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# Identification of aposporic embryo sac development in safflower (*Carthamus tinctorius* Linn)

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**Abstract**
The genotype 238-14-2 and derivatives of an interspecific cross [*C. palaestinus* X *C. tinctorius*] produced plants with cyto-exomorphic variations. The genotype 238-14-2 produced fused peripheral flowers in the capitula in addition to twin-embryo seeds and plants with higher ploidy levels at high frequency. The derivatives of the interspecific cross were either branched or unbranched and had flattened stems. The main capitulum in these plants was formed after fusion of many capitula and exhibited semi-circular shape. Flowers had single, bi- or trifurcated stigmas and produced single, twin or multipistillate ovaries with the seeds producing a high frequency of multiple seedlings. Cytological investigations of 238-14-2 and interspecific derivatives have revealed that besides unilocular ovaries containing single ovules, there exist fused uni- and bilocular ovaries with twin-ovulate condition in each locule of the bilocular ovary. Apomixis of somatic apospory-type and the development of embryo and endosperm through pseudogamy where pollination is needed for endosperm formation have been observed in the two genotypes in safflower.
Efforts to quantify the extent of apomixis in the two genotypes are in progress so that it can be used in improvement of safflower.

**Key words:** Apomixis – apospory – fascinated – twinning - safflower

## Introduction

Safflower [*Carthamus tinctorius* Linn.] plants can be classified as bushy, herbaceous annuals and produce several branches with each terminating into a globular structure called capitulum. Each capitulum produces several flowers ranging from 20-250. Safflower has a composite-type inflorescence and reproduces sexually. At the base of each flower is an inferior ovary, which develops into a single-seeded fruit or achene, which is commonly known as seed. Each flower produces a single seed. However, during the course of safflower improvement at NARI, the genotypes 238-14-2 and derivatives of an interspecific cross [*C. palaestinus* X *C. tinctorius*] produced plants with cyto-exomorphic variations. The genotype 238-14-2 produced fused peripheral flowers in the capitula, and gave twin-embryo seeds.

The exomorphic variations in derivatives of the interspecific cross *C. palaestinus* X *C. tinctorius* were in the form of flattened stems [Fig.1] and a fasciated main head with two to three capitula fused together to form a semi-circular structure [Fig.2]. However, the other capitula of the fasciated plants were normal. The flowers of the fasciated plants showed bi-or trifurcation of stigma [Fig.3] with twin and multipistillate ovaries. Similar exomorphic variations were reported earlier in safflower (Maheswari Devi and Pullaiah 1977) and in pearl millet (Hanna and Powell 1973). However, the fasciated plants in the present study exhibited the presence of a very high frequency of twin-embryo seeds (50-100%) [Fig.4]. Existence of multiple nucellar embryos has been reported earlier in the seeds of citrus (Das et al. 2007).

In view of the existence of high frequency of twin-embryo seeds in the two genotypes it was decided to study the origin of such seeds by employing suitable histological techniques and investigate reproductive behavior of such plants.

## Material and Methods

The embryological studies of pre-and post-fertilization stages were carried out through microtomy sectioning initially in the normal sexual genotypes and later in the fasciated derivatives of an interspecific cross *C. palaestinus* X *C. tinctorius* and in the genotype 238-14-2. Ovule, embryo sac and embryo development in them was studied. To study pre-fertilization stages such as ovule development, megasporogenesis and the development of embryo sac, capitula of both normal sexual genotypes and genotypes with apomixis-indicating traits were collected at successive stages from bud initiation to anthesis and were fixed in Allen-Bouins fluid for 4-12h or Randolph's solution for 12-24h (Johansen 1940) during 2006-07. For post-fertilization embryological study, the capitula were labeled at the time of anthesis and collected at intervals of 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours after pollination. The material was fixed in F.A.A. (Formaldehyde 5 ml + Acetic acid 5 ml + 70% Alcohol 90 ml) (Johansen 1940).

Somatic and meiotic chromosomal studies were also carried out in the two genotypes indicated as above.

