African Journal of Biotechnology Vol. 11(39), pp. 9336-9342, 15 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2196
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effect of colchicine on mitotic polyploidization and morphological characteristics of *Phlox drummondi*

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Accepted 7 December, 2011

The present work was undertaken to observe the response of *Phlox drummondi* to colchicine treatment. The survival rate and germination percentage is severely affected by various treatments of colchicine. In this investigation, doses related effects of the ploidy treatments on quantitative traits were noticed. Result indicates reduction in plant height, number of leaves per branch, but increase in number of branches. Stomatal size was negatively correlated with stomatal frequency. Stomatal size and stomatal frequency can be used as indirect methods for identification of ploidy level of *Phlox*. This finding demonstrates the existence of genetic variation for the morphological response to ploidy change in *P. drummondi.*

Key words: *Phlox drummondi*, polyploidy, floral abnormalities, survival rate.

INTRODUCTION

Phlox drummondi is a widely accepted annual ornamental flowering plant growing in winter season. The brilliance and clean colored appearance of this flower makes it favorite in the garden. Further, it is excellent for masses in borders and for solid beds. About all species of *P. drummondi* are diploid in nature having seven basic chromosome numbers (Raja et al., 1992). The polyploidization in basic chromosome numbers is a major source of evolution and quality production of flowering plants (Hick, 2003; Zlesak et al., 2005). Polyploidy in ornamentals results to an enlargement of flowers and leaves, sturdier stem, intensification of colors, hardier and more robust plants, thicker and more rigid foliage, apparent increase in the tolerance to different stresses, resistance to diseases and insects and reduction in fertility of flowering plants (Amiri et al., 2010). Thus, the development of polyploidy may crucial for quality production of flowers among ornamentals. Out of various chemicals tested for polyploidy production, colchicine is one of them that modifies plant shape, restores fertility and increases flower size (Amiri et al., 2010). Though, various concentrations of colchicine have different effects on variety of plant species (Stadler et al., 1989). Keeping these facts in consideration, the present investigation was carried out to find out the response of *P. drummondi* against various concentrations of colchicine with the optimism that the new species of *P. drummondi* with better suited ornamental qualities might be obtained.

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MATERIALS AND METHODS

Seeds collected from red color flowers *P. drummondi* were obtained from wild habitats of North Himalayas of India. In spring season well processed seeds kept in refrigerator for three days at 4°C to stimulate seed germination. The clean and healthy 60 germinated seeds were allocated for each treatment (12 seeds per replication). They were soaked in 0.01, 0.025 and 0.05% solutions of colchicine for two time scales of 24 and 36 h. After 24 or 36 h, the seeds were extracted from ploidy inducing (colchicine) solution and washed with tap water for about 6 h, and then cultured in greenhouse. Finally, seeds were planted in the field of floriculture section of College of Horticulture, Bhrasar, Pauri Garhwal, uttrakhand, India at 15 × 10 cm distance. All the standard cultural practices were practiced to raise the normal crop. Results obtained were statistical analyzed using two factorial arrangement of completed randomized design (STPR-2). A total of eight treatments (two time scale × 4 treatments) of each experiment were maintained with five replications. The graphic representations of results were also drawn with

