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Comparative Effects of Colchicine, Caffeine and Hydroquinone on Nodal Roots of *Callisia fragrans*

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Abstract. The effect of colchicine, caffeine and hydroquinone on nodal meristems of *Callisia fragrans* has been studied. The polyploidy have been induced following 4 and 8 hours of treatment in 0.5% colchicine. The persistence of polyploidy in emerging roots even after 60 d. of recovery in the soil indicates that the colchicine affected not only the nodal roots but also the nodal tissue of the plant. The occurence of both diploid and polyploid roots in the same node has been attributed to the differential penetration of the compound to the different zones of tissue. The increase in the division frequency following certain period of recovery was also observed.

Caffeine induced only different subnarcotic effects. The formation of the binucleate cells. (which is very common in *Vicia faba*) has been found to be very rare in the present material. No significant results have been obtained following the hydroquinone treatment excepting some common subnarcotic effects.

The use of colchicine in cytogenetical procedures has been well established. Its remarkable capacity of inducing polyploidy has been advantageously employed in inducing giganticism in characters and in facilitating the interspecific crosses (EIGSTI and DUSTIN 1957).

In addition to colchicine several other compound like caffeine or hydroquinone have been applied on plant tissue to secure chromosomal changes (KIHLMAN 1966) and their action have been referred to as subnarcotic (LEVAN 1949). Though a large amount of work has been done to study their effects on young shoot tips, seeds, young seedlings or occasionelly on the flower or on the pollen, no attempt has been given to study the effects on nodal regions of the stem. This region has got a special significance in plants, where the reproduction is principally vegetative. There are a number of species with a trailing habit, reproducing vegetatively with roots coming out at the nodes.

It is worthwhile to study the effect of different compounds on these nodal regions as any change thus produced would result ultimately into the changed shoot characteristics. *Callisia fragrans* of *Commelinaceae* is one of such species where this method of propagation is extensive. In the present work the treatment of colchicine, caffeine and hydroquinone was carried out under natural conditions in the field and the observations was recorded even upto 60 d. of recovery. This long period of recovery was chosen to note the persistence of the chemical effects.

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Material and Methods

Small cuttings from the apical portion of the prostrate shoot were grown in the earthenware pots containing a mixture of sand and soil. Within a few days, young and healthy roots were formed from the nodes of the plants. These plants with 1 to 1.5 cm long roots were then placed in small tubes containing different concentrations of colchicine, caffeine and hydroquinone, so that the nodes with their roots were completely immersed in the solutions. The period of treatment were mainly 4 and 24 h excepting in 0.5% colchicine, where 8 and 16 h were also applied. After the treatment the plants were transferred to the soil for a recovery.

Corresponding control sets for each experiment were kept in the distilled water and then transferred to the soil for a recovery.

Root-tips from treated plants were fixed in acetic acid — ethyl alcohol mixture (1 : 2) for one h, heated for a few seconds in a mixture of 2% aceto-orcein and 1N HCl (9 : 1) and finally squashed in 1% aceto-orcein solution. The preparations were made permanent by inverting the slides in acetic acid- ethyl alcohol (1 : 1), followed by butyl alcohol grades and finally mounting in euparal.

Results

Two series of observations were performed: 1) the roots being directly in contact with a chemical were observed at intervals of 24 h, 2) fresh roots coming out from the treated nodes were observed.

Colchicine

The effect of aqueous solutions of 1% and 0.5% concentrations were studied. In the case of 1%, only 4 and 24 h treatment showed a higher frequency of division (2—3 times more than the control), after one or two days of recovery. After that time the divisional frequency decreased to normal (Table 1). A high frequency of polyploidy along with other abnormalities such as diplochromosomes, stickiness, persistance of nucleolus at metaphase, clumping, lagging, very high chromosome numbers *etc.* were also observed (Fig. 1—7). The percentage of multinucleate cells were three to four times higher in 24 h treatment than in 4 h treatment. No fresh roots were developed from the treated nodes (Table 1).

