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Research Article

Karyomorphology, Meiotic Behaviours and Pollen Fertility of Calendula officinalis L. (Calenduleae- Asteraceae)

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Abstract

The present study includes chromosome number, karyomorphology, meiotic behaviors and pollen fertility of the species *Calendula officinalis* L.collected from central Nepal was cytologically carried out using two percent aceto-orcein for somatic chromosome determination and one percent aceto-carmine for meiotic study. Pollen fertility was based on stainability test using Muntzing solution. Somatic chromosome number determined for this taxon were 2n=28, haploid chromosome number n=14 and pollen fertility found to be 84.4 percent in the present study. In karyotype analysis chromosome length ranged from 0.4 to 2.6 µm and mean length was 1.5 µm. Likewise, absolute length found to be 21.9 µm and karyotype formula obtained was $M_{16}+m_{4+}$ $sm_{8.}$ Three types of chromosomes centromere at middle point, at median region and at sub –median region were observed. Meiosis with regular and irregular behaviors was observed in this study. The variations in chromosome numbers, irregularities in meiotic behaviors, variations in karyomorphological structure and high fertility rate shows evolve nature of this species which play a great role in evolution. Pollen fertility was also affected by meiotic abnormalities.

Keywords: Karyomorphology; meiosis; pollen fertility; Asteraceae; Aceto-orcein

Introduction

The taxa *Calendula officinalis* is the flowering plants of the family Asteraceae and placed in the tribe Calenduleae. The tribe Calenduleae has eight genera and over 110 species, mostly found in South Africa (Judd *et al.,* 2008). Only one species *Calendula officinalis* found in Nepal so far (Judd *et al.,* 2008). Economically the taxa *Calendula officinalis* is very important as it grows for ornamental purposes as well as for medicinal purposes. The leaves and flower of this is uses in wounds and burns. *Calendula officinalis* contained with many chemical constituents such as carotenoide, flavonoids, saponins, sterols, phenolic acids, lipids etc. (Ashlawyen, 2018). Karyomorphological study provides evolutionary characteristics of karyotypes, as well as the cytological mechanisms. Karyomorphological study is a fast and inexpensive approach to classify plant species by identifying the basic cytological parameters of a species, including chromosome number, ploidy level, karyotype asymmetry, and karyotype coefficient (Guerra, 2008). Chromosomes number and karyotype of a species are stable characteristics which can reflect its basic genetic information. Karyomorphological studies was done by different authors in different plant species time to time such

as Yano and Hashino (2007) studied four species of cyperaceae karyomorphologically. Seven species of the genus Salvia karyomorphologically studied by Martin *et al.*, (2015). Differences in chromosome numbers between populations are important evidence for determining reproductive isolation (Sun *et al.*, 2020). Meiosis is events of high evolutionary stability which reduce the chromosome number of chromosomes are one of the important determinants for the evolutional study. Pollen fertility is the ability of the pollen to perform its functions of delivering male gametes to embryo sac. Pollen is a critical stage in the life cycle of the plants as fertile pollen is the crucial for sexual plant reproduction. Commonly for pollen transport vectors are wind and different insects depending on the species. In seed formation fertile pollen is very necessary. Studies of pollen fertility are very helpful for recognition of wide range of variations existing within plants species and differentiating plant species with genera (Noor *et al.*, 2004). The main objective of this study is to determine chromosome number, karyotype analysis, meiotic behaviors and pollen fertility of the taxa *Calendula officinalis* L.

Materials and Methods

The plants were collected from Kathmandu, Nepal and transplanted in earthen pots at my home garden. Somatic chromosomes were prepared from healthy root tips. They were pretreated with aqueous solution of 0.002M 8-hydroxyquinoline for three hours. The root tip after pretreatment was fixed in mixture of absolute ethanol and glacial acetic acid (3:1) for 24 hours. The root tip materials were then hydrolyzed and stained in a mixture of 2% aceto-orcein and 1N HCl (9:1) contained in watch glass and warmed for few seconds and left for 30 minutes to 1 hour. Squashes were made in 45% acetic acid. Observed under compound microscope. The drawings were made with the help of 1366 Camera Lucida apparatus. Photomicrographs were taken by using digital camera of 12.1 megapixel using 10 x eye pieces and 100x objective of trinocular compound microscope. For karyotype studies at least three different preparations were made from root tips. Chromosomes were measured from the drawn figures. The methodology was followed as given by Levan *et al.* (1965).

