Journal of Positive School Psychology
2022, Vol. 6, No. 7, 2459-2466
http://journalppw.com

Manna Formation, Cytogenetic And Pollen Morphology In Echinops Polygamous Bunge. And E. Tenuisecta Rech

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Abstract

Echinops polygamous and E. tenuisecta are belong to Asteraceae family. In this article, we investigated the formation of manna, cytogenetics and pollen morphology in E. polygamous and E. tenuisecta. For cytogenetic studies, root tip meristems of seedlings were used. Meristem cells were stained with acetocarmine 2%. In these cells, the number and size of chromosomes were examined. Pollen grains were studied by light and scanning electron microscopy. These are rare plant species in which the Larinus forms its cocoons at the angles of the stems of them. Larinus cocoons (manna) have medicinal value. The number of chromosomes in both species was 2n= 28 and in both species aneuploidy was observed. In the karyotype, the four chromosomes were homolog. Therefore, it seems that both species are tetraploids with base chromosome number X= 7. The pollen grains of E. polygamous and E. tenuisecta were elliptical, with tricolporate, short spines and micro perforates.

Keywords: Echinops; Choromosome; Pollen; Manna; E. polygamous; E. tenuisecta.

Introduction

E. polygamous and E. tenuisecta are two plants belong to Asteraceae family that find abundantly in arid regions of Iran (Montazerolghaem et al., 2016). There are many species Echinops in many parts of Iran, some of which are able to produce manna (Mohammadi and Dini, 2003). Terhala manna produced on the Echinops plants, which is actually an insect cocoon, is called Shekrtighal in Iran. Trehala manna is one of the main manna produced in Iran, which is made by insect larvae between the petioles and stems of some Echinops species and has many medicinal properties (Dini et al., 2002; Mohammadi and Dini, 2003). Manna is a laxative, antitussive, anti-asthma, anti-infection and anti-fever and antioxidant (Amiri and Joharchi 2013). Manna trehala is obtained from the activity of larvae of Larinus vulpes on some species of the genus Echinops. Manna-producing insects feed on all species of Echinops, but can produce manna in only four species. Reports indicate that Echinops species have spherical capitol inflorescences, simillar stem length and grow in similar conditions produce manna. Also, the study of the life cycle of the Larinus showed that the life stages of the insect, including different larval, pupal and adult insect ages, take place inside the cocoon (Nasirzadeh et al.,

2005). Cytogenetic and pollen studies on Echinops species are scarce. The number of chromosomes reported for Echinops species is 2n= 26 to 36 chromosomes. Diploidic is the predominant condition and no reports of aneuploidy and polyploidy are available for species of Echinops genus (Sanchez-Jimenez et al., 2010). The number of chromosomes varied in different species of Echinops (Sanchez-Jimenez et al., 2012). A study on 18 populations of 5 species of Echinops showed that the chromosome numbers of these species are 2n= 30, 32, 34 and 36. Examination of the chromosomal formula has shown that most chromosomes are metacentric or submetacentric and their size is 41.4- 79.22 micrometers (Alijanpoor et al., 2019). Examination of chromosome number in 19 species of Echinops in Fars province of Iran has shown that the number of chromosomes in different species is 2n= 28, 30, 32 and 34. The size of the genome and the frequency of numbers of chromosomes 2n= 28, 30 and 32 between Echinops species indicate the ancestors of these species have become polyploid (Garantie et al., 2004).

The study of pollen morphology is one of the features that is important for the identification and genealogy of plants (Blaus et al., 2020). The pollen of E. sphaerocephalus was elliptical, with tricolporate with short spines. According report of Garnatje and Martin 2007, the pollens of most species of Echinops are similar. The ratio of polar (P) to equatorial (E) length (P/E) in most species of Echinops was1.06 - 1.46. The polar length was 14.5- 82.88 and the equatorial length was 12.75- 56.44 µm. Pollen grains in Echinops species are larger than other species of Asteraceae. The shape is prolate. The polar axis is up to about 110 µm and the equatorial axis is more than 60 µm in some species. Pollens are tricolporate (Tomsovic 1997). In this article, we have investigated the formation of mana, cytogenetics and pollen morphology in E. polygamous Bunge. and E. tenuisecta Rech.

