First Report of Intraspecific Polyploidy (2x, 4x) in *Physochlaina praealta* (Decne.) Miers. (Family: Solanaceae)

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Summary *Physochlaina praealta* (family: Solanaceae), a widely distributed species of Indian cold deserts, is studied for male meiotic studies. We here report for the first time the existence of intraspecific polyploidy (2x, 4x) in the species. Both the 2x and 4x individuals show perfectly normal meiotic behavior and nearly 100% fertile pollen grains. The 4x plants show allopolyploid like meiotic behavior characterized by normal chromosome pairing and regular segregation of chromosomes. The 2x and 4x plants grow under similar type of habitats but the 4x individuals were noticed to be more pubescent. The 4x plants can also be differentiated from the 2x on the basis of micro-characters like trichome (size, density), stomata (size and frequency) and pollen grain size which increases with ploidy.

Key words *Physochlaina praealta*, Intraspecific polyploidy (2x, 4x), Allopolyploid, Trichome, Pollen grain size.

Physochlaina praealta (Decne.) Miers., treated under various synonyms such as, *Hyoscyamus praealtus* (Decne.) Walp, *Physochlaina urceolata* Kuang and A.M. Lu, *Physochlaina grandiflora* Hook., and *Scopolia praealta* (Decne.) Dunal, grows as a tall perennial herb on high dry stony areas between 2660–4800m in India, Tibet and China. In India the species is found in the cold desert regions of Ladakh (Jammu and Kashmir), Lahaul-Spiti and Kinnaur districts of Himachal Pradesh and Nelong Valley in Uttarkashi district of Uttarakhand. The species is characterized by having a fruiting calyx which is contracted at the base and entire leaves. The light yellow colored flowers which are present in panicles appeared during April–September. The whole plant is poisonous and causes mental disturbance in human beings. However, the plant is used locally for preventing soil erosion and walls around local fields on steep slopes. Perusal of cytological literature reveals that the species is known to have a diploid chromosome count of $2n=42$ reported by Gu *et al.* (1993) and Gu and Sun (1996) from China. On the other hand, the Indian plants are known to have an altogether different chromosome count of $2n=82$ reported by Mehra and Sobti (1954) and Vasudevan (1975). Looking at its wide distribution/nature under hostile climatic conditions of dry cold deserts coupled with two different chromosome counts, the present cytological studies have been undertaken on individual plant basis from the phytogeographically differ-

ent and cytologically unworked out cold desert regions of Kinnaur district and Ladakh division. Consequent to cytomorphological explorations, intraspecific 2x and 4x cytotypes have been detected in the species. The aims of the study were (i) to work out the exact chromosome counts on individual plant basis, (ii) to know the distribution pattern of 2x and 4x individuals, (iii) to segregate the cytotypes through morphometric analysis, study in detail the male meiotic course, and (iv) to work out the pollen fertility.

Materials and methods

Plant material

For meiotic and pollen grain studies, floral buds of suitable sizes were fixed in freshly prepared Carnoy's fixative (mixture of alcohol, chloroform and acetic acid in a volume ratio 6:3:1) for 24h and preserved in 70% ethanol in a refrigerator.

Male meiosis and pollen grain analysis

Anthers from the developing floral buds were squashed in 1% acetocarmine and meiocyte preparations were made for detailed meiotic course at different stages of prophase-I, metaphase-I, anaphase-I/II, telophases-I/II and sporads. Pollen fertility was estimated through stainability tests by smearing the mature anthers from different flowers in glycerol–acetocarmine (1:1) mixture. Well-filled pollen grains with stained nuclei were scored as apparently fertile, while shriveled and flaccid pollen grains with unstained or poorly stained

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Fig. 1. a) Photographs of 2x accession of *Physochlaina praealta*. b) Trichome of 2x cytotype. c) Stomata of 2x cytotype. d) Photographs of 4x accession of *Physochlaina praealta*. e) Trichome of 4x cytotype. f) Stomata of 4x cytotype.

cytoplasm as sterile. The plants were identified by comparing them with the specimens lying in the Herbarium, Northern Circle, Botanical Survey of India, Dehra Dun. The duly identified specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN¹ 50967, 53786, 60343, 60344).

