Study on 24 Hour Root Tip Cell Division Mitotic and Mitotic Phase Index of *Allium chinense*

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**Abstract:** The plant material (*Allium chinense*) was collected from kitchen garden of Lumami village (Zunheboto district), Akuluto village (Zunheboto district) and from vegetable market of Mokokchung main town (Mokokchung district), Nagaland (North East India). The meristematic mitotic cells dividing as well as non-dividing of root tips were recorded per hour for 24 hours to characterize mitotic index and mitotic phase indices in *A. chinense*. The maximum values of all the indices analyzed were presented as mitotic index, MI (59.85%) and mitotic phases index, MPI [interphase, II (28.19%), prophase PI, (81.25%), metaphase, METAI (22.66%), anaphase, AI (5.23%), telophase, TI (7.99%), cytokinesis, CI (5.52%)]. The correlation between MI and MPI was recorded such as [MI was correlated with METAI (0.601**) at p=0.01, PI and TI was correlated with CI (0.428* and 0.522**) and time (0.631**) at p=0.05 and p=0.01, respectively]. ANOVA has not shown any significant differences among the indices at p=0.05. The goodness of fit regression line (MI, II, PI, METAI, AI, TI and CI) was found as follows R²=0.239, 0.0414, 0.089, 0.2011, 0.0772, 0.568 and 0.4345. The average movement of mitotic and phase index along with residuals (with not much variations) were recorded for the species (per hour/24 hour).

**Key words:** Mitotic stages - Mitotic index - Mitotic phase index - Correlation - Regression - Residuals

**INTRODUCTION**

*Allium chinense* and *A. bakeri* Regel are known as synonyms to each other and both belong to the Alliaceae family [1-2]. It has been reported that *A. chinense* supports sub-genus *cepa* in the section of sacculiferum [3]. It has been suggested that *A. chinense* is an edible uncultivated species of genus *Allium* [4]. *A. chinense* was recorded with different vernacular names around India such as morkhem (Sangtam tribe of Nagaland), tilhou (Meitei tribe of Manipur), rynsun (khasi tribe of Meghalaya) and the world as Chinese *Allium*, Chinese scallion, Japanese scallion or oriental onion. It has been reported that *A. chinense* is a tetraploid (2n=4x=32) plant but some other plants with deviation in chromosome numbers (2n=3x=24; 2n=24 and 2n=33) were also reported by Dutta, M. and M. Bandyopadhyay [3], Srivastava [4], Dubouzet et al. [5] and Gohil and Kaul [6].

In nature, the occurrence and gardening of *A. chinense* was accounted from eastern Asia [7]. Normally, in India, *A. chinense* was found in wild in nature in Himalayan region. Presently, *A. chinense* was grown in almost each and every one’s household kitchen garden and cultivated in field in entire North East region especially Nagaland, Manipur, Arunachal Pradesh, Tripura, Mizoram, Meghalaya, Assam, Sikkim (West Bengal), Darjeeling (West Bengal). In North East of India *A. chinense* has an important place in mount agriculture and used as vegetables [8-10], spices and condiments [11-13], pickles [14] flavors in various food (soups, curries) preparations [15], medicine [16] to cure ailments by local healers [17] and ornamental plant [18,12]. It was reported that a quantity of uncultivated species of *Allium* were used to introduce new desirable traits to fabricate transgenic plants against pest resistance [19]. The whole plant (*A. chinense*) was sold by the poor farmers and family in the confined local markets for generating income and their livelihood in the regions of North East India. The market value and the availability of whole plant, fibrous roots, fresh leaves and inflorescence depends upon the budding time and positive climatic environment of the plant life.

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The literature survey on the species showed that some extent of taxonomical, distribution, morphological and ethno-botanical work has been reported by Fritsch and Friesen [21]. Although a few karyomorphological studies were reported by Sharma and Gohil [22] and Mukherjee and Roy [23], but different authors suggested that data were not sufficient for complete description of the plant [13]. There was no report available on the physiological genetics, morphogenetic life cycle, cell division cycle, phase indices, nuclear and plant cell morphology in *A. chinense* which were important characteristics of a plant cell and helpful to understand the life cycle of a particular plant.

