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Research Article

STUDIES ON CYTOTAXONOMY AND STOMATA ON TWO VARIETIES OF *ALLIUM SATIVUM L.* COLLECTED FROM RANCHI, JHARKHAND

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ABSTRACT
Cytotaxonomic and stomatal studies were made in 2 varieties of *Allium sativum L.* (*Allium sativum L.* var. single clove and *Allium sativum L.* var. multiclove). These varieties were collected from local market of Ranchi, Jharkhand. These varieties were recorded with chromosome number 2n=2x=16. The total chromatin length of *Allium sativum L.* var. single clove observed was 53.76µ, mitotic index was 55.273, and karyotype formula is 7NM +1NSM. Whereas total chromatin length of *Allium sativum L.* var. multiclove observed was 63.48µ, mitotic index was 54.419, and karyotype formula was 7NM+1NSM.
In stomatal studies anomocytic type of stomata were found in both varieties. Maximum stomatal index was observed on apical region of ventral surface of leaf in both varieties.

Keywords: *Cytotaxonomy, Karyotype, Chromatin Length, Mitotic Index, Stomatal Index*

INTRODUCTION
Allium sativum L. belongs to Division Magnoliophyta, Class Liliopsida, order Liliales, and Family Liliaceae. It grows in tropical humid and temperate regions. It grows best in full Sun and fertile well drained soil. It is known to be cultivated in northern, eastern, southern and middle parts of country. Major garlic producing states are Punjab, Karnataka, Bihar, Tamilnadu, Haryana, Andhra Pradesh, Madhya Pradesh, Rajasthan, and Gujrat at smaller scale in Jharkhand. Punjab is the highest producer of garlic in India. It is known to be originated in China. In India, it has been known to originate in Himalayas. It is exclusively grown by vegetative means by planting bulbils (Verma and Mittal 1978). The bulbils are planted in the month of November and December in rabi season and harvested in February and March.
Allium sativum L. Var.single clove garlic is commonly known as monobulb garlic, single bulb garlic, pearl garlic, solo garlic, monoclove garlic, in Hindi it is known as ek kali ka lahsun, ek pot lahsun. Single clove garlic is formed as a result of failing to split into multiple cloves due to environmental factors. The size of the single clove garlic differs from approximately 25-30 mm in diameter. Plant height ranges between 1-1.5m. *Allium longicuspis* is considered as an endemic wild ancestor of *Allium sativum* (Siddiqui et al. 2007). *Allium sativum L.* is a popular condiment cultivated all over the world. Apart from its use as a condiment it is also used as a medicinal plant. It is rich in vitamin C, minerals, and other trace elements. Active medicinal chemical constituents of garlic are phenols, alkaloids, flavonoids, steroids, glycosides, and saponins etc. Phytochemicals are occurring in whole plants, leaves, bulbs, roots, and seeds.
Allium sativum L. is probably one of the earliest known medicinal plants (Lewis and Elvin-Lewis 2003). Garlic (*Allium sativum L.*) is widely consumed spices in food, while also consumed in form of drink. The fresh bulb contains an allin, allicin and volatile oils, when the garlic clove is crushed; the odorless compound allin is converted to allicin via enzyme allinase. Allicin gives garlic its characteristic pungent smell.
Allium sativum L. is an herbal medicine which is used for prevention and treatment of many diseases such as cold and flu symptoms through immune enhancement and exhibits anticancer, antioxidant, anti-inflammatory, antimicrobial, antithrombotic, hypochlolestrolemic, hypoglycemic, and hypertensive activities. And it is used to treat diabetes, atherosclerosis, hyperlipidema, thrombosis and hypertension. Also, it acts against stroke, gastrointestinal neoplasius, against blood clots (antiplatelet action) etc.

The chromosome number of *Allium* species is reported as 2n=16 (Levan, 1935; Mensinkai, 1939).
The main objective of this study was to investigate chromosome number, chromatin length, karyotype formula, mitotic index, and stomatal index; and Idiogram construction on the basis of position of centromere and length of short and long arm. On basis of these findings, analyze primitive and advance variety.

MATERIALS AND METHODS

Collection of bulbs

Bulbs of *Allium sativum L.* Var. single clove garlic were collected from local market of Ranchi, Jharkhand.