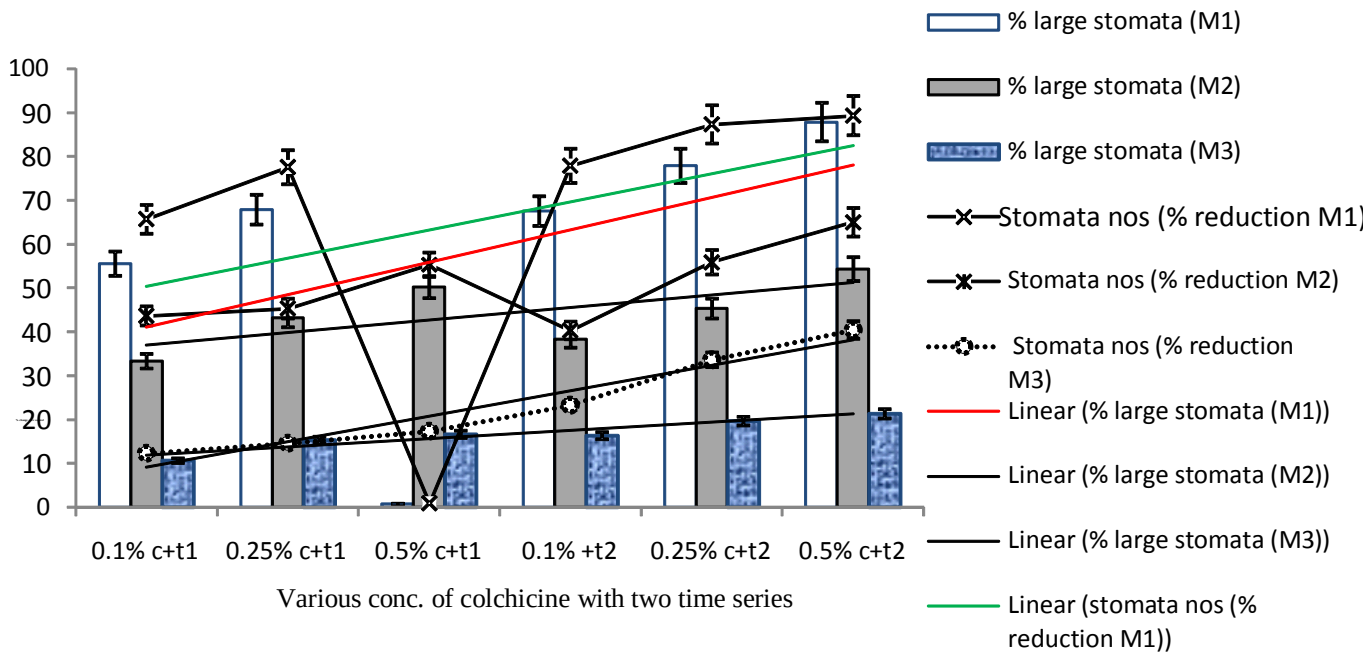

Figure 1. Germination % of seeds of *Phlox* as affected by colchicine concentration (SE bar with 5%).

5% standard error as suggested by Fisher (1954). The trial was conducted for three years (up to M_3 generation) to observe the stability of the induced traits before selecting as mutated plants.

15 days after planting (DAP), the numbers of seedling were counted and per cent germination was calculated. The numbers of surviving plant were also counted at 50 DAP. Five completely opened mature leaves were sampled from each plant aged two months old. Seedlings which contained very young and old leaves were not consider for sampling. A peel of epidermis was removed from the lower surface of the lamina with a fine scalpel and mounted on a drop of water between slide and cover slip. Stomatal lengths were measured by 400X light microscope equipped with stage and ocular micrometer. 10 measurements (each five measurements from both left and right sides of the mid vein) of stomata length in µm/leaf were made. Numbers of stomata /unit leaf area (stomata density) were further obtained using 40X light microscope. Surface cells of five leaves /colchicine treated and untreated plants were examined with 10 measurements/plant. The counting of surface cells was made from both left and right sides of each leaf. Observations on plant height, morphological abnormalities, the number of branches per plant, number of leaves per plant and days to flower were taken into consideration. Abnormal chlorophyll content was measured by a visible observation.

RESULTS

The germination per cent of seeds in M1 generation was decreased with increase doses of colchicine. However, non- significant effect of treatments in second generation onward were observed. Figure 1 reveals that germination per cent was reduced drastically with long time soaking of *Phlox* seeds of M1 generation. The seeds of M1 generation soaked with different time duration could not influence the germination per cent in second generation onward (Figure 1).

The concentrations and treatment durations of colchicine were inversely proportionate to survival per cent of 50 days old seedlings. At 0.5% concentration, per cent survival of *Phlox* plant was 25 and 5 at 24 and 36 h after being soaked, respectively. However, survival rate was non-significantly affected by treatments on second generation onward (Figure 2).

The average stomata sizes of the control group were found to be 21.17 and 21.02 µm for 24 and 36 h of soaking period, respectively. Treatments soaked in colchicines solution were separated into two groups, one with stomatal size less than or equal to 21 µm and the other with those greater than 21 µm stomatal size of the control. When statistically analyzed, it was found that the average of large stomatal size was significantly different from that of the control. Increasing concentration of colchicine, the treatment was also noticed to increase the numbers of plant with large stomatal size compared with that control. However, only M1 generation was significantly affected by concentration and duration of colchicines (Figure 3). As for stomatal frequency is concerned, the greater the stomatal size, the lesser, the stomatal frequency in M1 generation (Figure 3).

