The Bioscan 6(1) : 115-118, 2011
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

COLCHICINE INDUCED MORPHOLOGICAL VARIATION IN MULBERRY VARIETY M_5

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KEY WORDS
Colchicine
Mulberry
Stomata

Received on :
21.11.2010
Accepted on :
17.02.2011

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ABSTRACT

Mulberry variety M_5 is used for the induction of variation through colchicine treatment. Five different concentrations of aqueous colchicine [$C_{22}H_{25}NO_5$] viz., 0.1% - 0.5% were used to treat the vegetative buds. Sprouting%, rooting% and plant height were decreased with a linear increase in the percentage of colchicine used. Increase in leaf area was observed at 0.4% (166.94 cm^2) and 0.5% (165.20 cm^2) when compared to control (155.41 cm^2) plants. Thick, succulent and dark leaves were found associated with the increased leaf area. In the M_2 generation, the variant exhibited decreased number of stomata/unit area (39.57) and increased number of chloroplast (18 – 22) were observed when compared to control (49.42 and 11 – 14). Yield of the treated plants (0.573kg/plant) were considerably increased. The plants are grown in full maturity in order to test their potential for use in a breeding programme oriented at producing lines with quantitative quality of leaves.

INTRODUCTION

Mulberry is the exclusive food plant of silkworm *Bombyx mori* L. and mulberry variety M_5 is the most popular cultivar in Karnataka. Colchicine is a both polyploidising and mutagenic agent (Bragal, 1955) and this chemical has been used since from long time to produce polyploidy plants. Colchicine – a potent chemical can induce morphological variations in mulberry. Mulberry is a diploid plant with 28 chromosomes (2n = 28). Doubling of the diploid chromosome number may be achieved due to spindle inhibition. These mutagenic chemical disrupt mitosis by preventing microtubule polymerisation and hence polar migration of chromosomes at anaphase. Colchicine is one of the commonly used spindle inhibitors (Hancock, 1997) including some trees and other woody species (Dwivedi *et al*., 1986; Rose *et al*., 2000; Blakesley *et al*., 2002). The mutagenic effects on plant morphology, chlorophyll, sterility and yield have been confirmed earlier by Castro *et al*., (2003). The main objective of the present work is to induce tetraploidy in mulberry that have the potential to produce progenies with higher yield and nutritionally superior leaves.

MATERIALS AND METHODS

In the present investigation, mulberry variety M_5 was procured from mulberry germ plasm bank maintained at Jnana Bharathi, Bangalore University, Bangalore.

Morphological features of these taxa was critically examined and root, stem, leaf, inflorescence, floral pants, pollen grain, fruit and seeds were described following proper taxonomical yardsticks (Hotta, 1954; Das, 1984; Jolly and Dandin, 1986).

The mulberry genotype M_5 was used for the induction of variation through colchicine treatment. 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. Five buds in each plant were earmarked by tagging for the treatment. The selected buds were thoroughly washed in distilled water before the application of colchicine. The buds were covered with cotton swabs and colchicine solution was applied from 8am – 5pm for three consecutive days at an interval of 1 hr (Dwivedi *et al*., 1986). At the same time control buds were treated with distilled water. After the complete treatment, the cotton swabs were removed and the buds were thoroughly washed in distilled water. The buds were allowed to grow in the treated plants by providing needed agricultural inputs. The untreated axillary buds situated on the treated potion of the shoots were removed periodically so as to maintain only the treated axillary buds.

During the course of investigation, various propagation parameters viz., sprouting percentage, rooting percentage, survivability, height of the plant, branching pattern, internodal distance, leaf area, length of petiole, fresh weight of the leaves were recorded (Dandin and Jolly, 1986; Das *et al*., 1987; Shamachary and Jolly, 1988). Appropriate rearing techniques were adopted (Krishnaswami, 1971) and economic traits were also tabulated using adopting method of "Analysis of Variance" appropriate to the design of the experiment (Singh and Choudhary, 1979).

RESULTS AND DISCUSSION

Since the discovery of colchicines as a polyphoidising agent, artificial production of polyploids became an additional tool in the plant improvement programme. 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 (Verne Grant, 1984; Sybenger, 1992).

Survival and sprouting of axillary buds: Colchicine treated mulberry variety M_5 showed decreased survival percentage, delayed sprouting and exhibited slow growth. The survival percentage of treated axillary buds of the variety M_5 declined with the increase in the concentration of colchicines used. Similar observations were reported by Tojyo (1966) in Japanese mulberry genotypes, Dwivedi *et al*., (1986); Sikdar (1990) and Rao (1996) in tropical mulberry varieties namely, RFS_{135}, M_5, S_{30}, S_{36} and S_{41} genotypes. It is evident that the higher concentrations of colchicines (0.4% - 0.5%) not only drastically affect the sprouting and survivability but also result in the delayed emergence of buds. It may also be due to physico-chemical disturbances of cells and reduced rate of cell division and polyploidization (Table 1).

