IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
Volume 7 | Issue 2 | July, 2012 | 208-215

RESEARCH ARTICLE

A study of foliar epidermal pattern in colchiploid of *Zanthoxylum armatum*

■ RAMDAS, G.K. DHINGRA, PRERNA POKHRIYAL AND MUKESH KUMAR

SUMMARY

Zanthoxylum armatum was explored for the detailed colchicine induced variable characteristics. For the colchiploid plant study, leaves of colchicine treated seedlings were fixed in FAA. The analysis was carried out for control and treated plants using parameters like number of epidermal cells per mm², number of stomata per mm², guard cells index (GCI), size (length × breadth) of stomata (µm²), area of stomatal pore (µm²), pore area index (PAI), total pore area per mm² (TPA) etc. The mean number of epidermal cells in control plants ranged from 50.55 to 61.05; while in treated seedlings mean numbers of epidermal cells at different sites ranged from 35.10 to 44.50 in 1.0 M, 39.55 to 55.33 in 0.50 M and 39.10 to 58.90 in 0.05 M, respectively. During control (all sites), mean length and breadth of stomata ranged from 12.25 to 14.30 µm and 7.20 to 9.45 µm, respectively. In treated plants of all sites (Z-1 to Z-6), mean length and breadth of stomata ranged from 8.85 to 11.00 µm, 5.75 to 7.95 µm in 1.0 M; 13.70 to 18.40 µm, 9.35 to 12.05 µm in 0.50 M and 13.65 to 17.45 µm, 9.40 to 11.40 µm in 0.05 M, respectively. Reduced size of pore area was noticed in higher concentration (1.0 M) of colchicine. Guard cell index in control ranged from 27.90 to 38.59. For 1.0 M treated, range was observed from 23.89 to 33.33. For 0.50 M colchicine concentration, ranged from 37.59 to 45.22, while in 0.05 M treated plants, guard cell index ranged from 39.45 to 45.48, respectively.

Key Words : Colchicine, Colchiploids, Guard cells, Pore area, Total pore area

How to cite this article : Ramdas, Dhingra, G.K., Pokhriyal, Prerna and Kumar, Mukesh (2012). A study of foliar epidermal pattern in colchiploid of *Zanthoxylum armatum*. *Internat. J. Plant Sci.*, **7** (2) : 208-215.

Article chronicle : Received : 29.12.2011; **Revised :** 03.03.2012; **Accepted :** 06.04.2012

The genus *Zanthoxylum* is distributed worldwide from tropical to temperate zones. There are over 200 species from small shrubs to large trees. It has some other synonyms as *Z. planispinum, Z. alatum subtrifoliolatum* (French), etc. It is known as winged prickly ash, tejbal, tejphal, timroo or Nepali Dhaniya. It is widely distributed throughout the warmer region of the world, extending into temperate region of Europe, Asia and Australia. About 50 species among 20 genera are reported from India. Out of which 9 species are classed as commercial timbers (Pearson and Brown, 1932). The range of the plant is from Eastern Asia- China to the Himalayas. *Zanthoxylum* is recognized as having medicinal qualities for curing stomachache, toothache, intestinal worms, rheumatism, scabies, snakebites, fever, cholera and used as a flavoring agent or spice for preparation of certain traditional dishes. During winter, a soup made from the dried fruit (locally known as hag) is consumed by the entire family to keep warm in winter. A chutney (like a sauce), locally known as dunkcha, is also a popular food item (Kala *et al.*, 2004). The seed is ground into a powder and used as a condiment (Facciola, 1990). The fruit is rather small but is produced in clusters which make harvesting easy. Each fruit contains a single seed and young leaves are used as condiments (Gupta, 1945; Tanaka, 1976). The fruit contains 1.5 per cent essential oil (Chopra *et al.,* 1976).

The oil obtained from plant is known as Zanthoxylum oil or Nepali pepper oil. The essential oil is obtained by stem

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
RAMDAS, Department of Botany, Ram Chandra Uniyal P.G. College, UTTARKASHI (UTTARAKHAND) INDIA
Email: ram84.uki@gmail.com

Address of the Co-authors:
G. K. DHINGRA AND PRERNA POKHRIYAL, Department of Botany, Ram Chandra Uniyal P.G. College, UTTARKASHI (UTTARAKHAND) INDIA

MUKESH KUMAR, Survey of Medicinal Plant Unit, Regional Research Institute of Unani Medicine, BHADRAK (ODISHA) INDIA

distillation of the dried fruits. The oil being rich in linalool and also containing limonene, methyl cinnamate and cineole. It is used as anti infectious, sedative and for curing diseases like arthritis, cholera and toothache. It is also used as a spice and as pepper substitute (Gupta, 1945; Tanaka, 1976). All the plant parts like seeds, bark, fruits, branches, thorns are used in different ailments (Gupta, 1945; Uplof, 1959; Gamble, 1972; Usher, 1974; Chopra *et al.*, 1986).