As shown in Figure 4, the maximum plants height was in the control group and in the treatment 0.1 g/l-1/24 h of colchicine. The minimum plant height belonged to the treatment 0.5 g/l-1/36 h. There were significant differences between the applied treatments. Generally, an average height of plants was inversely proportionate to time period. As a matter of fact, plant height decreased with increment of incubation times and concentrations of colchicine. However, in second generation onward the

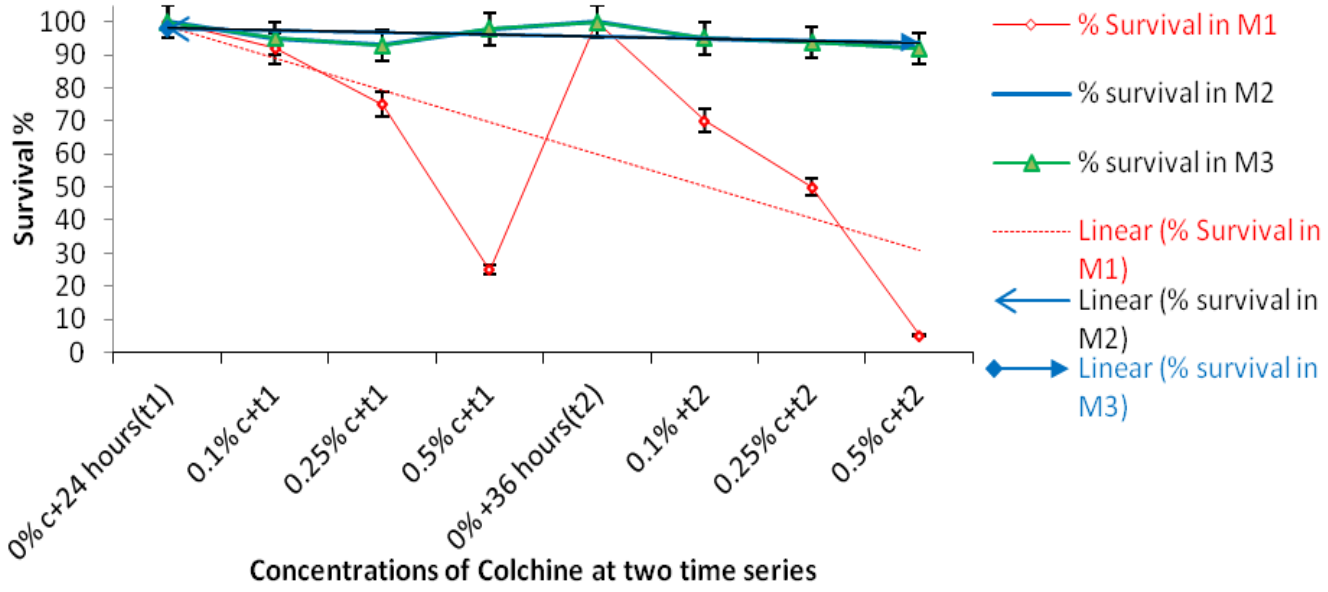

Figure 2. Survival rate of *Phlox* as affected by various concentrations of colchicine (SE bar with 5%).

