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Effect of colchicine treatment on plant growth and floral behaviour in cape gooseberry (*Physalis peruviana* L.)

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Abstract

Polyploidy induction is an important tool for development of desired fruits and vegetables. 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 trial was conducted under the Department of Horticulture, B.A.U., Kanke, Ranchi during two successive seasons (2017-18 and 2018-19) using colchicine concentrations as $0.10(C_1)$, $0.20(C_2)$ and $0.40(C_3)$ per cent for the duration of 12 (H_1) , 24 (H_2) and 36 (H_3) hours with seedlings apex dip method (M_1) , cotton plug method (M_2) and lanolin paste method (M₃). The results of both years showed that the colchicine treated flower buds @ 0.10 per cent for 12 hours with cotton plug method produced the minimum plant height of 70.90 cm and 73.60 cm, larger leaf length of 12.13cm and 11.70cm and breadth of 10.50cm and 10.55 cm, more taken time in flower bud emergence of 53.00 days and 54.67 days and anthesis of 19.33 days and 20.33 days, bigger flower size of 2.93cm² and 3.00 cm² and minimum percentage of pollen viability of 40.33 per cent and 40.67 per cent respectively in comparison to control. This finding demonstrated the existence of genetic variation for the plant growth and flowering characters response to ploidy change in Cape gooseberry (Physalis peruviana L.).

Keywords: Cape gooseberry, colchicine, polyploidy induction, plant growth, floral behaviour

Introduction

Cape gooseberry (*Physalis peruviana* L.) is an annual short duration minor fruit crop having potential to maximize the income of the farmers as a cash crop during the recent years. This belongs to the family Solanaceae 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. European settlers were introduced in India during beginning of nineteenth century. It is called as so many names in the world. In Hawaii it is commonly called as 'Poha', Golden Berry in South Africa and Rasbhari, Makoi or Tepari in India. The first description of Physalis genus was made by Linnaeus in 1753^[1]. The more than 100 species were identified under the genus *Physalis* 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. Cape gooseberry is tetraploid in nature and having chromosome number 2n = 48Muntzing (1951)^[2]. 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)^[3].

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)^[5]. 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)^[6],

hops (*Humulus lupulus* L.) (Roy *et al.*, 2001) ^[7], ginger (*Zingiber officinale* Roscoe) (Adaniya and Shirai, 2001) ^[8], tarragon (*Artemisia annua* L.) (Gonzalez and Weathers, 2003) ^[9] and feverfew (*Tanacetum parthenium* L.) (Saharkhiz, 2007) ^[10]. There are several target tissues for colchicine treatment, such as meristem and seed (Tamura *et al.*, 1996) ^[11].

In the present investigation 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 plant growth and floral behaviour of the plants of Cape gooseberry.

Materials and Methods

The present investigation was carried out in the experimental area of the Department of Horticulture, Birsa Agricultural University, Kanke, 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 (RBD) 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 $50 \text{ cm} \times 50 \text{ cmi}$. e. row to row and plant to plant respectively. The plot size was maintained two meter 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 per cent (C_1) , 0.20 per cent (C_2) and 0.40 per cent (C_3) for the duration 12 hours (H_1) , 24 hours (H_2) and 36 hours (H_3) with seedlings 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 0.5, 0.10 and 0.20 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

(i) Apex seedlings 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. Growth characters (a) Height of the plants

The height of main shoot from ground level to growing tip of each tagged plant was recorded at fortnightly intervals with the help of a meter scale. Each time the average height of five plants was calculated and ultimate height was represented.

(b) Size of leaves (Length and breadth)

The length and breadth of leaves were measured with the help scale at weekly interval with a view to study the effect of colchicine. The leaves were selected from the middle portion of five plants from each treatment for the measurement and average size was calculated and ultimate size of the leaf was represented.

2. Floral character

(i) Period of flower bud emergence

Each plant was closely watched for this purpose and as soon as the first flower bud emerged the date of its first appearance was recorded. The time required for appearance of flower bud was calculated from the date of transplanting. Five flower buds were selected in each plot for calculation of the period of flower bud emergence and it was represented in days.

