INFLUENCE OF COLCHICINE TREATMENTS ON THE MORPHOLOGICAL CHARACTERS OF THE OKRA SEEDLINGS

MÎNDRILĂ GHEORGHIȚA[1], BONEA DORINA[2], BOTU MIHAI[1], SOARE RODICA[2], DINU MARIA[1], BIZERA MIHAELA[3]

[1] University of Craiova, Faculty of Horticulture,13 A.I. Cuza Str., Craiova, Dolj, Romania
vioricamindrila@yahoo.com
[2] University of Craiova, Faculty of Agronomy,19 Libertății Str., Craiova, Dolj, Romania
[3] University of Craiova-SCDP Vâlcea, 464 Calea lui Traian Str., Rm. Vâlcea, Romania

ABSTRACT

The effect of the treatments with colchicine determines the doubling of the number of chromosomes, but can also induce mutations being often used in breeding works to induce variability. Abelmoschus esculentus (L.) Moench (syn. Hibiscus esculentus L.) is an economically important vegetable plant grown in tropical, sub-tropical and temperate areas with a warmer climate for multiple nutritional and medicinal properties. In the present paper, 0.1% colchicine treatments were performed on the okra seeds. Five variants with different exposure times were experimented and observations were made after seven days on the length and thickness of the roots and the hypocotyl. The treatment with colchicine induced the modification of the morphological characters, resulting in seedlings with short and thickened roots and hypocotyls. The maximum effect was recorded in variant no. 5 with 2 hours exposure.

INTRODUCTION

Colchicine is an alkaloid that occurs naturally in several plants belonging to the *Liliaceae f*amily and was isolated in 1857 of the seeds of autumn crocus or meadow saffron (*Colchicum autumnale*) (Sarin et al., 1974) and is used in the work of genetics and improvement to induce polyploidy. The effect of colchicine on plants depends on the concentration used and the exposure time (Trease and Evans, 1983).

Colchicine treatments mainly result in doubling the number of chromosomes, but may also induce mutations in the genome (Manzoor et al., 2019).

Different parts of the plant such as seeds, buds, roots, apical meristem can be used to induce polyploidy. However, the best results were achieved in the treatment of seeds (Sourour et al., 2014). The success of polyploid induction depends on the species, the organs of the plant used, the method of application of colchicine and the concentration and duration of exposure. The best results were obtained in the treatment of seeds (Pirkoohi et al., 2011).

Okra (*Abelmoschus esculentus*) (L.) Moench (syn. *Hibiscus esculentus* L.) is a species of the *Malvaceae* family, originating in Ethiopia and widely cultivated in North Africa, in the Mediterranean, in Arabia and India. (Sathish, and Eswar, 2013).

In Romania is less known, although it finds favorable conditions of culture especially in the south of the country.

Promoting the consumption of okra could provide important sources of macro and micronutrients, mineral elements and antioxidants (Shui and Peng, 2004).

The attention of researchers must be directed towards finding new varieties of okra with superior nutritional qualities and agro-biological properties allowing to expand into culture in less favorable areas.

The present paper addresses a study of okra capacities to react to colchicine treatments in order to establish a working methodology leading to obtaining a larger variability to be used into the future breeding programs.

MATERIAL AND METHODS

The biological material used consisted of okra seeds with germination of 98% of the 'Clemson spineless' variety.

250 seeds were used, 50 per each variant. The seeds were disinfected for 20′ with 5% sodium hypochlorite, washed with tap water and soaked for 60′.

Treatment with 0.1% colchicine was applied in four variants with different exposure times: (V2-30′; V3-60′; V4-90′ and V5-120′) plus control variant V1.

After the expiry of the exposure times, the seeds were washed with tap water and placed on the filter paper in plastic boxes with lids.

For seed germination, the Petri dishes were placed in the Binder germinator, at a temperature of 25ºC and a humidity of 80%.

After 7 days, measurements were carried on the length and diameter of the roots and hypocotyl.

In parallel, 100 seeds were also treated according to the same methodology and in the same variants to set up a field trial.

RESULTS AND DISCUSSIONS

Following the observations made, it was found that the seed germination was not influenced by the treatment, with all variants having the same percentage of germination (98%).

Differences were observed in the development of roots and hypocotyl, characters that were taken under study after 7 days.

The data obtained were processed using ANOVA and the results obtained are presented in Table 1.

Table 1

Results on biometric measurements recorded after 7 days

Variants	Root length (cm)	Root diameter (cm)	Hypocotyl length (cm)	Hypocotyl diameter (cm)
1	5.26 a	0.21 d	7.80 a	0.25 c
2	4.41 b	0.22 d	4.94 b	0.25 c
3	1.28 c	0.26 c	5.03 c	0.27 c
4	0.62 d	0.29 b	1.22 c	0.33 b
5	0.51 d	0.33 a	1.09 c	0.55 a

Means followed by the same letter do not differ significantly from one another according to Duncan's multiple range test, $p < 0.05$.

