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Effect of colchicine treatment on survival of seedlings and morphology in Cape gooseberry (*Physalis peruviana* L.)

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Abstract
Polyploidy plays an active role in evolution of plants. Thus, induction of polyploidy particularly at autotetraploid and triploid levels offers probably the best scope for seedless as well as quality fruits. So, this method is one of the best options for the chromosome manipulation. In the present study an attempt was made to induce polyploids in Cape gooseberry using colchicine with the objective of creating more genetic variability. The colchicine concentrations used were 0.10(C_1), 0.20(C_2) and 0.40(C_3) per cent for the duration 12 (H_1), 24 (H_2) and 36 (H_3) hours with seedling apex dip method (M_1), cotton plug method (M_2) and lanolin paste method (M_3). The growing apex of seedlings were treated with cotton swab C_1 (0.10), C_2 (0.20) and C_3 (0.40) per cent aqueous solution of colchicine for 12 (H_1), 24 (H_2) and 36(H_3) hours resulted into seedlings survival, maximum 87.50 per cent in 2017-18 and 89.58 per cent in 2018-19 with treatment $C_1M_2H_1$ (0.10% for 12 hours with cotton plug method) whereas expected ploidy levels 10.42 per cent and 12.50 per cent respectively in both the years with same treatment. On the basis of findings, seedling survival was less in apex dip method in comparison to the cotton plug method and lanolin paste method. During the morphological observation the results indicated that 0.10 per cent (C_1) of colchicine had more capacity to induce the polyploidy in seedlings rather than apex dip method (M_1) or cotton plug method (M_2).

Keywords: Cape gooseberry, polyploidy induction, survival, morphological character

Introduction
Cape gooseberry (*Physalis peruviana* L.) is a minor and a quick growing fruit which belongs to the family *Solanaceae* is catching the imagination of farmers for improved income during the recent years. This is native to Peru of South America but it is in cultivation in South Africa in the region of Cape of Good Hope from the early 19th century. It was introduced in India during nineteenth century by the early European settlers. It is commonly called as 'Poha' in Hawaii, Golden Berry in South Africa and Rasbhari, Makoi or Tepari in India. The first description of *Physalis* genus was made by Linnaeus in 1753. The genus *Physalis* has more than 100 species but only few of them are of economic value. One is the strawberry tomato, husk tomato or ground cherry, *Physalis pruinosa* L., grown for its small yellow fruits used for sauce, pies and preserves in mild-temperate climates. Muntzing (1951) suggested that, Cape gooseberry is tetraploid in nature and having chromosome number 2n = 48. The main stem is green, herbaceous and composed of 8 to 12 nodes, giving origin to productive ramifications by dichotomy. Flowers are unique, pedunculated and hermaphrodite, derived from the auxiliary bud with five yellow petals. Calyx is green, formed of five sepals which are about 5 cm in length, covering completely the fruit throughout its development stage. When the fruit is ripened, calyx shows a brown colour which is an indicator of maturity for harvest (Avila *et al.*, 2006) [2].

Nowadays, the high quality fruits are required for the burgeoning population. Breeding procedures for these plants are helping to meet the demand for such quality fruits (Bernath, 2002) [4]. The induction of artificial polyploidy may prove useful in enhancing the quality and quantity of important fruit crops (Dhawan and Lavania, 1996) [7].

Auto polyploidy can be induced by environmental factors as well as chemicals and efficient techniques are required for the enhancement of quality and quantity. The most widely applied and best studied chemical inducing polyploidy is colchicine, an alkaloid extracted from seeds or corms of the autumn crocus (*Colchicum autumnale* L.).

For improving the quality of the fruits colchicine was used for chromosome doubling of many crops including chickpea (*Cicer arietinum* L.) (Pundir *et al.*, 1983) [21], henbane (*Hyoscyamus niger* L.) (Lavania and Srivastava, 1991) [18] hops (*Humulus lupulus* L.) (Roy *et al*., 2001) [5], ginger (*Zingiber officinale* Roscoe) (Adaniya and Shirai, 2001) [1], tarragon (*Artemisia annua* L.) (Gonzalez and Weathers, 2003) [11] and feverfew (*Tanacetum parthenium* L.) (Saharkhiz, 2007) [26]. There are several target tissues for colchicine treatment, such as meristems and seeds (Tamura *et al.*, 1996) [29].

