STUDY OF THE CYTOLOGICAL AND MICRO-MORPHOLOGICAL CHARACTERISTICS OF SOME SPECIES OF THE GENUS EUPHORBIA L.BELONG TO EUPHORBIACEAE FAMILY, USING ELECTRON MICROSCOPE IN IRAQ

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ABSTRACT

This study was aimed to investigate micro-morphological and cytological characteristics for sex species of Euphorbiaceae family in Baghdad during the two growing seasons 2018-2019. The species are: Euphorbia granulate Forssk., E.helioscopia L., E. hirta L., E. hypericifolia L., E. milli Des Moul., E. puplus L. Which involved with morphological characters of pollen grains and seeds, as well the chromosomes number, and the study managed to count chromosome number for the above species sequentially: n=11, n=21, n=9, n=16, n=14,18, n=7,8. The morphological characters of pollen grains surface has been studied by using scanning electron microscope (SEM), shape and size, polar and equatorial axes, spines length, dimensions of ora and colpi, and the ornamentations on pollen surface, and it has been observed that all the species are tri-zono-corporate, spinulose and spinate (echinated), and spinolophate fruite, and importance of the pollen grains in taxonomy. The study includes morphological characteristics of seeds by using (SEM) In terms of size, shape, color, and appearance of the seed surface(configuration). It has been shown that the seeds are significantly different from species to another. Species help to isolate and classify, and through relying on these characteristics, classification limits could be set, whether at the level of species or genus within each family.

Keywords: Euphobiaceae, seeds, pollen grains, chromosomes number, pollen surface.

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INTRODUCTION
Euphorbiaceae considered one of the largest economically important plant family of Anthophyta with about 300 genera and 7500 species, cosmopolitan distribution in the humid tropics and Subtropics of both hemisphers (32). This family distinguished by the milky sap which may caused toxicity and can cause blind when entered to the eyes (12). In Iraq, this family contained about 7 genus and 51 species (34). Euphorbia L. is the largest genus in the spurge family with about 2100 cosmopolitan species (14). The genus comprises remarkable life form variability from annual to perennial herbs, shrubs, trees, succulent and xerophyte forms (19,42). All those species, belongs to this genus characterized by the prescerce of latex and have unique flower structures (1). Euphorbia species considered source of many active biological components such as tannins, flavonoids, unsaturated steroles, carbohydrates, diterperoids and triterpenoids (6,49). Due to the presence of these biologically compounds, Euphorbia species used for treatment of various ailments such as : skin disease, intestinal parasites and gonorrhea (10). Other studies showed the important of this genus as antitumor, antibacterial, antileishmanial and antiviral (13,23,25,27,41). The first who studied this family cytologically (1). Several Euphorbia species have basic chromosome number of X=8, whereas other species in clude X=6,7,9 and 10 of chromosome number, which is related to both aneuploidy and polyploidy (21,36). The previous study indicated cytologically various ploidy levels ranging from diploid, tetraploid, hexaploid to actaploid (2n=12-120). This indicate a significant role of polyploidy in evolution and speciation (14,15,21,22). The lowest chromosome number in Euphorbiaceae family is 2n=12 E.cornuta, while the highest was 2n=234 recorded in Antides mabuninus (51). Palynology has a strong relationship with taxonomy, it provide a taxonomic evidence which help in separate and identification of many genus and species (18). However, the palynological analysis may provide valuable in formation in further understanding the evolutionary relationship between species. Studies by previous researchers (35,37,43). Provided informations of the pollen of different species belong to Euphorbia genus. Seeds feature provide useful characters for the separation of closely related species and in the deduction of phylogenetic relationship. Seed characteristics of subgenera (40) and species (30,33) .In Iraq there are several studies on pollen grains, seeds and chromosome numbers but on another families such as the study of Sadeq (52) about Asteraceae family, as well as (3,4) for Amaranthaceae family, in addition to study (11) in fabaceae family. This study was focused on characterize six species of Euphorbia (E.granulata, E.helioscopia, E.hirta, E.hypericifolia, E.milli, E.puples). in order to determine their chromosome number, pollen grains and seeds by using light and scanning electron microscopy with the aim of providing additional information as possible about these species with the aim of providing useful taxonomic data that would give further informations into proper classification and identification.

MATERIALS AND METHODS
Fresh plant samples were gently collected from different areas 2018,2019 of Baghdad during Jan, Feb and March 2019, randomly between 8:00 am and 1:00 pm. They in cluded all sizes without consideration to the maturation stage, stored in farmens fixative immediately and stored in dark for 24 hr. (17). The taxonomic identification of the plants were characterized by Dr. Sukayna A. Aliwy and Dr. Zubaida A. Ismaeel. For cytological study, the procedure of sadeq (44) was done. The morphology of mature dried seeds and fresh pollen grains were studied by sing SEM. These samples were coated with gdd and examined by SEM at AL-Kufa College Najaf. The size were measured by using the program Imagei. The terminology of pollen grain patterns was adopted by palynology (31), While terminology of cypsela surface patterns adopted from (20,24).