In 4 h and 8 h treatment with 0.5% colchicine, followed by a recovery in the soil for different periods, the root cell showed polyploidy and usual abnormalities mentioned above. In some of the fresh roots a high frequency of polyploidy after 45 d was found, whereas in other fresh roots coming from the same nodes a frequency of polyploidy was very low. This indicates a clear case of polysomaty in the meristematic region of the nodes.

With 16 and 24 h of treatment, the percentage of polyploidy was high as expected but no fresh roots were formed from the treated nodes (Table 1).

Caffeine

Different dilutions of the saturated solution in distilled water were used. Up to 1% dilution, the solutions were toxic to the plants. In these concentrations the roots and nodes became flaccid and the plants died within a few days. After the treatment with lower concentrations (0.5%, 0.25%, 0.1%—4 or 24 h) only an occasional occurrence of binucleate cells was observed. Without any recovery the percentage of dividing cells was very low in 4 h treatment while it was totally absent in 24 h of treatment (Table 2). In addition to this other abnormalities like stickiness, erosion of chromosomes, fragmentation, well scattered plates, heavy concentrations of chromosomes *etc.* were also found (Figs 8 and 9).

TABLE 1

EFFECTS OBSERVED IN ROOTS of *Callisia fragrans* treated with colchicine solution

Concentration used [%]	Period of treatment [h]	Interval after treatment [d]	Dividing cells [%]	Metaphase arrest [%]	Polyploid cells [%]	Multinucleate cells [%]
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	4	1	15	15	15	4.5
1	4	2	18	17.9	15.9	4.8
1	4	3	9.0	9.0	9.0	2.5
			no new roots formed			
1	24	1	2.5	2.5	1.5	23.0
1	24	2	17.0	17.0	17.0	12.5
1	24	3	13.5	13.0	12.8	10.3
			no fresh roots developed			
0.5	4	1	20.9	19.9		
0.5	4	2	30.0	28.5	10.9	
0.5	4	3	25.0	23.1	13.0	
0.5	4	30	9.3	6.7	2.8	
0.5	4	45	6.4	5.4	0.3	
0.5	4	45	6.1	2.8	2.3	
0.5	4	45	8.1	4.3	2.6	
0.5	4	60	6.2	3.9	2.3	
0.5	8	1	26.0	17.3	2.5	
0.5	8	2	27.0	19.4	6.9	
0.5	8	3	30.3	25.5	19.1	
0.5	8	4	21.5	23.2	16.6	
0.5	8	30	14.9	6.5	4.7	
0.5	8	45	9.7	8.2	4.5	
0.5	8	60	9.0	6.0	3.0	
0.5	16	1	18.8	16.5	15.0	
0.5	16	2	21.6	20.8	20.8	
0.5	16	3	20.8	20.8	20.8	
			no new roots formed			
0.5	24	1	12.7	10.1	10.4	
0.5	24	2	15.6	13.8	13.6	
0.5	24	3	20.0	9.0	9.0	
0.5	24	4	13.0	9.0	7.5	
			no new roots			

After 4 h treatment at 5% solution, the division frequency was slightly lower than in the control; occasional fragments together with stickiness, pycnoses, spiralised prophase chromosomes *etc.* were found. After 3 days of recovery the treated roots failed to survive. The fresh roots after 15 d of recovery showed scattered metaphases and occasional sticky bridges. The root cells after 30 days showed normal division frequency without any abnormalities. In 24 h of treatment at the same concentration the roots became flaccid and the plants failed to survive (Table 2).