Materials

Cytological preparations were made by using following chemicals: Ethyl alcohol, Glacial acetic acid, Distilled water, Ferric chloride, 2% Acetocarmine, 1, 4-Paradichlorobenzene, Glycerin, Saffranin.
Following apparatus were used during project work: Slides, Cover slips, Needle, Tapper, Spirit lamp, Watch glasses, Petri plates, Microscope, Stage, Micrometry, Ocular, and Digital Camera.

Methods

Cytotaxonomical methods

Mitotic studies: Bulbs were first allowed to dry properly under Sunlight for 3-4 days. Bulbs are then placed in glass of water just touching the rooting disc under indirect Sunlight. Root starts growing in 2-3 days, root tips of measure 1-1.5 cm were cut between 1:30-2:00pm under sunlight condition. The cut root tips were first pretreated in 1, 4-Paradichlorobenzene for 4 hours at room temperature. The pretreated root tips were thoroughly washed with water and transferred to fixative 1:3 acetoalcohol (Carnoy's fluid) for 24 hours. After 24 hours fixed root tips were transferred to 70% ethanol for preservation.
For mitotic studies, slides were prepared by 2% acetocarmine squash technique. The root apices were first of all warmed in 2% acetocarmine solution for about 1 hour. The squashed preparations were made by smearing the warmed root apices in 45% glacial acetic acid solutions. The root tips were sandwiched in between a clean, lint free slide and cover slip and then slightly pressed for the mitotic studies. Three plates with well separated chromosomes were taken for experimental purposes and then photographed in Digital SLR camera.
The microphotographs of well separated chromosomes plate of metaphase stage were taken. The well separated chromosomes were measured with the help of ocular and stage micrometer. The data were statistically analyzed and idiograms were prepared.
The statistical analysis of data were made with the help of

1. Mean
2. Standard Deviation
3. Standard Error

The statistical analysis was made by following formula:

1. **Mean:-**

$\bar{X}=\frac{x1+x2+x3.....x}{n}$

Or, $\bar{X}=\frac{\sum x}{n}$

Where, X=Arithmetic mean
$\sum$x=Sum of all values of the variable
X=x1, x2, x3etc.
n=number of observations.

2. **Standard deviation:-**

There are 2 methods for the calculation of standard deviation.

(a) Method-1

Standard deviation (s) = $\sqrt{\frac{\sum(x-\overline{X})^2}{n-1}}$

$= \sqrt{\frac{\sum(d\bar{X})^2}{n-1}}$

(b) Method-2

$$S = \sqrt{\frac{\sum x^2 - \frac{(\sum x^2)}{n}}{n \text{ or } n-1}}$$

3. **Standard Error:-**

The following formula was used for standard error.

Standard error = $\frac{S.D}{\sqrt{n}}$

Where, S.D = Standard Deviation of sample

n = Size of sample.

4. **Mitotic index (MI):-**

$$MI = \frac{Total\ no.\ of\ dividing\ cell}{Total\ no.of\ cell\ observed} \times 100$$

Stomatal studies

Fresh Green leaves of *Allium sativum L.*Var. Single Clove and *Allium sativum L.*Var.Multiclove were collected and kept in water in petriplates and slides for stomatal studies was prepared by scratch method. Epidermal layer obtained after scratching were stained in saffranin and mounted with glycerin and glass cover slip. Slides of ventral and dorsal surface were prepared with its apex, middle, and base region. Stomata length and width of ventral and dorsal surface were calculated in apex, middle, and base region using ocular.

Stomatal index

Stomatal index of apex, middle, and base region of ventral and dorsal surface were calculated using the following formula:

Stomatal index (SI) = $\frac{S}{S+E} \times 100$

Where, S = Total no. of stomata cells, E = Total no. of epidermal cells

RESULTS AND DISCUSSION

Cytotaxonomic studies

Mitotic studies: Two varieties of *Allium sativum L.*var. single clove and *Allium sativum L.*var. multiclove were recorded with chromosome number 2n=2x=16 as shown in Fig.1and 2.In *Allium sativum L.*var. single clove , total chromatin length observed was 53.76µ (Table1), karyotype formula was 7NM+1NSM (7 nearly median and 1 nearly sub median) and mitotic index 55.273(Table2).