Meterial and Methods

Plant material: E. polygamous Bunge. and E. tenuisecta Rech. were examined in this study. E. polygamous was collected from Saveh (35 ° 36 ″ North, 50 ° 13 ′ 12 ″ East)) and E. tenuisecta from Sarpol-e- Zahab (34°27′40″ North, 45 ° 51′46″ East), from Iran.

Cytogenetic: Seeds of E. polygamous and E. tenuisecta were isolated from dry and complete inflorescences. To remove dormancy, the seeds were kept at 8 ° C in the dark for one month. Then, 5 seeds were placed on a paper towel with 2 ml of distilled water in a petri dish and after closing, it was placed in a dark condition at a temperature of 25 degrees. The seeds germinated after three days. 5-day-old seed tip meristems were used for cytogenetic study. 5-day-old seedlings were fixed in fixator (ethanol: acetic acid (3: 1)). Some seedlings were pretreated with colchicine 0.01 mg ml^{-1} for 6 hours before fixation. The seedlings were placed in 0.2 N hydrochloric acid for 1 minute. The seedlings were washed with water and placed on a slide. The tip of the root meristem is separated from the seedling in a drop of acetocarmine 2% (2g carmine in 100 ml boiling acetic acid 45%) on the slide and crushed by a sharp needle. The glass cover was placed on it and after squashing it was studied with Zeiss light microscope at magnifications of 40, 100, 400 and 1000.

Pollen: Pollen grains were obtained from dried inflorescences and shaking them on a plate. To study with a light microscope (Zeiss), some pollen grains were taken with the tip of a wet needle and was placed on a slide in a drop of water. After insertion a glass cover, it was studied with magnifications of 100 and 400 times. For scanning electron microscopy (Philip microscope XL30) study, pollen grains

were mixed with a drop of water and transferred directly to a metallic chip. In Sputtering Chamber (BAL-TEC, SCOOD), the surface of the pollen was covered with gold. The diameter of the gold coating was 100 angstroms. The study was performed with magnifications of 1250 times to 17500. Polar axis, equatorial and pore diameter were measured.

Static analysis: All the experiments repeated three times. All the treatments were statistically analyzed using Minitab software and the means were compared by Turkey's test ($p \leq 0.05$).

Results and discussion

Morphology: E. polygamous and E. tenuisecta are two species of Asteraceae family. E. polygamous and E. tenuisectus were perennial, with blue spherical capitol inflorescences and gray cut leaves with barbed ends at the incisions. E. polygamous was a plant on the ground with a large compact inflorescence and sparse leaf spines but E. tenuisecta stands, smaller sparse inflorescence and compact leaf spines (Fig. 1 A, B). In both species, manna was produced by larvae of Larinus (Fig 1C, D). There are 54 species of Echinops in Iran, which only a few species produce manna. It has been reported that the Larinus is active on E. endotichus, E. dichrous, E. persepolitans and E. tenuisectus (Nasirzadeh et al., 2005) and E. cephaotes, E. orientalis and E. robustus (Dini et al., 2002) and it form manna (cocoon). The formation of manna on shoots of E. polygamous has not been reported (Fig 2 C).