TABLE 2
EFFECTS OF CAFFEINE SOLUTION on roots of *Callisia fragrans*

Concentration used [%]	Period of treatment [d]	Interval after treatment [d]	Dividing cells [%]	Metaphase arrest [%]	Binucleate cells [%]	Polyploid cells [%]
0.5	4		5.2	3.6		
0.25	4		8.5	4.5		
0.1	4		9.3	4.7		
0.5	24		nil		5.3	
0.25	24		nil		6.5	
0.5	4	1	3.0	2.7		
0.5	4	2	6.8	3.8		
0.5	4	3	4.3	2.3		
0.5	4	15	7.0	5.0		
0.5	4	30	9.0	4.8		
0.25	4	1	8.1	5.7		
0.25	4	2	8.4	5.6		
0.25	4	3	7.6	5.0		
0.25	4	4	6.1	3.9		
0.25	3	7	6.2	4.0		
0.25	4	15	5.0	4.4		
0.25	4	30	5.0	3.1		
0.25	24	1	4.4	1.8		1.8
0.25	24	2	6.5	4.2		1.1
0.25	24	3	8.7	7.0		1.6
0.25	24	7	6.4	5.1		1.1
0.25	24	15	7.0	3.2		
0.25	24	30	7.5	4.0		
0.1	4	1	7.6	4.2		1.2
0.1	4	2	5.3	3.4		
0.1	4	3	5.3	3.9		
0.1	4	7	6.8	4.5		
0.1	4	15	7.2	4.8		
0.1	4	30	8.0	5.0		
0.1	24	1	5.3	2.6		1.6
0.1	24	2	7.0	3.4		0.8
0.1	24	3	5.9	3.8		
0.1	24	7	6.1	3.7		
0.1	24	15	6.5	4.0		
0.1	24	30	7.2	4.0		

At 0.25% the root cells showed, after 4 h of treatment, well scattered metaphase plates along with other abnormalities. Sticky bridges with many strands were very common. The percentage of division gradually slowed down up to the stable point similar to the control even after 12 d of recovery (Table 2).

24 h of treatment at 0.25% concentration showed some polyploid cells which continued up to 7 d of recovery in addition to other abnormalities. After 15 d of recovery no abnormalities were recorded. At 0.1% and 4 h of treatment, stickiness, bipolarity, metaphase grouping and gradual reduction in the division frequency were seen in addition to some polyploid cells after

one day. Similar effects were observed after 24 h treatment with the same concentration.

Hydroquinone

Aqueous solutions of 2% and 1% were tried and found to be toxic as the roots became flaccid and the plants failed to survive. Roots treated in 0.5% solution for 4 h and for 24 h, as well as in 0.25% and 0.05% for 4 h became also flaccid and died. The fresh roots coming out from the same node showed sticky bridges, granulation of chromosomes, frequent metaphase plates, few polyploid cells (0.05%), occasional breaks in primary constriction *etc.* The division frequency was also lower than in the control (Table 4) and gradually decreased (Table 3).

TABLE 3

EFFECTS OF HYDROQUINONE on roots of *Callisia fragrans*

Concentration used [%]	Period of treatment [h]	Interval after treatment [d]	Dividing cells [%]	Metaphase arrest [%]	Polyploid cells [%]	Remarks
0.5	4					Roots flaccid and dead
0.5	4	2	5.1	4.0		
0.5	4	4	7.5	5.4		
0.5	4	7	6.8	5.0		
0.5	4	15	7.2	5.1		
0.5	4	30	7.5	5.2		
0.5	24					Plants fail to survive
0.25	4	3	5.2	4.0		
0.25	4	4	5.0	4.2		
0.25	4	7	6.0	4.6		
0.25	4	15	5.8	3.7		
0.25	4	30	6.5	4.5		
0.25	24					Plants fail to survive
0.05	4	2	6.4	5.0		
0.05	4	4	7.7	5.6	2.8	
0.05	4	7	5.0	1.8		
0.05	4	15	5.6	2.0		
0.05	4	30	6.5	3.2		
0.05	24					Roots flaccid and dead
0.001	4	1	9.4	6.6		
0.001	4	2	8.3	5.0		
0.001	4	3	9.7	5.4		
0.001	4	7	9.5	5.0		
0.001	4	15	10.0	5.3		
0.001	4	30	10.2	5.5		
0.001	24	1	5.3	3.9		
0.001	24	4	4.3	1.6		
0.001	24	7	4.8	2.0		
0.001	24	15	5.0	2.0		
0.001	24	30	6.5	3.0		

In the 0.001% solution the treated roots remained healthy both at 4 and 24 h treatment. In both cases heavy fragmentation was found along with diplochromosomes, clumping, well clarified metaphases *etc.* The division frequency in 4 h treatment was the same as in the control. The abnormalities did not persist in fresh roots after 15 days of recovery (Table 3).