The meiotic behavior of pollen mother cells was observed from appropriate anthers of fixed flower buds. For this study buds of suitable sized are fixed in fixative prepared by one-part acetic acid and three parts ethanol (1:3) for 24 hours. Suitable sized anthers were dissected from the buds and teased with a needle in a 1-2 % aceto-carmine. The desired stages of meiosis were photographed under the compound microscope. Pollen fertility of the taxa was estimated on the basis of stainability test. It was determined by using Muntzing (1941) solution made by one-part aceto-carmine and one-part glycerine (1:1). Well inflated, uniformly stained and healthy grains were considered as fertile ones.

Results and Discussion

The plant is a short living, aromatic and erect annual herb, growing up to 80 cm tall with sparsely branched stem. It is commonly called pot marigold. The stem is angular, glandular and hairy. The leaves are oblong, lanceolate and hairy on both sides, margins entire. The head is yellow comprising a thick capitulum. Flowers are bright orange yellow, 3-toothed, tube hairy. In the wild form they have a single ring of ray florets surrounding the central disc florets. The disc florets are tubular and hermaphrodite, and generally of a more intense orange-yellow color than the ray florets. Peripheral ray florets tridentate. The flowers may appear throughout year where conditions are suitable. The fruit is a thorny curved achene. Achenes longer than the involucres, curved boat-shaped dorsally muricate not beaked, outer longer ventrally crested, beaked (Fig.1).

Somatic chromosome number determined for this taxon is 2n=28. Reproductive chromosome number or haploid number n=14 determined for this taxon. The somatic chromosome number determined is shown in Fig. 2 and camera lucida drawing in Fig. 3. Its ideogram is represented in Fig.4. The chromosome measurements are given in Table 1.

Chromosome of metaphase plate shows three types with centromere at median points, median regions and sub median regions. The chromosomes length ranged from 0.4 to 2.6 µm with mean length 1.5 µm and absolute length 21.9 µm. Karyotype formula is $M_{16}+m_{4+}\ sm_{8.}$

Meiotic study shows both regular as well as irregular behavior in this taxon. Normal diakinensis with rod bivalents were observed. Metaphase exhibit normal behavior. Anaphase with non- synchronous division and non-oriented chromosomes were observed which shows slightly irregular meiosis. Sticky chromosomes with laggar are found in metaphase-I stage. Formation of non oriented chromosomes may be due to early disjunctions of bivalents. Normal telophase was occurred. Diakinensis shown in Figs. 5 and 6. Metaphase-I is shown in Fig. 7-10 are noted. Telophase-I is in Fig. 11. Metaphase- II (Fig. 12-14). Anaphase-II with non- synchronous division (Fig. 15) and Anaphase-II with non-oriented chromosomes (Fig. 16) have been observed. Telophase-II abnormal shown in Fig. 16. Tetrads (Fig. 18) and circular, round, echinate large pollens (Fig. 19) are observed. Pollen fertility determined was 84.4 percent.

