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Comparative cyto-morphometric analysis of three *physalis* species (*Solanaceae*) from northwestern India

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ABSTRACT

Cape gooseberry (Physalis sp.) a widely recognized fruit from the Solanaceae family, is valued both commercially and culinarily. Various Physalis species have long been used in traditional medicine due to their significant therapeutic potential. These plants are known for a wide array of bioactivities, including immunomodulatory, cytotoxic, anti-diabetic, antioxidant, anti-cholesteric, and antimicrobial properties, largely attributed to their secondary metabolites. In the present study, Physalis specimens collected from different regions of Himachal Pradesh were analysed for their meiotic behaviour to assess ploidy levels using conventional chromosome counting techniques. This research aimed to contribute to future breeding efforts by identifying diverse cytotypes, morphotypes, and chemotypes, with the goal of developing high-medicinal-value and stress-tolerant varieties. Three species of Physalis were examined using morphological and cytogenetic methods and the results showed that Physalis longifolia Nutt. is a diploid with a chromosome count of 2n = 2x = 24, whereas Physalis peruviana L. and Physalis angulata L., both with 2n = 4x = 48, are tetraploid.

Keywords: Cytomorphology, Meiosis, Ploidy, *Physalis*

INTRODUCTION

The nightshade (Solanaceae) family comprises a diverse group of plants, including agricultural crops, medicinal herbs, and various weed species. This plant family encompasses a wide range of growth forms, from annual and perennial herbs to shrubs and trees, and is known for its adaptability to different habitats, morphologies, and ecological conditions (Rohini *et al.* 2024). It includes approximately 98 genera with 2,700 species. A significant number of these species, particularly over 100 belonging to the genus *Physalis*, commonly referred to as rasbhari, are predominantly found in warm and temperate regions across Africa, Asia, and the America, Smith (1991); Bastos *et al.* (2008). The *Physalis* genus includes small, upright herbs that may be annual or perennial in nature. Traditionally, these plants have been used in herbal medicine due to their rich composition of bioactive compounds such as physalins, withanolides, saponins, fatty acids, flavonoids, steroids, and essential vitamins. These constituents contribute to the plant's therapeutic use in managing various health conditions, including diabetes, cancer, inflammation, kidney and bladder disorders, jaundice, and gout, García-Barriga (1975); Glotter *et al.* (1975); Gilmore (1977); Glotter (1991); Gengaihi *et al.* (2013). Among these, physalins have drawn particular scientific interest for their potential cytotoxic and anti-tumour effects by Gharib *et al.* (2008), Mericli (2011). These plants are characterized by their prominent fruiting calyces, which enclose an orangish-yellow berry. They serve as valuable sources of compounds used in chemotherapy (Mier-Giraldo *et al.* 2017) Polyploidy has been observed in four species of the genus, with chromosome numbers ranging from 2n = 24 to 72, where 2n = 24 is the most frequently reported by Ganapathi *et al.* (1991). The genus exhibits three basic chromosome numbers, such as: x = 10, 11, and 12, with x = 12 being the most common as recorded by Tuteja and Bhatt (1984). Cytological studies play a critical role in conserving the genetic diversity of medicinal plants, especially by preserving germplasm from unique cytotypes for future research. Phytochemical analysis done by Chiang *et al.* (1992a & 1992b), Ismail and Alam (2001), Soares *et al.* (2003), highlight the need to identify additional chemotypes. This is essential for breeding efforts aimed at developing high-medicinal-yield varieties that are also stress-resistant, ultimately contributing to the creation of both herbal and conventional medicinal formulations (Deachen *et al.* 2024).

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The primary objective of the present study was to identify any novel cyto-morphotypes and to evaluate their potential for future breeding programs. Although some cytomorphological studies have been conducted individually on these species, comprehensive comparative analyses across all three have been lacking. This study presents the first comparative cyto-morphological investigation aimed at understanding the distinctions among these *Physalis* species.