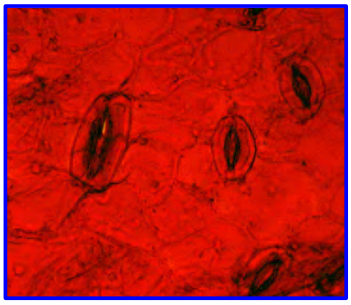
MATERIALS AND METHODS

For the foliar epidermal study, the leaves of *Z. armatum* were collected from the research sites (Z-1 to Z – 6) in the day time and fixed in formic acetic acid. Leaves of colchicine treated seedlings were also fixed in FAA. The middle portion of the fixed leaves of about 1cm² were washed thoroughly with water, then placed in 1:1 hydrogen peroxide and glacial acetic acid solution and kept in an oven at 60°C. When bristles came out, the adaxial and abaxial epidermises were separated out carefully. The epidermis was stained in 1 per cent safranin and washed with water and then mounted in 4 per cent glycerine. The number of stomata and epidermal cells were calculated in X 45 applying the generally followed formula of Metcalfe and Chalk (1979). Size of stomata and pore area were measured in X 100. For pore area, camera lucida drawing was used. Stomatal size, epidermal cells, guard cells, stomatal anomalies were studied with the help of binocular microscope and there photomicrographs were captured using Canon Cyber Shot Camera. The length and breadth of 20 stomata were measured at random in each concentration and their length/breadth ratio was also calculated separately to find out the relative effect of colchicine. For the present study, the stomata were defined as the structures comprising slits guarded by two guard cells each. Following epidermal parameters were explored: Number of epidermal cells per mm² number of stomata per mm² gurd cells index (GCI), size (length × breadth) of stomata (µm²), area of stomatal pore (µm²), pore area index (PAI), total pore area per mm² (TPA).

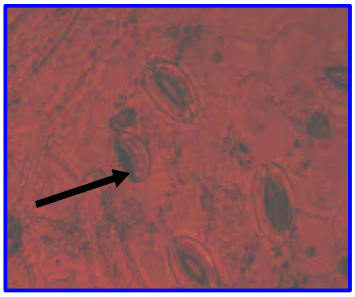
The total pore area (TPA) is defined as the per cent of the total area of the pores per mm². The radiuses between the frequencies of epidermal cells and stomata length and breadth of stomata, pore area, guard cells index (GCI), pore area index (PAI), and total pore area (TPA) of abaxial epidermis over that of adaxial epidermis for different sites were also calculated.

RESULTS AND DISCUSSION

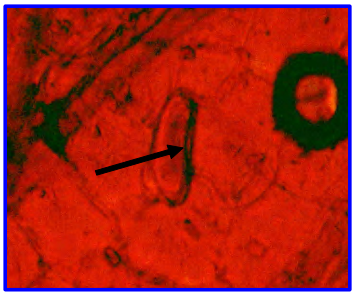
Control and colchicines treated plants of various sites were identified with foliar epidermal pattern variations. For this study, a series of parameters listed in materials and methods were analyzed in both control and colchicine treated abaxial as well as adaxial epidermis. Control and treated plants (seedlings) of different sites differed significantly in the above discussed parameters (Plate 1).



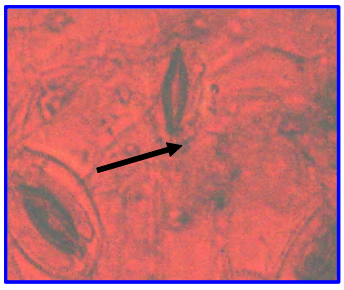
(a) Stomata showing size variation in 0.50 M treatment (Z-1)



(c) Degeneration of one guard cell in 1.0 M colchicine treated leaf (Z-5)



(b) Degeneration of whole stomata in 1.0 M treatment of colchicine (Z-3)



(d) Degeneration of both guard cells in 1.0 M colchicine treated leaf (Z-6)

Plate 1 : Variation in stomatal size and degeneration (a and b), degeneration of guard cell in colchicine treated leaf (c and d)

In lower concentrations *i.e.* 0.50 M and 0.05 M, larger and thicker leaves, darker green colouration, larger stomata, lower density of stomata across the lower leaf epidermis and increased number of chloroplast per stomata and guard cells were reported as compared to control. On analyzing the leaves of control and treated (1.0 M 0.50 M and 0.05) seedlings, these were found to be hypostomatic, so the stomatal study was done only in abaxial side of the leaf. In control plants of all the sites (Z-1 to Z-6), the number of epidermal cells and the number of stomata were found higher in number as compared to treated plants. Sizes of stomata on leaves varied largely even among those of the same leaf, but statistically significant mean size difference were reported between control and treated plants. There were no noticeable morphological differences in shape of the stomata among the control and treated plants however, on lower portion (abaxial) of treated leaves; larger size stomata were reported.

