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Cover: *Saproamanita praeclara*: Sporocarp in habitat © Kantharaja. R.



Cytotaxonomy and palynology study of some weed species from the state of Punjab, India

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Abstract: The present study was conducted in Malwa region of Punjab, India in 2019–2020. A total of 10 weed species belonging to seven genera and four families were recorded from different crops. Meiotic analysis has revealed the chromosome numbers in different weed species as *Datura innoxia* (n= 12), *Erigeron bonariensis* (n= 13), *Nicotiana plumbaginifolia* (n= 10), *Physalis angulata* (n= 24), *Sesbania bispinosa* (n= 6), *Sida cordifolia* (n= 8), *Solanum americanum* (n= 12), *Solanum nigrum* (n= 36), *Solanum villosum* (n= 24), and *Solanum virginianum* (n= 12). Chromosome numbers of *Solanum americanum* and *S. villosum* have been worked out for the first time from the state of Punjab, India. Morphological features along with the chromosome numbers have authenticated the identity of weed species. Similarly, pollen fertility analysis has suggested the potential of seed production by the weed species and their subsequent invasiveness.

Keywords: Angiosperm, invasion, morphology, meiosis, pollen, taxonomy.

Punjabi: ਮੌਜੂਦਾ ਅਧਿਐਨ 2019-2020 ਦੌਰਾਨ ਪੰਜਾਬ, ਭਾਰਤ ਦੇ ਮਾਲਵਾ ਖੇਤਰ ਵਿੱਚ ਕੀਤਾ ਗਿਆ ਸੀ। ਵੱਖ-ਵੱਖ ਫਸਲਾਂ ਤੋਂ 7 ਪੀੜ੍ਹੀਆਂ ਅਤੇ 4 ਪਰਿਵਾਰਾਂ ਨਾਲ ਸਬੰਧਤ ਕੁੱਲ 10 ਨਦੀਨਾਂ ਦੀਆਂ ਕਿਸਮਾਂ ਦਰਜ ਕੀਤੀਆਂ ਗਈਆਂ ਸਨ। ਮੀਓਟਿਕ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਵੱਖ-ਵੱਖ ਨਦੀਨ ਪ੍ਰਜਾਤੀਆਂ ਵਿੱਚ ਕ੍ਰੋਮੋਸੋਮ ਨੰਬਰਾਂ ਦਾ ਖੁਲਾਸਾ ਕੀਤਾ ਹੈ ਜਿਵੇਂ ਕਿ ਦਤੁਰਾ ਇਨੋਕਸੀਆ (n=12); ਐਰੀਗੇਰੋਨ ਬੋਨਾਰੀਅਨਸਿਸ (n=13); ਨਿਕੋਟੀਆਨਾ ਪਲੰਬੇਜੀਨੀਫੋਲੀਆ (n=10); ਫਿਸਾਲਿਸ ਅੰਗੁਲਤਾ (n=24); ਸੇਸਬਾਨੀਆ ਬਿਸਪੀਨੋਸਾ (n=6); ਸਿਡਾ ਕੋਰਡੀਫੋਲੀਆ (n=8); ਸੋਲਾਨਮ ਅਮਰੀਕਨਮ (n=12); ਸੋਲਾਨਮ ਨਿਗਮ (n=36); ਸੋਲਾਨਮ ਵਿਲੋਸਮ (n=24) ਅਤੇ ਸੋਲਾਨਮ ਵਰਜੀਨੀਅਨਮ (n=12)। ਸੋਲਾਨਮ ਅਮਰੀਕਨਮ ਅਤੇ ਸੋਲਾਨਮ ਵਿਲੋਸਮ ਦੇ ਕ੍ਰੋਮੋਸੋਮ ਨੰਬਰ ਪਹਿਲੀ ਵਾਰ ਭਾਰਤ ਦੇ ਪੰਜਾਬ ਰਾਜ ਤੋਂ ਤਿਆਰ ਕੀਤੇ ਗਏ ਹਨ। ਕ੍ਰੋਮੋਸੋਮ ਨੰਬਰਾਂ ਦੇ ਨਾਲ ਰੂਪ-ਵਿਗਿਆਨਕ ਵਿਸ਼ੇਸ਼ਤਾਵਾਂ ਨੇ ਨਦੀਨ ਪ੍ਰਜਾਤੀਆਂ ਦੀ ਪਛਾਣ ਨੂੰ ਪ੍ਰਮਾਣਿਤ ਕੀਤਾ ਹੈ। ਇਸੇ ਤਰ੍ਹਾਂ ਪਰਾਗ ਕਣਾਂ ਦੀ ਉਪਜਾਊ ਸ਼ਕਤੀ ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਨਦੀਨਾਂ ਦੀਆਂ ਕਿਸਮਾਂ ਦੁਆਰਾ ਬੀਜ ਦੇ ਉਤਪਾਦਨ ਦੀ ਸੰਭਾਵਨਾ ਅਤੇ ਉਹਨਾਂ ਦੇ ਬਾਅਦ ਦੇ ਧਾੜਵੀਪਣ ਦਾ ਸੁਝਾਅ ਦਿੱਤਾ ਹੈ।