MATERIALS AND METHODS

Plant materials

Extensive fieldwork was carried out across several northwestern districts of Himachal Pradesh, India, covering altitudinal ranges from 656 meters to 3,647 meters, as detailed in Table 1. Voucher specimens of the collected species are *Physalis angulata* L. (PLP#22085), *P. longifolia* Nutt. (PLP#22087), and *P. peruviana* L. (PLP#22086), have been preserved in the herbarium of CSIR-IHBT, Palampur, Himachal Pradesh. Additionally, live plant accessions are being maintained at the Eternal university's Herbal-cum-Botanical Garden, located at an elevation of 1,027 meters (30.5628°N, 77.42702°E).

Morphological studies

A range of morphometric traits (Tables 1 and 2) was analysed using standard measurement techniques, including metric scales and hand lenses, across an average of 10 accessions per species.

Table 1: Comparing the mean and standard deviation of key physical traits across three species of the *Physalis*

S. No.	Characters*	Plant Species		
		P. angulata	*P. longifolia*	*P. peruviana*
1.	Plant height (cm)	61.4±10.8	108.4±16.4	139.0± 41.1
2.	Leaf width (cm)	4.3±1.0	5.8±1.0	10.9±1.6
3.	Leaf length (cm)	6.3± 1.2	9.9 ±1.1	11.8± 2.1
4.	Petiole length (cm)	2.6± 0.7	1.8± 0.2	4.9±1.6
5.	Number of flowers per plant	19.6±5.2	44.2±8.5	69.3± 20.1
6.	Pedicle length (cm)	1.9± 0.2	3.2±1.2	1.3± 0.1
7.	Flower diameter (cm)	1.3± 0.2	1.3± 0.1	1.6± 0.2
8.	Calyx length (cm)	2.7± 0.3	3.0± 0.2	3.7± 0.2
9.	Calyx width (cm)	2.7± 0.4	3.2± 0.2	4.0± 0.3
10.	Number of seeds per fruit	131.1±11.3	147.0± 20.3	81.7±16.1
11.	Fruit weight (mg)	0.4± 0.1	1.1± 0.3	1.1± 0.2
12.	Fruit diameter (cm)	0.9± 0.2	1.4± 0.2	1.1± 0.4

**The average characteristic features of plants were calculated by using 10 accessions for each species*

Cytological Experiments

The study was conducted in the Department of Botany at Eternal University, Baru Sahib, District Sirmour, Himachal Pradesh. For each species, more than ten replicates were used. The seeds were sown and grown under natural environmental conditions in the university's Herbal-cum-Botanical Garden between April and October 2022. For slide preparation, flower buds at various developmental stages were collected and fixed in Carnoy's solution (a mixture of 6 parts ethanol, 3 parts chloroform, and 1part acetic acid) for 24 hours, following the protocol of Ramanpreet and Gupta (2015). Anthers were delicately removed using forceps and transferred to microscope slides containing 1% aceto-carmine stain. The material was gently squashed and sectioned to release pollen mother cells (PMCs), which were then covered with a coverslip, lightly heated, and examined under a Magnus microscope fitted with a MIPS USB 5MP digital imaging system. Photomicrographs of PMCs and pollen grains were taken from freshly prepared temporary slides.

RESULTS AND DISCUSSION

A total of ten accessions were selected for each *Physalis* species and cultivated under controlled conditions. The species displayed

Table 2: List of cytologically worked out species of genus *Physalis* (L.) with chromosome numbers, locality, habit and previous chromosomal number reports

