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RESEARCH ARTICLE

CYTOLOGICAL STUDIES IN *SARCOSTEMMA VIMINALE* (L.) R.BR. AND *CEROPEGIA BULBOSA* ROXB FROM RAJASTHAN, INDIA

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ABSTRACT

Main objective of our study is to describe cytological behavior in *Sarcostemma viminale* (L.) R.Br. and *Ceropegia bulbosa* Roxb. from Thar Desert of Rajasthan, India. The family Asclepiadaceae (approx. 290 genera) is a homogeneous complex with complicated floral structure. Little cytological data is available on most of the species in the family and both the plants under study are endangered. The present study deals with *Sarcostemma viminale* (L) R.Br. that is a shrub while *Ceropegia bulbosa* Roxb. var. *bulbosa* and var. *lushii* is a herb of milkweed family. Both plants are xerophytic and show CAM features. They grow in association of other shrubs. The microscopic slides of meristamatic cells were prepared by using the classical method and aceto-carmine staining. 2n=22 was observed in both the plants. Basic chromosome number in the family is frequently X=11. Polyploid taxa are also found in most of the genera in this family.

Key words: Asclepiadaceae, Chromosome no., Mitotic, Meiotic, Rajasthan, *Sarcostemma viminale*, *Ceropegia bulbosa* Roxb.

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INTRODUCTION

Rajasthan, situated at the northwestern part of India is the biggest state. It has a geographical area of 3,42,239 km^2 which constitutes 10.41% area of the country. It lies between 23°4' to 30°11' North latitude and 69°29' to 78°17' East longitude. The huge portion of the state is desiccated and houses the biggest Indian desert-the Thar Desert known as the 'Maru-kantar'. The oldest chain of fold mountains- the Aravali Range splits the state into two geographical zones-desert at one side and forest belt on the other. Only 9.36% of the total geographical region lies under forest vegetation. West of the Aravalli is desert or semi desert, characterized by high wind velocity, high temperature, low relative humidity and thorny vegetation while the area east of the Aravalli shows considerable variations in temperature and rainfall distribution and possesses the tropical type of forest vegetation. The Thar Desert or the Great Indian Desert encompasses about 70% of total landmass of Rajasthan and hence it is identified as the "Desert State of India". Asclepiadaceae (milkweed famiy) is a former plant family now treated as a subfamily (Asclepiadoideae) in the Apocynaceae (Endress and Bruyns, 2000). The former family Asclepiadaceae is included in Apocynaceae according to the APG III system (Angiosperm Phylogeny Group, 2009). The members are mostly herbs and shrubs with white sap comprising about 250 genera and 2000 species some of which are cactus like succulents with reduced leaves.

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Asclepiadaceae is under threat due to destructive collection or habitat degradation, poor seed viability and germination, the family remains distributed from South east Asia, India, Madagascar, Tropical Arabia, Canary Islands, Africa except Mediterranean region, New Guinea and Northern Australia (Bruyns, 2003; Mabberly, 2000; Dyer, 1983). The members of the family have great economic importance. Presence of pollinia is a special character of family, the pollinaria are directly attached to the corpusculum or attached to the corpusculum via caudicles, the pollinia in the pollen sacs are oriented upwardly. The best studied subtribe is the Ceropegiinae (Schumann, 1895) with its dominantly succulent members. Exactly half of the species are known karyologically. The basic chromosome number in the family is x = 11 and literature reveals few number deviating from this such as 10 and 12, in most of the genera polyploid taxa are also found (Albers, 1983). *Sarcostemma* is a genus of at least 35 species. These are commnally known as climbing milkweeds, caustic bushes, moon plant, smolata, khir-khimp and in veda it is known as "soma." The plants are well adapted to xeric conditions of Thar Desert. The flowers have a ring of thick tissue at the base which extends into hollow spherical appendages within the flower corolla (GRIN, 2011). It is more branched, leaves are either absent or highly reduced, grows always in association with *Euphorbia caducifolia* (Shetty and Singh, 1993). The existence of the speices is now under threat due to over exploitation. This herb is used in various diseases such as asthma, swelling, fever and cold, inflammatory infection and gastric problem etc. (Gupta *et al*., 2011).

