Studies on Induced Polyploidy in *Zinnia linearis* Benth

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Introduction

So far as it is known there are no reports of induced polyploid work in *Z. linearis*. Other reports on polyploid induction in *Zinnia*, apparently made on species other than *Z. linearis*, has been recently made by Srivastava (1963). Earlier, Elliott (1958) mentioned induction of tetraploidy in other species of *Zinnia*. Because of this, it was thought desirable to undertake induced 'polyploidy work in this species. In the present investigation two varieties of *Z. linearis* have been taken for the induction of polyploidy.

Materials and methods

Seeds of two varieties of *Z. linearis*, var. *Orange* and var. *White* were obtained from M/S. Sutton and Sons, Calcutta, and the method of colchicine treatment was as follows.

Seedlings having two leaves were taken for control and for 0.2 per cent colchicine treatment since this concentration was found to be most effective from previous experience in our laboratory (Hati 1963, Mukherjee 1965, Bose and Hati 1966). Thirty seedlings were used for control and the same number of seedlings were taken for 0.2 per cent colchicine treatment. Apical growing regions of these seedlings were treated with 0.2 per cent colchicine solution and daily treatment of four hours was given for three consequitive days making a total dose of twelve hours. Constant care was taken to see that the cotton wads, which were used for applying colchicine solution, did not dry up during the course of treatment. Both the control and the colchicine treated ones were transplanted in individual pots 37 days after sowing.

For meiotic studies, young buds of suitable size were fixed in 1:3, acetic acid : alcohol, for about 24 hours and then preserved in 70 per cent alcohol until use. Pollen mother cells were stained in 2 per cent aceto-orcein solution, while pollen grains were stained in 1 per cent aceto-carmine solution.

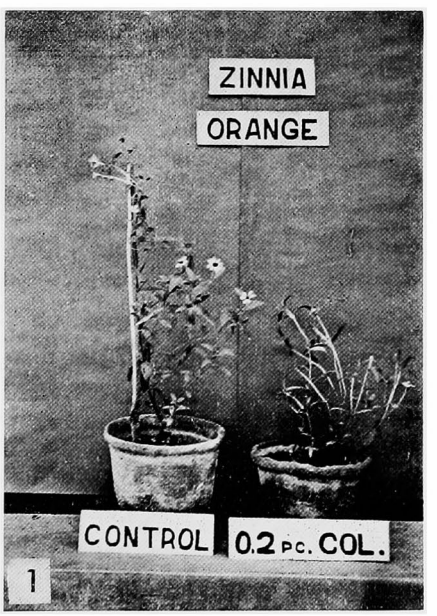
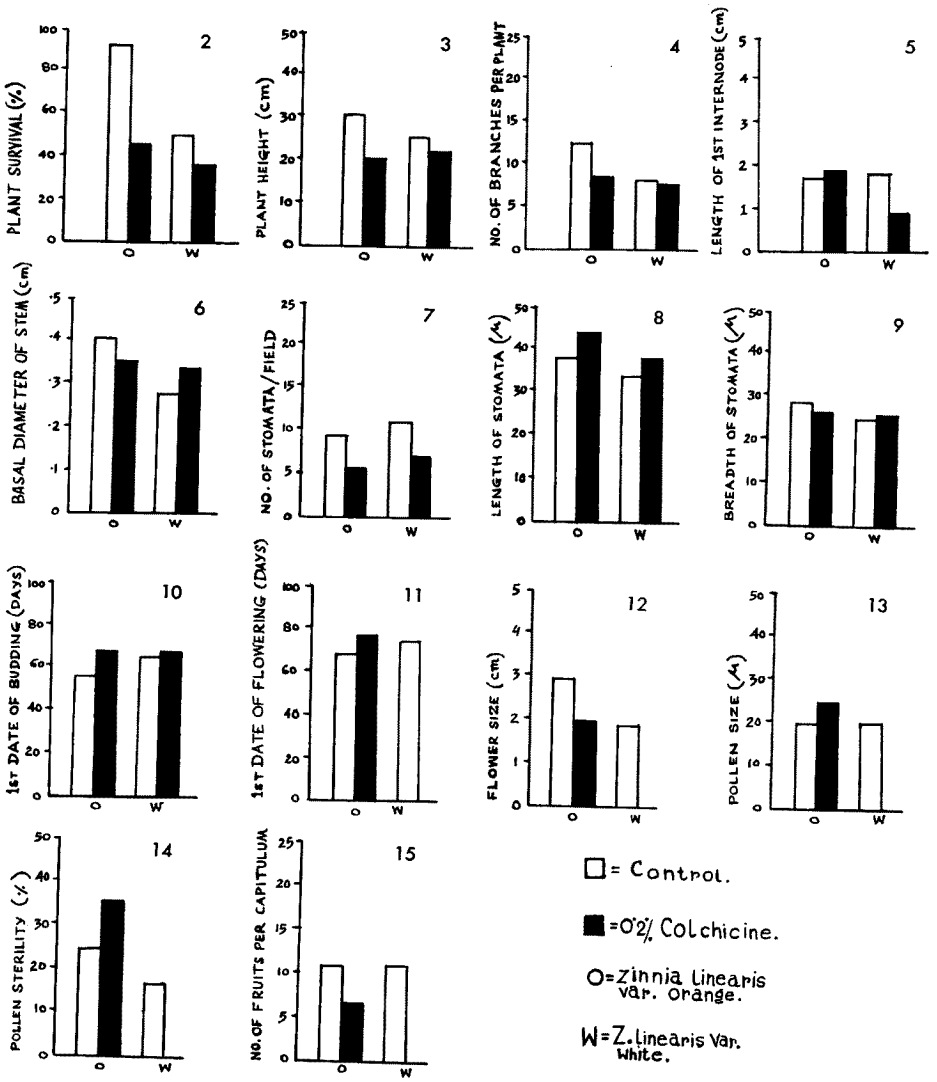