TABLE 4
CONTROL SET IN WATER

Period of treatment [h]	Period of recovery [d]	Dividing cells [%]
4	1	8.5
4	2	9.0
4	3	9.4
16	1	8.5
16	2	8.5
16	3	9.3
24	1	7.5
24	2	9.5
24	3	9.2

Discussion

The results obtained with colchicine, caffeine and hydroquinone reveal quite a number of interesting differences. So far as caffeine and hydroquinone are concerned, the effects noted on the growing nodes indicate the susceptibility of such tissues to narcotic action of these drugs. The subnarcotic actions of caffeine and hydroquinone have been reported by KIHLMAN and LEVAN (1949) and TJIO and LEVAN (1948). In the present work it has been observed that following hydroquinone treatment the division frequency does not undergo a marked change even after prolonged period of recovery (Table 3). Only in higher concentrations following treatment lethality has been observed. Therefore, so far as the rooting nodes are concerned, hydroquinone treatment does not show any narcotic effect. Subnarcotic action like bridges, fragmentation *etc.* have been recorded even in fresh roots emerging from the treated nodes. This indicates the adequate penetration of hydroquinone to the growing zones of the nodes where from the roots ultimately emerge.

Regarding caffeine, one of the interesting things has been the formation of binucleate cells in addition to several chromosomal abnormalities. Such binucleate cells have also been reported after caffeine treatment (KIHLMAN and LEVAN 1949, GIMENEZ-MARTIN *et al.* 1969) where the effect has been attributed to the inhibition of cytokinesis. The formation of binucleate cells as observed here, differs in certain respects from the data presented by previous authors. In the present case direct treatments with caffeine have resulted into very rare formation of binucleate cells whereas this behaviour has been found to be universal for all cells as reported by previous authors in *Vicia faba* (KIHLMAN and LEVAN 1949, GIMÉNEZ-MARTIN *et al.* 1969). This indicates the resistance of the nodal tissue to the action of caffeine as compared with the susceptibility of the root cells of *Vicia faba*. However, this differential effect is not attributed mainly to organ differences but also to the genotypic constitution. In direct treatment on *Callisia fragrans* where roots were presented from the beginning of the experiment, the data taken on such roots could be attributed to the effect on root tissue alone whereas the effects observed on the roots emerging later during the recovery may be attributed to the effect on the nodal tissue. In the former case such binucleate cells are rarely observed but in the latter binucleate cells were entirely absent. Such tissue differences in relation to susceptibility to colchicine was worked out by SHARMA and SARKAR (1957). Regarding the other changes induced by caffeine the present observation does not show much differences from the data presented by previous authors.

Interesting data has been obtained with regard to colchicine effect on growing nodal tissues and the nodal roots. Regarding the induction of polyploidy very successful results have been obtained following 4 and 8 h of treatment in 0.5% colchicine. Such high concentrations, as compared to the concentrations generally applied to roots, had to be used as lower concentrations did not yield any significant results. This may be attributed to the low rate of penetration in the nodal roots. That the effect of colchicine is confined not merely to the roots but also to the nodal tissue is exhibited by the emergence of polyploid roots even after 60 days of recovery in the soil (Table 2). All these facts clearly indicate that colchicine application in the nodal zones in trailing plants could be used as an effective means of securing rapid polyploidy.

Remarkable feature has been the occurrence of both diploid and polyploid roots from the same nodes emerging after a prolonged recovery. Such differential effect is rather different from the mixoploid effect often noted in shoot apices on branches following colchicine treatment. DAVIDSON *et al.* (1969), D'AMATO and NUTIRONCHI (1968) have suggested that the differential C-mitotic effect in root meristems of *Vicia faba* is principally dependent on the penetration of the compound to the different zones of the tissue. DAVIDSON (1966), however, claimed that the resistance is confined to the particular stage in the growth. In the nodal tissue of *Callisia fragrans* too, in view of the massive nature of tissues, it is likely that the occurrence of the diploid and polyploid roots coming out after prolonged recovery following colchicine treatment is the reflection of differential penetration in different zones of the nodes at the initial phase of treatment.