(ii) Duration of anthesis

The five flower buds were tagged in each treatment from selected plants. The period required from the date of first appearance of bud to full anthesis of flower was calculated and expressed in days.

(iii) Flower size

The five fully opened flowers were taken. They were measured cross wise with the help of a scale and average was worked out.

(iv) Pollen viability

It was assessed on the basis of stainability in acetocarmine (1%). The deeply stained ones were registered as viable while poorly stained ones were registered as non-viable.

Results

Plant height

During the first year (2017-18)of investigation, the minimum plant height of 70.90 cm was recorded by the effect of the treatment $C_1M_2H_1$ (Colchicine @ 0.10 % for 12 hours with cotton plug method) and the treatments $C_1M_1H_2$ (Colchicine @ 0.10 % for 24 hours with seedlings apex dip method) and $C_2M_1H_2$ (Colchicine @ 0.20% for 24 hours with seedlings apex dip method) were showed the statistically at par result with the having value of 72.90 cm and 73.10 cm plant height respectively. The maximum plant height 82.20 cm was noted under the treatment control ($C_0M_0H_0$). This is depicted graphically in Figure-1.

In the next year (2018-19) of investigation, more or less similar trend was observed as the result obtained in previous year. The minimum plant height 73.60 cm was recorded by the effect of the treatment $C_1M_2H_1$ (Colchicine @ 0.10 % for 12 hours with cotton plug method) and maximum 85.30 cm was under the control ($C_0M_0H_0$). The treatments 0.10% for 12 hours with seedlings apex dip method ($C_1M_1H_1$), colchicine @ 0.10% for 24 hours with seedlings apex dip method ($C_1M_1H_2$) and colchicine @ 0.20% for 12 hours with seedlings apex dip method ($C_2M_1H_2$) with having value of 75.40 cm, 75.60 cm and 75.77 cm respectively were found statistically at par with the treatment of colchicine @ 0.10 % for 12 hours with cotton plug method ($C_1M_2H_1$) [Figure-1].

Leaf size Leaf length

In the first year (2017-18) of experiment, the maximum leaf length 12.13 cm was recorded in the treatment $C_1M_2H_1$ (Colchicine @ 0.10 % for 12 hours with cotton plug method) and the treatment $C_1M_1H_1$ (Colchicine @ 0.10 % for 12 hours with seedlings apex dip method) showed statistically at par with having value of 11.40 cm, whereas; the minimum of 8.50 cm was registered under the treatment control ($C_0M_0H_0$). It is represented graphically in Figure-2.

During the second year (2018-19) of experimentation, it was also found more or less similar trend of the previous year. The treatment $C_1M_2H_1$ (Colchicine @ 0.10 % for 12 hours with cotton plug method)produced maximum leaf length of 11.70 cm and it was showed the statistically at par result with the treatments of $C_1M_1H_1$ (Colchicine @ 0.10 % for 12 hours with seedlings apex dip method), $C_1M_1H_2$ (Colchicine @ 0.10 % for 24 hours with seedlings apex dip method) and $C_1M_2H_2$ (Colchicine @ 0.10 % for 24 hours with cotton plug method) with the having value of 11.10 cm, 10.67 cm and 10.70 cm of leaf length respectively. The minimum of 8.33 cm leaf length was recorded in the treatment control ($C_0M_0H_0$). It is depicted graphically in Figure-2.

Leaf breadth

With considering the effect of different treatments on the first year (2017-18) of the experiment, it was evident that the treatment $C_1M_2H_1$ (Colchicine @ 0.10% for 12 hours with cotton plug method) gave the maximum of 10.50 cm leaf breadth, whereas; the minimum of 6.90 cm produced by the control ($C_0M_0H_0$). The remaining treatments were showed the statistically at par with the control ($C_0M_0H_0$). It is showed graphically in Figure-3.

