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OPTIMAL CONDITIONS FOR MAXIMUM MITOTIC INDEX IN ONION ROOT TIPS: INFLUENCE OF HARVESTING TIME AND ROOT AGE IN LABORATORY SETTINGS

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ABSTRACT

Utilizing the actively growing root tips of onions and sprouting lentils has become a standard practice in science laboratories for elucidating the various stages of mitotic cell division. Though the technique is widespread with well-established protocols, we noted inconsistencies in the distribution of cell division stages during morning and afternoon sample preparation done for lab practical sessions. This led us to investigate whether specific factors, such as the time of day or the age of the root, influence theprevalence of different mitotic stages. In this study, we sought to standardize the harvesting time, and age of onion root tips to obtain a maximum mitotic index. The roots were stained and meticulously observed three times a day from day 1 to 9 since root induction. Concurrently, root growth parameters were also recorded. There was no notable distinction in the mitotic index across days for both red onions and shallots. We noted a substantial presence of mitotic stages on day 6 in red onions. Frequency of mitotic stages were comparatively higher in the morning than afternoon and evening. Our findings indicate that the best time for observing peak mitotic stages is during the mornings of days 5 through 7.

1.INTRODUCTION

Somatic cells of eukaryotic organisms undergo mitotic cell division for an organism to grow and develop or to replace dead or lost cells by a process called mitosis (Walther Flemming 2023). Onion (*Allium cepa)* root tips have been widely used to demonstrate mitotic cell stages worldwide since the time mitosis was reported by Walther Flemming, a German biologist (Paweletz 2001; Yanagida 2014). In the beginning of the cell cycle, a cell takes maximum time to grow and multiply its genetic material to prepare it for the mitotic stage, which is called interphase. This is followed by mitotic division, which consists of four stages: prophase, metaphase, anaphase, and telophase (McIntosh and Hayes 2016; NHGRI 2024). However, the duration of mitosis varies based on cell type and species (Winter 1929). Previous studies have reported differences in the mitotic index at different time intervals on the same day in onion cells (Stephens Charles 1984). Though onion root tips are widely used by academic researchers to demonstrate cell division, there is no systematic study that focused on the age of the root and harvesting time to observe the optimum mitotic index., This makes it challenging to complete the experiments within scheduled time frames. Therefore, this study aims to provide an optimized harvesting time and age of onion roots, for teachers and educators who are seeking to achieve the maximum mitotic index. This optimization process provides more insights to effectively prepare for the demonstrations or engage the students in a reform-based learning environment, where they work together to resolve conceptual and procedural inconsistencies during experimentation by reasoning their hypotheses.

METHODS

ROOT INDUCTION

On day 0, three onion bulbs, each measuring approximately 5 cm in diameter, were kept for rooting in a 100 ml glass beaker by securing them with toothpicks in three different directions so that only the basal part of the onion bulb touches the water. Prior to immersion, the thin outer layer was removed from the basal portion and pricked in 5-6

places with the toothpick (Image. 1a). After 24 hours, (day 1), the number of roots, root length, and cell division stages were measured every day from day 1 till day 9. For each day these observations were recorded 3 times (9 am, 12.30 pm, and 4 pm) with an interval of 3.5 hours.

ONION ROOT TIP STAINING

To examine the cell division stages, onion root tips with meristematic cells measuring approximately 2 mm in length were collected in a watch glass containing acetocarmine (catalogue #A35671, Nice Chemical) stain. The underside of the watch glass was heated over a spirit lamp flame until the stain began to fume. The roots were left in the stain for 45 minutes at room temperature. Subsequently, the stained roots were transferred to a clean glass slide and covered with a coverslip. The slide was sandwiched between 2-3 layers of tissue paper, and the root tips were squashed to make a thin smear by gently rubbing the coverslip with the help of the blunt end of a pair of forceps. The sample slide was observed under an Olympus (CH20i) microscope, initially at lower magnification (10X air objective lens), followed by higher magnification (100X with oil immersion objective lens). The number of cells in interphases, prophase, metaphase, anaphase, or telophase stages were recorded per 100 cells (Image. 1b). This was repeated for sample slides prepared from 3 onion bulbs. A total of 300 cells from three independent onion bulb samples were counted at three-time points during the day – morning, afternoon, and evening. An average mitotic index across 300 cells was calculated for each time point.

