*J. Agric. Sci. Mansoura Univ., 25 (4): 2303 - 2313, 2000.*

# BREEDING STUDIES ON *Calendula SP.*

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## ABSTRACT

The present work aimed to study a breeding method for two *Calendul*a species, the cultivated species *Calendula officinalis* and the wild species *Calendula micrantha*. The study was concerned with the probability of hybridization between both species to obtain a hybrid plant with attractive flowers that needs less water requirements and maintenance, to be used as a ground cover or for flowerbeds in arid and semi-arid regions. This study was carried out in the Flowers and Ornamental Plants Research Gardens at the Faculty of Agriculture, Alexandria University from 1994 to 1997.

A comparison between the two used species was carried out (growth habit, vegetative growth, flowering, carotenoid content in flowers, stomata, anthers, pollen grains, pollen fertility and relative distribution of DNA content).

Crossing between both species took place and failed to produce hybrid seeds. Results indicated that adaptation had a significant effect on the growth and flowering of the wild *Calendula* species.

The comparison between the two *Calendula* species explained - to some extent - the failure of the crossing between both species regarding to their different genetical and anatomical structure.

## INTRODUCTION

*Calendula* is a genus that belongs to the Family *Compositae* with 15 species according to the studies along 57 years as shown in Table 1. In Egypt, there are two species; the common cultivated species *Calendula officinalis* and the wild type species *Calendula micrantha*. The common cultivated plant is one of the most universal garden flowers, running into many varieties, distinguishable by size, color, and degree of doubling. The color varies from white-yellow to deep orange. (Bailey, 1960).

The wild species is found in the deserts of Sinai and the Northern Coast of Egypt. This species has a prostrate habit of growth with small, orange flowers and can be used as a ground cover for a wide array of purposes, such as residential landscape plantings, parks, gardens, highway and street medians, industrial sites, and disturbed areas. An especially important need is groundcovers requiring little water and maintenance for arid and semi -arid regions with their limited water supply and with attractive flowers.

The ultimate aim of the present study was

(a) to adapt and cultivate the wild species *Calendula micrantha*,

(b) to compare between the wild and cultivated species in different means such as their vegetative and flowering characteristics, some anatomical and cytological characters and the carotenoid content in the ray florets, stomata number and size, anther size and pollen grain diameter and fertility, anther and pollen morphology by using Scanning Electron Microscope (SEM) was carried out to examine the possibility of their utility

as a mean of species identification in both *Calendula micrantha*. and *Calendula officinalis*. Determination of differential DNA content in root-tip cells in both species was done employing the Computerized Image Analysis for Scientific Application Systems (CIASA) as a mean of species identification and an indication for crossing ability.

(c) To carry out crosses between the two species to obtain a hybrid plant.

## MATERIALS AND METHODS

The experiments were carried out during the years from 1994 to 1997 in the Flowers and Ornamental Plants Research Gardens at the Faculty of Agriculture, Alexandria University, Egypt.

Seeds of the cultivated species were obtained from the Flowers and Ornamental Plants Research Gardens of the Faculty of Agriculture, Alexandria University while those of the wild species were obtained from the desert of the North Western Coast of Egypt.

500 seeds were specified to the field experiments, divided into 5 equal parts (100 seeds for each part) from each species for the germination rate experiment.

Seeds of both cultivated and wild species were sown on October 15$^{th}$, 1994 in the first season and on October 12$^{th}$, 1995 in the second one,

The first season was an evaluation trial to obtain hybrids from the different crosses between both species. Thus, there was no experimental design for the evaluation trail (Snedecor and Cochran, 1967). The second season was concerned with estimating the DNA content of both species to find out why the fertilization did not take place.

On November 25$^{th}$, 1994 and November 22$^{nd}$, 1995 in the first and second seasons respectively, seedlings were individually transplanted to 20 cm. diameter clay pots using clay soil. Germination percentage was determined at 25 $^{o}$C in the laboratory on wet filter papers in Petri dishes on October 25$^{th}$, 1994 in the first season and October 22$^{nd}$, 1995 in the second one.