In the next year (2018-19) also trend of leaf breadth was observed as previous year. The treatment $C_1M_2H_1$ (Colchicine @ 0.10% for 12 hours with cotton plug method) was registered maximum leaf breadth of 10.55 cm and minimum of 6.97 cm was noticed under the treatment control ($C_0M_0H_0$). The remaining other treatments were exhibited statistically at par value with the treatment control ($C_0M_0H_0$). It is graphically represented in Figure-3.

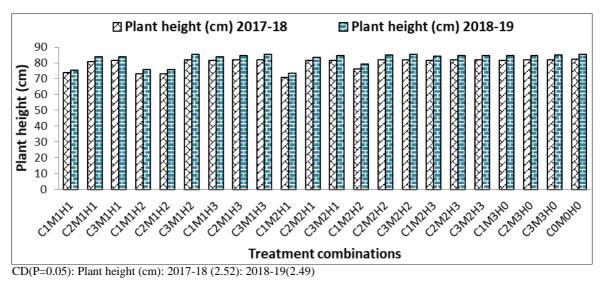


Fig 1: Plant height (cm)

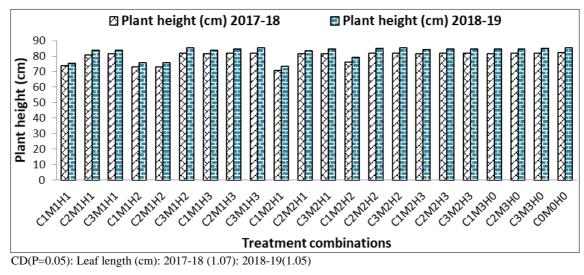


Fig 2: Leaf length (cm).

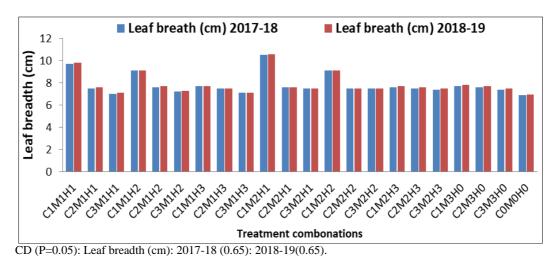


Fig 3: Leaf breadth (cm).

Floral behaviour

Period of flower bud emergence

In the first year (2017-18) of experiment, the more time of 53.00 days was taken in bud emergence from the date of transplanting by the effect of the treatments $C_1M_1H_1$ (Colchicine @ 0.10 % for 12 hours with seedlings apex dip method) and C1M2H1 (Colchicine @ 0.10 % for 12 hours with cotton plug method) and it was found statistically at par with the treatments of C2M1H1 (Colchicine @ 0.20 % for 12 hours with seedlings apex dip method), C₃M₁H₁ (Colchicine @ 0.40% for 12 hours with seedlings apex dip method), $C_1M_1H_3$ (Colchicine @ 0.10 % for 36 hours with seedlings apex dip method), $C_1M_2H_3$ (Colchicine @ 0.10 % for 36 hours with cotton plug method) and C₂M₂H₃ (Colchicine @ 0.20 % for 36 hours with cotton plug method) with the having value of 52.00 days, 51.00 days, 51.00 days, 52.00 and 51.00 days respectively, whereas; the minimum time for flower bud emergence of 46.00 days was taken by the treatment $control(C_0M_0H_0)$. The remaining treatments were varied from 48.00 days to 50.00 days in bud emergence, which was found statistically at par with the treatment control ($C_0M_0H_0$). The data represented in Table-1.

During the second year (2018-19) of experiment, the maximum of 54.67 days for first emergence of flower bud was also taken by the treatment $C_1M_2H_1$ (Colchicine @ 10% for 12 hours with cotton plug method) and it was found statistically at par with the treatments of $C_1M_1H_1$ (Colchicine @ 0.10% for 12 hours with seedlings apex dip method) and

 $C_1M_2H_3$ (Colchicine @ 0.10 % for 36 hours with cotton plug method) with the having value of 54.00 days and 53.00 days respectively. The minimum of 48 days was noted for first bud emergence with the effect of treatment control ($C_0M_0H_0$). In the remaining other treatments were taken time for flower bud emergence from 49.00 days to 52.00 days and statistically, it was noticed at par with the treatment control ($C_0M_0H_0$). The data showed in Table-1.