## Results

**Embryological studies:**

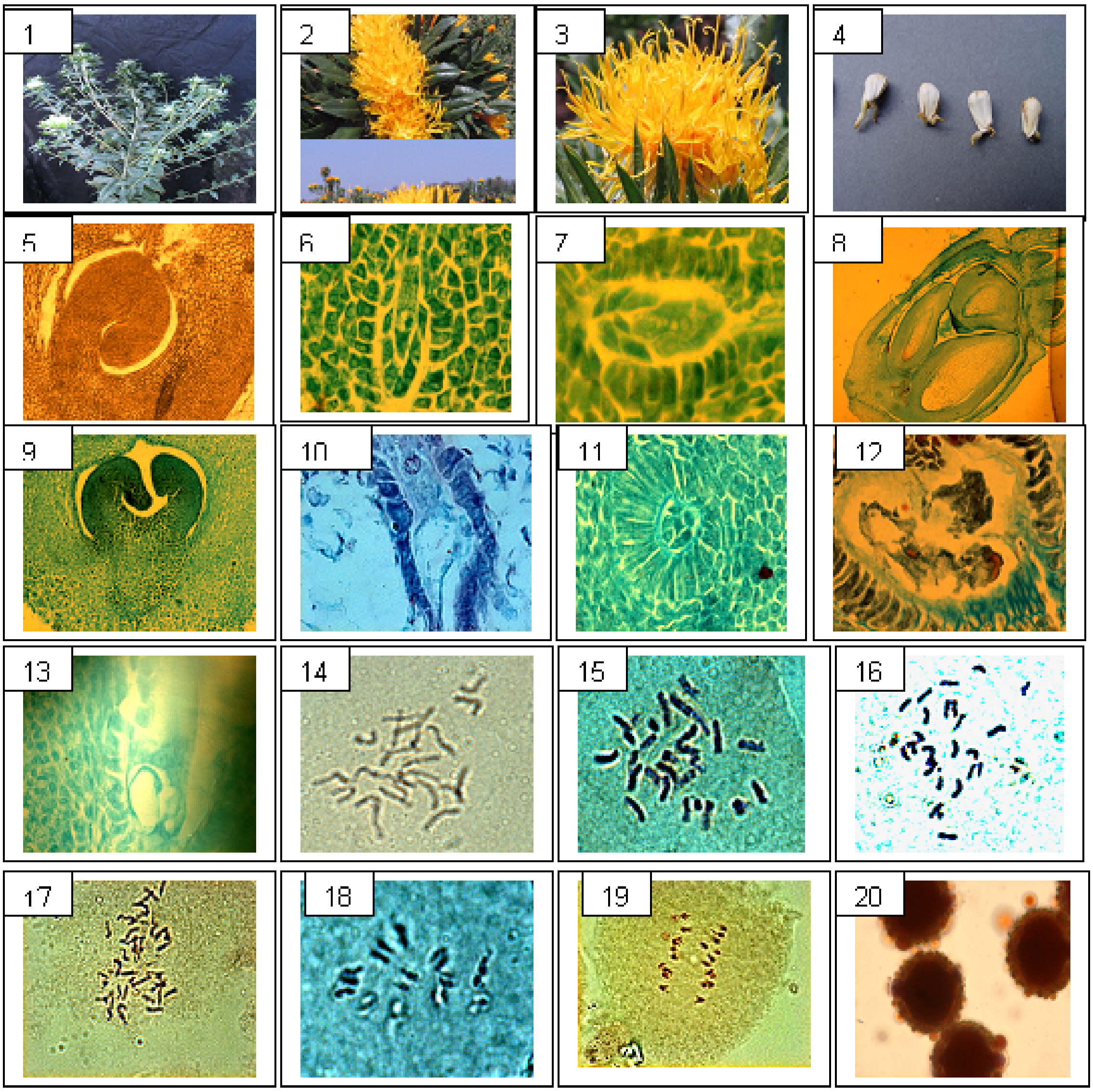
**(a)Ovule and gametophyte development in normal safflower plant:** The histological study of ovule and gametophytic development in normal safflower was done in order to know the regular process of development of the said plant parts so that any variation observed in the test genotypes could be identified. The studies showed that the flowers of normal safflower exhibited single pistils having inferior, syncarpous, unilocular ovaries with a single anatropous ovule in them [Fig.5]. The meiotic division of megaspore mother cell bordered by a single epidermal layer [Fig.6] gave rise to a linear tetrad of megaspores indicating the sexual nature of embryo sac development [Fig.7]. The fully developed embryo sac showed eight nuclei arranged in a ratio of three at micropylar end, two at center and three at chalazal end of embryo sac forming a mature sexual female gametophyte. In post-fertilization studies it was seen that embryo sac cavity enlarged much faster than the proembryo and was lined quickly with rapidly dividing cells of endosperm. These results are in conformity with the earlier findings in safflower (Carapetian and Rupert 1989).

**(b) Ovule and gametophyte development in fasciated-type derivatives of interspecific cross *C. palaestinus* X *C. tinctorius* and genotype 238-14-2:** Embryological studies in both the derivatives of the interspecific cross between *C. palaestinus* X *C. tinctorius* and in 238-14-2 showed some interesting deviations like the fusion of two to three ovaries to form a single unilocular ovary with two or three ovules in it [Fig.8]. The ovules are fused or separate as can be assessed from the condition of the funiculi [Fig.9]. Embryo sac development in these genotypes showed the presence of sexual embryo sacs in some ovules and multiple embryo sacs in others. The multiple embryo sacs were aposporic in nature as they originated from nucellar cells located inside the integumentary tapetum and also in some cases from the aposporic initial at chalazal end [Fig.10]. The presence of multiple embryo sacs in an ovule [Figs. 11 and 12] indicated the apomictic nature of embryo development in these safflower genotypes. The presence of both sexual and asexual embryo sacs in capitula of fasciated plants reveals the facultative nature of apomixis in safflower.