Ceropegia bulbosa Roxb. var. *bulbosa* is also one of the important and endangered plant of same family. The genus *Ceropegia* as a whole is under threat, owing to either destructive collection or habitat degradation. In India, approximately 50 species are present (Surveswaran *et al*., 2009). Among different species, *Ceropegia bulbosa* is one of the widely distributed species but still threatened (Yadav and Kamble, 2008). *Ceropegia bulbosa* is used to improve defense mechanism, tubers are used in the treatment of kidney stone, urinary tracts diseases and they are eaten by ladies to enhance fertility and viability. Two varieties are found locally, the broad leaved variety is known as *Ceropegia bulbosa* Roxb. var. *bulbosa* and the variety having thin and long leaves is *Ceropegia bulbosa* Roxb. var. *lushii* (Arora and Meena, 2016). Tuberous roots of the plant are edible and contains an alkaloid cerpegin (1, 1-dimethyl-5H-furo[3,4-c]pyridine-3,4-dione) and other components that form important ingredients in several conventional drug preparations (Mabberley, 1978). Cerpegin is known to possess analgesic properties (Sukumar *et al*., 1995). Recent studies report the antiurolithic activity of *Ceropegia* (Khan and Pradhan, 2012). Chromosomes have been considered as sources of valid taxonomic criteria. The classification of a number of families has been aided or substantiated by information on chromosome criteria or features (Moore, 1978; Jackson, 1984). At the generic level and below, the chromosomes provided a range of cytological possibilities for understanding the limitations, affinities and evolution of taxa (Badr *et al*., 2009; Kamel *et al*., 2009; Melahat *et al*., 2011; Tabur *et al*., 2012). Chromosome size determination and chromatin condensation pattern analysis, addressing of centromere locality (primary constriction) on chromosome is an essential part of the karyotype construction, that is one of the most important works in cytogenetic studies (Kulak *et al.*, 2002). The karyotype data also appears to be of taxonomic value in providing a logical basis for the redistribution of genera in tribes (Gregory, 1941). Karyotype studies were principally based on the bases that symmetrical karyotypes are more primitive than asymmetrical ones, longer chromosomes than shorter ones, median centromeres with chromosome arms of equal length were more primitive than chromosomes with arms of unequal length, low basic numbers had given rise to higher ones.

These features are based on the comparison between karyotypes of known relative antiquity, as determined through classical taxonomy (Sharma, 1990). The succulent Old World representatives of the genus *Sarcostemma* so far have been found to possess a base chromosome number of x = 11 (Sarkar *et al*., 1978; Liede and Meve, 1989). In the New World both x = 11 and x = 10 seems to occur. The Galapagos species *S. angustissima* (Anderss.) *R.* Holm has been reported to possess 2n = 22 chromosomes (Kliphuis, 1977) and *S. crispum* Benth. from the southwestern United States has been found to be tetraploid 44. *S. clausum Schultes, S. cynanchoides* Decne*, S.* cf. *odoratum (*Hemsl*.)* R. Holm and *S. pannosum* Decne have been found to possess 2n = 20 chromosomes. Cytotaxonomically, the best-studied subtribe is Ceropegiinae, which is, with few exceptions, well characterized by pollinia with a special germination area and winged caudicles (Schill and Jackel, 1978). It is represented in southern Africa by members of the genera *Orthanthera* Wight, Brachystelma R. Br., *Dichaelia* Harvey, *Ceropegia* L., *Riocreuxia* Decne. In this group karyological work was started by Reese (1971). Albers (1983) has reported tetraploidy in *Ceropegia woodii*. The present study is focused on analysis of mitotic and meiotic behavior to justify its placement and relationship with other taxa.