Crosses began in February 1995 after classifying the plants of *C officinalis* into four groups according to the color of the disc and ray florets as follows:

1- *Calendula officinalis* (yellow/ yellow form) x *Calendula micrantha*

2- *Calendula officinalis* (brown/ yellow form) x *Calendula micrantha*

3-*Calendula officinalis* (orange/orange form) x *Calendula micrantha*

4-*Calendula officinalis* (brown/ orange form) x *Calendula micrantha*

Both species were used once as maternal and another as parental in the previous crosses.

**Palynological Studies**

Leaf samples and flower buds were collected and stored in a solution of ethyl alcohol 70% at room temperature until further use according to (Sharma and Sharma, 1980)

The samples were then coated with gold using JEOL JFC E Sputter Coater alloy about 40-60 nm thickness and was then examined with a JEOL JSM- 5300 Scanning Electron Microscope at 25 kV.

Anthers morphology were prepared for Scanning Electron Microscope observations. Pollen grains of both species of *Calendula micrantha* and *Calendula officinalis* were morphologically studied using the Scanning Electron Microscope (SEM). The grain diameter of at least 50 random pollen grains were measured.

**Cytophotometric image analysis**

CIASA cell image processor (Computerized Image Analysis in Scientific Application Systems, Leica Company) was used to estimate and analyze the Grey values (G-values of the root tip cells and pollen mother cells as an indicator to DNA content of these cells

**Pollen Fertility**.

To determine pollen fertility dehiscent anthers were slightly tapped on a drop of acetocarmine on a slide. For each slide, ten microscopic fields were chosen at random and such pollen grains were counted in fertility and sterility classes.

All data of the present investigations were recorded and analyzed statistically according to Snedecor and Cochran (1967).

## RESULTS AND DISCUSSION

**Seed Germination percentage and growth characteristics**

Resuls of the comparison between *C. micrantha* and *C. officinalis* are shown in Table 2. Generally the adaptation of *C micrantha* increased growth characteristics (except for seed germination percentage because of the damping off infection).

**Hybridization**

The interspecific hybridization attempted in both *Calendula* species failed to produce hybrid seeds. This result raised many questions concerning the relationship between the species and their evolution. According to (Heyn and Joel, 1983) the annual Calendula species, which are selfers, succeeded in producing fertile F1 and F2 hybrids from crosses between parents with similar chromosome numbers: *C. palaestina x C. pachysperma* and crosses of different morphological forms of *C.arvensis* but species with different chromosome number at least partly fertile F1 hybrids were obtained from *C.tripterocarpa x C. stellata* and *C.tripterocarpa x C.arvensis* and crosses of the latter with *C. palaestina*.

**Table 2 : A comparison between *Calendula micarantha* and *C. officinalis* in first (S1) and second (S2) seasons**

| Character | *C.micrantha* | *C.officinalis* |
|---|---|---|
| Seed Germination (%) | | |
| S1 | 62.8 | 64.6 |
| S2 | 15.6 | 55.1 |
| Plant height /diameter (cm) | | |
| S1 | 7.35 | 18.8 |
| S2 | 14.6 | 31.3 |
| No. of main branches | | |
| S1 | 4.1 | 6 |
| S2 | 5.9 | 7.9 |
| Flower diameter (cm) | | |
| S1 | 1.5 | 5 |
| S2 | 1.9 | 6.4 |
| Flowering date * | | |
| S1 | 79.6 | 59.6 |
| S2 | 69.9 | 41.8 |
| Carotenoid content (mg beta carotene/lb) | | |
| S1 | 1.49 | yellow 1.82<br>orange 3.35 |
| S2 | 1.51 | yellow 1.85<br>orange 3.37 |

* expressed as number of days from sowing to showing th

In the present study the failure to produce hybrid seeds could be contributed to one of the following reasons:

1- Different genome number of chromosomes in both species as the basic number (x) of *Calendula officinalis* is 7 or 8 , while that of *Calendula micrantha* is 11.

2- Different types of ploidy, as *Calendula officinalis* are allotetraploid or amphidiploid (Ohle,1974 and 1975 a, b ) as follows:

$P_1$ *Calendula maroccana* (2n=18) x *Calendula stellata* (2n=14)

$F_1$ → 2n = 16

↓

Doubling

↓

*Calendula officinalis* (2n = 32)

On the other hand, *C. micrantha* is allopolyploid (4x11 =44 chromosomes).