Fig. 1

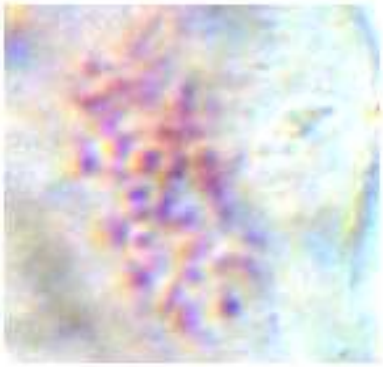

Fig. 2

Fig. 3

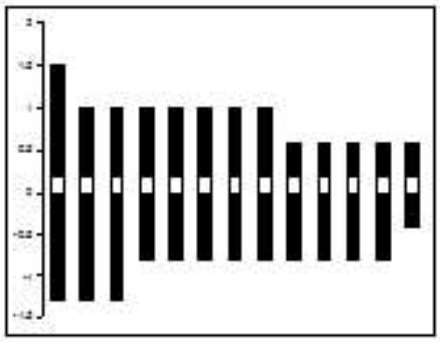

Fig. 4

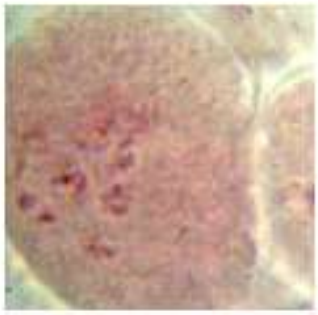

Fig. 5

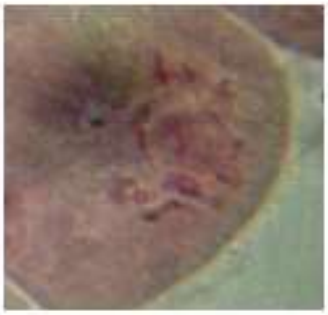

Fig. 6

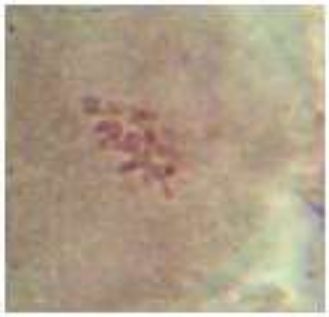

Fig. 7

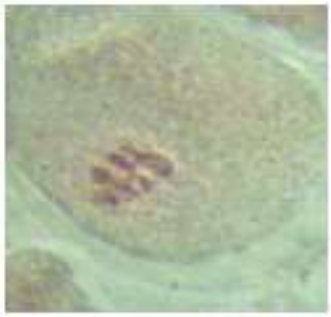

Fig. 8

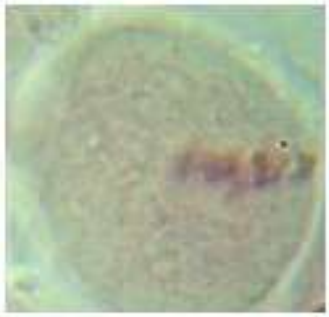

Fig. 9

Fig.1. Photograph of living plant. **Fig.2.** Photomicrograph of somatic metaphase plate.
Fig.3. Camera lucida drawing of the same. **Fig. 4.** Ideogram of the above. **Fig.5-6.** Diakinesis.
Fig. 7-9 Metaphase-I . **Fig .9,** metaphase-I with sticky chromosomes and laggars.