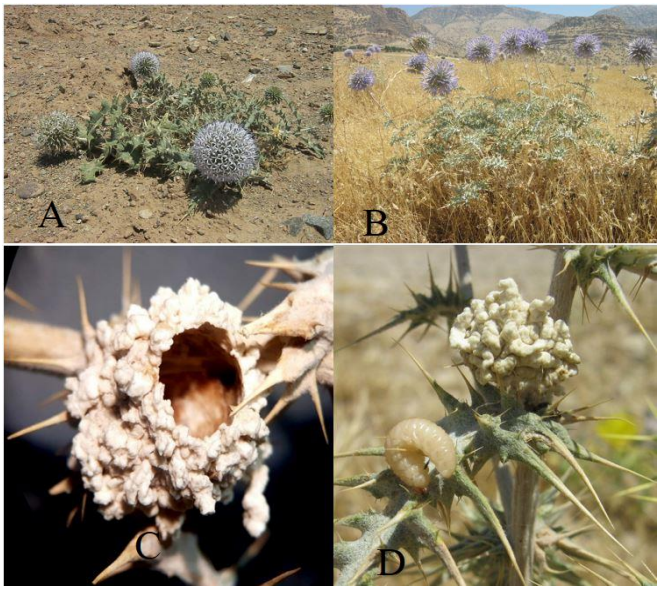

Figure 1. A: E. polygamous,B: E. tenuisectus, C:manna (insect cocoon) on shoots of E. polygamous and D:manna and Larinus larvae on E. tenuisectus.

Cytology: Cytogenetic study of root apical meristem of seedlings of E. polygamous and E. tenuisecta showed that both species have 28 chromosomes (Table 1, Fig 2 A, B). Study of division stages in both species showed that abnormalities do not occur in division stages, however, aneuploidy was rarely observed in cells with a reduction of three chromosomes (Table 1, Fig 3C). There was more aneuploidy in E. tenuisectus with a significant difference. The number of chromosomes reported for Echinops species is 2n= 26 to 36 chromosomes. The number of chromosomes in E. Ivaschenko was reported to be 2n = 28 (Garnat et al., 2004). The number of chromosomes varied in different species of Echinops, for example in E. graecus

2n = 32, E. sphaerocephalus 2n = 30, 32 and reported in E. spinosissimus 2n = 28 (Sanchez-Jimenez et al., 2012). A study on 18 populations of 5 species of Echinops showed that the chromosome numbers of these species are 2n= 30, 32, 34 and 36 (Alijanpoor et al., 2019). Examination of chromosome number in 19 species of Echinops in Fars province of Iran has shown that the number of chromosomes in different species is 2n= 28, 30, 32 and 34 (Garantie et al., 2004). The number of chromosomes reported for E. tenuisecta is similar to the 2n=28 chromosomes of Sarpole Zahab obtained from our results (Sheidai 2000). Our results show 2n=28 chromosomes for E. polygamous from Saveh, which is different from the 2n=32 chromosomes reported for polygamous from Semnan (Alijanpor et al., 2019). A special feature was observed in the mitotic division of both species, which included the sticking of homologs similar to meiosis (Fig 2D).

Table 1. Number of chromosomes in E. polygamous and E. tenuisecta

Choromosome set	E. pol		E.ten	
2n=4x	28	99%a	28	98.5%a
X	7	-	7	-
2n= 4X-3	25	1%b	25	1.5%a

Examination of the total size of chromosomes of the two species (Table 2) showed a significant difference. The difference in size of the largest chromosome of E. tenuisecta with E. polygamous was not significant but there was a significant difference between the smallest chromosomes (Table 2). There are reports that the chromosomal size of Echinops species was 41.4-79.22 µm (Alijanpoor et al., 2019) and 29.5 µm for E. tenuisecta (Sheidai, 2000).The total length of chromosomes of E. polygamous 66.3 µm (Alijanpor et al., 2019). In this study, the chromosomal size of E. polygamous was 43.53 µm and for E. tenuisecta was 41.223 µm in the end of prophase.

Table 2. Chromosome number, chromosome length and karyotype formula of E. tenuisecta and E. polygamous

Species	Local	Chromosome Number	Total Length (µm)	Longest Chromosome (µm)	Shortest Chromosome (µm)	Karyotype Formula (KF)
E. polygamous	Saveh	28	43.53a	2.51a	0.978a	12M+8 SM+ 8A
E. tenuisectus	Sarpol Zahb	28	41.223b	2.52a	0.672b	4M+12SM+12A

Means with different letter in a column are statistically different (Tukey's test, $p \leq 0.05$).