MATERIALS AND METHODS

Germplasm was collected from natural habitat of xeric conditions of Thar Desert. Somatic chromosome studies were carried out using root tips excised from in vitro germinating seed on bright sunny days between 8:30 am to 9:30 am. Root tips were washed in tap water and fixed in Carnoy's fluid (1: 3 acetic-alcohol). The fixative was changed at an interval of two days. One drop of ferric acetate was added to get better staining of the chromosomes. Micropreparations were made using the standard acetocarmine squash technique after a fixation period of about 10 days. Root tips were treated with colchicine (0.5%) for 2:30- 3:00 hrs to capture metaphase. A karyogram for each taxon was constructed by arranging the chromosomes in homologous pairs in order of decreasing length. Measurements of chromosomal arms was recorded to prepare karyotype. For meiotic studies, young inflorescences were fixed in Carnoy's fixative (alcohol: chloroform : acetic acid in 6 : 3 : 1) for 24 h and were transferred to 70% alcohol for preservation at 4°C. Meiotic studies were carried out by preparing smears of pollen mother cells (PMCs) in 1% acetocarmine. Photomicrographs of chromosome counts were made from freshly prepared slides using Trinocular Research Microscope (Olympus BX60F).

RESULTS AND DISCUSSION

The tribe Asclepiadeae is represented in this study by 3 accessions of *Sarcostemma viminale* (L.) R.Br., *Ceropegia bulbosa* Roxb. var. *bulbosa* and var. *lushii*. Chromosome counts and karyotype prepration was done. All accessions were found to have a basic chromosome number of x = 11. All accessions shows somatic chromosome number of 2n =22. The small size and presences of milky latex in the plants makes it difficult to study the chromosome and preparing the karyotype. Although the chromosomes remain reduced in size, some chromosomes were slightly larger than the others. In the New World both x=11 and x=10 seem to occur. The Galapagos species *S. angustissima* (Anderess.) R. Holm has been reported to possess 2n=22 chromosomes (Kliphuis, 1977) and *S. crispum* Benth. from the south western United States has been found to be tetraploid with 2n=44.

Sarcostemma viminale (L.) R.Br.

"Sarco" is Greek word meaning fleshy while "Stemma" refers to the fleshy inner corona. The 'caustic' in the common name is referring to the burning power of the released latex. It is a congeneric species i.e. when traits have diverged within genus, the competition is relaxed and that allows the coexistence of species. Flowers look like embossed star with umbel inflorescence. The main axis ends at a summit of peduncle. Fruit is a pair of follicles and it is smooth. Seeds oblong, margin is uneven with pappus (coma) at conical end (Arora and Meena, 2016). During the present investigation similar (n=11) chromosome numbers were observed in all accessions of *Sarcostemma viminale* (L.) R.Br. The result suggests that chromosomes have a basic number of x=11. 2n=22 is observed in all accessions under study with laggards with anaphase II. These results are in accordance with the earlier reports of 2n=22 for this species (Kliphuis, 1977) (Fig. 1). Karyotype was prepared by measuring length of both the arms of chromosome (Fig. 2).