In the present study an attempt was made to induce autotetraploidy in Cape gooseberry using colchicine with the objective of creating more genetic variability. The derived tetraploid plants were traced by studying the survival of seedlings and morphological characteristics of the plants.

Materials and Methods

The present investigation was carried out in the experimental area of the Department of Horticulture, Birsa Agricultural University, Kanke, and Ranchi during two successive seasons (2017-18 and 2018-19). The experimental site comes under VIIth Agro-Climatic Region i.e., Eastern Plateau and Hills. It is situated between 23^{0}17' North latitude and 85^{0}19' East longitude and the height from the mean sea level is 625m. The soil of the experimental plot was Sandy loam in texture with average fertility and thus considered suitable for cultivation of Cape gooseberry. The Randomized Block Design was adopted for the trial. The number of treatment combinations was 22 with three replications during both the years.

Field preparation

The field was prepared thoroughly. The required area was marked for experiment and land was again prepared thoroughly by spading to bring a fine tilth suitable for Cape gooseberry cultivation. A basal dressing of well rotten farm yard manure at the rate of two tractor trolley full load per acre was applied and was thoroughly incorporated in the soil. The sub-plots were then divided into different blocks according to the layout plan.

Nursery bed

Seeds were sown on the raised bed with suitable mixture of garden soil and well rotten farm yard manure. Germination started visible after nine days of sowing. The seedlings were ready for transplanting after a month of germination. Seedlings attained a height of 5-6 centimeter at the time of transplanting.

Seedling transplanting

Seedlings were transplanted in the field in the afternoon which was done manually in each sub-plot according to the layout plan with a planting distance of 50 cm × 50 cm i.e. row to row and plant to plant respectively. The plot size was maintained 2m in both sides with accommodation of 16 plants per plot. To overcome the shock of transplanting, the transplanted seedlings were irrigated immediately with the help of a watering rose can. This practice was continued up to seven days in both morning and evening hours.

Treatment details

The colchicine concentrations were taken as 0.10 (C_1), 0.20 (C_2) and 0.40 (C_3) per cent for the duration 12 (H_1), 24 (H_2) and 36 (H_3) hours with seedling apex dip method (M_1), cotton plug method (M_2) and lanolin paste method (M_3).

Preparation of chemicals

Colchicine solution

Colchicine solutions of different concentrations were prepared with distilled water. For making 0.10, 0.20 and 0.40 per cent concentration, 50mg, 100mg and 200 mg of Colchicine were dissolved in separate glass beaker respectively in small quantity of absolute alcohol and then transferred to 100ml measuring flask and distilled water was added to make required volume. The care was taken to keep the solution in dark place.

Lanolin paste

The required amount of Colchicine was measured and transferred to a Petridis containing the required quantity of melted lanolin. Then it was mixed thoroughly with the help of a glass rod. The paste was allowed to cool before application.

Methods of Treatment

(A)Polyploidy

(i) Apex dip method:

Apex of one month old seedlings was dipped in known concentration of Colchicine for a specific period. Roots of seedlings were protected by wrapping cotton swab. Water was poured on roots after some interval with the help of a dropper.

(ii) Cotton plugs method

Small quantity of cotton was soaked in aqueous solution of Colchicine of different concentrations with the help of glass rod. Soaked cotton or wool in different concentrations was applied over the growing apex of young and established seedlings for required duration. Treatments were repeated by dripping the solution with the help of a dropper after short interval.

(iii) Lanolin paste

The paste containing different concentrations of Colchicine was applied to the growing point of seedlings. The hairs and scales were removed from the growing point prior to application.

Observations recorded

1. **Survival percentage of seedling (after treatment):** The number of survived seedlings in each sub-plot was counted 15 days after first treatment. The percentage of survival of seedlings in each treatment was calculated from the total number of seedlings already transplanted.
2. **Morphological abnormalities**: An observation regarding development of leaves along with abnormal characters and growth of the plant was made for comparative study which might serve as an index of polyploidy.
3. **Ploidy analysis:** Selection of tetraploid plants was done on the basis of morphology of the plants. The putative tetraploids were examined two months later to validate ploidy stability.

Results and Discussion

Polyploidy: Polyploidy plays an active role in evolution of plants. Majority of choicest cultivars of apple, guava and

banana are natural triploids. Thus, induction of polyploidy particularly at auto tetraploid and triploid levels offers probably the best scope for seedless i.e. quality fruits. So far as the chromosomes manipulation method is concerned. This is illustrated by work on watermelon by Kihara (1951) [16] in Japan. This method although admirable in many ways offers considerable difficulties in some perennial fruit crops.