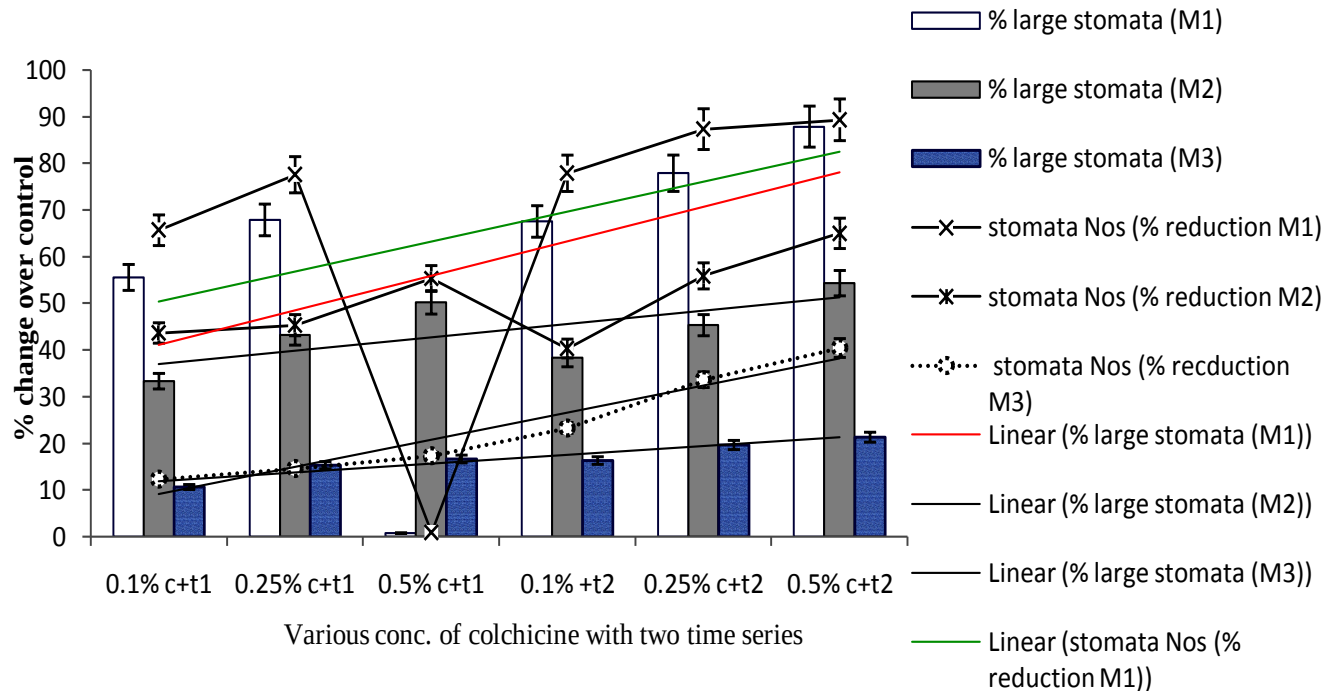

Figure 3. Change in stomata numbers and size as affected by colchicines treatments (SE bar at 5 %).

effects of treatments were non-significant. Aside from variation in height, the plants with large stomata also produced some morphological difference from the control, thick and course leaves, and heavy branching (Figure 7). The maximum number of branches was observed in the treatment containing 0.5 g/l-1/36 h and then in the treatment containing 0.25 g/l-1/36 h. The minimum were observed in control group of M1 generation. However, numbers of branches were non-significantly affected by treatment with colchicines in second generation onward (Figure 5).

The maximum number of leaves per branch was observed in the treatment containing 0.5 g/l in time period of 36 h. There were significant differences among all the treatments. The lower doses of colchicine resulted in relatively less number of leaves per plant for the treatment

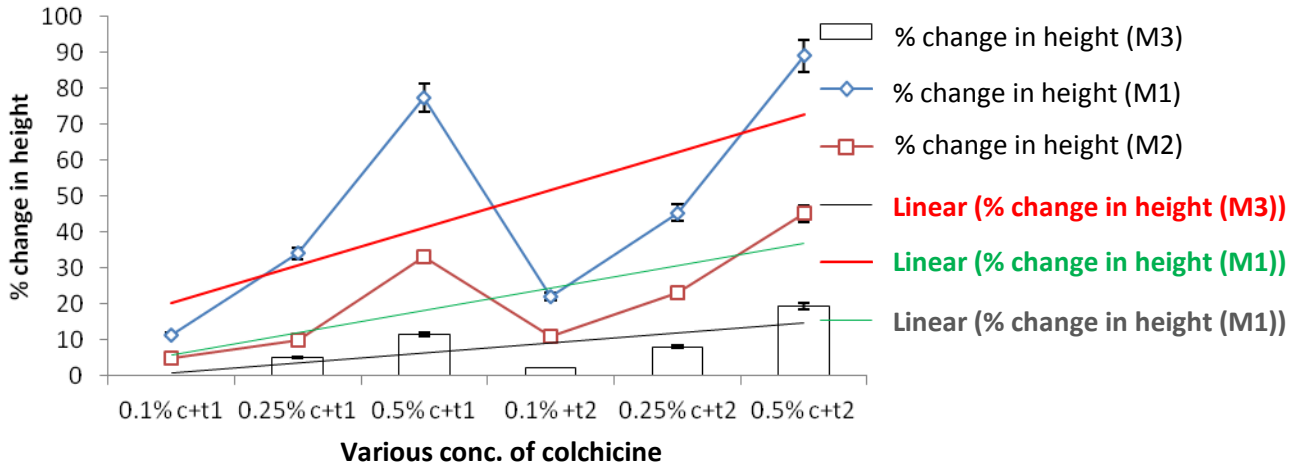

Figure 4. Change in height over control as affected by doses of colchicine (SE bar at 5%).