S. No.	Accession Name with number	Locality /Longitude, Latitude	Trade name	Morphology						Chromosomes (Ploidy levels)	Previous Reports	
				Habit	Plant height (cm)	Leaves	Fruit	Flower	Stem		Indian report	World report
1	*P. longifolia* (Nutt.); PLP#22087	Kalka (Haryana)-656 meters	Wild tomatillo, Longleaf groundcherry	Annual herb	120.0–150.0	Opposite, velvety, heart-shaped, entire, wavy, acute, and cordate with dark green color, grows up to 7.0-15cm.	7.0-8.0cm round berry with smooth and glossy Orangish-yellow skin.	Hermaphrodite with five, large, dark brown spots within, 1.0-1.5cm long.	Densely glandular-hairy.	Light yellow-orange	24 (2x)	2x, 4x (Tuteja and Bhatt, 1984)	2x,4x (Hinton, 1976)
2	*P. angulata* (L.); PLP#22085	Hamirpur (H.P.)-738 meters	Mullaca, Cut-leaved ground cherry, Wild-gooseberry	Annual herb	40.0–80.0	Simple, alternate with toothed margins, ovate to oblong, acuminate at the base, grows up to 5.0-10cm.	4.0-5.0 cm, Yellowish-green or Greenish-brown berry.	Zygomorphic (5-petaled), fused calyx inside the corolla, pale yellow with five indistinct brown spots, 0.5-0.6cm.	Erect branched with hollow stems.	Light brown	48 (4x)	2x,4x (Ganapathi *et al.,* 1991, Bir *et al.,* 1978; 1978a)	2x, 4x (Husaini and Iwo, 1990; Lydia and Rao, 1982)
3	*P. peruviana* (L.); PLP#22086	Sirmour (H.P.)-3647 meters	Cape-gooseberry, Peruvian groundcherry	Perennial herb	150.0–170.0	Simple, alternate, elliptic lamina, entire to slightly toothed, cuneate at the base with light green color, grows up to 8.0-16cm.	Dark brown or Greenish brown berry grows up to 5.0-7.0cm.	Greenish-yellow with light brown spots between anthers rotate corolla, 10-12cm long.	Glabrous or sparsely pubescent.	Light brown yellow	48 (4x)	2x,4x (Ganapathi *et al.,* 1991; Rao, 1979; Panda and Rao, 1983)	4x,6x (Quiros, 1984)

The average characteristic features of plants were calculated by using 10 accessions for each species

distinct visual differences, such as brown markings within the calyx throats, prominent glandular hairs, and five-angled fruiting calyces. The observations aligned with known morphological descriptions of economically important species like *P. peruviana*, which is characterized by hermaphroditic yellow flowers with brown blotches, smooth and shiny fruits, and the presence of 100–200 light yellow seeds per fruit. Among the traits assessed such as plant height, calyx length and width, flower count per plant, leaf measurements, and fruit weight of *Physalis longifolia* recorded the highest average number of seeds per fruit (147.0 ± 20.3). On the other hand, *Physalis peruviana* produced the heaviest fruits (1.19 ± 0.20 mg) but had the lowest seed count per fruit. Additional average morphological values are summarized in Table 1.

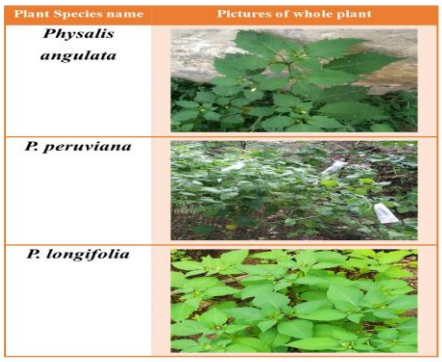

Fig. 1. Morphology of different *Physalis* species.

Meiotic cells (meiocytes) were analysed, and the number of cells observed at various stages of meiosis I and II including metaphase, anaphase, telophase as well as the formation of tetrads, was recorded. Pollen mother cells (PMCs) lacking bivalent chromosome configurations were classified as irregular. Further irregularities observed during the post-metaphase I and II stages included chromosomal stickiness (Fig. 2B), premature and delayed disjunction (Fig. 2D), interconnections between bivalents (Fig. 2F), and instances of chromatin transfer (Fig. 2I). Samples of *Physalis angulata* gathered from various regions in Himachal Pradesh were confirmed to be tetraploid, exhibiting a chromosome count of 2n = 4x = 48 during metaphase I (Fig. 2A, B). These observations are in agreement with earlier studies Bir (1978), Lydia and Rao (1982), Husaini and Iwo (1990)). Unlike some other species, *Physalis longifolia* was found to have a diploid chromosome number of 2n = 2x = 24 in the current cytological analysis. However, a previous study by Tuteja and Bhatt (1994) identified this species as a tetraploid in populations from Varoda, Gujarat. Earlier chromosome counts of n = 12 for *P. longifolia* var. *longifolia, P. pumila* ssp. *pumila*, and *P. pumila* ssp. *hispida* and were also reported by Hinton (1976).