3- Different anatomical and physiological characteristics of the reproductive organs in the flowers (anthers).

4- Different size and shape of pollen grains in both species.

5- Difference in the relative distribution of cellular DNA content in the cell cycles of both species.

6- Different growth habit of both species as *Calendula micrantha* is considered as an annual with creeping growth, while *Calendula officinalis* is considered as a perennial with erect growth and these differences were genetical , which means different genetical structure which may affect the germination of the pollen grains on the stigma.

**Palynological studies**

The stomata of *Calendula officinalis* are larger than the stomata of *Calendula micrantha* and the arrangement of stomata on the lower surface of the leaves of both species differs as the stomata are arranged parallel in *Calendula officinalis*, while they are arranged randomly in *Calendula micrantha* (Fig. 1). The frequency (stomata/$cm^2$) was higher in *Calendula micrantha* than in *Calendula officinalis*. Stomata size, distribution and frequency can be also used as a tool for species identification.

Figure 2 shows the difference between the anther morphology of both *Calendula* species. *Calendula officinalis* has larger anther compared with the anther of *Calendula micrantha*. Anther morphology could be considered as a mean of species identification. The large size of the anthers in *Calendula officinalis* can be as a result of its allopolyploidy genetic structure.

**Figure 1: Scanning Electron Microscope photograph showing the stomata morphology of both *Calendula* species: A. Stomata of *Calendula***

***micrantha.*** **B. Stomata of** ***Calendula officinalis.***

**Figure 2: Scanning Electron Microscope photograph showing the anther morphology in both *Calendula* species: A. Anther Morphology of *Calendula micrantha.* B. Anther Morphology of *Calendula officinalis.***

**Figure 3: Scanning Electron Microscope photograph of the pollen grain morphology of both *Calendula* species. A. Pollen grain of *Calendula micrantha.* B. Pollen grain of *Calendula officinalis.***

Pollen grains of both *Calendula* species as shown in Figure 3 show a great variation in size and shape of the pollens and this result could be an explanation for why the crossing between both species did not take place.

As a conclusion, the differences in pollen morphology among *Calendula* species apparently corresponds with ploidy levels of the species is considered a mean of species identification. The large pollen grain of *Calendula officinalis* can be attributed to its allopolyploidy genetic structure.

**Relative distribution of cellular DNA content in both *Calendula* species using Cytophotometric Image Analysis**

Figure 4 shows a comparison between *C. officinalis* and *C. micrantha*. In the cultivated species *Calendula officinalis*; 24.43 % of the whole cell population contained DNA equal to 2C, this refraction was in the G1 phase (a pre-replicative stage of interphase). At the beginning of the interphase, 38.8% of the whole cell population were considered non-dividing cells in S phase (replicative stage of nuclear division). While, 9% of the cells were in G2 phase and represented a relatively small portion of cell population.

In *Calendula micrantha*, It is supposed that 24.13 % of the whole cell population which contained DNA equal to 2C was in the G1 phase (a pre-replicative stage of interphase) and in the beginning of the interphase. The cells which contained DNA between 2c and 4c represented 38 % of the whole cell population and were considered to be non-dividing cells in S phase. whereas 10.3 % of the whole cell population were in the G2 phase.

Both species should be considered as diploids with different basic chromosome numbers as the cultivated *Calendula officinalis* has the basic number (x) of 8, while the wild species has the basic number of 11. This result could however explain the failure of producing hybrid seeds among other reasons mentioned previously.

Variations in relative distribution of DNA content in interphase nuclei in random samples of cell-population of root-tip cells of both *Calendula* species could possibly be attributed to one of the following reasons:

1- Heterochromatin amounts.

2- Variation in the number of chromosome strands and/or chromosome numbers.

Chromosome number has often been found to be significantly correlated with nuclear DNA content. (Black and Beckmann, 1983).

The present findings of the Cytophotometric studies agree with the different chromosome numbers of both *Calendula* species as the cultivated species "*Calendula officinalis*" chromosome number was stated by Negodi (1936), Darlington and Ammal (1948) and Murin (1993) as 2n=28 and by (Ohle,1974;1975) as 2n = 32 while "*Calendula micrantha*" has chromosome number of 2=44 (Meusel and Ohle,1966 and Nordenstam, 1972).