Fig. 1. A control and a 0.2% colchicine treated plant in *Z. linearis* var. *Orange*. Note stunted growth, many branches and few flowers in the latter.

Observations

It can be observed from Table 1 that the percentage of survival of the colchicine treated plants of both the varieties were lower than the respective controls but the number of colchicine treated plants surviving in the var. *Orange* was more than that in the var. *White* (Fig. 2). Colchicine treated plants of both the varieties exhibited slow growth during the early stages of development and the height of colchicine treated plants of both the varieties were shorter than the respective controls (Fig. 3). The leaves of some of the colchicine treated plants were thicker, leathery and dark green in colour. A few plants in the var. *Orange* had wrinkled leaves.



Figs. 2-15. Explanations in the text.

Number of branches in the colchicine treated ones of both the varieties were less than that in the control (Fig. 4). Significant decrease in the length

Table 1. Effect of colchicine on survival of plants and plant height in two varieties of *Zinnia linearis* Benth

Treatment	No. of plants treated	No. of plants surviving till maturity	Percent of control	Plant height (cm.)	
				Range	Mean±S.E.
			var. *Orange*		
Control	30	28	100.00	19-47	31.85±1.294
Colchicine (0.2%)	30	14	50.00	7.5-34.5	20.39±2.354*
			var. *White*		
Control	30	15	100.00	19.0-34.0	45.46±1.000
Colchicine (0.2%)	30	11	73.33	10.0-27.5	22.22±1.393*

* Significant at 5%

Table 2. Number of branches, length of Ist internode and basal diameter in two varieties of *Z. linearis* Benth following colchicine treatment of seedlings

Treatment	No. of branches per plant		Length of Ist internode		Basal diameter	
	Range	Mean±S.E.	Range	Mean±S.E.	Range	Mean±S.E.
			var. *Orange*			
Control	5-17	12.53±0.535	1.2-2.6	1.75±0.055	0.29-0.52	0.41±0.009
Colchicine (0.2%)	2-14	8.5 ±1.157*	1.8-2.1	1.94±0.053	0.27-0.42	0.36±0.018*
			var. *White*			
Control	4-12	8.2 ±0.506	1.5-2.2	1.86±0.063	0.22-0.32	0.28±0.215
Colchicine (0.2%)	4-11	7.72±0.648*	0.6-1.5	0.96±0.077*	0.22-0.48	0.34±0.143