Morphometric analysis

The morphometric analysis focused on the size and

frequency of stomata and trichomes, and size of pollen grains. Trichomes and stomatal studies were made from the abaxial epidermal peels obtained from the middle portion of mature leaves through KOH treatment. Thoroughly washed peels were mounted in glycerol. Stomatal index was calculated by using the following formula: $\{SI = [S/(S+E)] \times 100\}$, where SI stands for stomatal index, S is the number of stomata and E is the number of epidermal cells. Pollen grain size was measured through micrometry.

¹ PUN is the Herbarium Code of Department of Botany, Punjabi University, Patiala as per Index Herbariorum by Holmgren and Holmgren (1998)

Fig. 2. Meiosis in *Physochlaina praealta*. a) A PMC showing 21 bivalents at M-I. b) A PMC showing 21:21 distribution of chromosomes at A-I. c) Pollen grains of $2x$. d) A PMCs showing 42 bivalents at M-I. e) A PMC showing 42:42 equal distribution of chromosomes at A-I. f) A PMC showing cytomixis at early prophase (arrowed). g) Heterogeneous sized pollen grains of $4x$ cytotype. Scale bar= $10\mu\text{m}$.

Results

While exploring dicotyledonous flora for cytological studies from the cold desert regions of Kinnaur district (Himachal Pradesh) and Ladakh division (Jammu and Kashmir), we have reported the existence of $2x$ and $4x$ plants in *Physochlaina praealta* for the first time from India. Outside of India only diploid cytotype with $n=21$ is known from China (Gu *et al.* 1993, Gu and Sun 1996). The plants examined from Ladakh division (Jammu and Kashmir) exist at tetraploid level ($n=42$), while plants studied from Kinnaur district (Himachal Pradesh) depicted a chromosome count of $n=21$. The detailed study covering cytology and morphometric analysis including distribution of two cytotypes are given as below.

Cytology

The diploid ($n=21$)

The male meiotic studies carried out on the wild plants collected from Namgia Village (3050 m) and Nako Lake (3660 m) in Kinnaur district (Himachal Pradesh)

depicted the diploid chromosome count of $n=21$ as confirmed from the presence of 21 bivalents at M-I (Fig. 2a) and 21:21 chromosomal segregation at A-I (Fig. 2b). The sporad formation in both the accessions was found to be perfectly normal resulting into 100% pollen fertility and uniform sized pollen grains (Fig. 2c).

The tetraploid ($n=42$)

The plants collected from the Sankoo Village (3020 m) and Panikhar Village (3150 m) in Ladakh division (Jammu and Kashmir) were found to exist at tetraploid level with a meiotic chromosome count of $n=42$ as confirmed from the presence 42 bivalents at M-I (Fig. 2d) and 42:42 equal segregation of chromosomes at A-I (Fig. 2e). Further meiotic course including sporad formation in such $4x$ individuals was perfectly normal leading to very high pollen fertility (94–96%). However, a few PMCs in the accession from Panikhar Village (3150 m) showed the phenomenon of chromatin transfer during the early stages of Prophase-I (Fig. 2f). Also, the fertile pollen grains in the accession were noticed to be of heterogeneous size (Fig. 2g).