Time of anthesis

In the first year (2017-18) of experiment, the time of anthesis was counted after flower bud emergence and it was recorded maximum time of 19.33 days in anthesis by the effect of the treatment $C_1M_2H_1$ (Colchicine @ 0.10 % for 12 hours with cotton plug method) followed by the treatment of $C_1M_2H_3$ (Colchicine @ 0.10 % for 36 hours with cotton plug method), which was observed statistically at par with this treatment, whereas; the minimum time of 13.67 days was taken for anthesis by the treatment control ($C_0M_0H_0$). The remaining rest treatments were consumed time in between 14.33 days to 18.33 days in anthesis of Cape gooseberry flower bud. The data represented in Table-1.

During the next year (2018-19) of the experiment, it was also observed the similar results under the different treatments as the result of previous year. In this year also the maximum time for anthesis was recorded as 20.33 days by the effect of the treatment $C_1M_2H_1$ (Colchicine @ 0.10% for 12 hours with cotton plug method), which was found statistically superior

over the remaining rest treatments as well as control $(C_0M_0H_0)$. The minimum time of 14.67 days was taken for anthesis by the treatment of control $(C_0M_0H_0)$. In the rest of the treatments it was found from 15.00 days to 19.00 days for the anthesis of flower buds in Cape gooseberry. The data showed in Table-1.

Flower size

In the first year (2017-18) of investigation, the larger flower size 2.93 cm² was weighed with the effect of the treatment $C_1M_2H_1$ (Colchicine @ 0.10 % for 12 hours with cotton plug method) followed by the treatment $C_1M_1H_1$ (Colchicine @ 0.10 % for 12 hours with seedlings apex dip method) with having flower size of 2.87 cm², whereas; the small flower size of 2.37 cm² was noted under the treatment control ($C_0M_0H_0$). The data represented inTable-1.

In the second year (2018-19) of experiment, the bigger flower size of 3.00 cm² was recorded by the effect of the treatment $C_1M_2H_1$ (Colchicine @ 0.10 % for 12 hours with cotton plug method) which, was noticed statistically at par with the treatment $C_1M_1H_1$ (Colchicine @ 0.10 % for 12 hours with seedlings apex dip method) with flower size of 2.93 cm². The lower flower size of 2.47 cm² was produced by the treatment control ($C_0M_0H_0$). The data showed in Table-1.

Pollen viability

During the first year (2017-18) of observation, the maximum pollen viability of 70.33 per cent was recorded under the treatment control ($C_0M_0H_0$), whereas; the minimum of 40.33 per cent pollen viability was registered by the effect of the treatment $C_1M_2H_1$ (Colchicine @ 0.10 % for 12 hours with cotton plug method), which was found statistically superior over the remaining rest treatments as well as control ($C_0M_0H_0$). The data represented in Table-1.

In the next year (2018-19) of investigation, the trend of the results was recorded more or less similar as the results of

previous year. The minimum pollen viability of 40.67 per cent was observed statistically superior results in comparison to remaining treatments as well as control, this was exhibited by the effect of the treatment $C_1M_2H_1$ (Colchicine @ 0.10 % for 12 hours with cotton plug method), whereas; the maximum of 72.33 per cent pollen viability was noted under the treatment control($C_0M_0H_0$). The data showed in Table-1.