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Author contributions: RS—field survey, collection, laboratory practice and prepared a manuscript. MCS—review the manuscript and suggested some corrections. After corrections, the present manuscript is finalized by both the authors.

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INTRODUCTION

Angiosperms are one of the major group of plants with around 2,95,383 species in the world and 18,666 in India (Christenhusz & Byng 2016; Dash & Kumar 2019). Flowering plants supported various life forms including human for various basic needs including food, fodder, shelter, and medicine. Various angiosperm species are growing as weeds both in rabi and kharif crops. According to Dangwal et al. (2010), weed is a plant that competes with crop plants for nutrition, space, and light. Subsequently, they affect the quality and yield of the concerned crops. Weed species have high potential to grow even under unfavorable conditions. They develop some special modifications such as thick cuticle, sunken stomata, and waxy coating to retain water during drought conditions (Ram & Gupta 1997).

Identity of weed species can be established using different tools including morphology, cytology, palynology, phytochemistry, and molecular. Being simple and cost effective, morphological study is still commonly practiced for species identification. Morphological features like leaf shape and color, flower type and color, number and position of stamens, number and structure of gynoecium, shape and type of fruit and seeds are used for identification of species (Rieseberg 1992; Singh & Dey 2005).

Meiotic study has provided information about the number and arrangement of chromosomes which can be used for identification of species. According to Raven (1975) different species of the same genus may show variation in chromosome numbers. The chromosomal changes are also useful in taxonomic and evolutionary studies of plants (Jones 1979; Kaur & Sidhu 2014). Palynology deals with study of pollens and spores. The pollen fertility is directly related to seed production and yield. The more pollen fertility, the more chances of pollination and production of seeds. Acetocarmine staining method is simple and the most suitable to study the pollen fertility (Gaaliche et al. 2013).

The present study was conducted in Malwa region of Punjab. Earlier, Sharma & Bir (1978), Meenakshi & Sharma (1985), Sharma et al. (1987), Sharma (1990), Sidhu (1991), and Singh & Singh (2019) documented the angiosperm diversity in general and weed diversity in particular. Keeping this in view, the present study was planned to establish the identity of selected weed species based on morphological, cytological, and palynological studies.

MATERIALS AND METHODS

Study area

The state of Punjab covers an area of 50,362 km² and located in northern India. It is divided into Majha, Malwa, and Doaba zones. During present study, weeds were recorded from kharif and rabi crops growing in Malwa region including Bathinda, Faridkot, Fazilka, Ferozpur, Moga, and Muktsar in the years 2019 and 2020. Malwa is the largest zone, where temperature varies from 3°C to 47°C and average rainfall ranges 480–960 mm. Weed species were recorded from kharif crops (Cotton, Maize, Sorghum, Sugarcane, Rice) and rabi crops (Berseem, Gram, Mustard, Sugarcane, Wheat). Sugarcane crop was considered both in rabi and kharif season.