The aim of the present paper is to understand the cell’s life cycle (per hour index/24 hour) of *A. chinense* in term of mitotic and phase indices (mitotic index, interphase index, prophase index, metaphase index, anaphase index, telophase index and cytokinesis index).

**MATERIALS AND METHODS**

The material of whole plant *A. chinense* was collected from kitchen garden of Lumami village, Akuluto village and from vegetable market of Mokokchung town. The plants were checked at regular interval of time (5 d) for growth of the root tips (2-3 cm) long. Root tips were stained with 2% carmine (30 minutes), squashed using conventional root tip squash technique and observed under microscope (Leica) for various mitotic stages (interphase, prophase, metaphase, anaphase, telophase and cytokinesis) and capture them with the help of digital Leica microscope.

The total number of interphase, prophase, metaphase, anaphase, telophase and cytokinesis were recorded from the five different slides for each hour up to 24 hour (number of stages/h/24 h). The mitotic index (MI) and other mitotic phase indices, MPI (interphase index, prophase index, metaphase index, anaphase index, telophase index and cytokinesis index) were calculated from the mean number of stages from five slides up to 24 hours. Mitotic index and other indices were calculated according to the formulae given below.

\[
\text{Mitotic Phase Index} \% = \frac{\text{Number of dividing cells of phases}}{\text{Total number of dividing cells}} \times 100
\]

\[
\text{Mitotic Index} \% = \frac{\text{Total number of dividing cells}}{\text{Total number of dividing cells} + \text{non-dividing cells}} \times 100
\]

The mean number of MI and MPI was supported by statistically such as Mean, Standard Error, Bar graphs, homogeneity test, Correlation, Regression, ANOVA, two periods average movement line at a time and Residual graphs using MS Excel, SPSS and curve expert.

**RESULTS**

The results of mitotic index (MI), mitotic phase indices [interphase index (II), prophase index (PI), metaphase index (Meta I), anaphase index (AI), telophase index (TI) and cytokinesis index (CI)] in *A. chinense* were presented in Figures 1-20 and Tables 1-2.
**Fig. 2:** Goodness of fit regression line (df=4) of mitotic index for 24 hours of *A. chinense*.

**Fig. 3:** Two periods at a time movement average line of total mean of mitotic index for 24 hours of *A. chinense*.

**Fig. 4:** Residual graph of total mean of mitotic index (%) for 24 hours in *A. chinense*.
Fig. 5: Interphase Index (%) for 24 hours in *A. chinense*

Fig. 6: Prophase Index (%) for 24 hours in *A. chinense*

Fig. 7: Metaphase Index (%) for 24 hours in *A. chinense*
Fig. 8: Anaphase Index (%) for 24 hours in *A. chinense*

Fig. 9: Telophase Index (%) for 24 hours in *A. chinense*

Fig. 10: Cytokinesis Index (%) for 24 hours in *A. chinense*
Fig. 11: Goodness of fit regression line (df=4) among interphase, prophase and metaphase indices for 24 hours of *A. chinense*.

Fig. 12: Goodness of fit regression line (df=4) among anaphase, telophase and cytokinesis indices for 24 hours of *A. chinense*.

Fig. 13: Two periods at a time movement average line of total mean of interphase, prophase and metaphase indices for 24 hours of *A. chinense*.
Fig. 14: Two periods at a time movement average line of total mean of anaphase, telophase and cytokinesis indices for 24 hours of *A. chinense*.

Fig. 15: Residual graph of total mean of interphase index for 24 hours in *A.chinense*

Fig. 16: Residual graph of total mean of prophase index for 24 hours in *A.chinense*
Fig. 17: Residual graph of total mean of metaphase index for 24 hours in *A. chinense*.

Fig. 18: Residual graph of total mean of anaphase index for 24 hours in *A. chinense*.

Fig. 19: Residual graph of total mean of telophase index for 24 hours in *A. chinense*. 

A.chinense Residuals

Time (per hour)

Cytokinesis Index

Table 1: Pearson correlation of indices (%) and time for 24 hours in A.chinense.

