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RESEARCH ARTICLE

Induction of Colchiploids in Mulberry (*Morus*) variety Kajali in C_1 Generation

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Abstract

A number of mulberry varieties are available in nature, but they lack one or the other economic character required for silkworm *Bombyx mori* L. as food. **C**olchicine ($C_{22}H_{25}NO_6$) a known polyploidising agent used to induce morphological variation in plants. Efforts have been made to induce polyploidy in mulberry variety Kajali using colchicine. Mulberry variety Kajali is grown in mulberry germplasm bank and the vegetative buds are treated with aqueous solution (0.1%-0.5%) of colchicine. RBD Method with three replications/ treatment was followed. Agro-botanical traits such as sprouting, rooting and survivability percentages, plant height, leaf area and intermodal distance were encountered. Results revealed that, Kajali mulberry variety recorded decrease in the growth parameters with the increase in colchicine concentration. Plants recovered at C_1 (Colchicine treated plants in F_1 generation) generation showed beneficial characters only at 0.4% colchicine concentration, leaf area is considerably increased to 196.11cm^2 compared to control (178.27cm^2). Dwarf, stout, thick, greenish leaves, leaf yield increased to 10.11% and number of chloroplast ranged from 23-27 compared to control (44.00) at 0.4% concentration in C_1 generation. Mulberry plants recovered at 0.4% colchicine concentration require further systematic yield trials and evaluation of colchiploids over a period would establish their potentiality as cultivars
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Introduction

Presence of unique biochemical constituents such as morin, beta-sitosterol in mulberry leaves plays an exceptional role in the biosynthesis of silk by the silkworm Bombyx mori L. Colchicine is a widely accepted mutagenic agent to induce polyploidization in vegetatively propagated plants (Bragal, 1995). Mulberry is a diploid plant in nature and induction of tetraploid and triploid is an herculean task and this mutagenic agent is found to disrupt mitosis by preventing microtubule polymerization which is common in plant species (Luckett, 1989; Peterson *et al*., 2003; Casro *et al*., 2007; Deshpande *et al*., 2010).Dwarfing of plants, thick stem and receding of number of branches is a common phenomenon among the colchicine treated plants (Liu *et al*., 2007; Amiri *et al*., 2010; Yuxiang Wu *et al*., 2011). Colchicine is one of the commonly used spindle inhibitors (Hancock, 1997) and variations have been exploited in many woody species including mulberry and rose (Rose *et al*., 2000; Blakesley *et al*., 2002; Shao *et al*., 2003). Use of Colchicine on shoot meristems was one of the primary methods of somatic polyploidization in roses (Ma *et al*., 2004; Basye, 1990; Roberts *et al*., 1999). Mutagenic effects on plant morphology, chlorophyll sterility and yield have been confirmed earlier (Castro *et al*., 2003). Main objective of the present investigation is to improve the yield, produce nutritionally superior leaves which increase the palatability of silkworm and to evolve colchiploids acclimatize to the varied agro-climatic zones.

MATERIALS AND METHODS

Study Area

Mulberry variety Kajali procured from mulberry germplasm bank maintained at Jnana Bharathi Campus, Bangalore University, Bangalore and used for the induction of variation through colchicine treatment. Field experiment was conducted at mulberry germplasm bank maintained at Jnana Bharathi Campus and laboratory experiments were conducted at Moriculture Laboratory in the Department of Sericulture, Bangalore University.

Sampling and Sample Analysis

Mulberry bushes were given middle pruning to hasten the sprouting of axillary buds. Five different concentrations of aqueous colchicine ($C_{22}H_{25}NO_5$) viz., 0.1%-0.5% were used to treat the vegetative buds. Selected buds were thoroughly washed in distilled water before the application of colchicine. Buds were covered with cotton swabs and colchicine solution was applied from 8am-5pm for three consecutive days at an interval of one hour and control buds were treated with distilled water. Cotton swabs were removed after the treatment and buds were thoroughly washed in distilled water. Buds were allowed to grow by providing required agricultural inputs. Untreated axillary buds situated on the treated portion of shoots were removed periodically so as to maintain only treated axillary buds. Transplanted twigs were planted in randomized block design (RBD) with 90cmx90cm spacing (Kasiviswanathan and Iyengar, 1970; Das, 1984). Necessary cultural operations such as timely irrigation, weeding, intercultivation, manuring, protection against desiccation, diseases and pests were ensured. Suitable controls were maintained in similar conditions for comparative studies. Various propagation parameters viz., sprouting, rooting, survivability, plant height, branching pattern, internodal distance, stomata, number of chloroplast, pollen fertility, leaf area, fresh leaf weight, leaf yield were recorded as per the standard methods suggested by Jolly and Dandin, (1986); Dandin and Jolly, (1986); Shamachary and Jolly, (1988).