In *Allium sativum L.*var. multiclove, total chromatin length observed was 63.48µ (Table1), karyotype formula 7NM+1NSM (7 nearly median and 1 nearly sub median) and mitotic index 54.419 (Table3). Classifications of chromosomes were made on the basis of table given by Abraham and Prasad (1983). Idiogram prepared for representing long arm and short arm of chromosome on graph is depicted in Fig.5 and 6.

Stomatal studies: In this investigation, stomata observed were of anomocytic type of stomata that have guard cells that are surrounded by cells that have the same shape, size and arrangement as the rest of epidermal cells (Fig.3 and 4). Among the dorsal and ventral leaf surfaces of *Allium sativum* L.var. single clove, maximum stomatal index was observed in apex region of ventral side i.e. 16.316±0.34(Table4). And the lowest stomatal index was observed in base region of dorsal side i.e. 10.26±1.75 (Table4). The longest and widest stomata were reported on basal region of ventral surface with 4.48±0.14 and 2.64±0.16 respectively (Table 5).

In *Allium sativum* L.var. multiclove, maximum stomatal index was observed in apex region of ventral surface i.e. 17.394±1.05 (Table6) and the lowest stomatal index was observed in base region of dorsal side i.e.8.534±1.04 (Table 6). The longest and widest stomata were observed on middle region of ventral surface with 4.72±0.19 and 2.64±0.16 respectively (Table 7).

Discussion

The genus *Allium sativum* L. belongs to family Liliaceae with about 100 of subspecies. It is known to be native of central Asia and northeast Iran, and have originated in China. It is grown in tropical and temperate regions of world. It is mainly distributed in Asia, America and Europe. It is grown exclusively by vegetative means by planting bulbils. It is cultivated in well drained soil and full Sunlight. It is mainly cultivated for use as condiments and for its medicinal properties.

The study of karyotype is of great importance in modern taxonomy in large number of plant species. Karyotype data have been effectively employed to resolve taxonomic position and for authentic genotypic identification. The analytical data of karyotype of two varieties of *Allium sativum L*. of family Liliaceae were compared on the basis of total chromatin length and karyotype formula.

Chromosome number was recorded 2n=2X=16 which was also confirmed earlier by Mensikai in 1939, Brat Sharma, 1965; Konvicka and Albert Levan, 1972; Verma and Mittal, 1976; Deniz Yubasioglu and Fatmal Unal 2004; and Sharbani Mukhrjee and Kamini Kumar in 2007.

On the basis of Table 1, Idiogram prepared; the chromosomes varied in size and were nearly median and nearly sub median. Presence of nearly median and nearly sub median indicates symmetrical nature of karyotype. *Allium sativum L.*var. single clove was considered advanced due to its smaller total chromatin length, and *Allium sativum L.*var. Multiclove was considered primitive on the basis of its larger total chromatin length.

Table 1: Karyomorphological data of two varieties of *Allium sativum* L.

Variety	Chromosome Number	Arm length (µ)		Total length (µ)	LA/SA Arm ratio	Classification
		Long arm	Short arm			
Allium sativum L. var. single clove 2n=16	1	5.26±0.14	3.86±0.30	9.13±0.23	1.36	**NM**
	2	5.00±0.24	2.83±0.86	7.83±0.47	1.76	**NSM**
	3	4.46±0.14	3.40±0.34	7.86±0.42	1.31	**NM**
	4	4.20±0	2.66±0.23	6.86±0.35	1.57	**NM**
	5	3.60±0.18	2.66±0.23	6.26±0.38	1.35	**NM**
	6	3.20±0.26	2.33±0.28	5.56±0.47	1.37	**NM**
	7	3.00±0.24	2.60±0.34	5.60±0.58	1.15	**NM**
	8	2.25±0.14	2.06±0.14	4.66±0.21	1.09	**NM**
TCL (µ)				**53.76±3.11**		
Allium sativum L. var. multiclove 2n=16	1	8.13±0.10	4.80±0.24	12.93±0.14	1.69	**NSM**
	2	6.80±0.37	5.50±0.73	12.30±0.62	1.23	**NM**
	3	4.86±0.05	4.60±0.24	8.60±0.24	1.05	**NM**
	4	4.56±0.09	3.50±0.14	8.06±0.23	1.30	**NM**
	5	4.06±0.14	3.30±0.12	7.36±0.26	1.23	**NM**
	6	3.26±0.42	2.80±0.24	5.93±0.61	1.16	**NM**
	7	2.60±0.28	1.90±0.17	4.50±0.33	1.36	**NM**
	8	2.00±0.09	1.8±0.09	3.8±0.18	1.11	**NM**
TCL (µ)				**63.48±2.16**		

The data for total chromatin length indicated in Table 1shows remarkable changes in the two varieties under consideration. The differences in total chromatin content may be due to chromosomal aberrations possible due to deletion.