Alkaloid colchicine is considered effective in wide variety of plants which has been also used in the present investigation for inducing autotetraploid in Cape gooseberry. In the present studies survival of seedlings, differ in morphological characters of the plant from the normal growth after application of different treatments of colchicine by various methods and duration was observed, new leaves appeared were dark green, broader in size and other different floral parts were larger. These were characteristics in general which is helpful in identification, isolation and confirmation of polyploid plants. Although, Swaminathan (1958) [28] pointed out difficulties in inducing polyploidy in perennial fruit crops. The observations indicated that chromosome manipulation can be done in this annual fruit crops i.e. Cape gooseberry.

A number of plants were treated with different concentrations of colchicines which appeared quite effective in inducing chromosome doubling. Colchicine was also applied for different durations and by different methods to see their effects in inducing polyploidy. The results obtained are being presented below-

1. Seedling survival (%) [After colchicines treatment]

Forty eight seedlings of Cape gooseberry were subjected to various concentrations of colchicine in different media and variable duration. Their survival percentage is being presented in Table-1.

During first year (2017-18) from the perusal of Table-1, it is apparent that apex of seedlings when dipped for H_1 (12 hours), H_2 (24 hours) and H_3 (36 hours) in C1 (0.10%), C_2 (0.20%) and C_3 (0.40%) concentrations of colchicine, the result showed that the maximum of 75.00 per cent seedlings survived with $C_1M_1H_1$ (0.10 % for 12 hours) whereas, minimum of 25 per cent was observed with $C_3M_1H_3$ (0.40 % for 36 hours). Survival of seedlings in the remaining other treatments were registered from 31.25 to 66.67 per cent.

Similarly in cotton plug method (M_2) when growing apex of seedlings were treated with cotton swab for H_1 (12 hours), H_2 (24 hours) and H_3 (36 hours) in C1 (0.10%), C_2 (0.20%) and C_3 (0.40%) concentrations of aqueous solution of colchicine, survival was recorded maximum 87.50 per cent with $C_1M_2H_1$ (0.10 % for 12 hours) and minimum of 52.08 per cent with the treatment $C_3M_2H_3$ (0.40 % for 36 hours). The survival percentage seedlings of other remaining treatments were noticed from 54.17 to 83.33 per cent.

In lanolin paste (M_3) the survival of seedlings were found cent percent with C_1M_3 (0.10 %) whereas, minimum of 93.75 per cent with C_3M_3 (0.40%) was observed. In the rest of the treatment was recorded from 97.92 per cent.

In the next year (2018-19) when apex of the forty eight seedlings were again dipped in 0.10 (C_1), 0.20(C_2) and 0.40 (C_3) per cent concentration of colchicine for 12 hours (H_1), 24 hours (H_2) and 36 hours (H_3). The same trend was repeated i.e. the maximum 77.08 per cent of seedling was survived with the effect of the concentration $C_1M_1H_1$ (0.10 per cent for 12 hours with apex dips method). Whereas minimum 27.08 per cent survival of seedling was recorded with the effect of the treatment $C_3M_1H_3$ (0.40 per cent for 36 hours with apex dips method). Remaining other treatment indicated survival from 33.33 per cent to 68.75 per cent.

Similarly with cotton plug method (M_2) when growing apex of seedlings treated with cotton swab C_1 (0.10), C_2 (0.20) and C_3 (0.40) per cent concentrations of aqueous solution of colchicine 12 (H_1), 24 (H_2) and 36(H_3) hours results exhibited maximum 89.58 per cent survival of seedlings with treatment $C_1M_2H_1$ (0.10% for 12 hours with cotton plug method) whereas minimum 56.25 per cent survival was showed with the treatment $C_3M_2H_3$ (0.40 % for 36 hours). In the rest of the treatments indicated from 58.33 to 85.42 per cent.

In lanolin paste (M_3) survival of seedlings was found cent percent as per the previous year i.e. C_1M_3 (0.10%) whereas minimum of 95.83 per cent with C_3M_3 (0.40%). Remaining treatments it was noticed 97.92 per cent.