It was also observed that 1.0 M concentration of colchicine showed significant reduction in number of epidermal cells and number of stomata on abaxial side of epidermis per mm^2 1.0 M treated plants of all the sites (Z-1 to Z-6) showed less number of stomata. As compared to control, the colchicine treated seedlings showed various foliar epidermal pattern anomalies in *Z. armatum viz.,* thicker leaves (0.50 M), modified leaves morphology (1.0 M), size variation in epidermal cells and stomata (1.0 M and 0.50 M), degeneration of one and both guard cells and close type of stomata (1.0 M) etc.

Reduced numbers of epidermal cells with increased size were noticed in low concentrations of colchicine, while in higher concentration (1.0 M), more frequency of epidermal cell reduction was noticed in all the sites as compared to control. The mean number of epidermal cells in control plants ranged from 50.55 to 61.05; while in treated seedlings mean numbers of epidermal cells at different sites ranged from 35.10 to 44.50 in 1.0 M, 39.55 to 55.33 in 0.50 M and 39.10 to 58.90 in 0.05 M, respectively (Table 1 and Fig.1). During control,

Table 1 : Mean number of epidermal cell present on the adaxial surface of control and treated seedlings of *Z. armatum*

Sr. No.	parameters	mean	S.D.	+SE	Range
Z-1 Con.	NE	59.75	15.65	+ 3.50	38 - 83
1.0 M	NE	44.50	12.69	+ 2.83	33 - 88
0.50 M	NE	49.65	10.42	+ 2.33	28 - 66
0.05 M	NE	47.50	11.66	+ 2.61	30 - 70
Z-2 Con.	NE	61.05	17.42	+ 3.86	35 - 90
1.0 M	NE	42.67	7.30	+1.63	33 – 60
0.50 M	NE	55.33	18.41	+ 3.59	40- 110
0.05 M	NE	58.90	14.39	+3.22	35 - 80
Z-3 Con.	NE	57.80	13.13	+ 2.88	40 - 75
1.0 M	NE	42.15	9.26	+ 2.13	29 – 65
0.50 M	NE	51.00	8.96	+ 2.24	45 - 110
0.05 M	NE	44.45	12.73	+ 2.85	32 -85
Z-4 Con.	NE	52.90	11.41	+ 0.32	40-78
1.0 M	NE	43.50	11.23	+ 2.79	35-78
0.50 M	NE	50.55	15.31	+ 3.42	23 - 78
0.05 M	NE	51.00	13.27	+ 3.84	34- 110
Z-5 Con.	NE	50.55	15.31	+ 3.42	23 -78
1.0 M	NE	35.35	6.58	+ 0.88	10 - 23
0.50 M	NE	39.55	4.86	+3.84	34 -110
0.05 M	NE	43.75	15.00	+ 2.79	35 - 78
Z-6 Con.	NE	52.10	15.14	+ 3.38	23- 70
1.0 M	NE	35.10	7.12	+ 0.88	10 - 23
0.50 M	NE	42.95	6.39	+ 3.86	34 – 110
0.05 M	NE	39.10	6.12	+2.79	35-78

NE = Number of epidermal cells

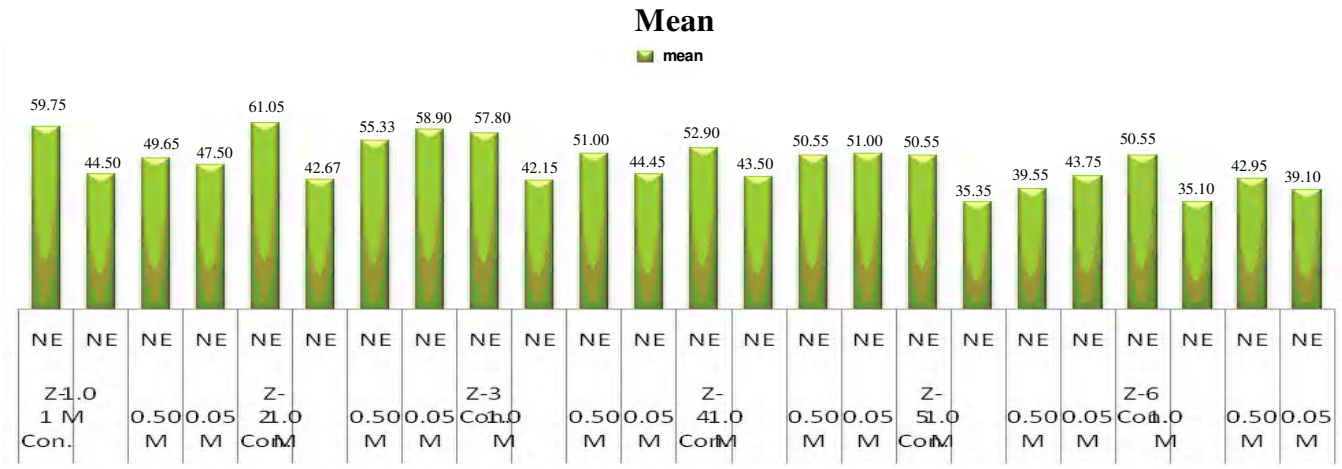


Fig. 1 : Number of epidermal cells (Adaxial side of leaf) in control and treated seedlings of *Z. armatum*