<table>
<thead>
<tr>
<th>Items</th>
<th>Mitotic index</th>
<th>Interphase index</th>
<th>Prophase index</th>
<th>Metaphase index</th>
<th>Anaphase index</th>
<th>Telophase index</th>
<th>Cytokinesis index</th>
<th>Time</th>
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</thead>
<tbody>
<tr>
<td>Mitotic Index</td>
<td>1</td>
<td>.063</td>
<td>.057</td>
<td>.601**</td>
<td>.229</td>
<td>.190</td>
<td>.053</td>
<td>.160</td>
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<tr>
<td>Interphase Index</td>
<td>1</td>
<td>.164</td>
<td>.043</td>
<td>.373</td>
<td>.078</td>
<td>.043</td>
<td>.136</td>
<td></td>
</tr>
<tr>
<td>Prophase Index</td>
<td>1</td>
<td>.063</td>
<td>.051</td>
<td>.241</td>
<td>.044</td>
<td>.164</td>
<td>.044</td>
<td></td>
</tr>
<tr>
<td>Metaphase Index</td>
<td>1</td>
<td>.172</td>
<td>.103</td>
<td>.043</td>
<td>.215</td>
<td>.343</td>
<td>.044</td>
<td></td>
</tr>
<tr>
<td>Anaphase Index</td>
<td>1</td>
<td>.172</td>
<td>.103</td>
<td>.215</td>
<td>.343</td>
<td>.044</td>
<td>.164</td>
<td></td>
</tr>
<tr>
<td>Telophase Index</td>
<td>1</td>
<td>.522**</td>
<td>.631**</td>
<td>.522**</td>
<td>.631**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokinesis Index</td>
<td>1</td>
<td>.323</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 2: Analysis of variance (ANOVA) among indices (%) for 24 hours of A.chinense.

<table>
<thead>
<tr>
<th>Items</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
</tr>
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<tr>
<td>Mitotic index</td>
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<td>1232.969</td>
<td>23</td>
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<tr>
<td></td>
<td>Within groups</td>
<td>.000</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1232.969</td>
<td>23</td>
</tr>
<tr>
<td>Interphase index</td>
<td>Between groups</td>
<td>986.186</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>.000</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>986.186</td>
<td>23</td>
</tr>
<tr>
<td>Prophase index</td>
<td>Between groups</td>
<td>6373.739</td>
<td>23</td>
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<tr>
<td></td>
<td>Within groups</td>
<td>.000</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6373.739</td>
<td>23</td>
</tr>
<tr>
<td>Metaphase index</td>
<td>Between groups</td>
<td>739.145</td>
<td>23</td>
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<td></td>
<td>Within groups</td>
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<tr>
<td></td>
<td>Total</td>
<td>739.145</td>
<td>23</td>
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<tr>
<td>Anaphase index</td>
<td>Between groups</td>
<td>69.688</td>
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<td></td>
<td>Within groups</td>
<td>.000</td>
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<td></td>
<td>Total</td>
<td>69.688</td>
<td>23</td>
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<tr>
<td>Telophase index</td>
<td>Between groups</td>
<td>107.009</td>
<td>23</td>
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<td></td>
<td>Within groups</td>
<td>.000</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>107.009</td>
<td>23</td>
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<tr>
<td>Cytokinesis index</td>
<td>Between groups</td>
<td>51.242</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>.000</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>51.242</td>
<td>23</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The plant cell’s life cycle were described and discussed in the form of mitotic and mitotic phases index per hour per 24 hour in A. chinense.

The growing root tip of a seedling i.e. the number of dividing meristematic cells may be associated with the actual time period of the mitosis in the cell division cycle. Also, the number of the mitotic cells in one of the division phases is related to the time used by this phase in relation to the total length of the mitosis. Therefore, mitotic index (number of cells in division as a percentage of the total meristematic cells) and phase index (percentage of the cells in the several phases of division) was used as a measure to know the actual time period and time utilized by the phase in mitosis in relation to the total length of cell division cycle.

