INTERNATIONAL JOURN AL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY © VISHWASHANTI MULTIPURPOSE SOCIETY (Global Peace Multipurpose Society) R. No. MH-659/13(N) www.vmsindia.org

MITOTIC INDEX (MI) AND PHASE INDEX (PI) IN ALLIUM SATIVUM LINN. (VARIETY AGRIFOUND WHITE AND YAMUNA SAFED)

# Sukeshini Deogade<sup>1</sup> and P. N. Nasare<sup>2</sup>

<sup>1</sup>De partment Of Botany, Hislop College, Civil Line, Nagpur (M.S.) India. <sup>2</sup>De partment Of Botany, Nilkanthrao Shinde Science And Art's College, Bhadrawati Dist.- Chandrapur (M.S.) India. sukeshinishahare@gmail.com

### Abstract:

Mitotic cell division was observed in root tips of varieties of *Allium sativum* L. i,e Agrifound white and Yamuna safed fixed in Carnoy's fixative (Glacial acetic acid : absolute alcohol, 1:3) at different intervals of time, i.e 8.00-8.30am, 8.30-9.00am, 9.00-9.30am, 9.30-10.00am, 10.00-10.30am, 10.30-11.00am. The number of semipermanent squash preparations were observed for the study of mitotic activity. For each time 20 slides were observed to scored prophase, metaphase, anaphase and telophase. In the varieties of Agrifound white, maximum metaphases (9.10%) and anaphases (5.15%) observed at the time 9.00-9.30am, and in Yamun safed, maximum metaphases (10.10%) and anaphases (7.30%) observed at the time 9.30-10.00am respectively. The purpose of present study is to know the mitotic index and phase index in *Allium sativum* L. varieties (Agrifound white and Yamuna safed) for proper study of the chromosomes for karyotype analysis.

Keywords: *Allium* sativumL., Mitotic cell division, Mitotic index and Phase index.

#### Introduction

The genus *Allium* comprises about 700 species. Several of them are important vegetables, spices and medicinal plants.

Allium sativum L. (garlic) a member of the Liliaceae family and a popular condiment is cultivated all over the world. The chromosome number of Allium species, is reported as 2n=16 (Levan, 1935, Mensinkal, 1939) and 2n=18 in two varieties of garlic (Sharma and Bal, 1959). Intraspecific variations in the size and type of chromosomes, positions of the secondary constrictions and positions and numbers of the heterochromatic bands occur frequently in most of plant species.

Aerial stems of Allium sativum L. are to + 1m tall, erect, simple, herbaceous, green, glabrous, terete mostly hollow. Bulb of many bulblets, with a papery coating and fibrous roots. Bulble with at least one flat side. Leaves present in the lower 1/3 to  $\frac{1}{2}$  of the plant. Leaves flat or very slight folded, to +/-30cm long, 7-10mm broad, glabrous, often glaucous, with a prominent midrib, sheathing. The ligule rounded ( "U"shaped), the free portion 1-2 mm tall (long ). Inflorescence dense capitate cluster of bulblets terminating the stem. Inflorescence covered in a papery spathe. Spathe with a long tip, splitting on one side at anthesis. Flowers mostly or entirely replaced by bulblets. Bulblets glabrous, whitish (more commonly) with a reddish tinge. If produced, the small flowers are greenish, whitish, or pinkish and tubular with acute lobes. Bulbs of Agrifound White (G-41) are compact, silvery and white with creamy flesh. A bigger elongated clove with 20-25 in number

and diameter of bulb is 3.5-4.5 cm. Recommended for Maharashtra and Madhya Pradesh. Chromosome number is 2n=18. The material was procured from Dr. Punjabrao Deshmukh Krishi Vidyapeeth, Akola. (M.S.).