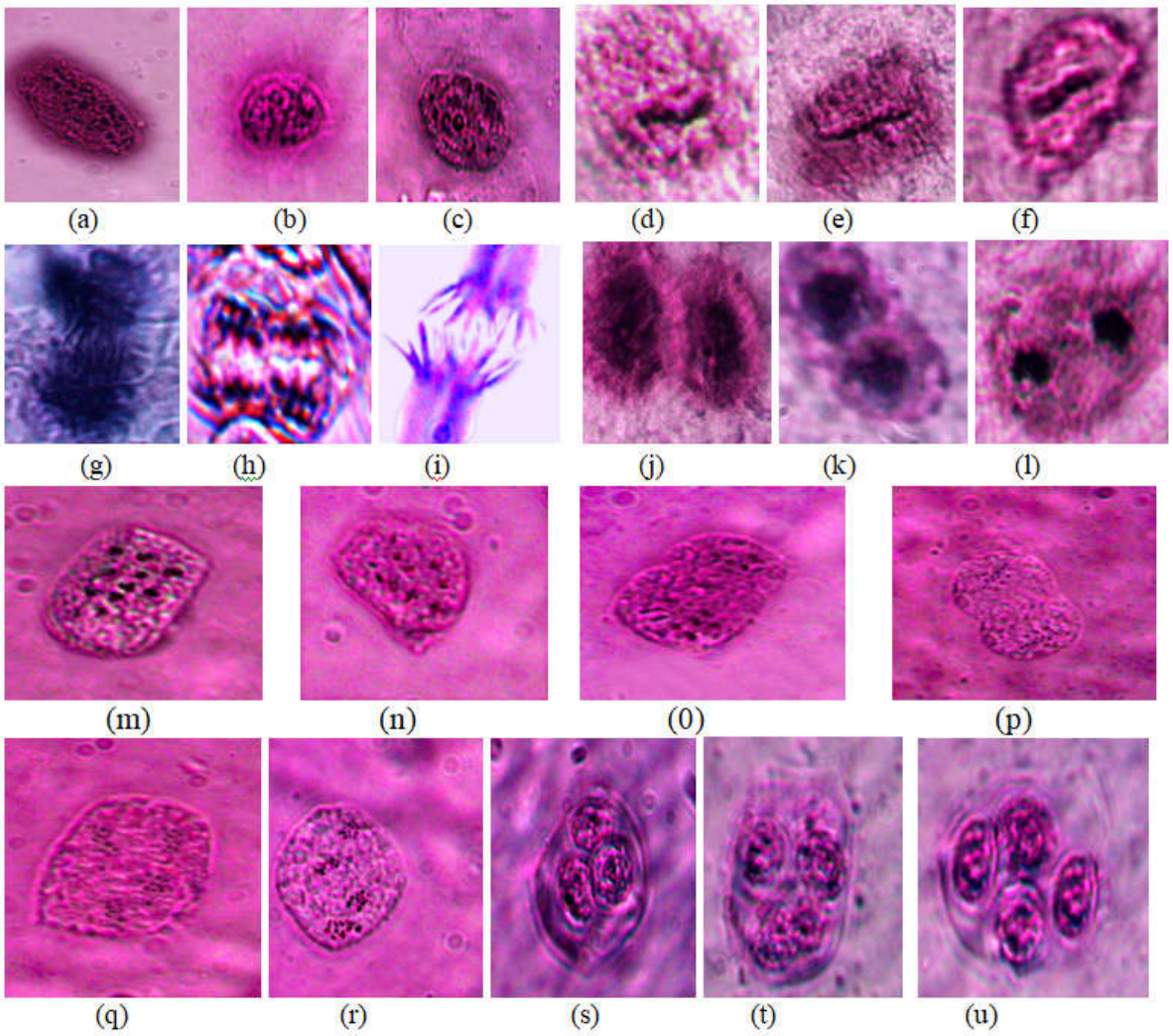

Figure-1. Mitotic abnormalities: (a-c) prophase, (d-f) normal metaphase (g) early anaphase showing chromatin bridge, (h-i) anaphase with spindle fibers, (j) late anaphase, (k-l) telophase; Meiotic abnormalities: (m-n) showing bivalents with diakinesis, (o-p) scattering at metaphase-II, (q) laggards at anaphase-II, (r) scattering at anaphase-II, (s) Triad, (t) early telophase-II and (u) telophase-II.

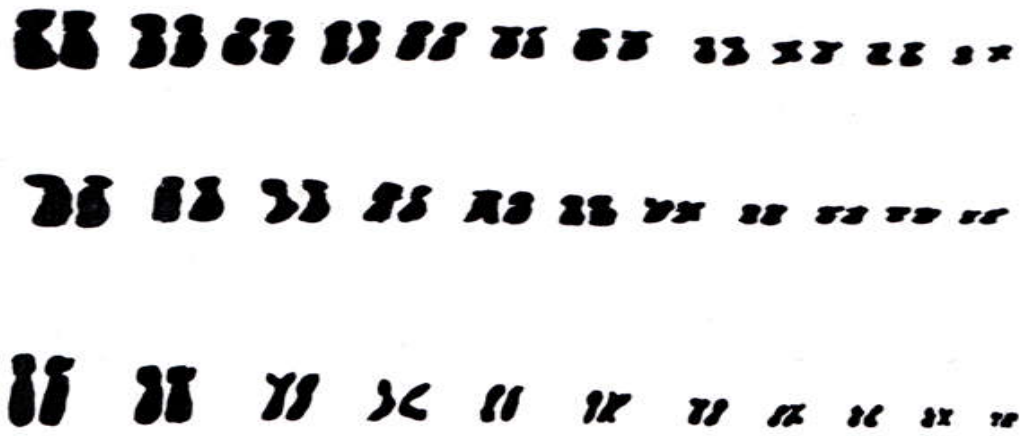

Figure 2. Karyograme of *Sarcostemma viminale* (L.) R.Br.