* Significant at 5%

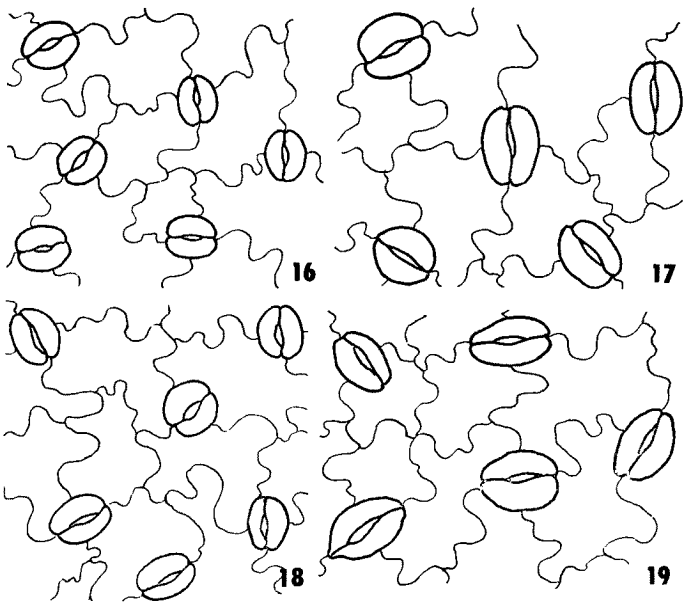
Table 3. Effect of colchicine on stomata frequency and size in two varieties of *Z. linearis* Benth

Treatment	No. of stomata per field		Length of stomata		Breadth of stomata	
	Range	Mean±S.E.	Range	Mean±S.E.	Range	Mean±S.E.
			var. *Orange*			
Control	7.8-11.2	9.58±0.843	35.6-40.4	37.92±1.197	24.0-31.6	28.56±1.439
Colchicine (0.2%)	4.6-6.4	5.63±0.536*	41.2-47.2	43.46±1.878	24.4-26.8	25.86±0.739
			var. *White*			
Control	10.6-11.6	11.00±0.244	32.0-36.0	34.10±0.920	22.8-28.0	24.80±1.140
Colchicine (0.2%)	6.4-7.7	6.97±0.870*	33.2-43.2	38.20±0.333	22.0-27.2	25.30±1.180

* Significant at 5%

of first internode was observed in the var. *White*, while basal diameter of the colchicine treated plants in the var. *Orange* was significantly higher than the control (Table 2, Figs. 5, 6).

It is evident from Table 3 that a significant decrease in the number of stomata per field was noticed in the colchicine treated plants of both the varieties. Increase in stomatal size was also noticed in the colchicine treated plants in the var. *White*, while the treated plants of the var. *Orange* showed increase in length (Figs. 7-9, 16-19).



Figs. 16-19. 16, stomatal size in *Z. linearis* var. *Orange* (control). ×500 approx. 17, stomatal size in *Z. linearis* var. *Orange* (0.2% colchicine). ×500 approx. 18, stomatal size in *Z. linearis* var. *White* (control). ×500 approx. 19, stomatal size in *Z. linearis* var. *White* (0.2% colchicine). ×500 approx.

Flower buds and flowers appeared much later in the colchicine treated plants of the var. *Orange* in comparison with the control and flower size in the treated ones were also shorter than the control (Table 4, Figs. 10-12). In the var. *White* all the buds in the colchicine treated plants dried up

Table 4. Effect of colchicine on flowering and flower size in two varieties of *Z. linearis* Benth

Treatment	Ist date of budding (days)		Ist date of flowering (days)		Size of flowers (cm)	
	Range	Mean±S.E.	Range	Mean±S.E.	Range	Mean±S.E.
	var. *Orange*					
Control	49-63	56.17±0.282	59-73	67.03±1.216	1.7-3.0	2.8 ±0.056
Colchicine (0.2%)	62-76	64.14±1.553*	72-85	76.2 ±4.310	1.5-2.2	1.95±0.082
	var. *White*					
Control	56-68	66.35±1.034	64-76	72.68±1.037	1.6-2.1	1.86±0.040
Colchicine (0.2%)	62-72	66.70±0.760	—	—	—	—