The stomata size and frequency are important parameters in selecting drought resistant genotypes maximum number of stomatal index was observed on ventral (adaxial) surface which can be correlated with the nature of plant *i.e.* it might be non xerophytes. Presence of maximum stomata on the ventral surface reveals that the transpiration rate is very high in comparison to xerophytes plants. Hence, these are not resistant to drought conditions.

A stomatal frequencies study along with karyotype studies plays an essential role in selecting suitable species for breeding programs for resolving taxonomic confusions and tracing the evolutionary tendencies (Venkatesh, 2017).

Table 2: Mitotic index of *Allium sativum* L. single clove

S. No.	No. of cell	Resting cell	Prophase	Metaphase	Anaphase	Telophase
1	49	26	21	2	0	0
2	58	18	36	3	0	1
3	50	16	32	1	1	0
4	50	20	26	2	1	1
5	48	26	21	0	0	1
6	46	26	19	1	0	0
7	48	19	27	2	0	0
8	46	20	25	1	0	0
9	67	30	31	3	1	2
10	50	28	21	1	0	0
Total	**512**	**203**	**259**	**16**	**3**	**5**

Total no. of dividing cell = Prophase + Metaphase + Anaphase + Telophase
= 259+16+3+5
= 283

Mitotic index (MI) $= \frac{Total\ no.of\ dividing\ cell}{Total\ no.of\ cell\ observed} \times 100$

$= \frac{\mathbf{283}}{\mathbf{512}} \times 100$

= 55.273

T**able 3: Mitotic index of *Allium sativum* L. var. multiclove**

S.NO.	No. of cell	Resting cell	Prophase	Metaphase	Anaphase	Telophase
1	25	17	7	1	0	0
2	53	25	22	3	1	2
3	48	13	29	6	0	0
4	50	18	29	2	0	1
5	52	21	26	1	1	3
6	75	38	36	1	0	0
7	67	31	34	2	0	0
8	58	27	25	2	0	4
9	68	34	31	1	2	0
10	81	39	38	3	0	1
Total	**577**	**263**	**277**	**22**	**4**	**11**

Total no. of dividing cell = Prophase + Metaphase + Anaphase + Telophase
= 277+22+4+11
=314

Mitotic index (MI) = $\frac{Total\ no.of\ dividing\ cell}{Total\ no.of\ cell\ observed} \times 100$

$= \frac{314}{577} \times 100$

$= 54.419$

Table 4: Stomatal index data of *Allium sativum* L. var. single clove.

Leaf portion	Ventral surface	Dorsal surface
Apex	16.316±0.34	11.24±2.05
Middle	14.594±0.37	11.05±1.03
Base	14.656±0.88	10.26±1.75

Table 5: Data related to measurement of stomata of *Allium sativum* L. var. single clove

Leaf portion	Ventral surface		Dorsal surface	
	Length (µ)	Width (µ)	Length (µ)	Width (µ)
Apex	4.32±0.14	2.40±0.17	4.48±0.14	2.40±0.12
Middle	4.24±0.16	2.40±0.17	4.32±0.14	2.72±0.14
Base	4.48±0.14	2.64±0.16	4.32±0.14	2.48±0.19

Table 6: Stomatal index data of *Allium sativum* L. var. multiclove.