Bulbs of Yamuna Safed are creamy white and bigger sized with 4.5-6 cm in diameter. Number of cloves/ bulbs is 15-16. Recommended for Madhya Pradesh, Maharashtra, Haryana, Gujarat, Punjab, Rajasthan, Uttar Pradesh and Chhattisgarh. Chromosome number is 2n=18. The material was procured from National Horticulture Research Development and Foundation, Nashik (M.S.).

Several investigators have worked on mitotic cell divisions in different plants Viz. Lens culinaris Medik (Yasar Kiran and Ahmed Sahni, 2006), Capsicum annum L.(Yadav and Yadav, 2010), Ocimum sanctum L. (Nasare and Choudhary, 2010), Triticum aestivum L. (Sanjaykumar et al,2010), Triticum aestivum L. (Sanjaykumar et al,2010), Treculia Africana Decne. (Osiji et al., 2010), Sacchanum spp. (Shrivastava and Jain, 2011), and Phaseolus vulgaris L. and Raphanus sativus L. (Elena et al, 2011), Allium cepa L. (Paul et al., 2013), Vigna unguiculata Linn. (Amirthalingam et al., 2013), Allium cepa L. ( Udo et al., 2014), Allium cepa L. (Pankaj et al., 2014).

The present study was designed to study the mitotic activity and phase index in the root meristems of *Allium sativum* L. to find out the maximum mitotic activity at specific time for each variety of *Allium*.

# **Materials and Methods**

Healthy cloves of Allium sativum L. varieties Agrifound white and Yamuna safed were selected and kept for germination on the jars filled with water. After 4 days, root tips were harvested with the help of blade that had attain the length of 1 - 1.5 cm and fixed in Carnoy's fixative (1:3, glacial acetic acid: absolute alcohol, V/V) for 24 hours in refrigerator. Fixed root tips were washed with distilled water and macerated in 1N HCl for 10-15 min. at 60° C in hot air oven. Macerated root tips were transfer to 2% acetocarmine stain for 20 min. for proper staining. Stained root tips were then squashed in stain and cover glass (cover slip) was sealed with paraffin wax. The chromosome spreads were then observed microscopically. The cells were scored for the different cell division stages, number of total dividing cells, number of prophases, number of metaphases, number of anaphases and number of telophases were scored. During mitotic cycles of the cell division at different intervals of time, data was prepared to calculate mitotic index (MI) and phases indices (PI) by following formulae (Bhatta and Sakya, 2008).

The different phases of mitosis were counted to calculate the mitotic index (MI) and phase indices, as following:

Mitotic Index = TDC x100/TC

PI% = prophase cells x 100/TDC

MI% = metaphase cells x 100/TDC

AI% = anaphase cells x 100/TDC

TC% = telophase cells x 100/TDC, where

TC = total cells (dividing and non-dividing), and TDC = total dividing cells.

## **Result and Discussion**

In the present study, mitotic activity and mitotic chromosomes were studied and the data for the same is presented in the table 1 and 2.

At the times around the clock, there was cell division (Table 1). The rate of division varied widely between the time periods of 8.00 am to 11.00 am. The percentage of dividing cells is higher in the time 9.00 am to 10.00 am than others. At about 8.00 am to 8.30 am a high percentage of prophase cells were recorded.

The cells that enter mitotic process start from the prophase stage as corroborated by the rise in the number of cells at this stage. Metaphase increased after prophase and continued mostly between 9.00 am to 10.00 am.

From the table number 1 there were always cells at the various stages of the mitotic division. The observation of the highest number of metaphase cells at the period between 9.00 am to 9.30 am. The rise in the number of cells in anaphase was after the rise in the number of those at metaphase. The highest number of anaphase cells at the period between 9.00 am to 9.30 am.

Hence there were always cells at the various stages of the mitotic division. The observation of the highest number of metaphase cells at the period between 9.30 am to 10.00 am. The number of cells at the period at anaphase started to rise at about 9.30 am to 10.00 am. The rise in the number of cells in anaphase was after the rise in the number of those at metaphase. The highest number of anaphase cells at the period between 9.30 am to 10.00 am.