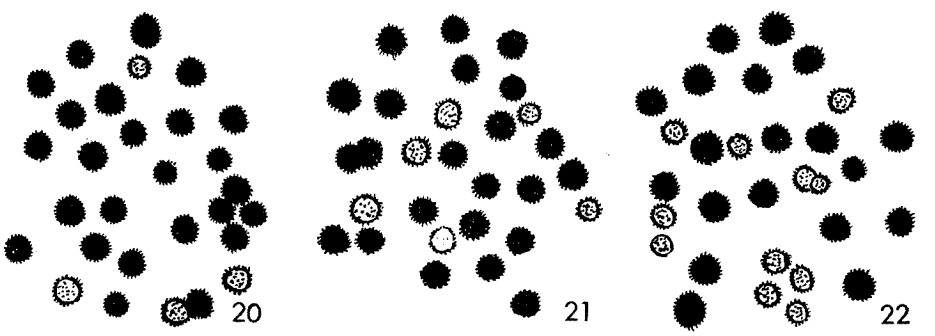
* Significant at 5%

before they came to flowering so no data could be secured in this respect. A significant increase in pollen size and sterility was observed in the colchicine treated plants of the var. *Orange* in comparison with the control (Table 5, Figs. 13, 14, 20–22).

Table 5. Effect of colchicine on pollen size and sterility in two varieties of *Z. linearis* Benth

Treatment	Pollen size (μ)		Pollen sterility (%)	
	Range	Mean±S.E.	Range	Mean±S.E.
	var. *Orange*			
Control	16.8-20.6	19.35±0.983	22.2-28.5	24.45±1.620
Colchicine (0.2%)	23.4-25.2	24.40±0.424*	28.9-39.4	35.75±2.422*
	var. *White*			
Control	20.0-20.2	20.1±0.400	13.6-20.0	16.80±3.177
Colchicine (0.2%)	—	—	—	—