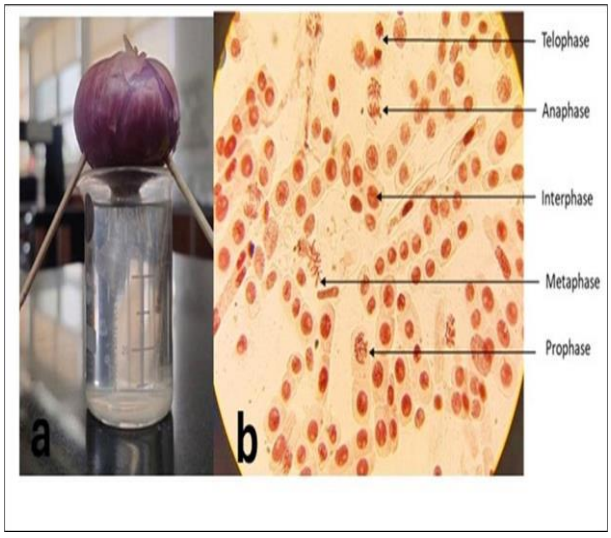


Image 1. a. Onion bulb in a beaker containing water for rooting; b. Cell division stages observed in the acetocarmine-stained root tip of red onion.

CALCULATION AND STATISTICAL ANALYSIS

The mitotic index per day was calculated by adding the number of cells in mitotic stages and dividing the sum by total number of cells counted – i.e. Number of cells in mitotic phase (prophase + metaphase + anaphase + telophase) / Total number of cells. Mean mitotic index across days (day 1 through 9) was compared using one-way ANOVA with days as factor, and at $p < 0.05$. Similarly for small onions (shallots), one-way ANOVA with days as factors was performed on mitotic index values obtained from day 5 – day 8 of experimental preparation. To determine the best day which has maximum occurrence of all the mitotic stages, Shannon diversity index ($H = -\Sigma pi*lnpi$) was calculated considering each mitotic stage as an individual species and each day as a plot.

RESULTS

The onion bulbs kept in the glass beaker containing water produced roots after 24hrs of incubation with an average root length of 1.19 cm. It was interesting to note that the root length increased only at night,

not during the daytime except for the 1st and 2nd days, which had a very negligible increase (0.1 to 0.2 cm). There was a constant increase in the root length from day 1 to day 4 with an approximate increase of 1 cm per day. The growth rate reduced from 5th day onwards and entered deterioration from day 9 onwards. The average root number after 24 hrs was 42 and the number was doubled after 48hrs. There onwards, there was reduction in the new root induction and maximum (106.3) roots were observed on the 6th day (Table 1).

Table 1: Average number of roots and root length observed in the red onions from day 1 to 9.

	Average number of roots (RN) and root length (RL) in cm																	
Time	Day1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9	
	RN	RL	RN	RL	RN	RL	RN	RL	RN	RL	RN	RL	RN	RL	RN	RL	RN	RL
Morning (9 am)	42	1.13	75.3	1.9	88	2.3	92.3	3.36	95	4.33	99.3	4.6	103	5.4	99.6	5.73	95.33	5.73
Afternoon (12.30 pm)	43.6	1.23	79	2.03	89	2.33	92.3	3.36	95	4.33	101	4.6	105.6	5.56	100	5.73	100.66	5.6
Evening (4pm)	43.6	1.23	79.3	2.2	89	2.33	95	3.36	95.6	4.33	101	4.6	106.3	5.63	108.3	5.73	100.66	5.46

Upon staining and observation, we found that mitotic stages were consistently present across all observed days and time points. However, there was a noticeable predominance (79.1 %) of cells in interphase compared to those undergoing mitotic division throughout the observation period. Maximum (14.77 %) of mitotic cells were in prophase followed by metaphase (3.01 %) and anaphase (1.83%). Telophase, conversely, exhibited the lowest (1.27 %) occurrence across all observed days (Fig 1).