Leaf portion	Ventral surface	Dorsal surface
Apex	17.39±1.05	10.008±0.58
Middle	16.24±0.91	9.43±1.13
Base	16.94±0.53	8.53±1.04

Table 7: Data related to measurement of stomata of *Allium sativum* L. var.multiclove

Leaf portion	Ventral surface		Dorsal surface	
	Length (µ)	Width (µ)	Length (µ)	Width (µ)
Apex	4.40±0.12	2.64±0.09	4.48±0.23	2.48±0.14
Middle	4.72±0.19	2.56±0.16	4.40±0.17	2.64±0.16
Base	4.16±0.09	2.88±0.14	4.48±0.14	2.72±0.14

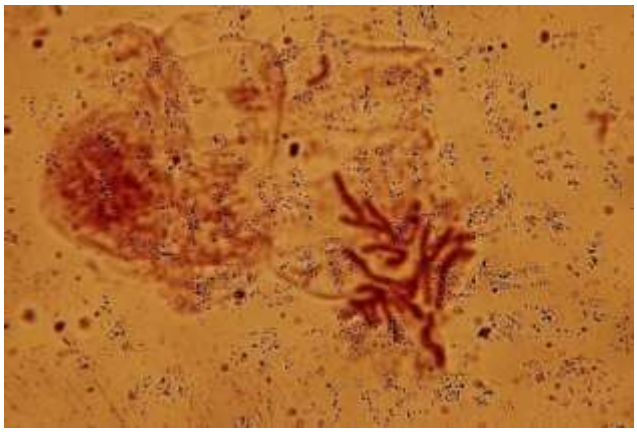

Figure 1: Mitotic metaphase chromosome of *Allium sativum L.*var. single clove.

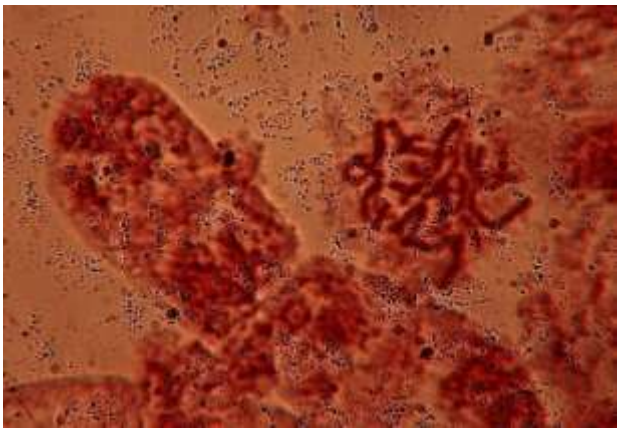

Figure 2: Mitotic metaphase chromosome of *Allium sativum L.*var. multiclove

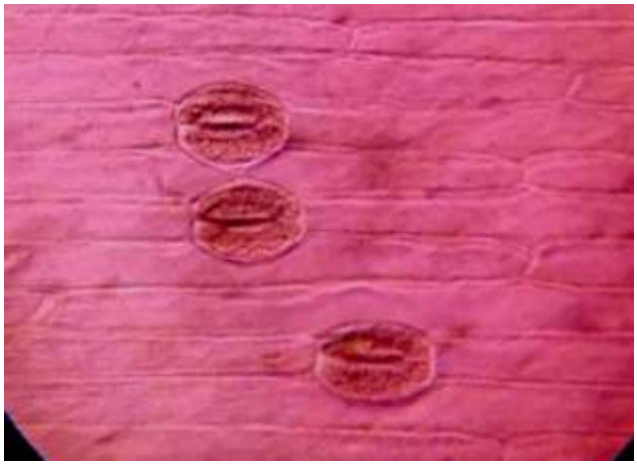

Figure 3: Microphotograph of stomata of *Allium sativum L.*var. single clove.