Discussion

The results of the both years (2017-18 & 2018-19) disclosed that the colchicine treated plants showed the minimum plant height in comparison to control (Fig.-1). It might be due to the effect of colchicine as antimitotic substances. It binds to cell protein tubulin and arrests mitosis in metaphase due to failure of spindle formation. It causes depolymerisation and disappearance of the fibrillar microtubules in granulocytes and other motile cells, inhibiting their migration as well as metabolic and phagocytic activity. This is caused the slow rate of growth and development due to physiological disturbance and reduced rate of cell division (Atichart, 2013) ^[12]. Amiri et al. (2010) ^[13] also reported the characteristics of colchicine treated plants with the slow growth, alter morphology and prolonged flowering of polyploids may, in part, result from slowed mitotic divisions and cell divisions of larger cells with more chromosomes. Total plant height was lower in both tetraploid lines compared to the three diploids and this reduced stature was partly due to shorter internodal distances. Several other researchers Gu et al. (2005) [14] in Zizyphus jujuba, He et al. (2016) ^[15] in Dendranthema indicum, Kazi (2015) ^[16] in Solanaceae crops, Kushwaha et al. (2018) ^[17] in Chrysanthemum carinatum L., Manawadu (2016)^[18] in radish and Manzoor et al. (2018)^[19] in Gladiolus grandiflorus reported that colchicine treatment had decreased the plant height. It is agreed with the results of our study.

Treatments	Period of flower bud emergence (Days)		Duration of anthesis (Days)		Flower Size (cm ²)		Pollen viability (%)	
	2017	2018	2017	2018	2017	2018	2017	2018
$C_1M_1H_1$	53.00	54.00	17.33	18.67	2.87	2.93	51.00	51.00
$C_2M_1H_1$	52.00	52.00	16.00	15.67	2.60	2.53	53.33	53.67
$C_3M_1H_1$	51.00	52.00	15.00	16.00	2.53	2.50	53.33	54.33
$C_1M_1H_2$	50.00	51.00	15.67	17.67	2.67	2.70	52.00	52.33
$C_2M_1H_2$	49.00	50.00	15.00	15.33	2.57	2.53	54.33	55.00
$C_3M_1H_2$	48.00	49.00	15.33	15.00	2.50	2.50	60.67	61.33
$C_1M_1H_3$	51.00	51.00	15.67	16.33	2.53	2.57	52.00	52.33
$C_2M_1H_3$	49.00	50.00	15.33	15.67	2.50	2.53	52.33	52.33
$C_3M_1H_3$	50.00	51.00	15.33	15.00	2.47	2.53	52.67	53.67
$C_1M_2H_1$	53.00	54.67	19.33	20.33	2.93	3.00	40.33	40.67
$C_2M_2H_1$	50.00	51.67	15.67	16.67	2.57	2.53	47.00	47.33
$C_3M_2H_1$	48.00	49.00	15.00	16.00	2.50	2.57	47.33	48.33
$C_1M_2H_2$	50.00	51.00	15.00	15.33	2.53	2.53	53.00	53.67
$C_2M_2H_2$	50.00	50.00	14.33	15.67	2.47	2.57	54.67	54.33
$C_3M_2H_2$	49.00	50.00	15.33	15.67	2.43	2.53	54.00	54.67
$C_1M_2H_3$	52.00	53.00	18.33	19.00	2.77	2.87	48.67	50.33
$C_2M_2H_3$	51.00	51.00	15.33	15.33	2.57	2.53	50.33	51.33
$C_3M_2H_3$	49.00	52.00	14.67	15.00	2.53	2.57	50.67	51.67
$C_1M_3H_0$	49.00	50.00	15.00	16.00	2.50	2.53	51.33	51.67
$C_2M_3H_0$	49.00	50.00	15.33	15.00	2.47	2.57	52.33	52.67
C ₃ M ₃ H ₀	49.00	49.00	14.33	15.33	2.40	2.53	53.33	53.67
$C_0M_0H_0$	46.00	48.00	13.67	14.67	2.37	2.47	70.33	72.33
SEm ±	0.89	0.84	0.43	0.39	0.04	0.04	1.83	1.96
CD (P=0.05)	2.54	2.40	1.23	1.11	0.13	0.10	5.22	5.58
CV %	3.09	2.86	4.79	4.18	2.97	2.45	6.03	6.37

Table 1: Floral behaviour.