Ceropegia bulbosa Roxb. var. bulbosa and var. lushii

Ceropegia bulbosa Roxb. var. *bulbosa* and var. *lushii* (Grah.) Hook.f. is a perennial,twining, herb of sandy substratum bearing tubers. Grows amongst thorny and spiny bushes of *Capparis decidua* and *Prosopis julifera* on which they climbs and also gets protected from grazing animals. It requires sandy soil mixed with some stony fraction. It was observed that both varieties

were growing together at same place (Arora and Meena, 2016). Flowering occurs during July-September. This genus has always been a lure to the researchers because of structural and adaptive strategies (Percival, 1969). Flower design, corolla size, shape and coloring pattern etc. are some special characters of this genus. Among different species *Ceropegia bulbosa* is one of the widely distributed species but still threatened (Yadav and Kamble, 2008). Two varieties of *Ceropegia bulbosa* were analyzed morphologically i.e. Ceropegia bulbosa Roxb. var.bulbosa and *Ceropegia bulbosa* Roxb. var. *lushii* (Grah.)Hook.f.

Book (Nayar and Sastry, 1987-89). Flowers are simple, umbel or cymose type, fruit is a pair of follicles that originate oppositely. Seeds are flat ovate-oblong and are crowned by a membranous margin, hairy; these hairs facilitate the dispersal of the seeds by wind. All accessions show diploid number of 2n=22 with a basic chromosome number of x=11 that agrees with earliyer findings (Albers, 1983). Irregular distribution of chromosomes was observed in anaphase. Leggards have been observed in *C. bulbosa* var. *bulbosa* . Karyograme shows meta and submetacentric nature of chromosome indicating a step ahead primitiveness (Fig. 3, 4).