maximum frequency of number of epidermal cells was found in Z-2 and minimum in Z-5 while in treated plants, maximum frequency of number of epidermal cells was found in Z- 2 (0.05 M) and minimum in Z- 6 (1.0 M).

In most of the treated seedlings, less number of epidermal cells and stomata with increased sized was reported as compared to control. In control plants of different sites (Z-1 to Z-6), number of epidermal cells on abaxial side ranged from 50.55 to 59.90 while in 1.0 M, 0.50 M and 0.05 M treated plants it ranged from 31.45 to 53.80, 44.70 to 56.50 and 42.00 to 55.85, respectively. Maximum frequency of epidermal cells was found in Z-2 and minimum in Z-5 (control) (Table 2). A significant variability between the control and treated seeds of different sites (Z-1 to Z-6) was reported for the mean number of stomata on abaxial side of seedling leaves. In control plants of all sites, mean number of stomata ranged from 17.65 to 23.60, while in treated seedlings it ranged from 12.60 to 17.35 in 1.0 M, 15.70 to 18.15 in 0.50 M and 14.85 to 20.35 in 0.05 M concentrations of colchicine. Characteristics variability was observed in the mean number of length and breadth of stomata of control and treated plants. In 1.0 M treated plants of all the sites (Z-1 to Z-6), size of stomata as well as area of stomatal pore was reported reduction as compared to control while size of stomata and pore area of stomata was increased in 0.50 M and 0.05 M concentrations as compared to control. During control (all sites), mean length and breadth of stomata ranged from 12.25 to 14.30 µm and 7.20 to 9.45 µm, respectively. In treated plants of all sites (Z-1 to Z-6), mean length and breadth of stomata was ranged from 8.85 to 11.00 µm, 5.75 to 7.95 µm in 1.0 M; 13.70 to 18.40 µm, 9.35 to 12.05 µm in 0.50 M and 13.65 to 17.45 µm, 9.40 to 11.40 µm in 0.05 M, respectively. Maximum frequency of length and breadth of stomata was found in Z-6 and minimum in Z-1 from all sites in control condition. In *Z. armatum* plants of all the sites (Z-1 to Z-6), control plants mostly showed imperipinnate leaf pattern with very rare peripinnate leaf pattern (Fig. 2). 1.0 M treated plants showed slight increase in peripinnate leaf pattern while low concentration of colchicine *i.e.* (0.50 M and 0.05 M) showed maximum increase in peripinnate leaf pattern.

In most of the treated (0.50 M and 0.05 M) seedlings, more pore area was reported as compared to control. Reduced size of pore area was noticed in higher concentration (1.0 M) of colchicine. In control plants, mean pore area ranged from 225.70 to 311.90 µm, while in 1.0 M, 0.50 M and 0.05 M treatment, mean pore area was ranged from 208.80 to 252.70 µm, 314.30 to 345.20 µm and 298.60 to 346.70 µm, respectively (Table 2). For the study of guard cell index on abaxial side of epidermis of all control and treated plants, it was observed that 1.0 M colchicine treated plants were reported a reduction in size of guard cells and less number of guard cell Index as compared to control while 0.50 M and 0.05 M treated plants had large sized guard cells and more number of guard cell Index. Guard cell index in control ranged from 27.90 to 38.59 with maximum and minimum frequency at Z-6 and Z-4, respectively. For 1.0 M treated, range was observed from 23.89 to 33.33 with maximum and minimum frequency at Z- 6 and Z-4, respectively. For 0.50 M colchicine concentration, ranged from 37.59 to 45.22 having maximum and minimum frequency