According to the previous results, both species have different cell cycles as the DNA cell cycle in *C. officinalis* begins at 500 arbitrary units and ends at 1600 arbitrary units, while the cell cycle in *C.micrantha* begins at 550 arbitrary units and ends at 1550 arbitrary units. Thus there is a difference of 50 arbitrary units between the two cell cycles.

**Figure 4. a: Relative Distribution of DNA Content in *Calendula officinalis***

**b: Relative Distribution of DNA Content in *Calendula micrantha***

**Pollen Fertility**

The pollen fertility as indicated by examining the morphology and stainability of pollen grains was estimated (Table 3). The fertility of pollen grains in *Calendula* species was found to be considerably different. The *Calendula officinalis* showed the higher percentage (87.29%), while *Calendula micrantha* showed the lower one (76.78 %).

**Table 3: Percentage of viable (stainable) and aborted pollen grains in random samples of the two *Calendula* species.**

| Species | Total No. of pollen examined | Viable pollen | | Aborted pollen | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| ***C. officinalis*** | 4605 | 4020 | 87.29 | 585 | 12.70 |
| ***C. micrantha*** | 4845 | 3720 | 76.78 | 1125 | 23.22 |

There is a relationship between the chromosomal behaviour at meiosis and pollen fertility. Amphidiploid plants have – generally- higher pollen fertility than allopolyploid plants because of the more regular chromosome configuration and separation by the meiosis. It seems probable that irregular chromosomal behavior and / or genetic background, beside physiological and pathological factors may cause the reduction of fertility.

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## دراسات في تربيـــة الأقحـــوان

**مصطفى بدر ، علا الشناوي و رانيا عبد الفتاح**

**قسم الزهور ونباتات الزينة وتنسيق الحدائق - كلية الزراعة - جامعة الإسكندرية**

كان هدف الدراسة الحالية هو البحث في تربية نبات الإقحوان باستخدام نوعين هما المنزرع واالبري ، وكان الهدف الأساسي هو محاولةالتعرف على صفات النوعين و اجراء تهجين بينهما بالإضافة إلى محاولة أقلمة النبات البري .

ويمكن تلخيص النتائج المتحصل عليها في هذا البحث فيما يلي :

1- تمت دراسة الأنواع التابعة لجنس الأقحوان ، والتعرف على أوصافها وطبيعة نموها وتركيبها الوراثي مما يساعد في أية دراسات لاحقة ..

2- اثرت عملية الأقلمة للنوع البري على صفات نموه الخضرية والزهرية .

3- وجدت اختلافات بين النوعين في نسبة الكاروتين في الأزهار .

4- وجدت اختلافات بين النوعين في حجم وشكل وطريقة توزيع الثغور .

5- أوضحت النتائج المتحصل عليها من دراسة متك أزهار النوعين المستعملين باستخدام المجهر الإلكتروني الماسح اختلافا في حجم وشكل المتك .

6- أوضحت نتائج الدراسة على حبوب لقاح النوعين بعد فحصها بالمجهر الإلكتروني الماسح أن هناك اختلافاً واضحاً بين النوعين في شكل و حجم حبوب اللقاح ، علاوة على التجاعيد الموجودة على الجدار الخارجي لحبة اللقاح وهذه خصائص مفيدة كوسيلة للتعرف على النوع وتمييزه .
7- أوضح تحليل النتائج المتحصل عليها من قياس كمية المادة الوراثية بالخلية أن هناك تفاوتاً في محتوى الأنوية من المادة الوراثية حيث ظهرت بعض الاختلافات بين التراكيب الوراثية للنوعين المستخدمين .
8- أظهر تقدير حيوية حبوب اللقاح للنوعين المستخدمين تبايناً واضحاً .
9- لم تنتج التهجينات بين النوعين المستخدمين اية بذور حية.
10- نتيجة لاختلاف النوعين المستخدمين في الصفات السابقة فقد أمكن إلى حد ما تفسير فشل حدوث التهجين بين النوعين .