The leaf size was obtained larger than that of control. The increase was seen not only in length but also in breadth. It might be due to increase in number of stomata chloroplast and larger size of component tissue, viz; epidermis palisade and spongy layers. The increase stomata components were the reason for having darker green leaves. The similar findings have been reported by the many researchers as Ascough and Staden (2008) ^[20] in Watsonia lepida, Biswas and Bhattacharyya (1976) ^[21] in French bean, Glowacka *et al.* (2010) in Miscanthus species, He et al. (2016) in chrysanthemum, Kushwaha et al. (2018) ^[17] in Chrysanthemum carinatum, Liu et al. (2007)^[22] in Platanu salicifolia, Qinghua et al. (2016) [23] in Sour Jujube, Tome et al. (2016)^[24] in Solanum commersonli ssp. and Zhang et al. (2016)^[25] in *Trollius chinensis*.

The period of flower bud emergence and duration of anthesis in Cape gooseberry, it was indicated in the present investigations that the application of colchicine extended the period of bud emergence and duration of opening of flowers as compared to untreated plant. Besides bigger stomata cells and a decrease in stomata number per unit area were observed in tetraploids. In recent year studies, most researchers examined the size of the stomata cells and reported bigger cells in polyploids Tulay and Unal (2010) [26]. Resulting in the upset of translocation of the food materials from the site of producing to the growing point has been attributed the possible delay in emergence of flower bud and also opening of flowers. The results obtained are in conformity with the findings of Gu et al. (2005) ^[14] in Zizyphus jujuba, Kushwaha et al. (2018) ^[17] in Chrysanthemum carinatum L. and Luo et *al.* (2018) ^[27] in rubber plant.

The size of flowers was considerably larger in colchicine treated plants than the untreated plant ($C_0M_0H_0$). It might be due to accumulation of more food materials in colchicine treated plant through larger size of leaf resulting in large flower in Cape gooseberry. The rate of photosynthesis depends upon the size of leaf and chlorophyll content. This results obtained are in agreement with the findings of Ascough and Staden (2008) ^[20] in *Watsonia lepida*, Gu *et al.* (2005) ^[14] in *Zizyphus jujuba*, He *et al.* (2016) ^[15] in chrysanthemum, Kwon *et al.* (2014) ^[28] in *Prunella vulgaris* for. *Albiflora* Nakai, Manzoor *et al.* (2018) ^[19] in *Gladiolus grandiflorus*, Muhammad *et al.* (2007) ^[29] in watermelon.

It was observed that the pollen grain viability percentage in Cape gooseberry flowers was decreased in the colchicine treated plant. The minimum viability percentage was caused by the application of aqueous solution of colchicine @ 0.10 for 12 hours with cotton plug method $(C_1M_2H_1)$ followed by the treatment colchicine @ 0.20 per cent for 12 hours with cotton plug method (C₂M₂H₁). The reduction in pollen fertility of colchicine treated plant can be attributed to multivalent association during synopsis of chromosome and the consequent production of unbalance gametes. Meiotic abnormalities are also one of the causes of low pollen grain viability. The viability in auto as well as allopolyploids influenced not only by the presence or absence of multivalent but also by other genetic factors (Sohoo et al., 1970)^[30]. The similar observations were earlier reported by Biswas and Bhattacharyya (1976)^[21] in French bean, Glowacka et al. (2010) ^[31] in *Miscanthus* species, Kadota and Niimi (2002) ^[32] in Japanese pear, Omidbaigia et al. (2010) [33] in dragonhead, Shao et al. (2003)^[34] in pomegranate and Zhang et al. (2016) ^[25] in Trollius chinensis.

Conclusion

The colchicine treated plants for minimum period with cotton plug method performed better in respect to minimum plant height (cm), more leaf length and breadth (cm), prolonged period of flower bud emergence (days)and anthesis of flower (days), larger flower size (cm²) and minimum pollen viability percentage in comparison to control. The plants treated with colchicine @ 0.10 per cent solution for 12 hours with cotton plug method had ability to induce polyploid in comparison to remaining concentrations of colchicine for variable durations with different methods.

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