Morphological study

Various leaf morphological features, like arrangement, shape, type, color, stem, flower colour, shape of stamens and shape of stigma were analyzed for identification of species. The collected weed species were identified on the basis of available literature such as Hooker (1872–1897), Bamber (1916), Nair (1978), and Singh & Singh (2019). Specimens from Herbarium, Botany Department, Panjab University Chandigarh and online herbaria were also consulted for identification of weed species.

Meiotic study

A meiotic study was carried out to determine the numbers and behavior of different weed species chromosomes. Young flower buds of different growth stages were collected from the study area and fixed (ethanol 3: glacial acetic acid 1) for 1–2 days then transferred to 70% ethanol till further use. Anthers were excised and squashed in drop of acetocarmine (1%) on the glass slide. Debris was removed and rest of the material was covered with a micro cover slip. After gently heating, the slide was pressed in two folds of filter paper by putting thumb pressure for spreading of the cells and to remove air bubbles. Slides were observed under the microscope and photographs of the cells with countable chromosomes were taken.

Pollen study

Pollen fertility of 100–150 pollen grains of each species was analyzed by stainability tests. Mature anthers were taken on the glass slide and squashed in glycerol acetocarmine (1:1) mixture. The slide was observed under the microscope after 24 hours. The stained pollen was considered fertile whereas poorly

stained/ unstained pollens were considered as sterile. (Pollen fertility= (Fertile pollen / Total pollens observed) x 100).

Photography and herbarium preparation

Field photographs of the weed species were clicked in natural habitats preferably during flowering or fruiting season. After collection, different plant materials were processed for herbarium preparation. After complete drying, the plant specimens were mounted on the herbarium sheets. Herbarium specimens were deposited in the Herbarium, Department of Botany, Panjab University Chandigarh (PAN No. 21986–21995).

RESULTS AND DISCUSSION

A total of 10 weed species belonging to seven genera and four families were documented and collected from different kharif and rabi crops in Malwa region of Punjab, India in 2019–2020. Morphological, cytological, and palynological details were worked out. These plant species were identified primarily based on their morphological features. Different plant groups or families or genera may have a unique basic chromosome number. Keeping this in view, species have been analyzed for their chromosome number to further strengthen the morphological identification. According to Sidhu et al. (2011) basic chromosome number informs about polyploidy and evolution of species. Palynological study has been conducted to record the percentage of fertile pollens. The seed setting percentage depends upon the pollination which is related to the amount of fertile pollen.

Erigeron bonariensis Linn. (Syn. *Conyza bonariensis* (L.) Cronquist) ... Asteraceae (Image 1b)

It is a perennial tall herb. The hairy stem is erect and rarely branched. Leaves are green, linear, narrow, lanceolate, hairy, and green. Inflorescence shows profuse branching and bear numerous heads (Capitulum). Each head consists of outer ray florets and central disc florets. Fruit is papose with linear and yellowish-brown. The course of meiosis was regular anaphase-II (13-13-13-13) (Image 2b). Gupta & Gill (1983) also worked out similar number of chromosomes ($n=13$). Pollen size range $18.75 \times 16.25\text{--}23.75 \times 21.25 \mu\text{m}$ with pollen fertility of 89.36% (Image 3b).

Sesbania bispinosa (Jacq.) W. Wight ... Fabaceae (Image 1e)

It is an erect annual plant having green, branched stem bearing spines. Leaves are pinnately compound, alternate, green; leaflets many, narrow, linear, and oblong with round apex. Racemes with bisexual and complete 4–8 flowers. Sepals are 5, green, hairy; Petals are 5, yellow, unequal; Stamens 10, diadelphous; Stigma 1, style long and curved. Pods are long, narrow linear with numerous seeds. Seeds are linear long, shining and brown to black. Meiotic study has witnessed six bivalents at metaphase-I (Image 2f). The chromosome count is similar to the earlier reports of Parihar & Zadoo (1987) and Jahan et al. (1994). Pollen size varies between $20 \times 18.75 \mu\text{m}$ to $22.5 \times 21.25 \mu\text{m}$ (Image 3e) and pollen fertility 91.66%.