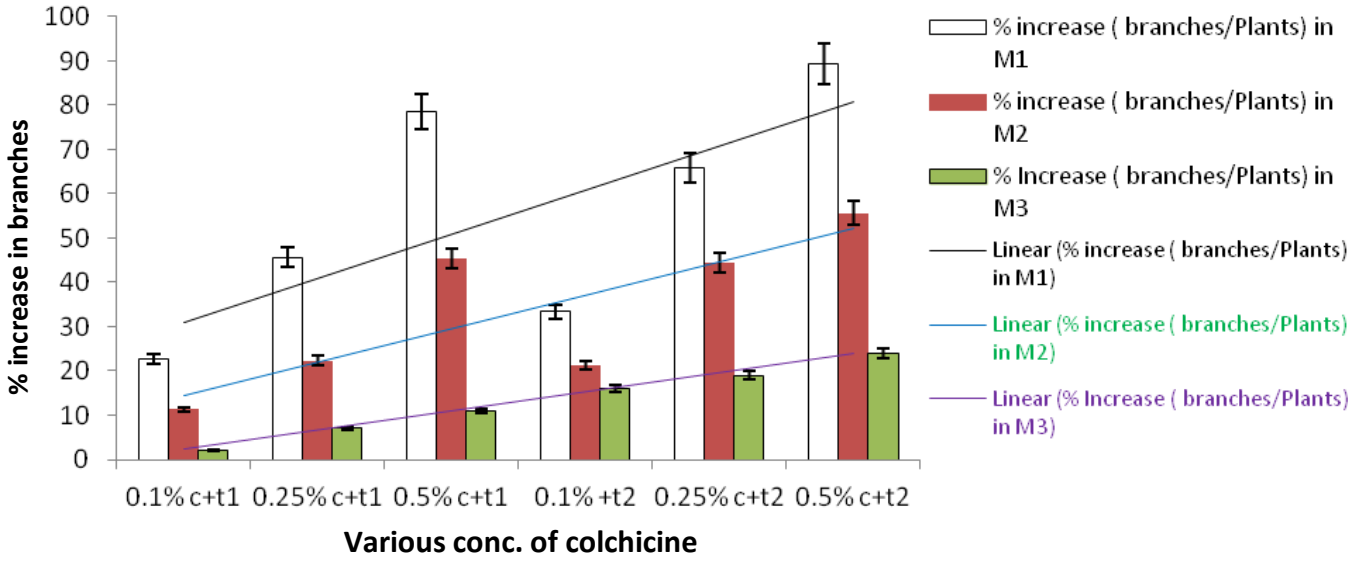

Figure 5. Percentage increase in no. of branches/plant by colchicine treatment (SE bar at 5%).

of 24h than time period of 36h. The minimum number of leaves was counted with control group. Generally, number of leaves increased by enhancement of colchicines concentration or incubation time (Figure 6).

Relationship of morphological characteristics with colchicine concentrations and time period treatment

Regression line for the effects of different colchicine treatments on germination and survival per cent are shown in Figures 1 and 2. Survival and germination of seed/plants per cent linearly decreased as colchicine concentration was increased. Plants germination and colchicine concentrations was negatively correlated (R^2 = 0.3103, 0.2755, 0.2252 in M1, M2 and M3 generation respectively). Regression equations were M1y = -2.6429x + 96.643, M2y = -1.2857x + 98.286, M3y = -0.6786x + 100.43 (Table 1), respectively. Similarly, survival rate and colchicine concentrations was negatively correlated (R^2 = 0.453, 0.25, 0.25 in M1, M2 and M3 generation, respectively). Regression equations of regression line were M1y = -9.7024x + 108.29, M2 y = -0.631x + 98.714, M3y = -0.631x + 98.714 (Table 1), respectively.