Statistical Analysis

Data collected on various parameters were tabulated using "Method of Analysis of Variance" appropriate to the experimental design (Sundararaj *et al*., 1972; Singh and Choudhary, 1979).

Results

Survivability, Sprouting and Rooting

Propagation parameters such as survivability, sprouting and rooting depict a dismal picture with abysmally poor results and showed non-significant results. Percentage increase in colchicine concentration narrows down the propagation traits which is an indication of subtle changes taking place aftermath the chemical treatment. Survivability receded from 78%-27% when concentration was increased (0.1%-0.5%) showing SEM at 0.19 and CD at 5% recorded non-significant values. Delay in sprouting was recorded and with the gradual increase in colchicine concentrations, buds of Kajali variety took longer duration to sprout and resume growth. Maximum duration was taken to sprout in the treated buds is 15-20 days compared to control (4-5 days). Concentration of colchicine has direct bearing on the root growth with SEM at 0.21 and CD at 5% revealed insignificant values (Table-1).

Growth parameters

Plant height is the demonstration of high yield, also genetically controlled. None of the concentrations of colchicine surpasses the control (152.43cm) though minor variations were noticed in treated plants. Visible appearance of stem depicts thick, stout and dwarfness were of common occurrence. Increase in the concentration of colchicine concurrently reduces the plant height and lowest concentration of the chemical (144.29cm) has minimum effect on the plant height. Highest concentration of chemical (0.4%) exhibited increased number of branches (5.72) when compared to control (5.51) and significant results in CD at 5% were observed. Internodal distance, one of the prime concerns in mulberry breeding and decreased internodal distance invariably signifies the increase in quantum yield of leaves. Considerable decrease in internodal distance was observed at 0.2% (3.64cm) compared to control (4.04cm) and CD @ 5% was measured 1.92 (Table-1). Another important growth parameter in the form of leaf area is also a measure of yield and all the treated plants exhibiting decreased leaf area with the increase in the concentration of colchicine except at 0.4% (196.11cm^2) and thick, dark, greenish leaves and compact growth habit were noticed. Leaf petioles tended to be slightly shorter and broader than found to be diploids. Polyploids lead to thicker and deep green coloured leaves, increased leaf-width ratio of leaves, large and heavy textured flowers and

more compact growth of plants. 100 leaves weight in treated plants at 0.4% yielded 422.14gm compared to control 363.24gm. Considerable reduction in stomatal number (44) compared to control (53) and increased number of chloroplast (23-27). Beneficial variant procured from 0.4% revealed polyploidy nature of the treated plants (Table-2).

Table 1: Effect of colchicine on propagation and growth parameters of Kajali mulberry variety at C_1 generation.

Treatment	Survivability (%)	Sprouting (%)	Rooting (%)	Plant height(cm)	No. of branches (No.)	Internodal distance (cm)	Leaf area (cm^2)
Control	100.00	92.10	89.00	152.43	5.51	4.04	178.37
0.1	78.00	82.70	81.37	144.29	4.29	3.80	165.27
0.2	69.00	84.19	76.29	129.14	4.64	3.64	167.31
0.3	58.00	69.21	78.40	131.88	4.87	3.75	158.43
0.4	36.00	73.88	67.10	119.10	5.72	3.82	196.11
0.5	27.00	62.42	56.21	106.76	5.28	3.72	174.29
SEM	0.19	0.32	0.21	0.27	0.55	0.09	1.31
CD@5%	NS	NS	NS	NS	1.69	1.92	2.04

Table 2: Leaf yield, stomatal frequency, stomatal chloroplasts and pollen fertility in polyploid variant of mulberry variety Kajali recovered at 0.4% colchicine treatment in C_1 generation.