To know the type of embryo development in apomictic safflower, some of the capitula of an apomictic plant were emasculated before flowering and bagged to avoid pollination by stray pollen so that the autonomous embryo development in them could be examined. Some of the emasculated capitula were pollinated on subsequent days with pollen from the remaining capitula of the same plants. The screening of pollinated and unpollinated emasculated flowers of the apomictic plants for seed setting after 10 days revealed that no seed setting had taken place in flowers which were bagged and unpollinated. However, full seed setting was observed in the flowers which were pollinated after emasculation. Thus the study suggested that the embryo development in apomictic plants in safflower is pseudogamous in nature [Fig.13], wherein endosperm development takes place only after fertilization for which pollination is needed.

**Somatic and meiotic chromosomal studies:** The somatic chromosomal studies carried out in safflower genotype 238-14-2 and fasciated derivatives which produced multi-embryo seeds revealed that the prophase, metaphase, anaphase and telophase in both the genotypes were normal. The metaphase plates of genotype 238-14-2 showed the chromosome number to be 2n=24 in single-embryo seeds [Fig.14], while in twin-embryo seeds it was 2n=24+2 indicating the presence of aneuploids in the genotype [Figs.15 and 16]. The presence of tetraploids (2n=48) was also observed in a few cases [Fig.17]. This genotype appeared to have a greater tendency to produce hyperploids and thus was thought to be apomictic.

The derivatives of *C. palaestinus* X *C. tinctorius* which had fasciated stem and main capitulum and which produced twin and multi-embryo seeds were subjected to meiotic investigations. The meiotic studies showed 12 normal bivalents at metaphase-I and at diakinesis [Fig. 18]. Equal distribution at both the poles with no laggards was observed at anaphase-I [Fig. 19] .This indicated that there were no cytological abnormalities during microsporogenesis in these derivatives. However, the interspecific derivatives showed variable seed setting, which ranged from 5-100%, which could at least partly be attributed to variable pollen fertility [Fig. 20]. In addition to non-fertilization, another possible reason for low seed set may be competition between multiple pistils, ovules and embryo sacs.




**Description of figures:**
(1) Plant with flattened stems (2) Semicircular structure of main capitulum (3) Bi- or trifurcation of stigmas in fasciated plants (4) Germinating seeds with multiple radicles. (5) Single sexual anatropous ovule (6) MMC bordered by a single epidermal layer in a sexual plant (7) Linear tetrad of megaspores formed after second meiotic division of MMC (8) Fusion of the ovules with each other depending on the development of the funicle,

whether common or independent (9) Twin ovules with a common funicle (10) Aposporous initial at the chalazal end of the ovule (11) Multiple (aposporous) embryo sacs (12) Multiple embryo sacs in a single locule (13) Pseudogamous twin embryo development (14) 2n=24 in normal plant (15&16) 2n=24+2 in twin seeds of 238-14-2 (17) 2n=48 (tetraploid) in 238-14-2 (18) $II^{12}$ at diakinesis in fasciated plants (19) Anaphase-I showing equal distribution of chromosomes with no laggards in fasciated plants (20) fertile pollens of fasciated plant.

**Discussion and Conclusion:**
Safflower (*Carthamus tinctorius* L.) belonging to family Asteraceae exhibits wide variation in exomorphic and embryological features. A normal safflower plant has characteristic inferior, bicarpellary, syncarpous, unilocular ovaries. However, the exomorphic and embryological characteristics in the fasciated derivatives of the interspecific cross *C. palaestinus* X *C. tinctorius* and in the genotype 238-14-2 differ widely from those of normal sexual types. Existence of high frequency of multipistillate uni- or bilocular ovaries with varied number of ovules in each locule and occurrence of multiple embryo sacs in a single ovule in the derivatives of the interspecific cross and in the genotype 238-14-2 point to possible existence of apomixis in them.

Similar observations have been made in safflower earlier (Maheswari Devi and Pullaiah 1977). They also reported aposporic development of embryo sac in some of the ovules of safflower. We also observed here that one or more cells at the chalazal end enlarge, become vacuolated and develop into unreduced embryo sacs. This can be called as somatic type of apospory in which somatic cells from nucellus and integument at the chalazal end are involved in the development of the unreduced embryo sacs. The embryo sac development is of hieracium-type where one or more cells at the chlazal region directly develop into the initials of a gametophyte after undergoing mitotic divisions thereby by-passing the formation of linear tetrads as in regular meiosis of sexual process. However, in the present material, it was found that both normal and aposporic embryo sacs develop simultaneously and become functional. Thus the development of embryo and endosperm seem to be pseudogamous. The studies done on microsporogenesis of the fasciated derivatives have shown normal production of pollens with 2n=24 chromosomes countable at diakinesis. However occurrence of tetraploids (2n=48) and aneuploids (2n=26) in multi-embryo seeds of the genotype 238-14-2 was an interesting feature. The presence of ploidy levels higher than normal in 238-14-2 further suggests the possible existence of apomixis in it (Grimanelli et al. 2001), since apomixis has been reported in the polyploid forms of *Tripsacum* (Grimanelli et al. 1998), *Paspalum notatum* (Quarin et al. 2001) and angiosperms (Galitski et al. 1999).

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