* Significant at 5%

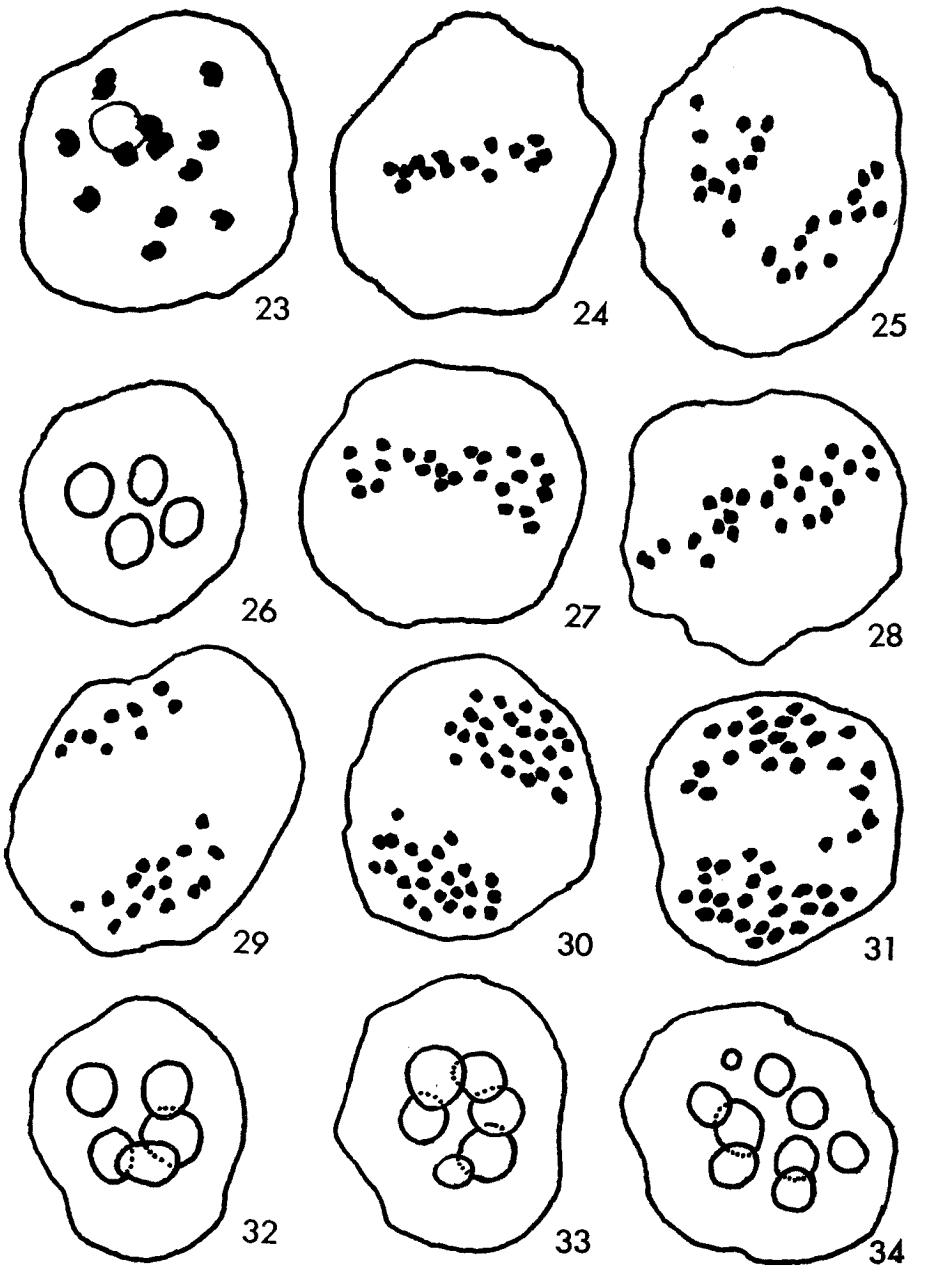


Figs. 20–22. 22, fertile and sterile pollen in *Z. linearis* var. *White* (control). ×500 approx. 21, fertile and sterile pollen in *Z. linearis* var. *Orange* (control). ×500 approx. 22, fertile and sterile pollen in *Z. linearis* var. *Orange* (0.2% colchicine). ×500 approx.

Table 6. Effect of colchicine on fruit size (capitulum) and number of seeds (fruits) per fruit (capitulum) in two varieties of *Z. linearis* Benth

Treatment	Size of fruit (capitulum) (cm)		No. of seeds (fruits) per capitulum	
	Range	Mean±S.E.	Range	Mean±S.E.
	var. *Orange*			
Control	0.6-0.9	0.73±0.013	9-14	11.0±0.033
Colchicine (0.2%)	0.5-0.9	0.67±0.039	3-9	6.7±0.558*
	var. *White*			
Control	0.6-0.9	0.71±0.03	9-13	11.1±0.433
Colchicine (0.2%)	—	—	—	—

* Significant at 5%



Figs. 23-34. 23, diakinesis showing 12 bivalents in *Z. linearis* var. *Orange* (control). ×1000 approx. 24, metaphase I showing 12 bivalents in *Z. linearis* var. *Orange* (control). ×1000 approx. 25, anaphase I showing n=12 chromosomes in each pole in *Z. linearis* var. *Orange* (control). ×1000 approx. 26, a tetrad from a control plant in *Z. linearis* var. *Orange*. ×1000 approx. 27, metaphase I showing 24 bivalents in *Z. linearis* var. *Orange* (0.2% colchicine). ×1000 approx. 28, another metaphase I showing 24 bivalents in *Z. linearis* var. *Orange* (0.2% colchicine). ×1000 approx. 29, anaphase I showing n=9 at one pole and n=15 at the other in *Z. linearis* var. *Orange*

Number of fruit (seed) set per capitulum in the control plants were significantly, higher than the colchicine treated plants of the var. *Orange*. The size of capitulum, however, did not differ significantly among the control and the colchicine treated ones (Table 6, Fig. 15).

Reciprocal crosses between control and colchicine treated plants showed that highest percentage of fruit set was found when control plants in the var. *Orange* was used as female parent and crossed with the colchicine treated ones while lowest percentage of fruit set was from colchicine treated plants of the var. *Orange* used as female parent and crossed with the control of the var. *White*.

Meiotic chromosome count from some of the control and colchicine treated ones in the var. *Orange* showed n = 12 chromosomes while n = 24 chromosomes were counted in a few of the colchicine treated ones. In addition to this, meiotic irregularities like unequal separation of chromosomes at anaphase I were noticed in some of the treated ones. Regular tetrad formation was noticed during pollen grain formation in the control ones while pentad, hexad and higher categories were observed in the treated ones, in addition to the tetrads (Figs. 23–34).

Discussion

Studies on the effect of 0.2 percent colchicine on two varieties of *Z. linearis* revealed that the colchicine treated plants were shorter in height, number of stomata per field was less, stomata and pollen grains were bigger and pollen sterility was higher in comparison with the control. No flowers were, however, observed to be bigger in the colchicine treated ones in the var. *Orange*. In the var. *White*, 0.2% colchicine treatment affected flower formation in that all the buds dried up before coming to flowering stage.