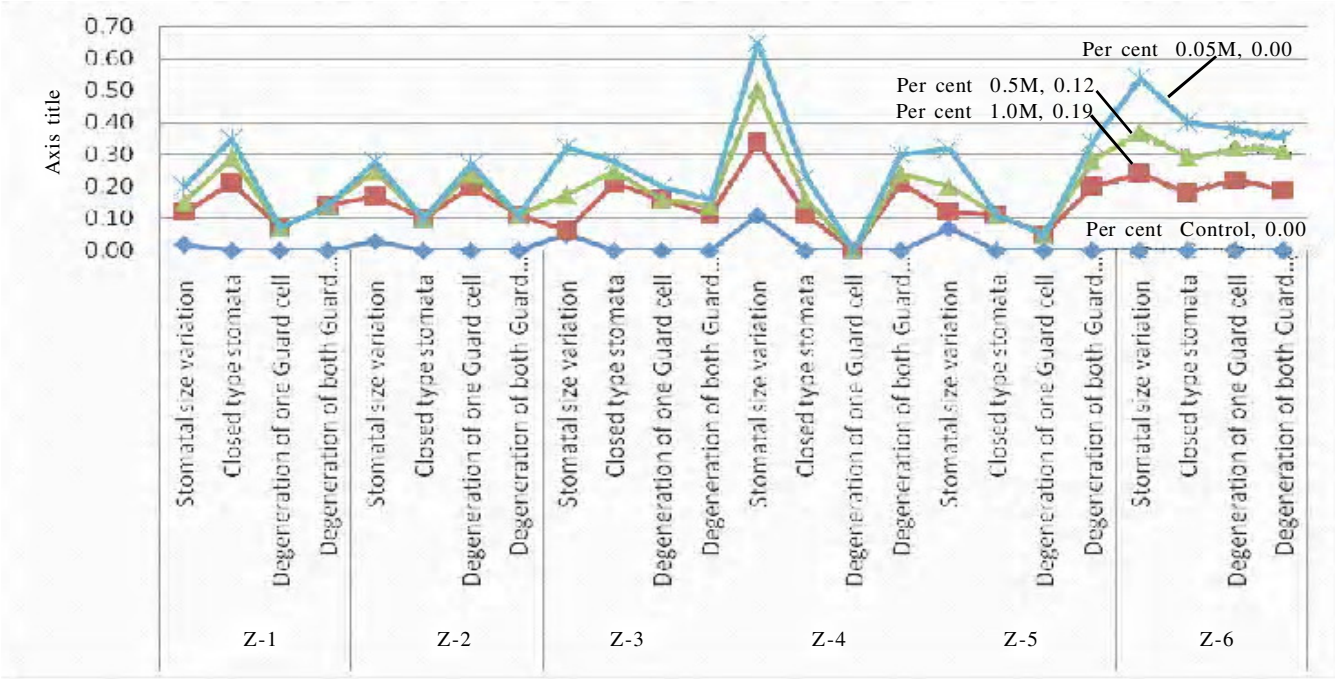


Fig. 2 : Frequency distribution (%) of stomatal abnormalities in control and treated seedlings of *Z. armatum*

Table 2 : Mean number of stomata and epidermal cells per mm², length and breadth of stomata and pore area on the abaxial surface of *Z. armatum* seedlings (control and treated)