Additionally, *P. peruviana* was found to exhibit a tetraploid chromosome number (n = x = 24) at metaphase I (Fig. 2E). Previous research carried out in India and abroad has documented both tetraploid (2n = 4x = 48) and hexaploidy (2n = 6x = 72) chromosome numbers for this species by Rao (1979), Panda and Rao (1983), Quiros (1984). Table 1 and Figure 2 illustrate various stages of meiosis, along with certain chromosomal abnormalities. Based on information available from the Index to Plant Chromosome Numbers database (accessible at http://mobot.mobot.org/W3T/Search/ipcn.html/), chromosome counts have been documented for 42 species within the genus. These counts range from 2n = 20 to 72, with 2n = 24 being the most frequently reported.

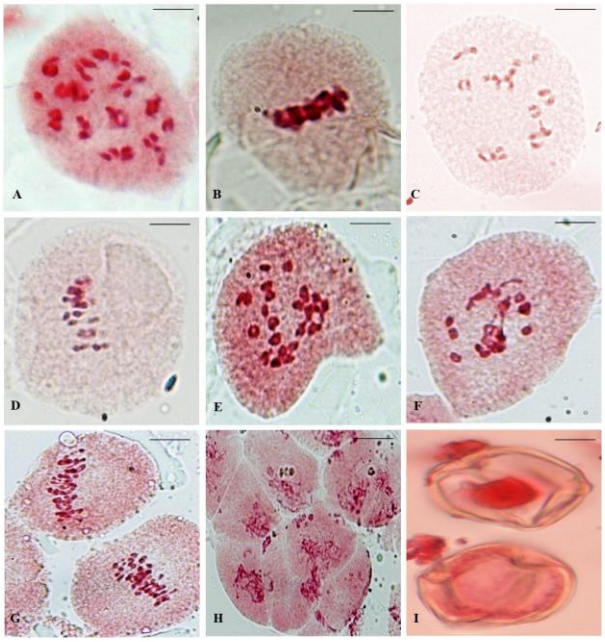

Fig 2. (A) *P. angulata;* PMC with 24_{II} at diakinesis (B) *P. angulata;* Chromosome stickiness (C) *P. longifolia;* PMC with 12_{II} at metaphase (D) *P. longifolia;* Early and Late disjunction (E) *P. peruviana;* PMC with 24_{II} at metaphase (F) *P. peruviana;* Inter-bivalent connections, (G) *P. peruviana;* Early and Late disjunction (H) *P. peruviana;* Chromatin transfer (I) Fertile and sterile pollen grains. Scale bars=10 µm

The chromosome number has been associated with diploid status in several *P. peruviana* populations from different countries. Studies on genetic material from Colombia have reported a chromosome count of 2n = 2x = 24 as

reported by Nohra *et al.* (2006), Liberato *et al.* (2014). Similarly, Azeez *et al.* (2019), Azeez and Faluyi (2019) also confirmed that *P. peruviana* possesses a diploid chromosome complement of 2n = 2x = 24. The genus *Physalis* is known to possess three basic chromosome numbers: x = 10, 11, and 12, with x = 12 being the most frequently observed. In Indian populations, reported chromosome counts include *P. angulata* (2n = 24, 48), Ganapathi *et al.* (1991), Brar and Gupta (2017); *P. longifolia* (2n = 24), Tuteja and Bhatt (1984); and *P. peruviana* (2n = 48, 72), Shibata (1962), Panda and Rao (1983). Azeez *et al.* (2019) reported that *P. angulata* is tetraploid (2n = 4x = 48), while other species such as *P. peruviana*, *P. micrantha*, and *P. pubescens* typically exhibit diploid chromosome numbers (2n = 2x = 24). All four *Physalis* species examined in this study displayed well-paired homologous chromosomes during the pachytene stage, forming either ring bivalents or rod bivalents, with synchronous cytokinesis observed in each case.

AUTHOR CONTRIBUTIONS

Rohita Singla: independently conducted all laboratory experiments as part of her research project, which involved cultivating wild seeds inside green-house, data collection and report preparation, data compilation for manuscript preparation. Due to her significant contribution, she is credited as the first author of this publication. Anjali Sharma: played an active role in coordinating the submission of this research article to the targeted journal. Vivek Sharma: Served as the principal advisor for this research project and provided guidance to the first author in conducting cytomorphological analysis.

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