Stomatal frequency and size, pollen sterility and irregularities in meiotic divisions have been taken as the criteria of juding polyploidy since it has not been possible to get meiotic chromosome count of most of the plants. Eigst and Dustin (1955) suggested that for the quick detection of polyploidy, slow growth of plants, thicker and darker green leaves, bigger size of stomata and pollen grains, high pollen sterility, bigger size of flowers and seeds should be taken into consideration. They, however, pointed out that detection of chromosome number in the colchicine treated ones and comparing them with the control was the most accurate test of the induction of polyploidy. Bali and Tandon (1957) observed that morphological changes, pollen sterility and pollen

(0.2% colchicine). ×1000 approx. 30, metaphase II showing n=24 chromosomes in each nucleus in *Z. linearis* var. *Orange* (0.2% colchicine). ×1000 approx. 31, another metaphase II stage showing n = 24 chromosomes in each nucleus in *Z. linearis* var. *Orange* (0.2% colchicine). ×1000 approx. 32, a cell showing pentad in *Z. linearis* var. *Orange* (0.2% colchicine). ×1000 approx. 33, a cell showing hexad in *Z. linearis* var. *Orange* (0.2% colchicine). ×1000 approx. 34, a cell showing nonad in *Z. linearis* var. *Orange* (0.2% colchicine). ×1000 approx.

size were more reliable criteria than stomatal size and frequency. It may be pointed out in this connection that Derman and Diller (1962) detected polyploids on the basis of pollen size and leaf characters in Bunch grape. They marked 4-4 for plants with larger stomata and pollen grains and 2-4 for plants which had only larger pollen grains, i.e., internal polyploidy. They also pointed out that true detection of polyploidy rested with the counting of chromosomes.

So far as the effect of colchicine treatment on growth and development is concerned, Swanson (1957) suggested that the slow growth of the colchicine treated plants were due to physiological disturbances, decrease in auxin supply, slower rates of cell division and respiration. Dwarfing of plants has been attributed to physiological causes by Gunckel (1957) who also considered that cytological factors were associated with physiological changes. Sen and Vidyabhusan (1960) thought that increase in basal circumference could be attributed to the increase in the size of constituent cells and also to some extent to the wider medullary cavity. Skoog (1935) has explained the increase in the number of branches on the basis of destruction of auxin while it has been explained by others to be due to direct bud stimulation (Johnson, 1948). Dwarfing of plants and increase in the number of branches have also been observed by Bose and Hati (1966), Bose and Mukherjee (1966) and Bose and Mukherjee (1967) in their studies on different ornamental plants in this laboratory.

The tetraploid number of $n = 24$ chromosomes observed in some of the colchicine treated plants in the var. *Orange*, gives lots of scope for continuing this study in the C_2 generation and selecting promising types as has been the experience of Bose and Mukherjee (1967) in their study on the C_2 generation in *Impatiens balsamina*.

Summary

Apical growing regions of two varieties of *Z. linearis*, *Orange* and *White*, were treated with 0.2% colchicine for 12 hours (four hours daily for three days).

Survival of plants till maturity was less in colchicine treated ones than in the controls. Typical effects of colchicine like slow growth of plants during early stages of development, thicker, leathery and deformed leaves, shorter internode, increased basal circumference and more number of branches were observed in the treated plants in comparison with the control. In addition to this, colchicine treated ones of both the varieties had less number and bigger sizes of stomata in comparison with the control.

In the colchicine treated plants of the var. *White* all the buds dried up before the flowers opend while the control plants flowered and had seed formation. Significant increase in pollen size and sterility was observed in the colchicine treated plants of the var. *Orange* in comparison with the control.

Meiotic count of n=12 chromosomes was made from some of the control and colchicine treated plants in the var. *Orange* while n = 24 chromosomes was counted in a few of the colchicine treated ones. Meiotic irregularities like unequal separation of chromosomes at anaphase I were noted in some of the treated ones while control plants were free from these. Irregularities during pollen grain formation were also noted in the colchicine treated ones. Number of fruit (seed) per capitulum was significantly higher in the control plants.

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