For this study, different stages of mitosis (Prophase, metaphase, anaphase, telophase) were counted to calculate mitotic index and phase indices.

From the table number 1, it was observed that mitotic index was found maximum  $(26.41\pm0.18)$  at the time 9.00 – 9.30 am and phase index was found maximum (421.63) at the time 10.30 – 11.00 am. While mitotic index was found minimum  $(23.72\pm0.14)$  at 10.30 – 11.00 am and phase index was found minimum (378.68) at the time 9.00 – 9.30 am.

From the table number 2, it was observed that mitotic index was found maximum  $(27.76\pm0.19)$  at the time 9.30-10.00 am and phase index was found maximum (387.82) at the time 10.00 - 10.30 am. While mitotic index was found minimum  $(25.79\pm0.19)$  at 10.00 - 10.30 am and phase index was found minimum (361.48) at the time 9.00 - 9.30 am. The data of mitotic index and phase index is also presented in following bar diagrams.

Several cytologist were studied the mitotic index and phase index in many plants viz., Foeniculum vulgare (Jahagirdar, 1975), Coriandrum sativum (Gaikwad, 1975), Carcum copticum (Khanolkar, 1977), Solanum nigrum (Kothekar, 1978), Cajanus cajan (Chary and Bhalla, 1987), Oryza sativa s(Ramesh, 1987), Vicia faba (Abraham and Nayar, 1989), Medicago sativa (Singh et al., 1990), Trigonella foenum-graecum (Devi, 1990), Pearl millet (Bansikar and Srivastava, 1992), Linum usitatissimum (Karpate, 1995), Cyamopsis tetragonoloba (Kanaklata, 1995), Lathyrus sativus (Girhe, 1999), Brassica napus (Lange, 2000), Lens culinary Medik (Yasar and Ahmet, 2006), Hordeum vulgare L. (2007), Triticum aestivum L. (Sanjay kumar et al, 2010), Capsicum annum (Yadav and Yadav, 2010),

Ocimum sanctum L. (Nasare and Choudhary, 2010), Hibiscus rosa-sinensis (Ozmen Ali, 2010), Treculia Africana Decne. (Osiji et al., 2010), Saccharum spp. (Shrivastava and Jain, 2011), Phaseolus vulgaris L. and Raphanus sativus L. (Elena et.al, 2011), *Allium cepa* L. (Paul et al., 2013), *Vigna unguiculata* Linn. (Amirthalingam et al., 2013), *Allium cepa* L. (Udo et al., 2014) and *Allium cepa* L. (Pankaj et al., 2014).

**Table1:** Shows Mitotic index (MI) and Phase Index (PI) in root tips cells of *Allium sativum* (Agrifound white) Linn.

Time	тс	TDC	Prophase	Metaphase	Anaphase	Telophase	Mitotic	Phase
Intervals			index	Index	Index	Index	Index ±SE	Index
8.00-8.30am	1683	413	11.17	6.18	4.63	2.73	24.54±0.20	407.50
8.30-9.00am	1667	440	11.82	8.33	4.08	2.10	26.39±0.19	378.86
9.00-9.30am	1670	441	10.54	9.10	5.15	2.40	26.41±0.17	378.68
9.30-10.00am	1628	407	7.31	8.85	5.10	3.75	25.00±0.13	400
10.00-10.30am	1691	413	6.86	8.28	4.90	4.02	24.42±0.17	409.44
10.30-11.00am	1657	393	6.46	7.97	5.07	4.47	23.72±0.14	421.63

SE = Standard Error (±)