Sr. No.	parameters	Mean	S.D.	±SE	Range
Z-1 Con.	NS	23.60	6.74	± 1.51	15- 39
	NE	58.10	20.62	± 4.61	28 - 119
	LS	12.25	1.07	± 0.24	10 - 12
	BS	7.20	0.89	± 0.20	06 - 08
	PA	250.80	53.10	± 16.79	150- 315
1.0 M	NS	17.35	4.49	± 1.00	10 -25
	NE	52.40	13.34	± 2.98	34 - 90
	LS	10.45	0.89	± 0.20	09 - 12
	BS	6.25	0.85	± 0.19	05 - 08
	PA	242.00	61.82	± 19.55	129- 343
0.50 M	NS	18.15	2.16	± 0.48	18 - 34
	NE	56.50	17.90	± 4.00	45 - 111
	LS	13.70	1.98	± 0.44	07 - 11
	BS	9.35	1.23	± 0.27	07 - 11
	PA	345.20	62.34	± 19.72	267 - 448
0.05 M	NS	20.00	3.40	± 0.76	14 - 27
	NE	55.80	12.00	± 2.68	39 - 74
	LS	13.65	1.46	± 0.33	11 - 17
	BS	9.90	1.21	± 0.27	07 - 12
	PA	332.70	35.16	± 11.12	288 - 392
Z-2 Con.	NS	22..45	5.66	± 1.27	11 - 35
	NE	59.90	19.92	± 4.45	35 - 119
	LS	13.45	1.64	± 0.37	10 - 16
	BS	7.80	1.44	± 0.32	05 – 10
	PA	260.10	52.82	± 16.70	179 - 328
1.0 M	NS	16.25	4.85	± 1.09	10 - 25
	NE	48.80	11.95	± 0.43	34 - 90
	LS	8.85	1.18	± 0.26	09 - 13
	BS	5.90	0.97	± 0.22	04 - 08
	PA	252.70	34.32	± 10.85	205 - 311
0.50 M	NS	17.35	2.50	± 0.85	18 - 34
	NE	48.20	8.25	± 4.23	47 - 111
	LS	15.70	2.11	± 0.47	15 - 20
	BS	9.90	1.12	± 0.05	09 - 12
	PA	314.30	47.18	± 3.70	258 - 440
0.05 M	NS	20.35	3.99	± 0.89	11 - 27
	NE	54.70	12.34	± 2.76	35 - 79
	LS	15.75	1.80	± 0.40	16 - 22
	BS	11.40	1.47	± 0.07	09 - 15
	PA	304.30	72.15	± 22.82	160 - 382
Z-3 Con.	NS	18.40	4.27	± 9.55	12 - 26
	NE	57 .95	12.80	± 3.66	35 - 110
	LS	13.45	1.28	± 0.29	11 - 16
	BS	7.50	1.00	± 0.22	05 - 09
	PA	311.90	49.15	± 15.54	198 - 347
1.0 M	NS	14.45	3.55	± 7.93	11 - 23
	NE	53.80	13.04	± 2.92	34 - 78
	LS	10.10	1.07	± 0.24	09 -12
	BS	5.75	0.91	± 0.20	05 - 07
	PA	255.30	35.04	± 11.08	200 - 285
0.50 M	NS	17.30	3.91	± 7.89	18 - 32
	NE	55.75	16.08	± 3.60	36 - 111
	LS	16.15	2.52	± 0.56	16 - 20
	BS	11.95	0.76	± 0.17	10 - 13
	PA	335.90	51.35	± 16.24	254 - 440
0.05 M	NS	15.80	2.28	± 8.48	13 - 27
	NE	55.85	15.11	± 3.38	33 - 68
	LS	17.45	1.93	± 0.43	16 - 20
	BS	10.50	1.15	± 0.26	09 - 12
	PA	346.70	49.44	± 15.64	250 - 416
Z-4 Con.	NS	17.65	3.15	± 0.70	10 - 23
	NE	52.10	15.14	± 4.38	23 - 68
	LS	13.80	1.85	± 0.09	09 - 17
	BS	7.70	1.45	± 0.33	05 -09
	PA	278.00	31.91	± 10.09	241 - 332
1.0 M	NS	12.60	3.62	± 0.81	10 - 23
	NE	41.60	10.22	± 2.29	29 - 67
	LS	9.55	1.10	± 0.25	09 - 12
	BS	7.95	1.43	± 0.32	05 - 09
	PA	231.90	35.13	± 11.11	194 - 298
0.50 M	NS	16.80	5.43	± 1.22	25 - 30
	NE	45.75	9.35	± 2.09	30 - 78
	LS	17.95	1.39	± 0.31	16 - 20
	BS	10.60	0.99	± 0.22	09 - 12
	PA	339.70	64.16	± 20.29	280 - 461
0.05 M	NS	14.85	2.98	± 0.67	10 - 20
	NE	42.00	6.91	± 1.54	30 - 50
	LS	15.90	1.17	± 0.26	15 - 19
	BS	10.25	0.91	± 0.20	09 - 12
	PA	298.60	80.78	± 25.55	159 - 407
Z-5 Con.	NS	19.45	3.72	± 0.88	11 - 23
	NE	50.55	15.31	± 3.42	23 - 78
	LS	13.80	1.79	± 0.40	09 - 17
	BS	7.55	1.19	± 0.27	05 - 09
	PA	225.70	79.33	± 25.09	177 -330

Table 2 : Contd….

Table 2 : Contd….

1.0 M	NS	12.85	2.58	± 0.58	10 - 19
	NE	41.30	9.95	± 2.23	30 - 65
	LS	11.00	2.71	± 0.61	07 - 17
	BS	7.90	1.29	± 0.29	05- 09
	PA	215.90	33.19	± 10.48	160 - 267
0.50 M	NS	15.70	2.85	± 0.01	12 - 30
	NE	44.70	9.83	± 3.84	34 - 110
	LS	16.50	1.47	± 0.33	15 - 21
	BS	11.20	1.15	± 0.26	10 - 16
	PA	315.40	37.61	± 4.88	280 - 400
0.05 M	NS	17.50	3.69	± 0.83	12 - 23
	NE	45.20	9.52	± 2.79	35 - 78
	LS	15.95	1.70	± 0.38	14 - 20
	BS	10.80	1.11	± 0.25	07 - 12
	PA	316.50	56.60	± 17.71	245 - 407
Z-6 Con.	NS	18.90	4.68	± 0.88	10 - 23
	NE	52.15	13.84	± 3.10	23 - 78
	LS	14.30	2.23	± 0.50	10 - 19
	BS	9.45	1.61	± 0.36	06 - 12
	PA	247.50	49.66	± 15.70	157 - 317
1.0 M	NS	14.30	1.72	± 0.38	10 - 17
	NE	31.45	7.10	± 0.88	10 - 23
	LS	10.55	1.85	± 0.41	09 - 16
	BS	7.10	1.21	± 0.27	06 - 09
	PA	208.80	32.38	± 10.24	160 - 267
0.50 M	NS	17.90	4.69	± 1.05	12 - 30
	NE	47.05	9.98	± 3.84	34 - 110
	LS	18.40	1.98	± 0.44	15 - 23
	BS	12.05	1.79	± 0.40	09 - 15
	PA	330.70	34.00	± 10.75	280 - 400
0.05 M	NS	15.50	3.35	± 0.83	12 - 23
	NE	46.25	15.58	± 2.79	35 - 77
	LS	16.90	1.80	± 0.40	14 -20
	BS	9.40	1.57	± 0.35	07 - 12
	PA	335.00	54.80	± 17.33	266 - 412