RESULTS AND DISCUSSIONS
1-Chromosomes number
A total of (120) samples were detected for 6 species that belongs to Euphorbiaceae family and the chromosomes number for each species was calculate as shows in Table 1.
Table 1. Chromosomes number of species

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>2n</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.granulata</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>E.helioscopia</td>
<td>21</td>
<td>42</td>
</tr>
<tr>
<td>E.hypericifolia</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>E.hirta</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>E.milli</td>
<td>14,18</td>
<td>28,36</td>
</tr>
<tr>
<td>E.puples</td>
<td>7,8</td>
<td>14,16</td>
</tr>
</tbody>
</table>

According to this study, E.granulata have revealed n=11, 2n=22, this results was supported by the same number. This was ensured that, the chromosomes number of the species was the same although the environmental was differed. Results of E.helioscopia agreed with the study of other researchers (16,45,48) they revealed that chromosomes number was n=21, 2n=24. Some researchers (26,38,50) pointed out that chromosomes number in E.hirta was n=9, 2n=18. This was supported the results of this scurvy, but it was not agreed with the findings of Alam (2), they revealed the number of chromosomes n=10, 2n=20. The results of thing study showed that the chromosomes number was n=16, 2n=32 in E.hypericifolia. This result was agreed with the study of Sadeq and Alewy (50). In E.milli, chromosomes number was n=14,18, 2n=28,36. The same result was noticed by Soontornchain(46). These results did not other researcher agreement with the study of other researcher(5,47). It was n=20, 2n=40. This species is cosmopolitan all over the world and to the different of the environmental factors. According to the present study, chromosomes number of E.puples was n=7,8, 2n=14,16. This findings was supported by the study of other's (7,19,28,29).plate.1
The results of this study show the important of the surface sculpturing which was found to be Reticulate. This finding was agreed Park (35). Measurement of the dimensions of the pollen grains is very important in differentiate between species and added a new important in formation in Taxonomy (39). A great number in this family have Zonocolporate. Grains have 3 colpi (Trizonocolporate) in *E.puples*, *E.hirta*, *E.helioscopia*, and *E.granulata*. According to the results, studied species can be classified into 3 groups:

1-First group: including pollen that have elongated shapers in *E.puples* and *E.helioscopia*

Plate 1. Phases of chromosomal division of the studied species (1000X)

2-Pollen grains

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1-First group: including pollen that have elongated shapers in *E.puples* and *E.helioscopia*
2-Second group: including grains have flat spherical shape. This group included only *E. hirta*

3-Third group: including grains which have elongated spherical shapes as in *E. granulata*. All these characters have an important taxonomic value (9). Results were showed that grains were isopolar, have simple apertures, trizonocolporate except in *E. granulata* which was zonocolporate. According to the results of LM, all the grains have yellowish color. In polar view, I-have circular to sub circular shape as in *E. granulata*, *E. helioscopia*, *E. hirta*, and *E. puples*. 2-Flat to ovate shape as in *E. milli*. 3-Have ovate to width ovate as in *E. hypericifolia*. In equatorial view: 1-Long to circular in *E. hirta*, *E. granulata* and *E. puples* 2-Flat spherical in *E. milli* and *E. helioscopia*. The sculptured was reticulate in *E. hirta*, pitted in *E. granulata* and *E. milli*, accurate reticulate in *E. helioscopia*, *E. puples* and *E. hypericifolia*. Table 2.3

<table>
<thead>
<tr>
<th>Pollen shape</th>
<th>E/P Um</th>
<th>Equatorial um</th>
<th>Polar um</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oblong</td>
<td>1.36</td>
<td>3.574</td>
<td>4.885</td>
<td><em>E. granulata</em></td>
</tr>
<tr>
<td>Oblong spherical</td>
<td>1.14</td>
<td>23.295</td>
<td>26.647</td>
<td><em>E. helioscopia</em></td>
</tr>
<tr>
<td>Flat spherical</td>
<td>0.99</td>
<td>8.975</td>
<td>8.910</td>
<td><em>E. hirta</em></td>
</tr>
<tr>
<td>Elongated spherical</td>
<td>1.13</td>
<td>4.285</td>
<td>4.854</td>
<td><em>E. puples</em></td>
</tr>
</tbody>
</table>

Table 2. Shows measurements of electron microscopy for pollen grains of species of Euphorbiaceae family