**Table 2:** Shows Mitotic index (MI) and Phase Index (PI) in root tips cells of *Allium sativum* (Yamuna safed) Linn.

Time Intervals	тс	TDC	Prophase	Metaphase	Anaphase	Telophase	Mitotic	Phase
			index	Index	Index	Index	Index ±SE	Index
8.00-8.30 am	1601	418	10.31	8.81	4.43	2.44	26.11±0.47	383.01
8.30-9.00 am	1571	422	11.84	8.98	4.39	1.97	26.86±0.26	372.27
9.00-9.30 am	1511	418	10.52	9.07	4.90	2.45	27.66±0.08	361.48
9.30-10.00 am	1567	435	7.85	10.15	7.21	2.62	27.76±0.19	360.23
10.00-10.30 am	1528	394	7.73	8.44	5.56	4.25	25.79±0.19	387.82
10.30-11.00 am	1471	405	8.84	8.91	5.03	4.69	27.53±0.18	363.21

SE = Standard Error (±)



# Allium sativum L.(Agrifound white)



# Allium sativum L. (Yamuna safed)



metaphase anaphase telophase

### Conclusion

The authors concludes the present investigation reveals that for karyotype analysis of Allium species, the pre-requisite study of mitotic and phase index is essential. Information and data presented in this work is expected to be valuable for further chromosomal study.

### Acknowledgement

Author's thanks to Dr. Mousmi Bhowl (Head) and Dr. K. J. Cherian (Ex-Head) Department of Botany, Hislop College, Civil Lines, Nagpur for providing all possible resource materials and facilities available in the department whenever required without any hesitation. Thanks to Dr. Dipti Christian, Principal, Hislop college, Nagpur for her timely help and kind cooperation. We acknowledge our gratitude to Dr. L. S. Ladke, Principal, Nilkanthrao Shinde Science And Art's College, Bhadrawati and Mr. N.Y. Shinde (Ex-MLA), Secretory, Bhadrawati Shikshan Sanstha, Bhadrawati for their valuable guidance kind cooperation.

### References

[1] Adegbite A. E. and Sanyaolu E. B. (2009): Cytotoxicity testing of aqueous

extract of bitter leaf (Vernonia amyglalina Del.) using the Allium cepa chromosome aberration assay. Scientific research and Essay Vol. 4(11), pp. 1311-1314.

[2] Adegbite A. E., Avodele M. S., Odunbaku K. R. and Idehen E. O. (2009): Mutagenic effect of neem leaf extract used in traditional medicine on Allium cepa (L.). Scientific Research and Essays Vol. 4(11), pp. 1315-1321.

[3] Ali O. (2010): Cvtotoxicity of Hibiscus rosasinensis flower extract. Caryologia Vol. 63, no. 2:157-161.

[4] Amirthalingam T., Velusamy G. and Pandian R. (2013): Cadmium- induced changes in mitotic index and genotoxicity on Vigna unguiculata (Linn.) Walp. Journal of Environmental Chemistry and Ecotoxicology. Vol. 5(3), pp. 57-62.

[5] Bansikar V. and Srivastava A. K. (1992): Mercury induced chromosomal inconstancy in Pearl millet. J. Cytol. Genet. 27: 79-90.

[6] Bhatta P. and Sakya S.R. (2008): Study of mitotic activity and chromosomal behavior in root meristem of Allium cepa L. treated with magnesium sulphate. Ecoprint, 15:83-88.

[7] Chary H.S. and Bhalla J. K. (1987). Chromosomal abnormalities induced by gamma rays magnetic fields and EMS in Pigeon pea, Cajanus cajan (L. Milli sp). In Proc. "First All India Conference on Cytology and Genetics" Banglore, India. 41.

[8] Devi P. (1990): Cvtological effects of chemical mutagen on Trigonella foenum-graecum L. J. Cytol. Genet. 25, 117-119.

[9] Elena Truta, Maria-Magdalena Zamfirache and Zenoviaolteanu (2011): Caffeine induced genotoxic effects in Phaseolus vulgaris L. and Raphanus sativus L. Botanical Serbica 35(1): 49-54.