Morphometric analysis and distribution

Both the diploid and tetraploid individuals grow in the same type of habitats under cold desert conditions between 2460–4800 m altitudes. The individuals of 2x and 4x cytotypes also look alike except that 4x plants were noticed to be more pubescent than the 2x (Fig. 1a and 1d). Detailed analysis revealed that trichome frequency per 1 cm² of leaf surface (Table 1) shows significantly higher numbers of trichomes in 4x (4.06 ± 1.60) compared to 2x (1.47 ± 0.69). Also, the trichomes in the 4x plants were noticed to be significantly larger ($184.23 \pm 26.43 \mu\text{m}$; Fig. 1e) compared to the diploid ($126.3 \pm 35.68 \mu\text{m}$; Fig. 1b). The two cytotypes could also be differentiated in other micro-characters like stomata and pollen grains. Stomata are significantly large sized in the 4x cytotype ($36.94 \pm 2.40 \times 31.16 \pm 2.41 \mu\text{m}$) compared to those in the 2x ($32.20 \pm 1.78 \times 27.37 \pm 2.41 \mu\text{m}$) (Figs. 1c and 1f). The fertile pollen grains in 4x individuals were noticed to be of three types (Fig. 2g) as, large ($46.96\text{--}52.68 \times 41.34\text{--}51.73 \mu\text{m}$, 29.84%), typical ($38.14\text{--}43.86 \times 39.34\text{--}43.47 \mu\text{m}$, 65.74%) and small ($25.52\text{--}39.91 \times 23.15\text{--}32.08 \mu\text{m}$, 4.42%) as compared to the 2x individuals where pollen grains are of uniform size ($39.44\text{--}49.95 \times 38.14\text{--}41.69 \mu\text{m}$; Fig. 2c). As far as the stomatal density is concerned, the values are significantly higher in the 2x individuals compared to 4x (Table 1). On the other hand, stomatal index in the two cytotypes are not significantly different.

Discussion

Polyploidy has long been recognized as a major evolutionary force driving both speciation and diversifica-

tion. Ever since the first report of natural polyploids in *Oenothera lamarckiana* (Lutz 1907, Gates 1909), several reviews on polyploidy were published from time to time (Müntzing 1936, Darlington 1937, Löve and Löve 1949, Stebbins 1950, 1971, Lewis 1980, Grant 1981, Chen and Birchler 1995, Levin 2002, Wendel and Doyle 2005, Clausen *et al.* 2010, Soltis *et al.* 2014). The estimates about frequency of polyploidy given by different workers varies considerably depending upon the criteria adopted. The role of polyploidy in the evolution of flowering plants is also evident from the fact that there are numerous cases where intraspecific polyploidy cytotypes are known at different levels. In the members of Solanaceae, we here add one more case of intraspecific polyploidy (2x, 4x) in *Physochlaina praealta*.

In general, polyploids are either classified as auto- or allopolyploids largely based on the mode of origin. Autopolyploids originate from the polyploidization event within or between populations of a single species, whereas allopolyploids are the result of a hybridization event between different species. The polyploids are also categorized as auto- or allopolyploids on the basis of chromosome pairing and gametic fertility. On the basis of chromosome pairing the 4x individuals behaved like an allopolyploid. Such allopolyploids have generally been considered to be much more common than autopolyploids mainly due to their adoptive superiority involving perfect chromosome segregation and gametic fertility. Intraspecific polyploids in plants were thought to have been evolved either through somatic gametic duplication (somatic polyploidization) or through the formation of 2n gametes (diplogametes) involving different mechanisms such as meiotic restitution (Bretagnolle

Table 1. Site of collection and comparison of microscopic characters in the two cytotypes of *Physochlaina praealta*.