NS = Number of stomata, NE = Number of epidermal cells
LS = Length of stomata (µm), BS = Breadth of stomata (µm)
PA = Pore area (µm)

at Z-6 and Z-1, respectively.

While in 0.05 M treated plants, guard cell index ranged from 39.45 to 45.48 having maximum and minimum frequency at Z- 4 and Z-1, respectively (Table 3). In 1.0 M treated plants of all the sites (Z-1 to Z-6), value for pore area index and value for total pore area per mm² was found to be reduced as compared to control. While value for pore area index and total pore area was increased in 0.50 M and 0.05 M concentrations as compared to control. In case of control of all sites, mean pore area index was found to be 17.89 to 23.09. Maximum frequency was noticed in Z-3 and minimum at Z-4. In colchicine treated seedlings, mean pore area index ranged from 13.56 to 19.00 in 1.0 M, 15.32 to 54.40 in 0.50 M and 15.60 to 24.60 in 0.05 M concentration. At different sites (Z-1 to Z-6), a significant variability between the control and treated seedlings and mean number of total pore area observed. Mean number of total pore area was ranged from 0.023 to 0.034 in control, while in treated seedlings total pore area was found to be 0.018 to 0.031 in 1.0 M, 0.019 to 0.053 in 0.50 M and 0.025 to 0.035 in 0.05 M concentration of colchicine from all sites. Maximum frequency of total pore area in control was found in Z-3 and minimum in Z-1. From all sites. In treated accessions, maximum frequency of mean total pore area was recorded in Z-5 (0.50 M) and minimum in Z-2 (1.0 M) .

Table 3: Guard cell index (GCI), pore area index (PAI) and total pore area (TPA) in *Z. armatum* (control and treated)

Site name	GCI	PAI	TPA
Z-1 Con.	30.57	18.90	0.023
1.0 M.	27.78	16.80	0.018
0.50 M	37.59	20.80	0.031
0.05 M	39.45	22.90	0.034
Z-2 Con.	37.03	18.49	0.024
1.0 M.	31.08	16.06	0.020
0.50 M	43.01	15.40	0.019
0.05 M	41.03	20.50	0.031
Z-3 Con.	38.05	23.09	0.034
1.0 M	32.67	19.00	0.031
0.50 M	43.36	54.40	0.053
0.05 M	43.04	24.60	0.029
Z-4 Con.	27.90	17.89	0.026
1.0 M	23.89	14.78	0.023
0.50 M	43.68	16.40	0.033
0.05 M	45.48	15.60	0.025
Z-5 Con.	33.32	19.32	0.024
1.0 M.	26.79	13.56	0.019
0.50 M	44.48	15.32	0.030
0.05 M	43.02	24.50	0.028
Z-6 Con.	38.59	21.90	0.032
1.0 M	33.33	17.33	0.026
0.50 M	45.22	19.00	0.034
0.05 M	44.60	23.70	0.035

GCI = Guard cell index, AI = Pore area index, TPA = Total pore area

Leaves occupy maximum portions of the aerial components of the terrestrial plants and these serve as interaction area of the shoot with the atmosphere. Foliar epidermis is the outer most skin of the leaves immediately in

contact with the atmosphere which helps in gaseous exchange and water evaporation. The stomata present on these layers operate, as inlets and outlets of gases like oxygen and carbon dioxide and water vapour. Stomata were analyzed in four leaf stages of development. The difference in their proportions indicated the difference in the genotype dependent pattern of genetic programme distribution within the cells of the same tissue. Differences within the values for pore area indices (PAIs) gave information about the proportion of the area of stomatal complex that could differentiate as pore. In control and treated accessions of different sites, dependent significant differences in the mean values of total pore area clearly indicated the genotypic control on the distribution of stomata.

The control and colchicine treated accessions of different sites differed significantly in the parameters like number of epidermal cells and stomata on abaxial and adaxial epidermis per mm^2. The mean values of total pore area clearly indicated the genotypic control on the distribution of stomata, in addition to the environmental control. Several treated accessions of different sites (Z-1 to Z-6) exhibited structural anomalies as the degeneration of one or both guard cells of the stomata, presence of one guard cell in the stomata, weavy thick walls of epidermal cells etc. No anomalies were reported in foliar epidermis in control accessions. Presence of numerous stomata interrupts the continuity of the lower epidermis. Stomata surrounded by average thickness of the inner or ventral face of each guard cell which are nearest to pore is considerably greater than that present in outer or dorsal wall.