Table 2: The mean proportion of cells (in percentage) in various mitotic stages observed during morning, afternoon, and evening periods from day 1 to day 9 of root induction in red onion.

	Percentage (%) of Cell division stages observed								
Mitotic Phases	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Interphase	84.11	80.55	80.11	79.11	79.11	76.44	74.11	74.33	84
Prophase	13.89	14.55	13.22	14.22	15.33	14.44	17.33	18.44	11.55
Metaphase	1.22	3.11	4.11	3.33	1.77	3.55	4.22	4.11	1.66
Anaphase	0.44	1.11	1.77	2.55	2.22	2.66	2.66	1.44	1.66
Telophase	0.33	0.66	0.77	0.77	1.55	2.88	1.66	1.66	1.11

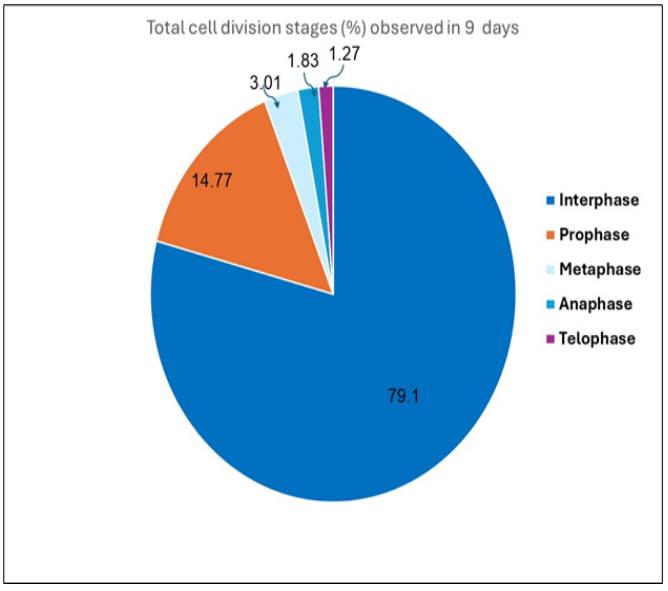


Fig 1: Average percentage of cell division stages observed across 9 days and all three-time points in red onion samples

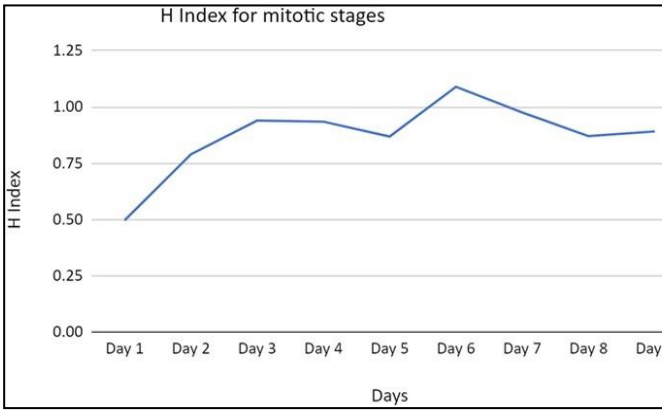
Table 3. Day wise mitotic index of red onion and shallot.

Data represented in the table are mean ± SE (Standard Error) scored at different days of observation from the 100 cells each from 3 onions, 3 times a day. Numbers with similar alphabets are not significantly different.

The mitotic index exhibited fluctuations across observed days, reaching its peak (25.8 %) on day 7, followed closely by day 8 (25.6 %). However, statistical analysis revealed that the mitotic index was not different between these peak days or when compared to all other observed days (one-way ANOVA with days as factor, p = 0.144466). The Shannon diversity index, employed to assess the prevalence of all four mitotic stages across different days, indicated a gradual increase in stage frequency from day 1 to day 4, with a slight decline noted on day 5. Notably, day 6 recorded the highest abundance of mitotic stages (Figure. 2).