Treatment	Stomata/unit area (No.)	Number of chloroplasts (No.)	Yield/plant (kg)	100 leaves weight(gm.)	Pollen fertility (%)
Control	53.00	8-9	0.478	363.24	88.81
Polyploid (variant)	44.00	23-27	0.601	422.14	57.64
SEM	6.68	8.22	0.101	39.74	29.83
CD@5%	8.04	10.04	0.127	51.87	35.19

Discussion

Rooting and root proliferation is an important genetic trait and results proved phenomenal decrease in rooting percentage. With the increase in colchicine concentration there was a corresponding decrease in rooting. Sprouting results are in conformity with the earlier findings of Sikdar, (1990) and Eswar Rao, (1996) in tropical mulberry varieties namely RFS_{135}, M_5, S_{30}, S_{36} and S_{41} genotypes. Das *et al*., (1987) observed that, treating mulberry apical buds with 0.4% and 0.6% aqueous solution is most effective in the induction of tetraploids. It was also opined that, higher concentrations of colchicine (0.4%-0.5%) not only delayed the emergence of buds but also severely affect survivability. It may also due to physico-chemical disturbances of cells and reduced rate of cell division or may be due to polyploidization in some of the treated vegetative buds. Poor rooting was observed in colchicine induced autotetraploids of S_{30} and S_{36} mulberry varieties compared to their diploid varieties (Dwivedi *et al*., 1989a). Similar results were noticed in mulberry varieties M_5 and S_{54} by Ramesh *et al*., (2011). Pollen fertility was considerably receded in the treated variants. In growth parameters, present findings are in agreement with the reports of earlier workers (Verma *et al*., 1986; Chakraborthi *et al*., 1998). Several investigators have succeeded in inducing polyploidy and utilizing these polyploids either directly for commercial purpose or for further breeding work in various crop plants (Sybenger, 1992). Different workers are of the opinion that abnormal cytological behaviour may

be the reason for reduced growth in treated plants. Stunted growth with deformities following the treatment is due to serious hormonal imbalance resulting in physiological disorder and sometimes it is also due to reduced rate of cell division (Bharathi Behera and Patnaik, 1975). 0.3% and 0.4% of colchicine applied for 6hrs and 8hrs for three consecutive days were more effective in the induction of tetraploids in mulberry variety $RFS_{135.}$ Branching pattern of treated progenies varied and the number of branches ranged between 4.29 and 5.72. Maximum branching was observed at 0.4% (5.72) and minimum at 0.1% (4.29) when compared to control (5.51) (Dwivedi *et al*., 1986). Ramesh and Munirajappa, (2001) observed that, Kosen mulberry variety treated with colchicine at 0.5% enhanced number of chloroplasts, leaf yield and leaf weight compared to control. Mensah *et al*., (2007) reported that, colchicine treatment enhanced number of branches in sesame. Tetraploid plants leaves were thick, large and dark green than diploid plants. Increase in leaf thickness is due to increased palisade, spongy tissues and cell size of the polyploid tissues. Occurrence of dark green colour leaves has been attributed to increase in chloroplasts number in tetraploids of Coriander and Foeniculum (Singh *et al*., 1987). Sikdar *et al*., (1986) observed stomatal chloroplasts/guard cell ranged from 8-11 in diploids and 16-21 in tetraploid forms of mulberry. Dwivedi *et al*., (1988b) have opined that, some irregularities observed in leaf size, shape, texture and colouration are attributed to differential rate of cell division intermixed with physiological disturbances in treated buds. Stomatal frequency, stomatal chloroplast counts and pollen fertility were adopted to ascertain polyploidization in colchicine treated populations (Susheelamma *et al*., 1991). Beck *et al*., (2003) and Cohen and Yao (1996) have observed and reported that, size and number of stomata may change significantly due to chromosome doubling compared to the diploids of nine *Zantedeschia* cultivars and *Acacia mearnsii* respectively.

Conclusion

Mulberry is polygenic in nature and evolving a variety is a tough task. Colchicine considerably reduces the sprouting and survivability percentages in mulberry variety Kajali. Deformities are observed due to treatment like reduced plant height, stout stem and dark, thick greenish leaves with shortened petioles. Increased leaf area and yield, reduced stomatal frequency and higher number of chloroplasts at 0.4% were noticed. Efforts have been made to induce polyploids in Kajali using colchicine, a potent spindle inhibitor.

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