<table>
<thead>
<tr>
<th>Surface configuration</th>
<th>Pollen grain in polar view</th>
<th>Pollen grain shape in equilateral view</th>
<th>Wall thickness rate</th>
<th>E/P</th>
<th>Equilatera l axis</th>
<th>Polar axis</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pitted</td>
<td>Spherical</td>
<td>Elongated spherical</td>
<td>1.7</td>
<td>0.94</td>
<td>18(21-16)</td>
<td>17(18-15)</td>
<td><em>E. granulata</em></td>
</tr>
<tr>
<td>Accurate reticulate</td>
<td>Spherical</td>
<td>Flattened spherical</td>
<td>1.6</td>
<td>1.14</td>
<td>41(42-38)</td>
<td>47(53-45)</td>
<td><em>E. helioscopia</em></td>
</tr>
<tr>
<td>Reticulate</td>
<td>Spherical</td>
<td>Elongated spherical</td>
<td>1.3</td>
<td>0.92</td>
<td>13(14-11)</td>
<td>12(13-10)</td>
<td><em>E. hirta</em></td>
</tr>
<tr>
<td>Accurate reticulate</td>
<td>Spherical</td>
<td>Semi-spherical</td>
<td>0.9</td>
<td>1.06</td>
<td>15(16-13)</td>
<td>16(17-12)</td>
<td><em>E. hypericifolia</em></td>
</tr>
<tr>
<td>Pitted</td>
<td>Flattened ovate spherical</td>
<td>Flattened spherical</td>
<td>2</td>
<td>0.96</td>
<td>31(33-29)</td>
<td>30(31-29)</td>
<td><em>E. milli</em></td>
</tr>
<tr>
<td>Accurate reticulate</td>
<td>Flattened spherical</td>
<td>Elongated spherical</td>
<td>1</td>
<td>0.43</td>
<td>29(31-25)</td>
<td>27(28-25)</td>
<td><em>E. puples</em></td>
</tr>
</tbody>
</table>
Plate 2. Scanning electron micrographs and Light micrographs (300X.) For pollen grains of species.
3-Seeds
SEM results showed two different shape
1-Oblong ovate and different in dimensions in E.puples , E.hirta and E.helioscopia
2-Three sided oblong pyramidal in E.granulata
The sculpture was undulated with concave as aribs in E.helioscopia and undulating zigzag in E.hirta while in E.puples , the sceptered was concave papillae widespread on the external surface of the seed. Theresults showed that dimensions ranged between 889.919 mm in E.granulata and 1.713 in E.puples. According to LM, all the studied species have oblong ovate seeds with undulating in E.granulata and reticulate undulating in E.hirta and have different shape in E.helioscopia. While in E.puples, it was circular concave like pits. Al-Bukhati (8). Showed that seeds sculptured was different for the species that he studied. All these results were first show in Iraq and revealed the important taxonomic value of the studied characters.

Table 4. Showing the characteristics and dimensions of seeds in an electron microscope

<table>
<thead>
<tr>
<th>Seeds shapes</th>
<th>Surface configuration</th>
<th>Dimensions</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oblong pyramidal</td>
<td>Reticulate undulated</td>
<td>462.237</td>
<td>889.919</td>
</tr>
<tr>
<td>Oblong ovate</td>
<td>Reticulate undulated</td>
<td>1.482</td>
<td>2.238</td>
</tr>
<tr>
<td>Oblong ovate</td>
<td>Reticulate undulating</td>
<td>1.082</td>
<td>2.074</td>
</tr>
<tr>
<td>Oblong ovate</td>
<td>Circular concave</td>
<td>1.940</td>
<td>1.713</td>
</tr>
</tbody>
</table>

Table 5. Showing the characteristics and dimensions of seeds by light microscopy(LM.)

<table>
<thead>
<tr>
<th>Seeds shapes</th>
<th>Surface configuration</th>
<th>Seeds dimensions</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oblong ovate with undulating</td>
<td>Reticulate undulated</td>
<td>0.75</td>
<td>0.95</td>
</tr>
<tr>
<td>Oblong sharp ovate</td>
<td>Reticulate</td>
<td>1.35</td>
<td>2.45</td>
</tr>
<tr>
<td>Oblong ovate with differenced ends</td>
<td>Reticulate zigzag</td>
<td>1.15</td>
<td>1.9</td>
</tr>
<tr>
<td>Little oblong sharped ovate</td>
<td>Circular concave like pits</td>
<td>1.1</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Plate 3. Scanning electron micrographs showing seeds configuration for the species

- E. granulata SEM
- E. granulata SEM
- E. helioscopia SEM
- E. helioscopia SEM
- E. hirta SEM
- E. hirta SEM
- E. puples SEM
- E. puples SEM
Plate 4. showing seeds of species by Dissect light microscope

REFERENCES
Master Thesis . College of Science . Baghdad University
44. Sadeq, Zainab Ghazi. 2019. Study of micromorphological and cytological characteristics for some Asteraceae family species grown in Al-Jadriya campus, University of Baghdad
46. Soontornchain aksaeng, P and K. chaiyasut.1999.cytogenetic in restigation of some Euphorbiaceae in Thailand. Cytologia 64;229-234