Regression line for the effects of different colchicine treatments on plant height is shown in Figure 4. Plants height was linearly decreased by enhancement of colchicine concentrations or incubation time. Plants height and colchicine concentrations was negative strongly correlated (R^2=0.5621, 0.5354 and 0.4057 in M1, M2 and M3 generation, respectively). Regression equation of regression line were M1y = 6.2251x - 0.5847, M2y = 2.752x –

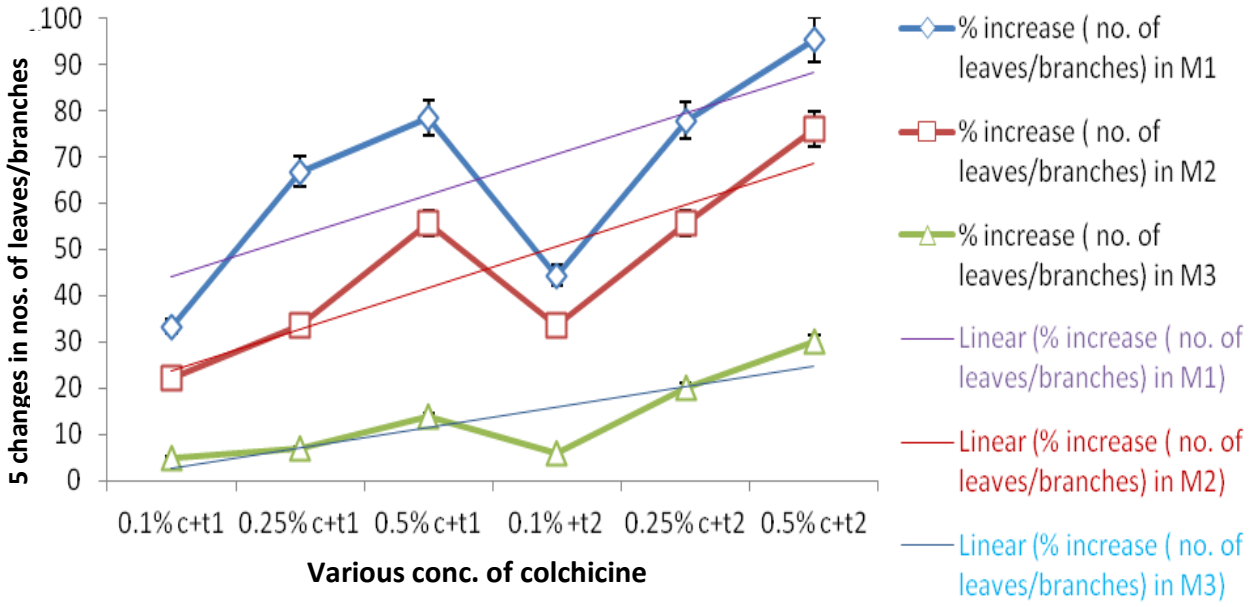

Figure 6. Numbers of leaves/branches as affected by colchicine treatments (SE bar 5%).

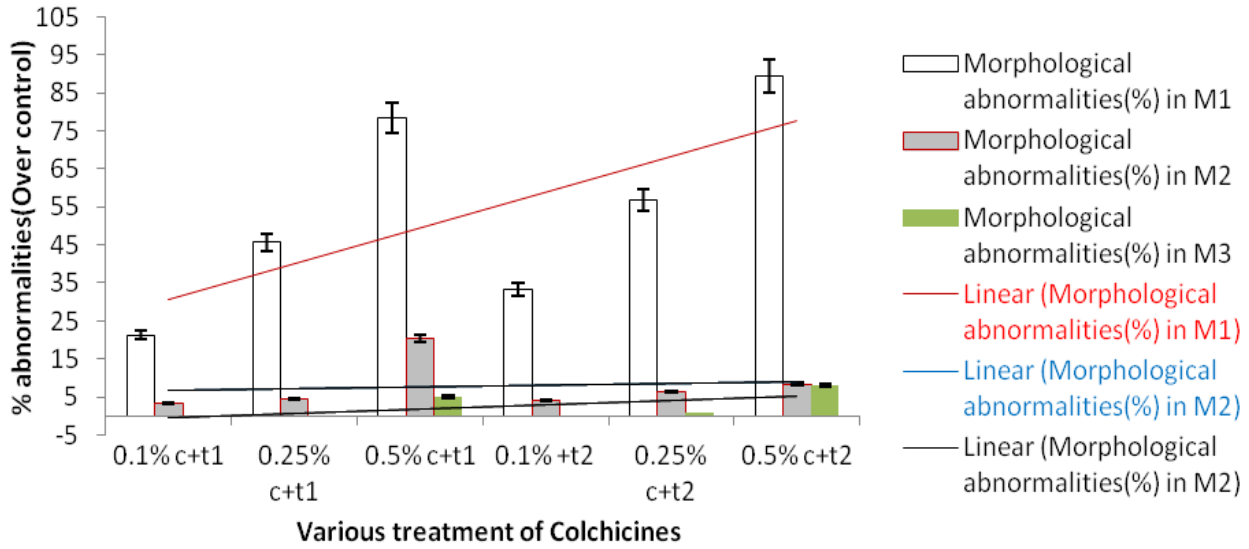

Figure 7. Morphological abnormalities in Phlox due to colchicines treatments (SE bar at 5%).