To discern whether various onion species demonstrated similar mitotic index trends, the roots of small onions or shallots were stained and examined from days 5 to 8 of root induction. The mitotic index rangedfrom 14% on day 5 to 16.7 % on day 8 (Table 3) but was not significantly different across days (one-way ANOVA with days as factor, p = 0.354808). In comparison to the H index of red onion and shallots, red onions exhibited a slightly higher abundance of mitotic stages (Figure. 3) across day 5 through day 8.

Given that day 6 was the best day to observe the abundance of all mitotic stages, we wanted to further refine the time of the day that was most suitable to harvest cells in other mitotic stages. Since most of the observed cells were in prophase (14 %) at all time points, we performed a comparative analysis of data collected during morning, afternoon, and evening periods for the three subsequent mitotic stages: metaphase, anaphase, and telophase. Our data shows that these mitotic stages were more abundant in the morning slot and less in the evening slot for all three days of observation from day 5 through day 8(Figure 4). Specifically, on day 5 and day 7, the highest frequency of mitotic cells was observed in the morning slot and only for day 6, the highest frequency of mitotic cells was observed in the afternoon slot (Figure 4).



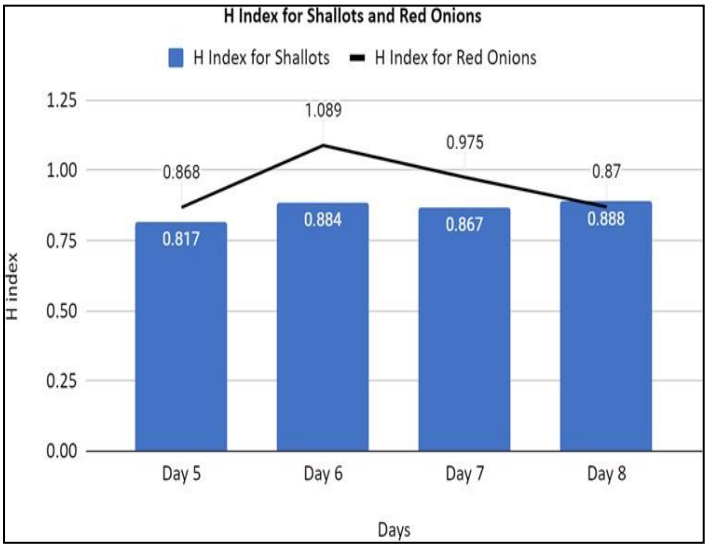


Figure 3: Comparison of Shannon's index for diversity of mitotic stages in red onions and shallots from day 5 to day

Day	Mitotic index	Mitotic index %	Mitotic index	Mitotic index %
	Red onion		Shallot	
DAY 1	0.16 ± 0.02 a	15.8	-	-
DAY 2	0.19 + 0.05 a	19.4	-	-
DAY 3	0.20 ± 0.05 a	19.8	-	-
DAY 4	0.21 ± 0.02 a	20.8	-	-
DAY 5	0.21 ± 0.02 a	20.8	0.14 ± 0.02 a	14
DAY 6	0.24 ± 0.02 a	23.5	0.20 ± 0.01 a	20.1
DAY 7	0.26 ± 0.01a	25.8	0.16 ± 0.03 a	16.3
DAY 8	0.26 ± 0.02 a	25.6	0.17 ± 0.02 a	16.7
DAY 9	0.16 ± 0.02 a	16	-	-
F value	1.793469		1.248598	-
P- value	0.144466		0.354808	-
∞ =0.05				-