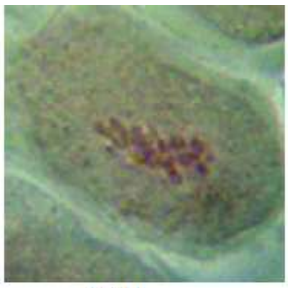
Fig. 10

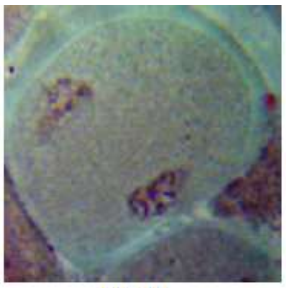
Fig. 11

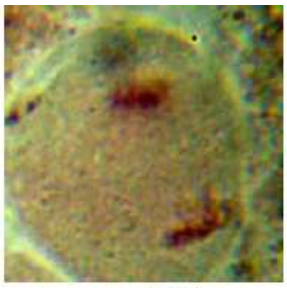
Fig. 12

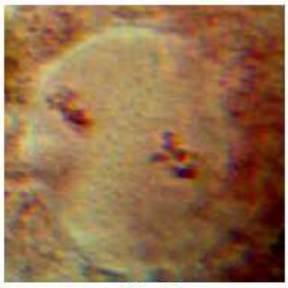
Fig. 13

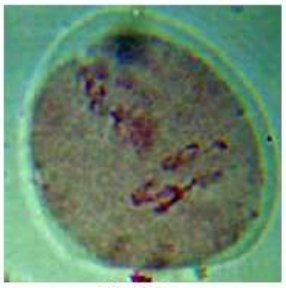
Fig. 14

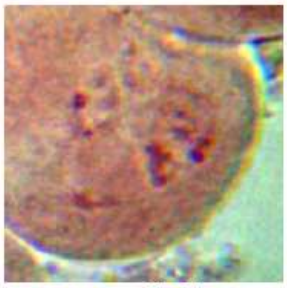
Fig. 15

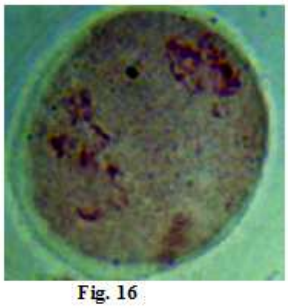
Fig. 16

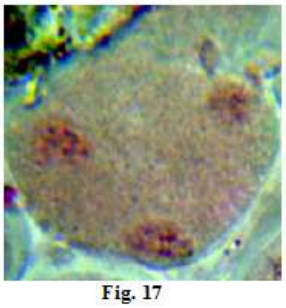
Fig. 17

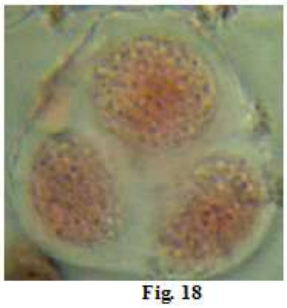
Fig. 18

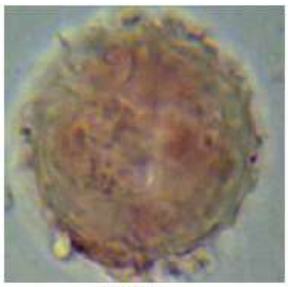
Fig. 19

Fig. 10 Metaphase-I. **Fig. 11. Telophase-I.** **Fig. 12-14** Metaphase-II.
Fig. 15. Anaphase-II with non synchronized division. **Fig. 16.** Anaphase-II with non-oriented chromosomes.
Fig.17. Telophase-II **Fig.18. Tetrad normal** **Fig. 19.** Pollen grain.

Table 1: Chromosome measurement in *Calendula officinalis* L.

Chrom. Pairs	Long Arm (µm)	Short Arm (µm)	Total Length (µm)	r-value	Relative Length (µm)	Position of centromere
I	1.3	1.3	2.6	1	11.8	M
II	1.3	0.8	2.1	1.6	9.8	m
III	1.3	0.8	2.1	1.6	9.8	m
IV	0.8	0.8	1.6	1	7.8	M
V	0.8	0.8	1.6	1	7.8	M
VI	0.8	0.8	1.6	1	7.8	M
VII	0.8	0.8	1.6	1	7.8	M
VIII	0.8	0.8	1.6	1	7.8	M
IX	0.8	0.4	1.2	2	5.9	Sm
X	0.8	0.4	1.2	2	5.9	Sm
XI	0.8	0.4	1.2	2	5.9	Sm
XII	0.8	0.4	1.2	2	5.9	Sm
XIII	0.4	0.4	0.8	1	3.9	M
XIV	0.2	0.2	0.4	1	1.9	M

Karyotype (M_{16}+m_{4+} sm_8) of *Calendula officinalis* shows slightly asymmetrical chromosomes with centromere at median point, median and sub-median regions. Fallahi *et al*. (2020) determined symmetrical karyotypes with metacentric and sub-metacentric chromosomes for these taxa. The somatic chromosomes number in present study was found to be 2n=28 but somatic chromosomes, 2n=32 found by Fallahi *et al.* (2020). So, this species found in two different cytotypes. The chromosome lengths ranged from 0.4 to 2.6 µm in this study. The longest and shortest chromosomes ratio indicates its advanceness. Annual habit, sparsely branched stem and large oblong- lanceolate leaves show advanceness of this taxon.

The haploid chromosome number n=14 for the taxa *Calendula officinalis* of the tribe Calenduleae is determined in present investigation. Earlier haploid number (n=16) was reported by Gupta (1969). So, *Calendula officinalis* may be existed with two haploid numbers. Meiosis in this taxon exhibited both regular as well as irregular behaviors. Regular meiosis was observed in this species by Gupta *et al.* (1972). Samatadze *et al.* (2019) determined sixteen bivalents for this species. Meiotic disorders like chaotic disjunction, laggards, bridges, chromosome fragments also revealed by them for this species. Darlington and Wylie (1955) suggested different basic numbers for this species such as x=7, 8 and 9. Basic number x=8 was reported for *Calendula officinalis* by Gupta *et al.* (1972). Thus, *Calendula officinalis* could be found in triadic forms.

Pollen fertility for *Calendula officinalis* 95 percent was determined by Noor *et al*. (2004). Pollen fertility determined for this species in present investigation is 84.4 percent. Pollen fertility was affected by development of abnormalities like laggards, non-orientations of chromosomes etc leading to the formation of deficient pollen grains.

Conclusions

The longest and shortest chromosomes ratio indicates its advanceness. The variations in chromosome numbers, in meiotic behaviors and karyomorphological structure and high fertility rate shows evolve nature of this species which play a great role in evolution.

Conflict of Interest

The author declares that there is no conflict of interest with present publication.

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