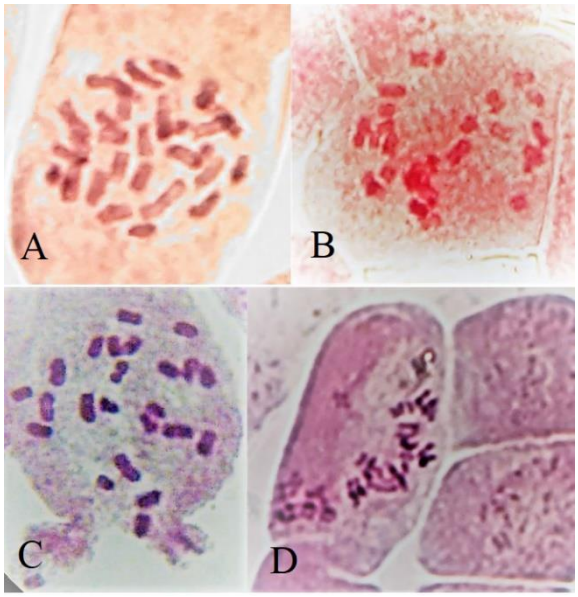

Figure 2. A: E. polygamous (2n=28), B: E. tenuisecta (2n=28), C: E. polygamous (2n=25) and D: the sticking of homologs similar to meiosis in E. polygamous.

The karyotypic formula was also different in the two species. In E. polygamous, metacentric chromosomes were more but sub-metacentric and acracentric were equal. In E. tenuisecta, metacentric chromosomes were very few, but sub-metacentric and acracentric were high and equal while other reports of the chromosomal formula of E. polygamous and E. tenuisecta have shown that most chromosomes are metacentric and submetacentric (Sheidai 2000; Alijanpoor et al., 2019).

Examination of E. polygamous and E. tenuisecta karyotypes (Fig 3, 4) showed that in both species, chromosomes could be divided into groups with 4 chromosomes. The chromosomes of each tetrad were similar in size and location of the centromere. This result indicates that the two species are tetraploid (Fig3, 4). Based on this finding, it is assumed that the base number of chromosomes of the two species is X=7. Despite the fact that Diploidic is the predominant condition and no reports of aneuploidy and polyploidy are available for species of Echinops genus (Sanchez-Jimenez et al., 2010), the results of this study showed that aneuploidic in both species there is a reduction of three chromosomes and both species are tetraploid. In line with these results, it has been reported that genome size and chromosome number diversity in Echinops species indicate polyploidy of the ancestors of these species (Garantie et al., 2004).

2.5	2.27	1.308	1.309	1.297	1.199	0.978
2.5	2.269	1.309	1.307	1.3	1.198	0.977
2.503	2.267	1.306	1.307	1.299	1.192	0.969
2.501	2.268	1.307	1.307	1.296	1.192	0.966

Figure 3. Karyotype of E. polygamous and size of chromosmes (µm).

2.52	1.82	1.69	1.65	1.102	0.88	0.67
2.51	1.82	1.687	1.651	1.1	0.879	0.672
2.52	1.81	1.701	1.648	1.103	0.878	0.669
2.518	1.802	1.703	1.649	1.102	0.881	0.668

Figure 4. Karyotype of E. tenuisecta and size of chromosmes (µm).

Pollen morphology

Examination with light and electron microscopy (Table 3, Fig 5) showed that pollen grains of both species are oval, tricolporate with short scattered spines (Fig 5), but the polar length of pollen grains in E. tenuisecta with a significant difference more, but its equatorial length with the difference was significantly less than that of E. polygamous. The ratio of polar to equatorial length was 1.97 in E. tenuisecta but less significant in E. polygamous. Micro perforate were observed on the exine surface. The diameter of the pollen surface micro perforate in E. polygamous was significantly higher.

Table 3. Pollen characters of E. polygamous and E. tenuisecta

Characters	E. pol	E. ten
Shape	Elliptical	Elliptical
Equatorial Axis Length (E)	53±1.02 µm	50.6± 0.8µm
Polar Axis Length (P)	90± 1.8 µm	100±1.51 µm
P/E	1.7	1.97
Micro perforate Diameter	261.33± 0.55nm	244± 0.73nm
Anagleph	Spinules	Spinules
Crack	Trizonocolpate	Trizonocolpate

(Tukey's test, $p \leq 0.05$).