Sida cordifolia Linn ... Malvaceae (Image 2f)

The plant body erect, branched, annual to perennial herb with woody base. Hairy leaves are alternate, ovate oblong to cordate with long petiole. Flowers are bisexual, axillary with small pedicel. Sepals 5, green and hairy, Petals 5, light yellow. Fruit with 5–8 mericarps with long awns. Seeds are ovoid or trigonous and brown to black in color. Chromosomal segregation was normal at anaphase-I (8-8) (Image 2g). Similar chromosome number ($2n=2x=16$) was also studied by Kumar et al. (2012) that supported the present chromosome analysis. Pollen size ($66.25 \times 62.5\text{--}73.75 \times 71.25 \mu\text{m}$) (Image 3f) of this species is maximum but pollen fertility comparatively less (61.16%) than the other recorded species.

Datura innoxia Mill ... Solanaceae (Image 1a)

The herbaceous plant has dichotomous branches. Leaves are ovate lanceolate to broad ovate, unequal at the base. Flowers large, white or dirty white, funnel-shaped with equal number of sepals and petals (5). Stamens 5, epipetalous; stigma 1 with long style. Capsule nodding, covered with green spines. Numerous, small seeds are brown to black in colour. Meiotic preparations have shown the presence of 12 bivalents at metaphase-I (Image 2a). El-Twab et al. (2010) reported $2n=2x=24$ chromosomes in this species from Egypt which tallies with the present study. It has shown the genetic stability of this species across the boundaries in term of chromosome number. Pollen size of this species is $48.75 \times 46.25\text{--}51.25 \times 47.5 \mu\text{m}$ (Image 3a). Pollen fertility of this species is 87.05%.

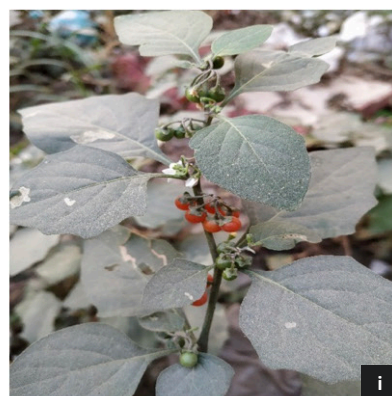
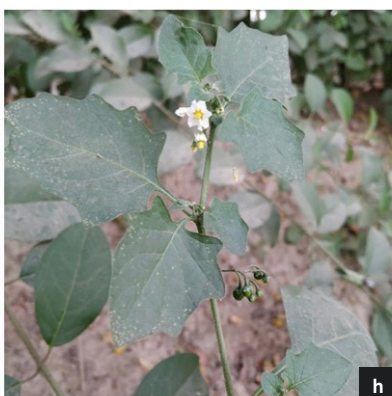
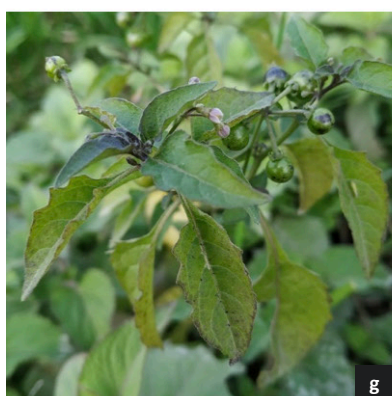
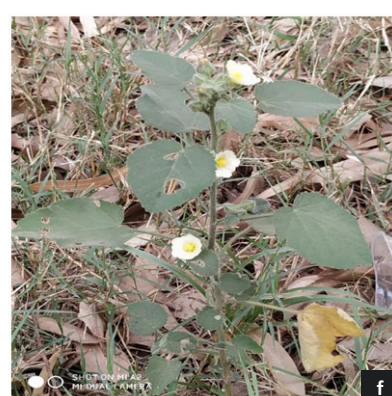
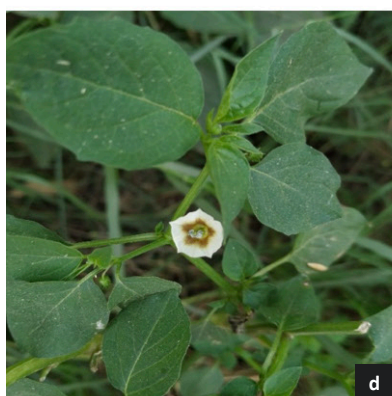
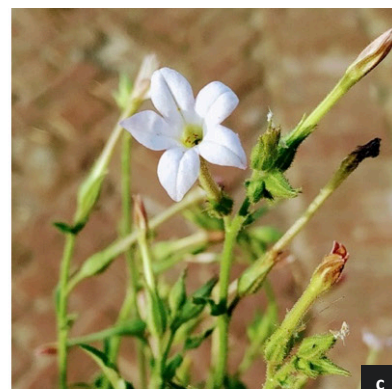
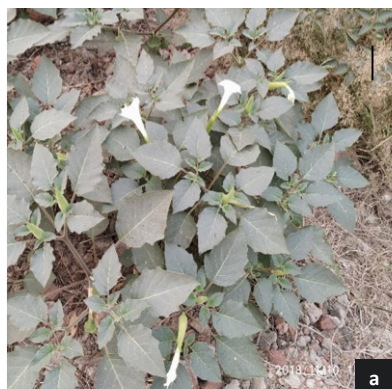