In both the control and treated plants of same variety of *Z. armatum,* epidermal cells are bigger polygonal with sinuous wall on both surfaces of treated and untreated leaves and their frequency increased gradually in control than the colchicine at all the sites. Similar results were recorded by Ahmadi *et al.* (2008) and Oiyama and Okudai (1986) in *Hyoscyamus muticus* plant. The stomata were hypostomatic in all cases on abaxial surface of leaf. Several variations like stomata with single guard cell and unequal accessory cells were observed in the treated leaves at higher concentration (1.0 M). In case of control, all stomata were observed open and had large stomatal aperture but the size of aperture gradually increased in the leaves of treated plants. In case of higher concentration, some close stomata were observed. Increased size of stomata was also found by the work of Oiyama and Okudai (1986), Cohen and Yao (1996), Takamura and Miyajima (1996), Beck *et al.*, (2003) Hansen *et al.,* (2007), Nilanthi *et al.,* (2009) and Rauf *et al.* (2006) in colchicine treated plants. The stomatal frequency, length and breadth, stomatal index on lower surfaces showed gradual decrease up to 1.0 M and increased with 0.05 M to 0.05 M. The length/ breadth ratios increased against control at all concentrations. From the above observations, it is clear that the colchicine affected stomatal structure, frequency, length and breadth of stomata variously. Guard cell index, pore area index and total pore area was reported maximum in treated seedlings of all the sites. Increased size guard cell was also found by the work of Beck *et al.* (2003), Hansen *et al.* (2007). Nilanthi *et al.* (2009) and Rauf *et al.* (2006).

Acknowledgement :

The authors are thankful to the UGC, Delhi for the providing financial support in the form of Rajiv Gandhi National Fellowship during this research work.

REFERENCES

Ahmadi, S., Dehghan, F.E., Farsi, M. and Azizi, M. (2008). Tetraploid induction of *Hyoscyamus muticus* L. using colchicine treatment. *Pakistan J. Biological Sci.,* **11**: 2653-2659.

Beck ,S.L., Dunlop, R.W. and Fossey, A.(2003). Evaluation of induced polyploidy in *Acacia mearsnii* through stomatal counts and guard cell measurements. *South African J. Bot.,* **69** (4): 563- 567.

Cohen, D. and Yao, J.L. (1996). *In vitro* chromosome doubling of nine *Zantedeschia* cultures. *Plant Cell, Tissue Organ Culture*, **47**: 43-49.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. (1986). *Glossary of Indian medicinal plants including the supplement.* CSIR, Pub., New Delhi **4.**

Facciola, S. (1990). *Cornucopia-A source book of edible plants.* Kampong Publications.

Gupta, B.L. (1945). *Forest flora of Chakrata, Dehradun and Saharanpur.* Forest Research Institute Press.

Gamble. J.S. (1972). *A mannual of Indian timbers.* Bishen Singh Mahendra Pal Singh.

Hansen, D.L., Lambertini, C., Jampeetong, A. and Brix, H. (2007). Clone-specific differences in *Hragmites australis*: Effects of ploidy level and geographic origin. *Aquat. Bot*., **86** (3): 269-279.

Kala, C.P., Farooquee, N.A. and Dhar, U. (2004). Prioritization of medicinal plants on the basis of available knowledge, existing practices and value status in Uttaranchal (India).

Nilanthi, D., Xiao-Lu Chen, Fu-Cheng Zhao, Yue-Sheng Yang and Hong Wu (2009). Induction of tetraploids from petiole explants through colchicine treatments in *Echinacea purpurea* L. *J. Biomedicine and Biotechno.,* **2009**: 343-485.

Oiyama, I. and Okudai, N. (1986). Production of colchicine- induced autotetraploid plants through micro grafting in monoembryonic citrus cultivars. *Japanese J. Breed.,* **36:** 371-376.

Pearson, R.S. and Brown, H.P. (1932). Commercial timbers of India, **2**: 1150.

Rauf, S., Khan, I.A. and Khan, F.A. (2006). Colchicine induced tetraploidy and changes in allele frequencies in colchicine treated populations of diploids assessed with RAPD markers in *Gossypium arboretum* L. *Turk. J. Biol.,* **30**: 93-100.

Takamura, T. and Miyajima, I.(1996). Colchicine induced tetraploids in yellow- flowered Cyclamens and their characteristics. *Sci. Hort.,* **65**: 305-312.

Tanaka, T. (1976). *Tanaka's encyclopedia of edible plants of the world*. Keigaku Publishing.

Uphof, J.C. Th. (1959). *Dictionary of economic plants*. Weinheim.

Usher G.A. (1974). *Dictionary of plants used by man*. Constable. ISBN 0094579202.

****
*****