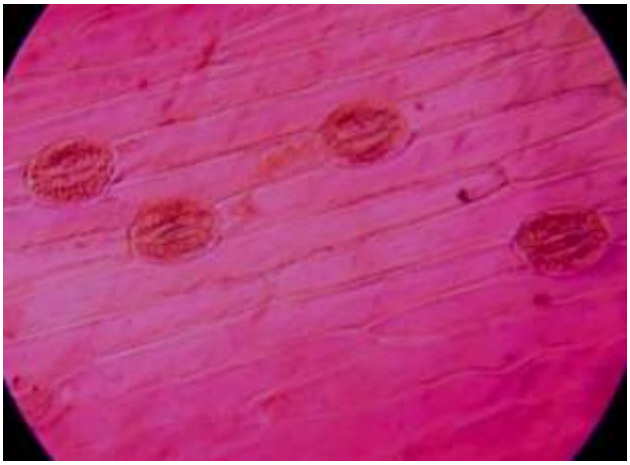

Figure 4: Microphotograph of stomata of *Allium sativum L.*var. multiclove

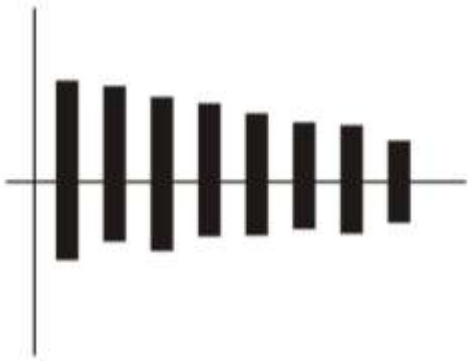

Figure 5: Idiogram of *Allium sativum L.*var. single clove

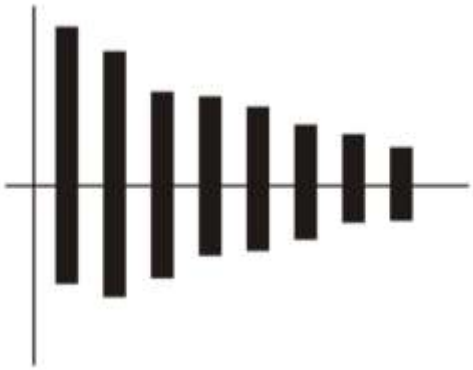

Figure 6: Idiogram of *Allium sativum L.*var multiclove

Figure 7: Comparative Histograms of *Allium sativum L.*var. single clove and *Allium sativum L.*var. multiclove

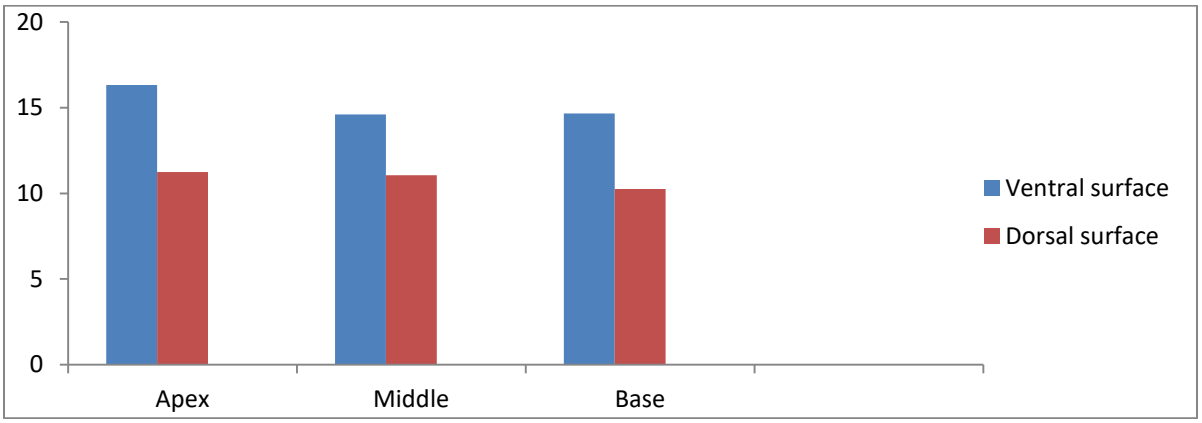

Figure 8: Column graph showing the stomatal index of *Allium sativum L.*var. single clove