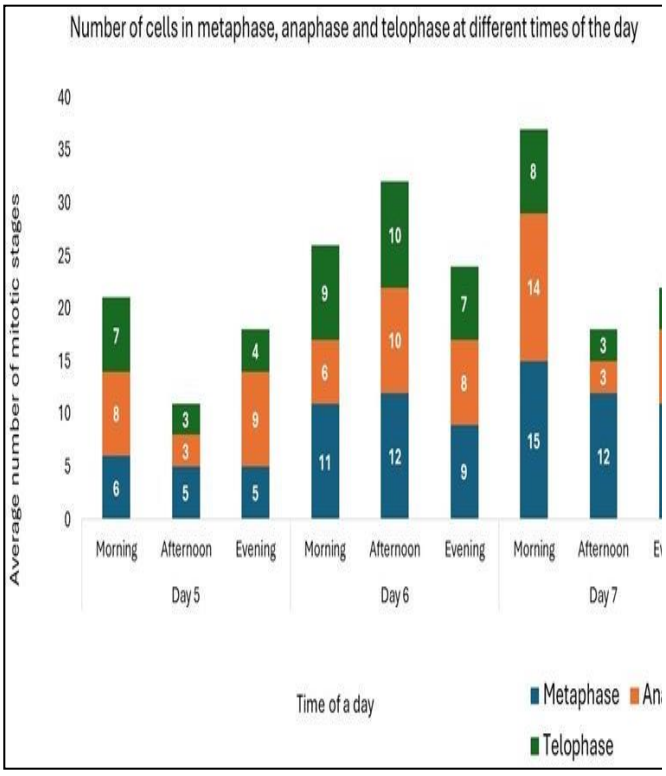


Figure 4: Number of cells in metaphase, anaphase, and telophase on the 5th, 6th, and 7th day with respect to time.

Figure 2: Shannon's H- index diversity for mitotic stages in red onions from day 1 to day 9.

DISCUSSION

The optimal timing of mitotic division in plants remains a subject of debate among researchers, with varying rates observed throughout the day across different species (Winter 1929). Various factors can influence the cell division rate –such as temperature, light, stress, nutrition, endogenous hormones, etc. In our study, we observed a consistent average increase of 1 cm in root length per day for up to 4 dayswith prophase being the dominant phase of the cell cycle. These findings align with previous observationsby Tylar and Kang 2021, and Alfy and Leblond 1987 who reported a growth rate of 0.94 cm per day over a 3-day period and where prophase also constituted the highest percentage in their study. Another studyby Soltys et al., 2011 reported onion root tip growth of 2.3 cm across 6 days viz 0.38 cm per day. The variation in root length observed across different studies may be attributed to fluctuations in rooting conditions affected by physical parameters. The initial growth of germinated *A. cepa* seeds, as observed by Birdsall and Macleod (1990) and McIntosh and Hays (2016) showed maximum root induction followed by a slight decrease, aligning with our findings where the root number doubled in the initial two days before slowing down. Over a span of 9 days in our investigation, there was no notable disparity inthe mitotic index. Likewise, rapidly dividing onion root cells exhibited a consistent division rate in different times of the day and in the initial days, as noted by Lewis (1901) and Bryant (969b). Interphase predominated over other cell cycle stages (~ 80 %), emphasizing its role as the synthetic phase preparing cells for division, and is notably the longest phase of the cell cycle. Similarly, Stephens (1984) reported interphase predominance in *A. cepa*. Most studies examining cell division in onion root tip meristematic cells have primarily focused on analyzing the mitotic index across various time periods within a day, rather than emphasizing the abundance of different stages on a specific day. In our observations, we foundthat all mitotic stages were notably abundant on day 6. Further, by performing minute analysis of the best time slot to observe all mitotic stages, we report that different mitotic stages were more abundant in the morning than in the evening.

In conclusion, we recommend that the study of mitotic cell stages using onion root tip samples should be ideally performed within day 5 to day 7 of root induction, and preferably in the morning slots. We hope that this research offers valuable insights for educators to enhance their instructional strategies and align their timetables to maximize the learning outcome from the study of mitotic stages. This researchstemmed from our observations that there was fluctuation in mitotic stages observed in practical sessions held in the morning vs afternoon timings. These simple, and reproducible set of experiments can be adopted in the science labs to empower students to engage in projects where they can explore, modify, and test various parameters such as temperature and light, instead of merely showing them the cell division stages.

AUTHOR CONTRIBUTIONS: BDB conceptualized and designed the experiments, performed analysis, and wrote the manuscript. AK and AGM carried out the experiments and arranged the data.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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