2.022 and M3y = 10.489x + 9.8047 (Table 1), respectively.

The number of branches had significantly positive-strong correlation with different incubation times and concentrations of colchicine (R^2=0.5029, 0.6548 and 0.9966 in M1, M2 and M3 generation respectively). In Figure 5, regression was linear, and the regression equation was M1y = 9.9631x + 21.051, M2y = 7.5297x + 7.0127 and M3y =4.3143x - 1.9333 (Table 1).

As shown in Figure 6, there were positive relation between the change in number of leaves per branch and applied colchicine treatments. Also their correlation was R^2 = 0.5071, 0.7141 and 0.7163 in M1, M2 and M3 generation, respectively. Resultant regression equation was linear and was as: M1y = 8.8349x + 35.091, M2y = 8.9543x +14.763 and M3y = 4.4571x - 1.9333. The reduced number of stomata /leaves enhanced by colchicine concentrations and incubation times, is a trait which had linear relation and very strong correlation with different incubation times and colchicines concentrations (R^2 = 0.1307 and 0.9379 in M1, M2 and M3 generation, respectively). Regression equation were M1y = 6.417x + 43.981, M2y = 5.8157x + 3.27 and M3y = 5.8157x + 3.27, respectively for number of stomata (Figure 3 and Table 1).

Table 1. Linear regression between traits and different incubation times and concentrations of colchicine.

Trait	Regression equation			R^2		
	M1	M2	M3	M1	M2	M3
Germination % of *Phlox* seeds	y = -2.6429x + 96.643	y = -1.2857x + 98.286	y = -0.6786x + 100.43	0.3103	0.2755	0.2252
Survival rate	y = -9.7024x + 108.29	y = -0.631x + 98.714	y = -0.631x + 98.714	0.453	0.25	0.25
Stomata size	y = 7.3804x + 33.756	y = 2.8411x + 34.183	y = 1.886x + 10.061	0.2011	0.481	0.9063
Stomata numbers	y = 6.417x + 43.981	y = 5.8157x + 3.27	y = 5.8157x + 3.27	0.1307	0.9379	0.9379
Change in plant height	y = 6.2251x - 0.5847	y = 2.752x - 2.022	y = 10.489x + 9.8047	0.5621	0.5354	0.4057
Changes in nos. of branches	y = 9.9631x + 21.051	y = 7.5297x + 7.0127	y = 4.3143x - 1.9333	0.5029	0.6548	0.9966
Change in nos. of leaves/ branches	y = 8.8349x + 35.091	y = 8.9543x + 14.763	y = 4.4571x - 1.9333	0.5071	0.7141	0.7163
Morphological abnormalities (%)	y = 9.3911x + 21.276	y = 0.416x + 6.4107	y = 1.0857x - 1.4667	0.4523	0.0147	0.3598

There was rising linearly relation between size of stomata/leaves and different times and concentrations of colchicine. This correlation was medium (R^2 = 0.2011, 0.481and 0.9063 in M1, M2 and M3 generation, respectively). The regression equation was M1y = 7.3804x + 33.756, M2y = 2.8411x + 34.183 and M3y = 1.886x + 10.061as well (Figure 3 and Table 1).

DISCUSSION

Germination % of *Phlox* (M1) seeds soaked in colchicine solution at different levels of concentration; it was found that % seed germination decreased with the increasing doses of colchicine. Figure 1 shows greater % germination reduction when the soaking duration was higher. The results agrees with Raphiphan (2002) who reported that the duration of soaking seeds and colchicine concentrations had significant effects on the germination per cent of *Ipomoea quamolic* Linn. The highest colchicine concentration showed the least germination percentage. In addition, among the surviving seedlings, some were noticed to gradually die, especially seedlings in the treatments employing high colchicine doses along with long soaking period. The results are similar to those reported by Parakarn et al. (2002) which indicated the rate of survival to be inversely related with colchicine concentration and soaking duration. The linear trend of treatment duration and concentration of colchicines, on germination and survival rate was observed (Figures 1 and 2). In most cases, the mortality appeared to be due to poor seedling vigor resulting in an ability to overcome the toxic effect of colchicine (Zlesak et al., 2005). Addink (2002) stated that high concentration of colchicine could inhibit the development of living part resulting in mortality of organism.