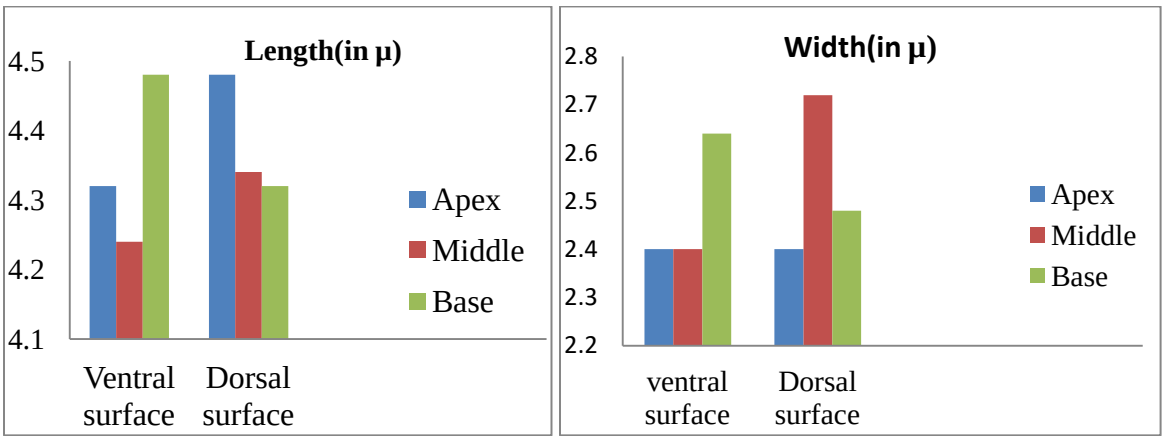

Figure 9: Column graph showing the stomatal length and width (in µ) of *Allium sativum L.*var. single clove

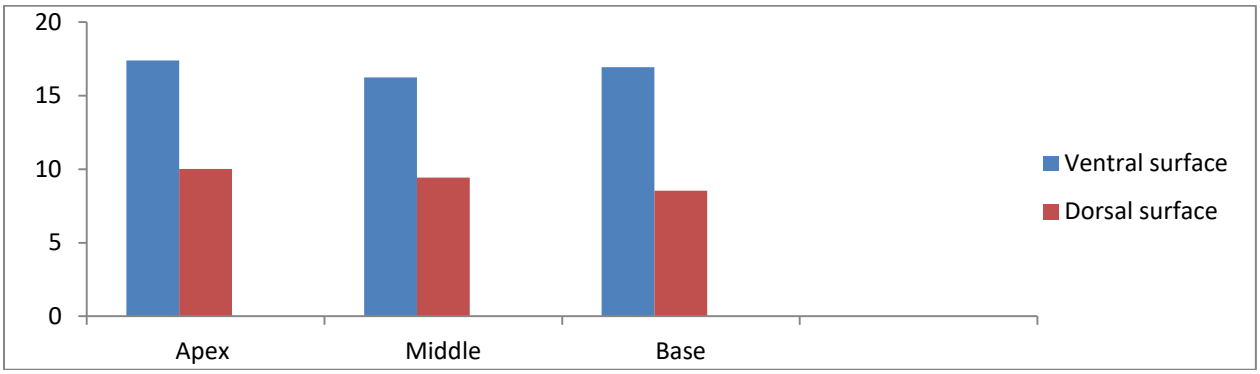

Figure 10: Column graph showing the stomatal index of *Allium sativum L.*var. multiclove.

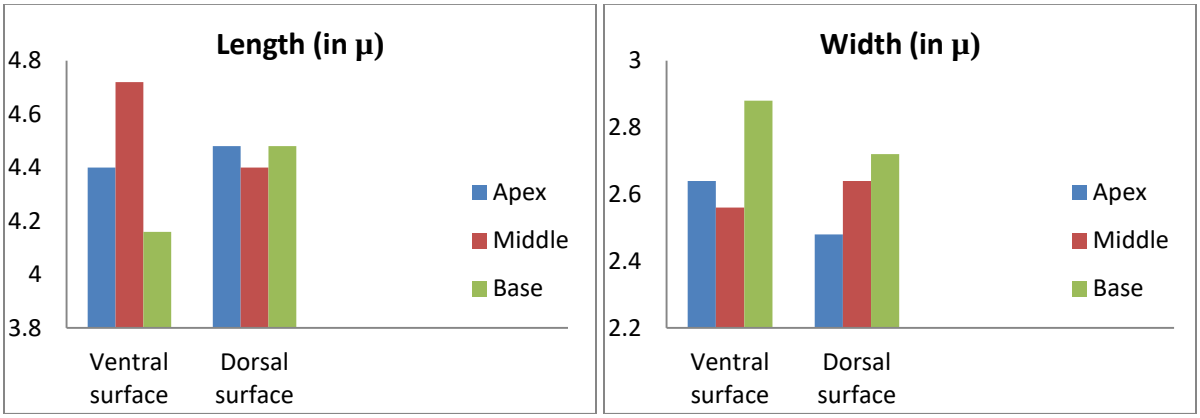

Figure 11: Column graph showing the stomatal length and width (in µ) of *Allium sativum L.*var. multiclove.

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