Image 1a-j. Habitat of weed species: a—*Datura innoxia* | b—*Erigeron bonariensis* | c—*Nicotiana glauca* | d—*Physalis angulata* | e—*Sesbania bispinosa* | f—*Sida cordifolia* | g—*Solanum americanum* | h—*Solanum nigrum* | i—*Solanum villosum* | j—*Solanum virginianum*. © Rai Singh.

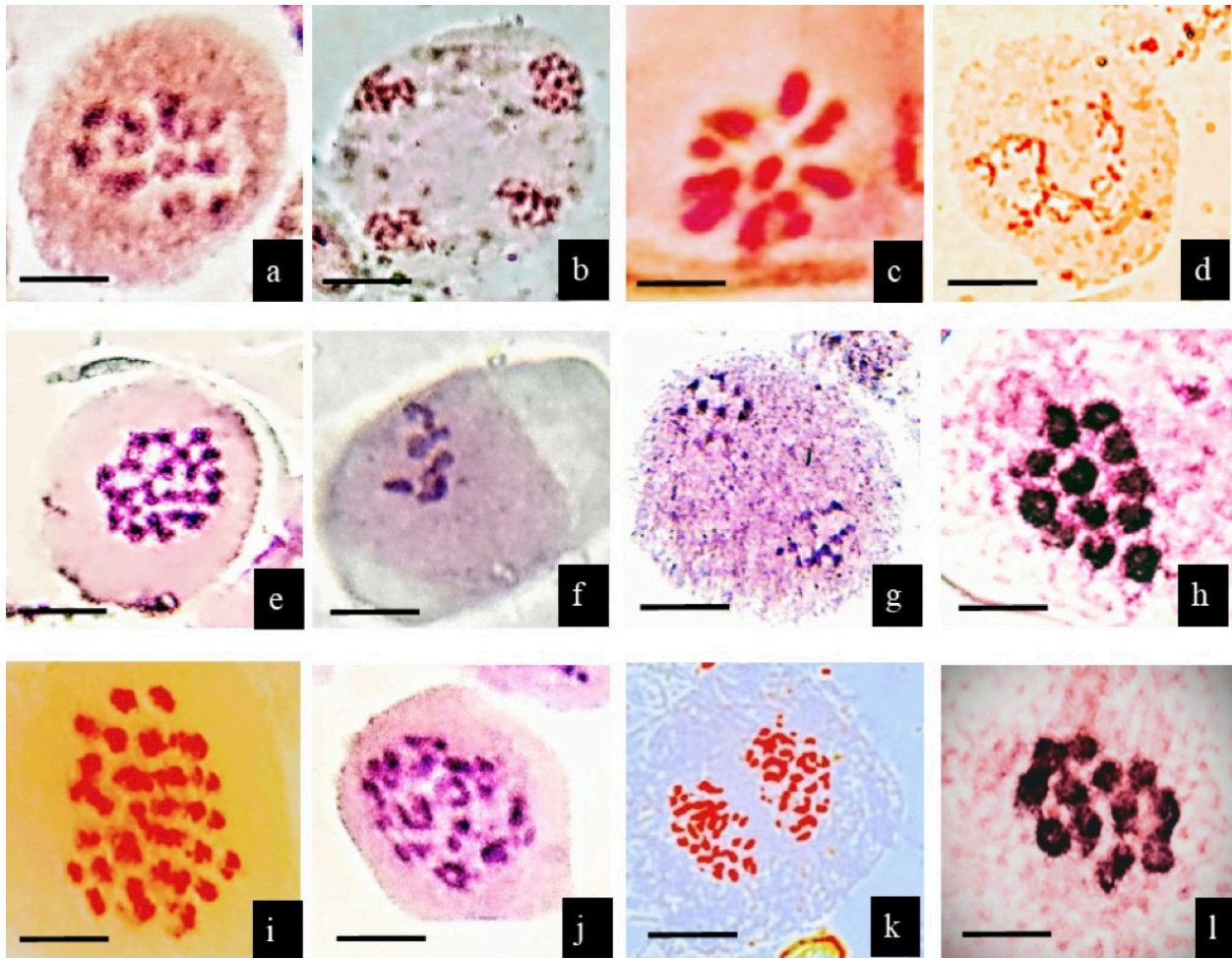


Image 2a–l. Chromosomal details of weed species: a—*Datura innoxia* (n= 12) | b—*Erigeron bonariensis* (n= 13) | c–d—*Nicotiana plumbaginifolia* (n= 10) | e—*Physalis angulata* (n= 24) | f—*Sesbania bispinosa* (n= 6) | g—*Sida cordifolia* (n= 8) | h—*Solanum americanum* (n= 12) | i—*Solanum nigrum* (n= 36) | j–k—*Solanum villosum* (n= 24) | l—*Solanum virginianum* (n= 12). (Scale= 10 μ m). © Rai Singh.

***Nicotiana plumbaginifolia* Viv ... Solanaceae (Image 1c)**

It is an annual erect herb. Stem is branched, slender, herbaceous at young stage then turns woody at maturity. Leaves are simple, alternate, and ovate to elliptic or lanceolate. Lower leaves form a rosette at the base and these leaves are comparatively larger than the upper ones. Flowers are bisexual, tubular, complete, creamy white, pale yellow to purplish in colour. Fruits are small, round berries. Seeds are small, round, many, and brown to black in colour. Meiotic analysis