The other feature worth discussion is the frequency of dividing cells following colchicine treatment. A sharp rise in the frequency of division was observed following colchicine treatment after 1—3 d of recovery (Table 2). Colchicine, even though it is an agent in causing metaphase arrest, has recently been shown by DAVIDSON *et al.* (1966) to increase the mitotic indices of *Vicia faba* root meristems. In their material this increase continued up to 36 h after which a steady fall could be recorded. In nodal meristems of *Callisia fragrans*, this increase has been found to continue up to 2 and 3 d after which there is a steady fall. This increase in division frequency is no doubt in part due to the entering of the accumulated polyploid cells in the division. DAVIDSON *et al.* (1966) suggested that colchicine has a stimulatory effect during the mitotic cycle which causes a heavy increase in the number of cells seen in the division following recovery. In the nodal meristems of *Callisia fragrans* too, the heavy increase in division frequency is possibly attributed to two factors *i.e.* division of accumulated polyploid cells and the stimulatory effect (DAVIDSON *et al.* 1966) of colchicine.

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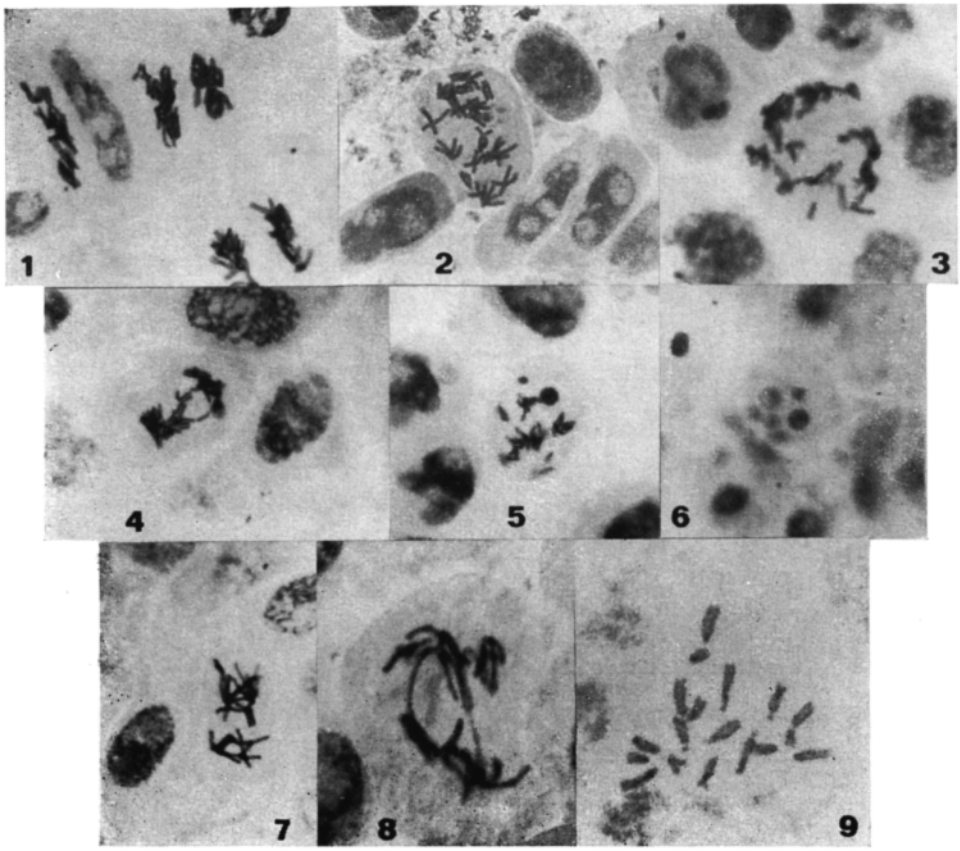
Figures at the end of the issue.

SATYESH CHANDRA ROY, Cytogenetická laboratoř Botanického Oddělení, University v Kalkatě, Indie: **Srovnání účinků kolchicinu, kofeinu a hydrochinonu na nodální kořeny *Callisia fragrans*.** — Biol. Plant. **15** : 383—390, 1973.

