.27	4.85	1.14	0.03	46.80	m
XVII	3.07	1.71	4.78	1.80	0.03	35.77	sm
	3.05	1.71	4.76	1.78	0.03	35.92	sm
XVIII	2.87	1.62	4.49	1.77	0.03	36.08	sm
	2.82	1.62	4.44	1.74	0.03	36.49	sm
XIX	2.42	2.02	4.44	1.20	0.03	45.50	m
	2.40	2.04	4.44	1.18	0.03	45.95	m
XX	2.35	1.86	4.21	1.26	0.03	44.18	m
	2.35	1.86	4.21	1.26	0.03	44.18	m
XXI	2.02	1.93	3.95	1.05	0.03	48.86	m
	1.95	1.93	3.88	1.01	0.03	49.74	m
XXII	1.94	1.93	3.87	1.01	0.03	49.87	m
	1.93	1.90	3.83	1.02	0.03	49.61	m
XXIII	1.93	1.73	3.66	1.12	0.02	47.27	m
	1.88	1.73	3.61	1.09	0.02	47.92	m
XXIV	1.50	1.42	2.92	1.06	0.02	48.63	m
	1.50	1.30	2.80	1.15	0.02	46.43	m
XXV	1.36	1.30	2.66	1.05	0.02	48.87	m
	1.34	1.30	2.64	1.03	0.02	49.24	m
		GT=	**291.94**				

m=metacentric. sm=sub-metacentric, st=sub-telocentric

This may be attributed to pre-fertilization incompatibility in *H. littoralis*, potentially explaining the lack of fruit formation. In contrast, a few other species within the Liliaceae family, such as *Allium tuberosum* Rottler *ex* Spreng, show significant pollen germination and pollen tube development within 24-30 h after both self- and cross-pollination. During open pollination, certain pistils exhibit pollen with fully developed tubes, indicating successful fertilization pathways, while others lack pollen tubes altogether, suggesting variability in pollination success.

In the Amaryllidaceae family, studies on *Narcissus triandrus* and *Hippeastrum advenum* have shown that self-pollination results in fewer seeds compared to cross-pollination (Saavedra *et al.*, 1996; Sage *et al*., 1999). However, *Zephyranthes atamasco*, another member of this family, produces an equal number of seeds through both self- and cross-pollination (Broyles and Wyatt, 1991). The female pistil plays a vital role by providing essential nutrients and guidance cues that facilitate pollen tube growth across different cellular environments. Simultaneously, it acts as a barrier, preventing incompatible pollen from accessing the ovules, including that from other species (Swanson *et al*., 2004). Hiscock *et al*. (2002) found that stigma types, particularly the presence of dry versus wet stigmas, influences cross-compatibility and self-incompatibility in *Senecio squalidus* (Asteraceae), which partially aligns with our findings. Our study suggests that the absence of a wet-type stigma and essential nutrients likely contributed to the failure of pollen tube formation in *Hymenocallis littoralis*. However, a more detailed investigation into the pollen morphology and viability of this species is needed to gain a comprehensive understanding of its reproductive mechanisms and compatibility.

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