has revealed the presence of 10 bivalents at diakinesis and metaphase-I (Image 2c,d). Chromosomal stickiness has also been observed in some pollen mother cells. Kaur et al. (2015) also reported similar chromosome number ($2n= 20$) for this species from Rajasthan, India. Size of pollen grains of this species ranges $20 \times 18.7\text{--}23.75 \times 21.25 \mu\text{m}$ (Image 3c) and pollen fertility 92.85%.

***Physalis angulata* Linn ... Solanaceae (Image 1d)**

It is a common herbaceous prostrate or erect weed of paddy fields. The stem is woody green possessing petiolate, ovate to lanceolate and green leaves. Flowers yellow, bisexual, complete and solitary. Fruit is berry, globose, green and enclosed within the enlarged, reticulate veined calyx. Seeds are numerous, small, and yellowish-white. The meiotic analysis has described 24 bivalents at metaphase-I (Image 2e). Similar chromosome number ($2n= 4x= 48$) were also reported by Azeez et al. (2019) from Nigeria. Their study has supported the presently studied chromosome number and suggested the conservation of chromosome number of this species in different parts of the world. Pollen size vary from $17.5 \times 16.25 \mu\text{m}$ to $20 \times 18.75 \mu\text{m}$ (Image 3d) and pollen fertility is 75.24%.