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Differences were observed in the development of roots and hypocotyl,

characters that were taken under study after 7 days (fig 1,2).

The data obtained were processed using ANOVA and the results obtained are presented in table 1.

Following the analysis of the results obtained it was found that the effect of treatment with 0.1% colchicine was inversely proportional to the exposure time, inducing a reduction in the roots and hypocotyl lengths with the increase in exposure time.

Thus, in variant 5, the values obtained were 10 times lower (0.51 cm) from the control variant (5.26 cm), characteristic effect of colchicine treatments that inhibit the rate of cell division (Eigsti, 1947), leading to shortening of the roots.

The same phenomenon was also recorded for the hypocotyl length of the 2nd (5.03 cm), 4th (1.22 cm) and 5th (1.09 cm) comparatively with control (7.80 cm).

The diameter of the roots recorded values directly proportional to the exposure time, leading to the formation of short and thicked roots, (variant 5 with 0.33 cm in diameter and 0.51 cm in length).

Similar results were also obtained by Khoshoo (1959) in the study performed on Gymnosperms, where polyploid plantlets were short, thickened and with slow growth.

The diameter of the hypocotyl manifested the same way of development, recording maximum values for the 5th variant (0.55 cm), compared to control, where the diameter of the hypocotyl did not exceed 0.25 cm.

In the field trial were followed the number of emerged plants and the number of plants that survived and matured, thus settling the lethal exposure time to 0.1% colchicine concentration. Although the germination was not influenced in the first 10 days, part of the seedlings did not survive; due to inhibition of the development of the roots.The data obtained is presented in table 2.

Table 2

Number of plants emerged and matured

Variants	No. seed	No. of plantules after 10 days	%	No. of mature plants obtained	%
1-CONTROL	100	98	98	96	96
2-30′	100	76	76	73	73
3-60′	100	57	57	53	53
4-90′	100	50	50	46	46
5-120′	100	32	32	22	22

In the case of 4th variant, 50% of the seeds have germinated, indicating that at the concentration of 0.1% colchicine the tethal dose (LD50) of exposure is 90 minutes (fig. 3).

The seeds of this variant will be set together with the control variant in order to detect any mutations that might arise in X_2 generation.

CONCLUSIONS

The concentration of 0.1% colchicine, with 30', 60' and 120' exposure times, induced the most significant changes in the morphology of plantlets; this protocol of work could be applied in inducing mutagenesis to okra seeds.

The seeds obtained from 4th and 5th variants can represent a source of variability that might be useful for future studies and eventually breeding works.

BIBLIOGRAPHY

1**. Eigsti, O.J.** (1947). *The pollen tube method for making comparisons of differences in mitotic rates between diploid and tetraploid.* Genetics 32: 85.

2. **Khoshoo, T.N**. (1959). *Polyploidy in gymnosperms*. *Evolution*. 13: 4–39.

3. **Manzoor, A., Ahmad, T., Bashir, M.A., Hafiz, I.A., Silvestri, C.** (2019). *Studies on Colchicine Induced Chromosome Doubling for Enhancement of Quality Traits in Ornamental Plants. Plants*, *8*(7),194. https://doi.org/10.3390/plants8070194.

4. **Pirkoohi M.H., Keyvanloo M., Hassanpur M**., (2011). *Colchicine induced polyploidy in mint by seed treatment* International journal of agriculture and crop sciences 3-4: 102-104.

5. ***Sarin*, *Y.K.*, *P.S. Jamwal*, *P.K. Gupta* and *C.K. Atal*.** (1974). *Colchicine from the seeds of Gloriosa superba*. Current Science 43 (3): 87.

6. **Sathish, D., and Eswar, A.** (2013). *A Review on: Abelmoschus esculentus ("Okra")*. International Research Journal Pharmaceutical Applied Sciecces, 3(4):129-132.

7. **Shui, G., & Peng L.L.** (2004). *An improved method for the analysis of major antioxidants of Hibiscus esculentus Linn.* Journal of Chromatography A, 1048, pp. 17–24.

8**. Sourour, A., Ameni B., Mejda C.** (2014). *Efficient production of tetraploid barley (Hordeum vulgare L.) by colchicine treatment of diploid barley*. Journal Exp. Biol. 2(1S): 113-119.

9. **Trease, S.E.** and **Evans, D.** (1983). *Textbook of pharmacognosy*.12th Edition, Tindall and Co., London, 343-383.

FIG. 1,2 - Roots and hypocotyl, characters that were taken under study after 7 days.