The total mean number of mitotic index (%) were calculated (mitotic index/hour/24 hour) and maximum (59.85±) and minimum (30.14±) values at (6 AM) and (4 AM) were recorded with approximate mitotic index range (=30 % mitotic index =60%), respectively. The mean number of mitotic index were analyzed for the homogeneity of the data collection (mitotic index/hour/24 hour).
hour) and it was observed that all the data were homogeneous and similar (not diversified) (alphabets, a, b, c). The high mitotic index suggests that cells were dividing at very fast rate and highly active (6 AM) or moderate rate at other time. The high mitotic index also supports the increase in number of mitotic cells which contributes to the growth and development of the plant tissues as well as whole plant (Fig. 1). The results are in agreement with those reported by Khodjakov and Rieder [24]. The total mean number of mitotic index (mitotic index/hour/24 hour) was correlated with other mitotic phase indexes and it was moderately correlated (0.601**) with metaphase index at p=0.05 which supports the number of metaphase stage available at all the 24 hour time and active participation of metaphase index for mitotic index in cell division cycle (Table 1). The analysis of variance (ANOVA) of total mean number of mitotic index was found non significant with mean square value (53.607) and sum of square values between (986.186, 6373.739, 739.145, 69.688, 107.009 and 32.137, 3.03, 4.653 and 2.228) and sum of square values within (0.00) group at p=0.05 respectively (Table 2). The goodness of fit regression line (df=4) was drawn and total mean number of mitotic phase indexes (interphase index, prophase index, metaphase index, anaphase index, telophase index and cytokinesis index) was found non significant with mean square value (42.878, 277,119, 32.137, 3.03, 4.653 and 2.228) and sum of square values between (986.186, 6373.739, 739.145, 69.688, 107.009 and 51.242) and within (0.00) group at p=0.05 respectively (Table 2). The correlation suggested that number of cytokines is cells (production of two daughter cells) has significant role for prophase index as well as telophase index. Also, the maximum number of telophase cells may be observed all the time which contributes for the telophase index (telophase stage/hour/24 hour). It also ensures that once prophase mitotic cells progress through the mitotic cell cycle stages and achieves telophase stage it must undergo for cytokinesis and which completes the mitotic cell cycle (Table 1). The analysis of variance (ANOVA) of total mean number of mitotic phase indexes (interphase index, prophase index, metaphase index, anaphase index, telophase index and cytokinesis index) was found non significant with mean square value (42.878, 277,119, 32.137, 3.03, 4.653 and 2.228) and sum of square values between (986.186, 6373.739, 739.145, 69.688, 107.009 and 51.242) and within (0.00) group at p=0.05, respectively (Table 2). The goodness of fit regression line (df=4) was drawn and total mean number of mitotic phase indexes (telophase index) have shown maximum fit (56.80%) of the line \( y=0.0002x^2-0.0125x+0.2546x^{2}+1.5837x+4.7969 \) followed by cytokinesis index (43.45%), metaphase index.
(20.11%), prophase index (8.9%), anaphase index (7.72%), interphase index (4.14%) of the line \(y = 0.0001x^4 - 0.0066x^2 - 0.0003x + 0.0135x - 0.2248x + 1.443x + 7.3188\), respectively. The regression line of interphase index have shown less percentage of fitting value (4.14%) the reason may be because of the scattered collection of the values of the interphase cells per hour per 24 hour (Fig. 11-12). The two period’s average line at a time movement was drawn and the movement of the mitotic phase indexes (interphase index, prophase index, metaphase index, anaphase index, telophase index and cytokinesis index) was mixed as low, high or elevated and sometimes almost constant. The constant nature of mitotic phase indexes (interphase indexes and metaphase) movement supports that most of the mitotic cells were in resting stage \((G_0, G_1, S \text{ and } G_2)\) and almost constant or less in number (as in metaphase index) for the synthesis and preparation for the next cell division cycle (resting stage/hour/24 hour) (Fig. 13-14). The residual graph showed that most of our collected data (mitotic phase index per hour per 24 hour) more or less near to the residual line and showed constancy or similarity in data collection. The residuals of mitotic phase index were less disturbed or more acceptable as all the residual points were very near or close to the residual line. Also, some more standardized data collection method required to convert into perfect standardized data which may be very near or close or overlap to the residual line (Fig. 15-20).

**CONCLUSION**

It can be concluded that the study of *A. chinense* life cycle may provide the information of its growth pattern and best time of cell division which could be utilized (by selection of best timing and high indexes) to cultivate the species at large scale in North East as well other parts of India. Also, it can be utilized to increase the number of plants at kitchen garden which is a source of generating income and their livelihood for poor people.

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**REFERENCES**