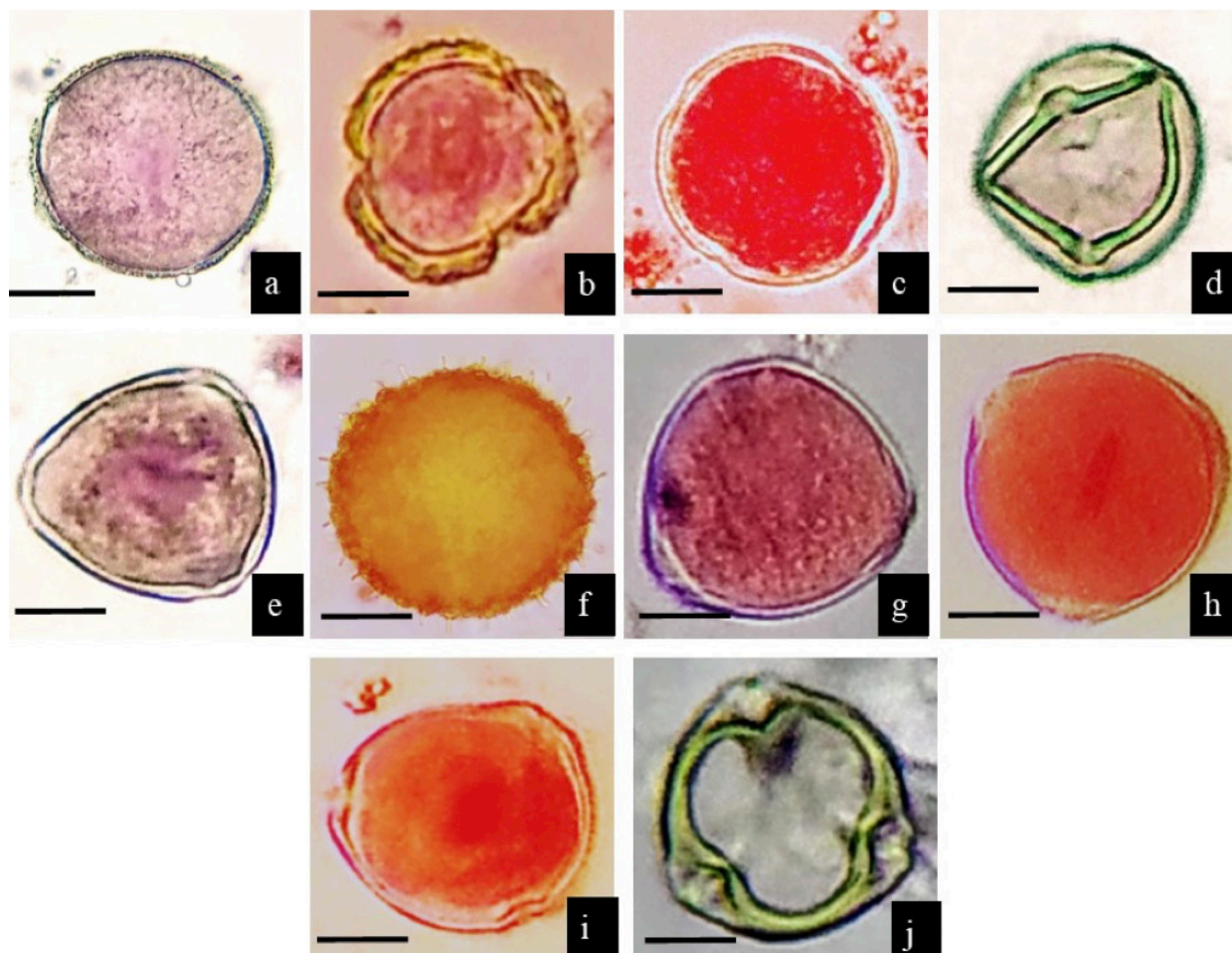


Image 3a-j. Pollen size and viability: a—*Datura innoxia* | b—*Erigeron bonariensis* | c—*Nicotiana plumbaginifolia* | d—*Physalis angulata* | e—*Sesbania bispinosa* | f—*Sida cordifolia* | g—*Solanum americanum* | h—*Solanum nigrum* | i—*Solanum villosum* | j—*Solanum virginianum*. (Scale 10 µm). © Rai Singh.