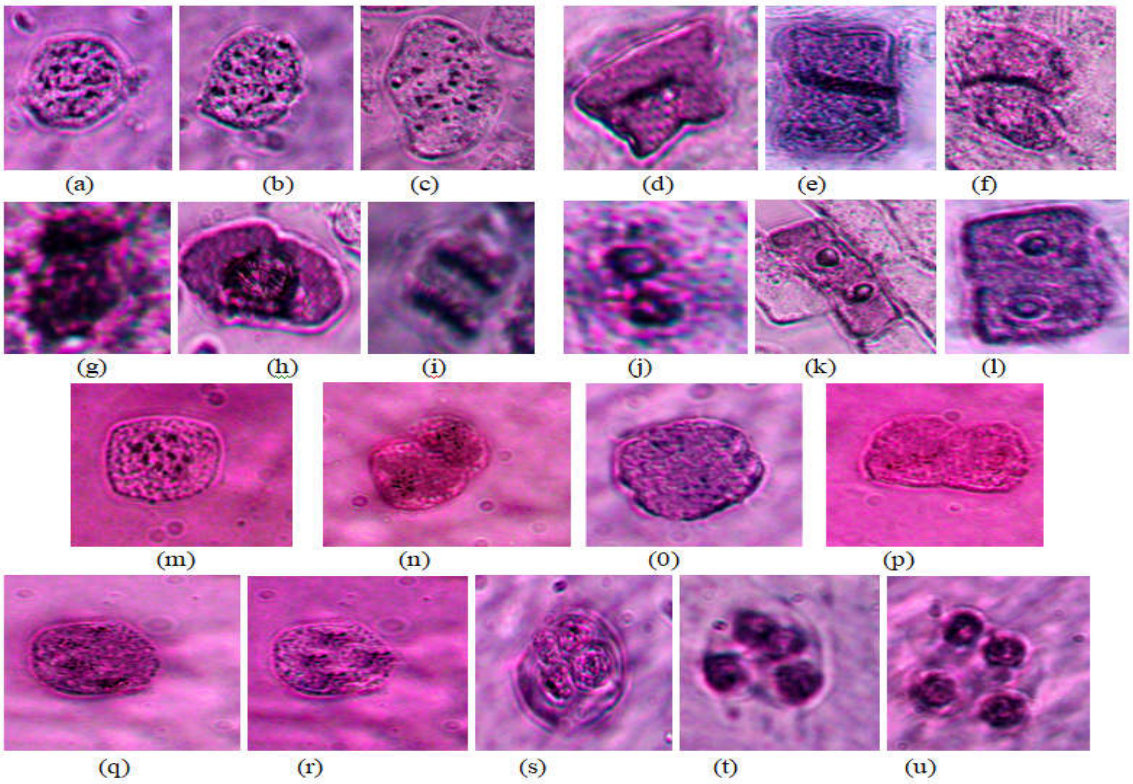

Figure 3. Mitotic abnormalities: (a-c) prophase (d-f) normal metaphase, (g) early anaphase, (h) anaphase showing spindle fiber bridge, (i) anaphase, (j) early telophase, (k-l) normal telophase; Meiotic abnormalities: (m) diakinesis with bivelants (n-p) metaphase II, (q) laggards at anaphase-II, (r) anaphase II, (s) late anaphase II, (t) early telophase, (u) four daughter cell – telophase II.

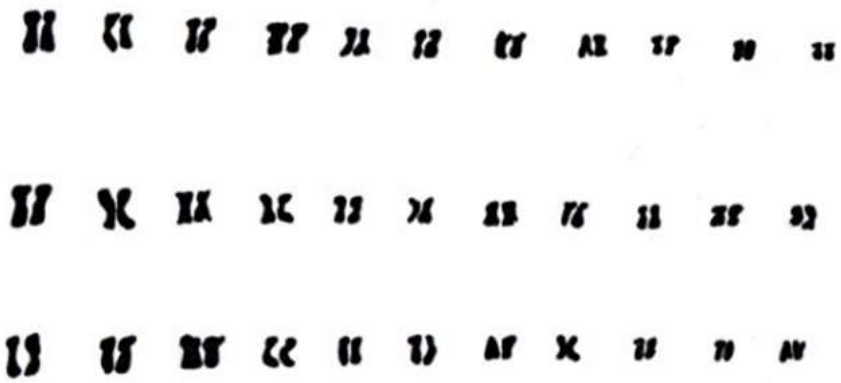

Figure 4 Karyogram of *Ceropegia bulbosa* Roxb. var. *bulbosa*

The former one is characterized by broad leaves while the later is a narrow leaved variety (Cooke, 1958). Several species of *Ceropegia* are facing different threats and almost 16 species have been recorded under different categories in Red Data

Conclusion

In the present investigation chromosome behavior was observed in some accession of *Sarcostemma viminale* (L.)

R.Br., *Ceropegia bulbosa* Roxb. var. *bulbosa* and var. *lushii*. The results match with earlier findings except some distributional patterns. The karyotype features are useful in establishing relationship between both plants and variety. Most of the data obtained from karyotype analysis confirm the systematic relationships within tribes of subfamily Asclepiadoideae. Both the plants under study are characterized by presence of the same basic number (x=11). Chromosomes base-numbers are usually considered as having a great evolutionary significance and taxonomic value. The base-numbers is providing important diagnostic characters of plants. In the Asclepiadaceae x = 11 is not only the most frequently encountered base chromosome number (Albers, 1983), but also probably the primitive condition. The same base number is the primary condition in the Apocynaceae (Van der Laan and Arends, 1985). In *Sarcostemma* sensu Holm, x = 11, 10 and 9 is recorded, following a geographical distribution from Africa (x = l l), Central America (x = 11→ 10) and South America (x = 11→ 10→ 9) (Albers *et al*., 1993).

The reduction of chromosomes seems to have taken place independently several times within this family as well as within the Asclepiadaceae. The small size of Asclepiadaceous chromosomes does not permit the use of more elaborate techniques to distinguish between auto- and allotetraploid taxa in the karyotype; an isozyme study is needed to clarify the nature of the tetraploid taxa. Allotetraploids are preadapted to conquer new habitats by the increased variability in their gene pool, while autotetraploids commonly display a better physiological performance (Ehrendorfer, 1980). Karyotypes of these species were constructed for comparative analysis to justify species relationships, and to speculate chromosomal evolution in the *Sarcostemma viminale* and both variety of *Ceropegia bulbosa.* Although further chromosome study is necessary to see intraspecific differentiation with euploid chromosome variation, chromosome counting seems to be good item to discriminate between these two species until now. Immediate steps are to be taken for their conservation and sustainable utilization.

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