The pollen of E. sphaerocephalus was elliptical, with tricolporate with short spines. According report of Garnatje and Martin 2007, the pollens of most species of Echinops are similar. The ratio of polar (P) to equatorial (E) length (P/E) in most species of Echinops was1.06 - 1.46. The polar length was 14.5- 82.88 and the equatorial length was 12.75- 56.44 µm. Pollen grains in Echinops species are larger than other species of Asteraceae. The shape is prolate. The polar axis is up to about 110 µm and the equatorial axis is more than 60 µm in some species. Pollens are tricolporate (Tomsovic 1997). Our results showed that the pollens of both species were larger than the reported samples because the equatorial length, polar length and P/E of the two species were bigger than the reported ones.

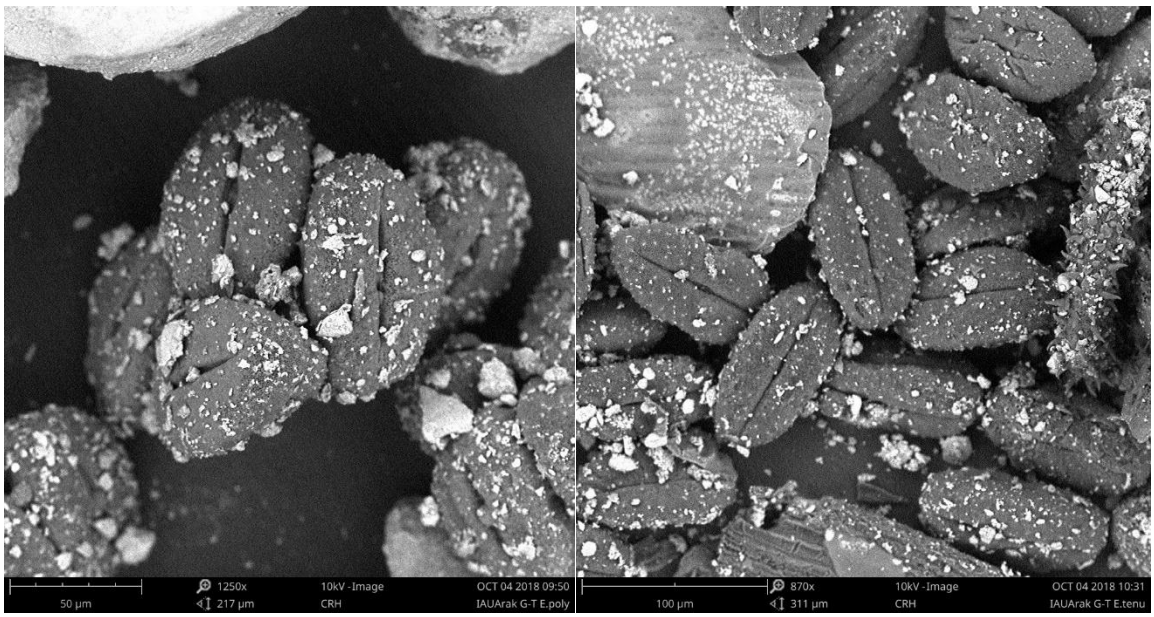

Figure 5. Scanning electron microscope images of pollen: Left: E. polygamous, Right: E. tenuisecta.

Conclusion

The E. polygamous and E. tenuisecta inflorescences were spherical capitols. The inflorescence of E. polygamous was compact but it was sparse in E. tenuisecta. These are rare plant species in which the Larinus forms its cocoons at the angle of the stems and petioles of them. Larinus cocoons called mana have medicinal value. The number of chromosomes in both species was 2n= 28 and in both species aneuploidy was observed with a decrease of three chromosomes. In the karyotype, the four to four chromosomes were similar in size and location. Therefore, it seems that both species of tetraploids are base chromosome number X= 7. The pollen grains of E. polygamous and E. tenuisecta were elliptical, with tricolporate, short spines and micro perforate.

Acknowledgments: Thanks to Mr. Esharati and Mr. Fattahi, researchers at Saveh University and Farhangian University, for helping us with this project.

Disclosure Statement: No possible conflict of interest has been reported by the authors.

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