Rooting behaviour: Root initiation and development in the colchicines treated population of M_5 showed a general reduced trend. Lower concentration of colchicine (0.1%, 0.2% and 0.3%) did not affect the rooting behaviour. However, the number of roots developed was considerably decreased in the progeny treated with higher concentration of colchicine (0.4% and 0.5%). Slightly improved rooting was recorded in M_5 at 0.1%. Dwivedi *et al*., (1989) observed poor rooting behaviour in colchicine induced autotetraphoids of S_{30} and S_{36} varieties compared to their diploid genotypes.

Growth parameters: M_5 mulberry variety showed decreased height when treated with different concentration of colchicine except at 0.1% at which the height was slightly increased when compared to control. The height of the plants was gradually decreased with an increase in the concentration of colchicine used.

These findings are in agreement with the reports of earlier workers (Dwivedi *et al*., 1986; Verma *et al*., 1986). The investigators are of the opinion that the reason for reduced growth in colchicines treated material is due to abnormal cytological behaviour. The stunted growth with deformities following colchicine treatment is probably due to serious hormonal imbalances resulting in physiological disorder (Behera and Patnaik, 1975). It is also due to reduced rate of cell division (Swanson, 1965).

Colchicine treated plants showed marginal reduction in the number of branches over their controls. Internodal distance is another genotypic attribute which determines the total foliage produced. Short internodes were noticed in some population of M_5 treated with 0.4% and 0.5% of colchicines respectively (Fig. 1).

The main aim of mulberry breeding is to increase the leaf yield and the leaf area was considerably increased in treated populations. In M_5, leaf area was increased in 0.4% (166.94 cm^2) and 0.5% (165.20 cm^2) colchicine treated plants compared to control (155.41cm^2). In addition, beneficial traits such as thick, succulent and dark green leaves with prominent veins were also found associated with the increased leaf area in the variants of M_5 (Fig. 1). These results prove that colchicine is very effective at 0.4% and 0.5% in evolving elite mulberry varieties. Das *et al*., (1987) reported 0.4% and 0.6% aqueous colchicines solution is most effective in the induction of tetraploids by treating the growing apical buds of the mulberry seedlings. Dwivedi *et al*., (1986) found that 0.3% and 0.4% colchicines applied for 6 hours and 8 hours for three consecutive days were effective in the induction of tetraploids in *Morus alba* variety RFS_{135}.

In tetraploids of Coriander and Foeniculum, the dark green colour of the leaves has been attributed to the increase in the number of chloroplasts by Raghuvanshi and Joshi (1964). Johnson and Sass (1944) noticed an increased leaf thickness in polyploid species of Mellilotus, which they attributed to the increased Palisade and spongy tissues. They opined that the increased leaf area is due to the increase in the cell size of the polyploid tissues.

Table 1: Effect of Colchicine on propagation and growth traits of M_5 mulberry variety at C_1 generation

Treatment	Sprouting%	Rooting%	Survival%	Height of the Plant (cm)	Number of branches	Internodal distance (cm)	Leaf area (cm^2)
Control	90.96	88.56	100.00	145.54	5.21	4.69	155.41
0.1 %	83.53	91.63	89.00	147.82	5.17	4.58	143.28
0.2 %	77.80	81.28	81.00	134.28	5.23	4.61	147.49
0.3 %	77.56	74.41	62.00	121.47	4.78	4.38	139.23
0.4 %	62.86	56.44	43.00	136.29	4.67	4.09	166.94
0.5 %	59.80	51.29	32.00	123.33	4.71	4.14	165.20
SEM	—	1.68	—	0.78	0.11	—	2.69
CD at 5%	NS	2.01	NS	1.73	0.28	NS	4.07

Table 2: Leaf yield, Stomatal frequency and Stomatal chloroplasts in polyploid variant of M_5 mulberry variety (Recovered at 0.4% Colchicine treatment)

Treatment	Stomata/unit area (no)	Number of Chloroplasts	Leaf yield/ plant (kg)	Weight of 100 leaves (g)
Control	49.42	11 – 14	0.429	322.00
Polyploid variant	39.57	18 – 22	0.573	377.00
SEM	7.62	5.89	0.098	31.44
CD at 5%	9.91	7.35	0.152	56.98

Figure 1: Plant treated at 0.4% colchicine showing thick, dark green and succulent leaves