Increasing the concentration of colchicines, the treatment was also noticed to increase the numbers of plant with large stomatal size compared with that control. 36 h of soaking, the treatments gave more percentages of plant with large stomatal sizes which indicated that both concentrations and soaking durations tended to increase efficacy in chromosomal increase of *Phlox*. However, only M1 generation was significantly affected by concentration and duration of colchicines (Figure 3). As for stomatal frequency, Figure 3 shows that the greater the stomatal size, the lesser, the stomatal frequency in M1 generation. *Phlox* with large stomatal size and reduced stomatal frequency had potential to be polyploidy or had increased chromosome numbers. The experiment found that 24 h of seed soaking in 0.1 and 0.5% colchicine solution yielded plants with 55.56 and 78.57% large stomata (Figure 3) and 87 and 25% seedling survival of the control. The experiment found that 24 h of soaking seed in 0.1 and 0.5% colchicine solution yielded plants with 55.56 and 78.57% large stomata (Figure 3), and 87 and 25% seedling survival of the control, respectively (Figure 2). While soaking seed for 36 h gave quite increasing stomatal size, yet percentage survival was very low (Figure 3) . Amir et al. (2010) also reported that length of stomata was the accurate indicator of polyploidy levels in many plant species. As a result, the stomatal size and stomatal frequency could be used as the indicators of polyploidy level for *Phlox*. The increases in dimensions and area were probably due to the fact that cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasm to nuclear volume, and express more proteins with the presence of more genes. This increase in size may translate to an increase in plant and its organs (Raufe et al., 2006). However, the counting of chromosome number of *Phlox* with large stomatal size which will be further conducted will accurately

indicate whether such speculation is accordingly.

The high concentration of colchicine solution plus long soaking duration was noticed to cause the treated seeds to give low height plants (Figure 4). It works by disrupting the polymerization of microtubules which in turn disrupts spindle fibre development and mitosis. Cells arrested at metaphase may recover and enter the next mitotic cycle with twice as many chromosomes (Zlesak et al., 2005). Jensen (1974) mentioned that in addition to the negative side effects of colchicine, such as mitotic irregularities, growth retardation, etc, other mutagenic effects including quantitative changes have been reported for various crops in.

Polyploidy usually leads to thicker leaves, a deeper green color, increased width-to-length ratio of leaves, larger and heavily textured flowers, and a more compact growth habit (Amir et al., 2010). In other hand, polyploidy plants usually have thicker roots and stems (Rose et al., 2000). In this study plants under ploidy inducer agents differed from diploid plants (controls plant) in growth rate and morphology with more branches. Similar phenotypic variations, such as larger plant organs in polyploid compared with diploid plants, were reported by other workers (Raufe et al., 2006). Also, the characteristic slowed growth, altered phenology, and prolonged flowering of polyploids may, in part, result from slowed mitotic divisions and cell divisions of larger cells with more chromosomes (Stebbins, 1971). Total plant height was lower in treated plants compared to the diploids (control), and this reduced stature was partly due to shorter internodal distances. Raufe et al. (2006) also reported that colchicine treatment decreased the plant height which agrees with the results of our study. In this study, we observed the number of branches increased by increment of incubation times and concentrations of ploidy inducer agents. Increased branching (bushy habit) has been reported with colchicines treatment in other studies (Hewawasam et al., 2004).

Conclusion

According to the experiment, the following conclusions were made:

1. With increasing concentration of colchicine solution and soaking duration, per cent germination and survival of seedling decreased while % plants with large stomata increased.
2. Stomatal size was negatively correlated with stomatal frequency.
3. Stomatal size and stomatal frequency can be used as indirect methods for identification of ploidy level of *Phlox*.
4. Total plant height was lower in treated plants compared to the diploids (control) and as concentration and duration increases, plant height decreases.
5. Morphological abnormalities were positively correlated with concentration and duration of treatment.
6. Only M1 generation had significant effect of concentration and duration of colchicines treatments.

ACKNOWLEDGEMENT

Thanks are due to the Dean, Vir Chandra Singh Garhwali College of Horticulture, Govind Ballabh Pant University of Agriculture and Tech., Bharsar, Pauri Garhwal for providing the financial aid to carry out this experiment.

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