***Solanum americanum* Mill ... Solanaceae (Image 1g)**

The plant is an annual erect, branched and glabrous herb. Stem is herbaceous during early stages and becomes woody in mature plants. Leaves are alternate, ovate or ovate-lanceolate, glabrous, thin, apex sub-acute or acuminate and light green. White flowers possess equal number of sepals and petals (5); stamens 5; stigma 1. Fruits are berries globose, green, and purplish-black at maturity. Seeds are numerous, disc shaped and yellow. Pollen mother cells contain 12 bivalents at metaphase-I (Image 2h). Ganapathi & Rao (1986) suggested that *Solanum americanum* Mill possess small flowers; globose, bluish-black fruit, and chromosome number $2n = 24$. Size of ranges $21.25 \times 20-23.75 \times 21.25 \mu\text{m}$ (Image 3g) and pollen viability is 93.65%.

***Solanum nigrum* L ... Solanaceae (Image 1h)**

It is an erect, branched, glabrous annual herb. The stem bear alternate, ovate-lanceolate, glabrous, apex

sub-acute or acuminate and dark green. Flowers are white, sepals (5) and petals (5), stamens 5; stigma 1. Fruits are berries globose, green, and purplish-black at maturity. Seeds are numerous and yellowish-white. Meiotic analysis has revealed 36 bivalents at metaphase-I (Image 2i). Similar chromosome numbers ($2n = 6x = 72$) were also studied by Ganapathi & Rao (1986) and Sidhu & Sharma (2016). Pollen size of this taxa varies $22.5 \times 21.25-28.75 \times 27.5 \mu\text{m}$ (Image 3h) and pollen viability 91.51%.

***Solanum villosum* Mill ... Solanaceae (Image 1i)**

It is an annual, erect, branched, and glabrous herb. The glabrous stem possesses leaves which are alternate with reticulate venation, hairy, ovate with lobed margin and acute apex and dark green. Flowers white, bisexual; sepals and petals 5; stamens 5; stigma 1. Fruits are berries, globose, green, and orange red when mature. Seeds are numerous, yellowish-white in colour. Meiotic

preparations have shown possess 24 bivalents at metaphase-I and equal distribution of chromosomes at anaphase-I (24-24) in some PMCs (Image 2j,k). Ganapathi & Rao (1986) also recorded $2n=4x=48$ chromosome numbers in this tetraploid cytotype having orange red berries. Presently studied chromosome count is also to the tune of the findings of Sidhu & Sharma (2016) in this species. Pollen size ranges $25 \times 22.5\text{--}26.25 \times 25 \mu\text{m}$ (Image 3i) and pollen viability 97.34%.

***Solanum virginianum* L. (Syn. *Solanum xanthocarpum* Schrad. & Wendl.) (Image 1j)**

It is a prostrate herb. Stem is woody at the base, branched and bear spines. Leaves are alternate, elliptic oblong, deeply lobed, dark green and covered with spines. Flowers are bluish-purple, few, in extra-axillary cymes. Berries globose with white lines; green when young and yellow at maturity. Seeds are smooth and brown to black in colour. This species has shown the presence of 12 bivalents at metaphase-I (Image 2l). Pollens are smaller than other studied species ($13.75 \times 12.5\text{--}16.25 \times 13.77 \mu\text{m}$) (Image 3j) and pollen viability is 77.66%.

CONCLUSION

Morphology is one of the most preferred and commonly used tool for identification of plant species. During present investigations, 10 weed species have been described based on morphological features. The morphological characterization of the weed species has been further strengthened by the chromosome study. Morphological characterizations will be useful for researchers in general and taxonomists and plant breeders. Similarly, the chromosomal study recorded species will be useful to understand genetic variations and stability of these species. The pollen fertility related information of the weed species has pointed out towards the productivity, seed setting, and dominance of the weed species.

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