Sledován byl účinek kolchicinu, kofeinu a hydrochinonu na nodální meristémy *Callisia fragrans*. 0.5% koncentrace kolchicinu aplikovaná po 4 či 8 h indukovala polyplodii. Perzistence polyplodie v později vzniklých kořenech i po 60 dnech po působení ukazuje, že kolchicin zasáhl nejen nodální kořeny, ale také nodální pletiva, rostliny. Výskyt jak diploidních tak polyploidních kořenů vzniklých ze stejného nodu je vysvětlen odlišnou penetrací sloučeniny do různých zón pletiva. Zjištěna byla rovněž zvýšená frekvence dělení po určitém období od doby působení, v němž byly ovlivněné rostliny pěstovány v půdě.

Kofein vyvolal pouze různé subnarkotické účinky. Tvorba dvoujaderných buněk, tak častá u *Vicia faba*, byla zjištěna pouze ojediněle. Hydrochinon nevyvolal žádné pozoruhodné účinky, s výjimkou některých běžných subnarkotických efektů.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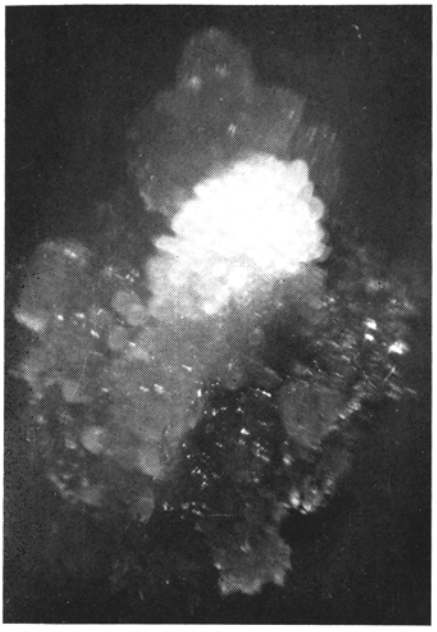

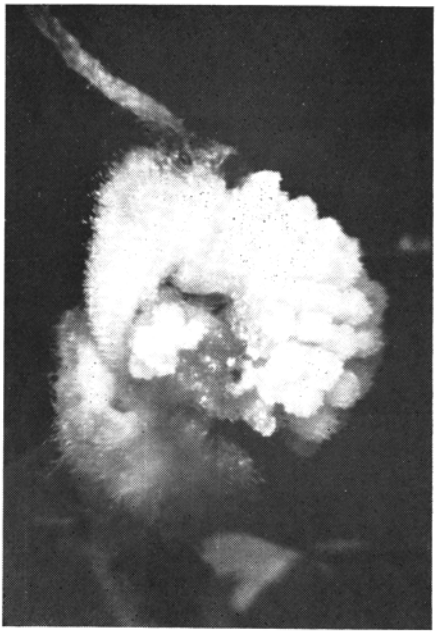

Fig. 1. An intensive cell proliferation on the placentae two weeks following placental pollination *in vitro* (about 26×).
Fig. 2. The development of seeds two weeks following stigmatal pollination *in vitro* (about 11×).

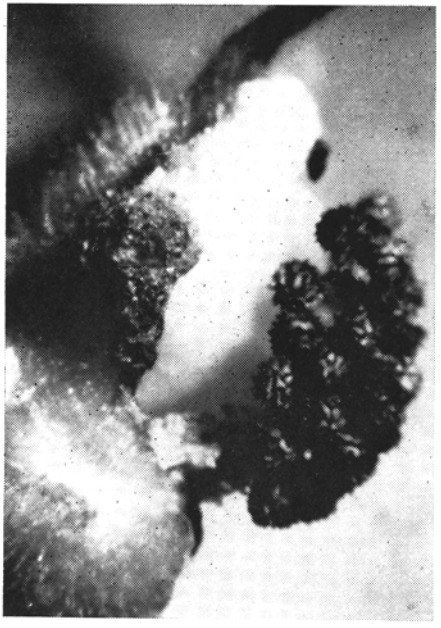

Fig. 3. Mature, fully differentiated seeds 21 days following stigmatal pollination *in vitro* (about 19×).
Fig. 4. Abnormal development of all ovules on an uncovered placenta 4 days following stigmatal pollination *in vitro* (about 11×).