On the basis of above findings it can be concluded that seedling survival was less in Apex dip method as compared to the cotton plug method and lanolin paste method. With increasing concentration of colchicine and treatment duration, lethality of seedlings was increased with all the methods. Reduction in the percentage of seedlings survival after treatment, which indicates that it was directly correlated with the increase in colchicine concentrations and duration of treatment as well as method of treatment. These findings are in confirmation with the results of Atichart (2013) [3] in *Dendrobium chrysotoxum*, Chopra and Swaminathan (1960) [6] in watermelon, Elyazid and Shereif (2014) [8] in "Balady" mandarins, Glowacka *et al*. (2010) [9] in the genotype of *M. sinensis* and *M. giganteus*, Grosser *et al.* (2014) [10] in pummelo, Kainth and Grosser (2010) [14] in pummelo, Miguel and Leonhard (2011) [20] in orchids and Rodrigues *et al.* (2011) [24] in banana.

Table 1: Seedling survival percentage after colchicines treatment (2017-18).

Method/ Hour	Concentration (%)	Total number of seedlings		Survival (%)	Affected seedlings	Expected Polyploids (%)
		Treated	Survived			
Apex dip method (M_1)						
12 hr (H_1)	0.10 (C_1)	48	36	75.00	2	4.17
	0.20(C_2)	48	32	66.67	0	0.00
	0.40(C_3)	48	30	62.50	0	0.00
24hr(H_2)	0.10 (C_1)	48	30	62.50	0	0.00
	0.20(C_2)	48	28	58.33	4	8.33
	0.40(C_3)	48	27	56.25	0	0.00
36hr(H_3)	0.10 (C_1)	48	18	37.50	0	0.00
	0.20(C_2)	48	15	31.25	0	0.00
	0.40(C_3)	48	12	25.00	0	0.00
Cotton plug (M_2)						
12 hr(H_1)	0.10 (C_1)	48	42	87.50	5	10.42

	0.20(C_2)	48	40	83.33	0	0.00
	0.40(C_3)	48	39	81.25	0	0.00
24hr(H_2)	0.10 (C_1)	48	36	75.00	4	8.33
	0.20(C_2)	48	32	66.67	0	0.00
	0.40(C_3)	48	30	62.50	0	0.00
36hr(H_3)	0.10 (C_1)	48	28	58.33	2	4.17
	0.20(C_2)	48	26	54.17	0	0.00
	0.40(C_3)	48	25	52.08	0	0.00
Lanolin paste (M_3)						
	0.10 (C_1)	48	48	100.00	0	0
	0.20(C_2)	48	47	97.92	0	0
	0.40(C_3)	48	45	93.75	0	0

Table 2: Seedling survival percentage after colchicines treatment (2018-19)

Method/	Concentration	Total number of seedlings		Survival	Affected	Expected
Hour	(%)	Treated	Survived	(%)	seedlings	Polyploids (%)
Apex dip method (M_1)						
12 hr(H_1)	0.10 (C1)	48	37	77.08	3	6.25
	0.20 (C2)	48	33	68.75	0	0.00
	0.40 (C3)	48	31	64.58	0	0.00
24hr(H_2)	0.10 (C1)	48	29	60.42	0	0.00
	0.20 (C2)	48	27	56.25	5	10.42
	0.40 (C3)	48	26	54.17	0	0.00
36hr(H_3)	0.10 (C1)	48	19	39.58	0	0.00
	0.20 (C2)	48	16	33.33	0	0.00
	0.40 (C3)	48	13	27.08	0	0.00
Cotton plug (M_2)						
12 hr(H_1)	0.10 (C1)	48	43	89.58	6	12.50
	0.20 (C2)	48	41	85.42	0	0.00
	0.40 (C3)	48	40	83.33	0	0.00
24hr(H_2)	0.10 (C1)	48	38	79.17	5	10.42
	0.20 (C2)	48	33	68.75	0	0.00
	0.40 (C3)	48	31	64.58	0	0.00
36hr(H_3)	0.10 (C1)	48	29	60.42	3	6.25
	0.20 (C2)	48	28	58.33	0	0.00
	0.40 (C3)	48	27	56.25	0	0.00
Lanolin paste (M_3)						
	0.10 (C1)	48	48	100.00	0	0
	0.20 (C2)	48	47	97.92	0	0
	0.40 (C3)	48	46	95.83	0	0

2. Evaluation of different techniques

During the year (2017-18) from the perusal of Table-1& 2, it is clear that apex of forty eight seedlings were dipped for 12 (H_1), 24 (H_2) and 36 (H_3) hours in C_1(0.10%), C_2(0.20%) and C_3(0.40%) concentrations of colchicine and the results indicated that only two seedlings have been presented the characters of polyploid (4.17%) with $C_1M_1H_1$ (0.10 % for 12 hours) whereas, four seedlings showed similar characters with $C_2M_1H_2$ (0.20 % for 24 hours) i.e. 8.33 per cent. No seedlings with polyploid characters were found when apex of seedlings was dipped for 36 hours in various concentrations.