[10] Gaikwad P. D. (1975): Cytogenetic studies in Coriandrum sativum Linn.Ph.D. Thesis in Botany, Faculty of Science, RTM Nagpur University, Nagpur, India.

[11] Girhe S. (1999): Mutational Studies in Lathyrus sativus L. Ph.D.

Thesis in Botany, Faculty of Science, RTM Nagpur University, Nagpur,

India.

[12] Heiner R. E. (1971): Alternations in the nuclear cvcle, mitotic index and chromosomes of *Vicia* as affected by diethyl sulphate. Mut. Res. **12**: 249-254.

[13] Jahagirdar H. A. (1975): Cvtogenetic studies in *Foeniculum vulgare* Mill, Ph.D. Thesis in Botany, Faculty of Science, RTM Nagpur University, Nagpur, India.

[14] Kanaklata (1995): Mutational studies in *Cyamopsis tetragonoloba* Taub. Ph.D. Thesis in Botany, Faculty of Science, RTM Nagpur university, Nagpur, India.

[15] Karapate R. R. (1995): Mutational studies in Linum usitatisissum L. Ph.D. Thesis in Botany, Faculty of Science, RTM Nagpur University, Nagpur, India.

[16] Khanolkar S. M. (1977): Cvtogenetic studies in *Carcum copticum*. Ph.D. Thesis in Botany, Faculty of Science, RTM Nagpur University, Nagpur, India.

[17] Kothekar V. S. (1978): Mutational studies in Solanum nignum L. Ph. D. Thesis in Botany, Faculty of Science, RTM Nagpur University, Nagpur, India.

[18] Landge S. P. (2000): Induced mutations in *Brassica napus* (L.) C.V. Westar. Ph.D. Thesis in Botany, Faculty of Science, RTM Nagpur University, Nagpur, India.

[19] Nasare P. N. and Choudhary A. D. (2000): Effect of physical and chemical mutagens on mitotic activity in root tips of *Ocimum sanctum* Linn. Int. J. Mendel, Vol. **27**: 53-55. [20] Osuii, Julian O. and Owei, Sweet D. Jnr. (2010). Mitotic index studies on *Treculia Africana* Decne. In Nigeria. Australian Journal of Agricultural Engineering. **1(1)**: 25-28.

[21] Pankai P. P., Kumari N. and Priadarshini A. (2014): Evaluation of cytotoxic in *Allium cepa* L. root tips cells. International Journal of Pharmaceutical and Clinical Research. **6(1)**: 36-39.

[22] Paul A., Nag S. and Sinha K. (2013): Cvtological effects of Blitox on root mitosis of *Allium cepa* L. International Journal of Scientific and Research Publications. Vol. **3** Issue 5.

[23] Saniavkumar, Arva S. K., Rov B. K. and Singh A. (2011): The effects of 2,4dichlorophenoxy acetic acid and isoproturon herbicides on the mitotic activity of Wheat (*Triticum aestivum* L.) root tips. Turk J Biol. **34**: 55-66.

[24] Srivastava S. and Jain R. (2011): In-situ monitoring of chromium cvtotoxicity in Sugercane J. Environ. Biol. **32**: 759-763.

[25] Udo I. J., Akpan G. A., and Esenowo I. K. (2014): Cvtotoxic effects of (5) medicinal plants on mitosis in *Allium cepa* root tips. Current Research Journal of Biological Sciences. 6(2): 71-75.

[26] Yasar Kiran and Ahmet Sahin (2006): The effect of cadmium on seed germination, root development and mitotic of root tip cells of Lentic (*Lens culinaris* Medik). World Journal of Agricultural sciences 2 (2): 196-200.

[27] Yasemin E., Halil E. E. and Ali I. I. (2007): Gamma ray reduces mitotic index in embryonic roots of *Hordeum vulgare* L. Advances in Biological Research 1(1-2): 26-28.