S. No.	Characters/Site of collection	Cytotype	
		Diploid (n=21)	Tetraploid (n=42)
1.	Site of collection	Namgia Village (3050 m) Nako Lake (3660 m)	Sankoo Village (3350 m) Panikhar Village (3250 m)
2.	Distribution	Less common	More common
3.	Habit	Perennial herb	Perennial herb
4.	Habitat	In dry stony areas	Along road sides and dry stony areas
5.	Flowering period	April–September	April–September
6.	Stomatas Size (μm)	$29.10\text{--}35.58 \times 23.66\text{--}30.77$ (32.20 ± 1.78) $\times$ (27.37 ± 2.41)	$33.42\text{--}40.53 \times 26.31\text{--}35.46$ (36.94 ± 2.40) $\times$ (31.16 ± 2.84)
	Density (1 cm ²)	$11.57\text{--}15.78$ (14.10 ± 1.76)	$4.21\text{--}9.47$ (6.31 ± 1.33)
	Stomatal index	$24.24\text{--}38.23$ (30.13 ± 4.56)	$23.68\text{--}35.71$ (28.60 ± 3.87)
7.	Trichomes Size (μm)	$91.88\text{--}199.6$ (126.3 ± 35.68)	$142.1\text{--}201.5$ (184.23 ± 26.43)
	Density (1 cm ²)	$1.05\text{--}3.15$ (1.47 ± 0.69)	$2.10\text{--}6.31$ (4.06 ± 1.60)
8.	Pollen size (μm)	$39.44\text{--}42.95 \times 38.14\text{--}41.69$ (41.16 ± 1.18) $\times$ (39.88 ± 1.23)	Large $46.96\text{--}52.68 \times 41.34\text{--}51.73$ (50.21 ± 2.07) $\times$ (47.12 ± 3.15) Typical $38.14\text{--}43.86 \times 39.34\text{--}43.47$ (41.34 ± 1.69) $\times$ (41.57 ± 1.52) Small $25.52\text{--}39.91 \times 23.15\text{--}32.08$ (29.48 ± 2.61) $\times$ (28.02 ± 2.46)
9.	Pollen fertility	100%	94–96%

and Thompson 1995, Andreuzza and Siddiqi 2008, Dewitte *et al.* 2010), abnormal spindle activity (Rana *et al.* 2013) or as a product of pre-meiotic fusion of meiocytes/whole chromatin transfer during cytomixis (Singhal and Kumar 2008, 2010, Kim *et al.* 2009, Singhal *et al.* 2011, 2012, Kumar and Singhal 2012, De Storme and Geelen 2013).

In *Physochlaina praealta*, the intraspecific 4x cytotype shows typical allopolyploid meiotic behavior depicting regular chromosome pairing and segregation, resulting into normal sporads and high pollen fertility. There are numerous reports in literature where intraspecific polyploid cytotypes exist in nature and polyploid individuals show normal diploid-like behavior. Genome duplication (auto- or allopolyploids) has played an important part in speciation through shaping phenotype and ecological diversity, altering habitat use, life histories, competitive abilities, interaction with herbivores and pathogens and pollinator mediated reproduction (Thompson *et al.* 2004, Oswald and Nuismer 2007, Thompson and Merg 2008, Arvanitis *et al.* 2010, Boalt *et al.* 2010, Lavanaia 2015, Ramsay and Ramsay 2014), and it has been observed that changes in morphological characters in polyploids having multiple set of chromosomes are often associated with plant vigor and ability to different environments. In the present case the 4x individuals not only are more pubescent but also possess significantly larger stomata, trichomes and pollen grains. All these factors could enable polyploids to be more competitive and potentially more invasive in a new environment (Pandit *et al.* 2011, 2014). Such an increase in the size of various vegetative and reproductive parts including micro-characters like stomata, trichome and pollen grains in the 4x individuals seems to be directly correlated with polyploidy and has been reported in a number of species, such as *Terminalia chebula* (Gill *et al.* 1982), *Syzygium cumini* (Gill *et al.* 1991), *Centaurea stoebe* (Spaniel *et al.* 2008, Mráz *et al.* 2011), *Ranunculus hirtellus* (Kumar and Singhal 2011), *Galinsoga parviflora* (Bala *et al.* 2011), *Plantago depressa* (Bala and Gupta 2011), *Spergularia diandra* (Kaur and Singhal 2012), *Agrimonia eupatoria* (Kumar *et al.* 2011, 2014a), *Silene vulgaris* (Kumar *et al.* 2014b), and *Tordyliopsis brunonis* (Kumar *et al.* 2014c).

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