Similarly, in cotton plug method (M_2) five seedlings indicated polyploid characters (10.42 %) when treated with 0.10 per cent aqueous solution of colchicine for 12 hours (H_1) whereas four seedlings were observed 8.33 per cent polyploid characters with the treatment of 0.10 per cent for 24 hours. Only two seedlings were found with polyploid characters (4.17 %) with the concentrations 0.10 per cent for 36 hours.

The seedling treated with different concentrations of colchicine in lanoline paste (M_3) was found ineffective without showing any sign of polyploid.

The experiment was repeated again in year (2018-19) and the results indicated the similar trends as previous year. Among the forty-eight seedlings, only three seedlings indicated symptoms of polyploid (6.25%) in apex dip method (M_1) for 12 hours in aqueous solution 0.10(C_1) per cent of colchicine, whereas five seedlings produced the similar symptoms i.e. 10.42 per cent of polyploid character with 0.20 (C_2) per cent of colchicine solution for 24 hours (H_2).

In cotton plug method (M_2) six, five and three seedlings were indicated polyploidy characters i.e. 12.50 per cent, 10.42 per cent and 6.25 per cent upon treatment with 0.10 (C_1) per cent for 12 hours (H_1), 24 hours (H_2) and 36 hours (H_3) respectively.

The results showed that 0.10 per cent of colchicine had higher capacity to induce the polyploid in seedlings applied either by apex dip method or cotton plug method. It indicates that plants with young shoots absorb more colchicine gradually rather than sudden application of this chemical. Twelve hours was found to be the best duration when aqueous solution of colchicine 0.10 per cent was applied with cotton plug method. Several workers like Biswas and Bhattacharyya (1976) [5] in the cultivated varieties of *Phaseolus vulgaris* L., Glowacka *et al*. (2010) [9] in the genotype of *M. sinensis* and *M. giganteus,* Gu *et al. (*2005) in *Zizyphus jujuba* Mill. cv. Zhanhua, He *et al*. (2016) in chrysanthemum, Kazi (2015) [15] in tomato, Kwon *et al.* (2014) [17] in *Prunella vulgaris,* Qinghua *et al.* (2016) [23] in sour jujube, Shanko (2017) [27] in cowpea and Yang *et al*. (2006) [30] in grapes have found similar results. In view of the difficulty encountered by

different methods for inducing polyploidy, it seems worthwhile to try this method on large scale which may lead to isolate autotetraploid plants. It is obvious that the genetic effects associated with the altered chromosome number may affect the economic characters of the plant in an undesirable direction. However, in case of Cape gooseberry (2n=48) loss or rearrangement of chromatic materials even at a relatively small level of polyploidy is expected.

Table 3: Characters of polyploid seedlings observed during both the year (2017-18 & 2018-19) of the experiment

Sl. No.	Treatment combination	Leaf texture	Growth
1.	$C_1M_1H_1$	Light dark green, broader, upper surface less hairy and veinal ridges less distinct.	Stunted growth, internodal distance less, less branching and stem thick.
2.	$C_2M_1H_2$	Light green, upper surface not rough, leaf less broad and less hairy on upper surface.	Stunted growth, normal branching, internodal distance less and normal stem thickness.
3.	$C_1M_2H_1$	Dark green, broader, upper surface thick, rough, more hairy and distinct veinal ridges	Stunted growth, less branching, thick stem and less internodal distance.
4.	$C_1M_2H_3$	Light green, less veinal ridges, normal upper surface and less hairy.	Less growth, normal branching, normal internodal distance and less thickness.
5.	Control	Normal green, upper surface smooth, non hairy and thin.	Normal growth, more internodal distance, more branching and thin stems.

Conclusion

The cotton plug method gave maximum percentage of seedling survival when compared with apex dip method. However, with increase in concentration of colchicine and treatment duration more mortality was observed. Plants treated with 0.10 per cent colchicine solution had higher capacity of inducing polyploid rather than apex dip or cotton plug method**.**

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