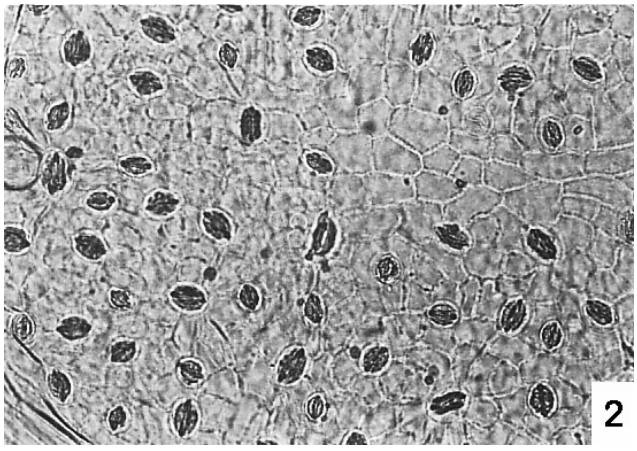

Figure 2: Lower epidermal peel showing stomata/unit area (control)

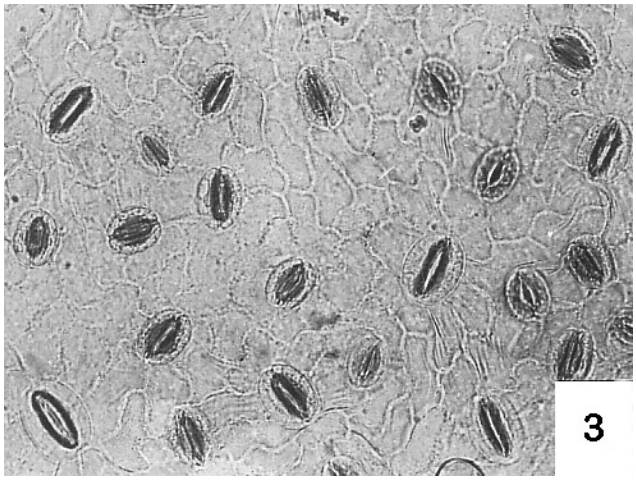

Figure 3: Lower epidermal peel showing reduced number of stomata/ unit area at 0.4% colchicines treatment

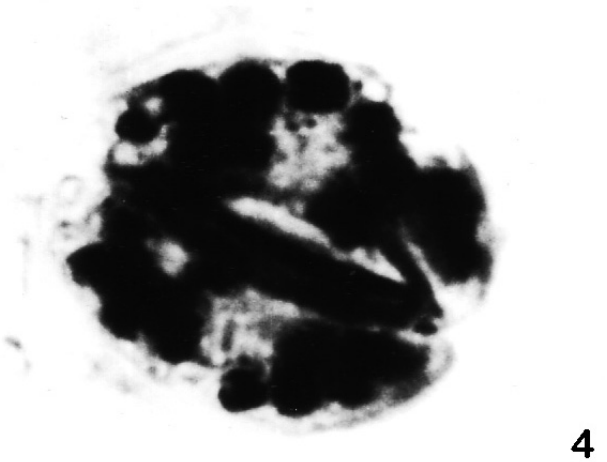

Figure 4: Increased chloroplast number observed in stomata of 0.4% colchicine treated leaves

Some of the irregularities observed in leaf size, shape, texture and colouration are attributed to the differential rate of cell division coupled with physiological disturbances in the treated buds. Such irregularities are commonly noticed in colchicines treated populations (Dwiwedi *et al*., 1988; Sikdar *et al*., 1986).

The beneficial variant procured from 0.4% colchicine treatment, the stomatal frequency and stomatal chloroplast counts revealed to be polyploidy nature (Table 2). In the variant, decreased stomata per unit area and increased number of chloroplast per stoma were encountered. Reduced pollen fertility was also observed (Fig. 2, 3 and 4).

Similar methods considering stomatal frequency, stomatal chloroplast counts and pollen fertility were adopted by other workers to ascertain the polyploidization in colchicine treated populations (Sikdar *et al*., 1986; Susheelamma *et al*., 1991). Sikdar *et al*., (1986) also studied the stomatal chloroplasts per guard cell ranged from 8 - 11 in diploids and 16 - 21 in tetraploid forms.

The beneficial variants of M_5 increased number of stomatal chloroplast secured in the present investigation seem to be at tetraploid level. Attempted cytological studies in these variants were not fruitful owing to small size and stickiness of the chromosome. However, the cytological evidences to prove their polyploidy nature are desired.

Total foliage yield per plant and average weight of leaves were found to be increased in the beneficial induced polyploidy variant of M_5 over control counterparts in C_2 generation. Increased leaf yield of 24.28% was recorded in M_5 variant. The leaf weight recorded in the variant is significant with an increase of 15% – 17% in comparison to the control.

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