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Effect of Induced Polyploidy on Morphology, Antioxidant Activity, and Dissolved Sugars in *Allium cepa* L.

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Abstract: The role of onion as the second most-consumed and cultivated vegetable around the world and its renowned qualities that lead it to be called the "queen of the kitchen" have positioned it as a vital source of nutritional and economic contributions around the world. Polyploidy serves as a groundbreaking innovation in plant breeding, improving the yield and vigor of plants. This study was conducted to determine the effects of applying different concentrations of colchicine to onion seedlings on their morphology, antioxidant activity, and dissolved sugars. The mutagen was applied to the onion seeds at three different concentrations (0.05, 0.1, and 0.2% *w*/*v*) for an exposure period of 24 h. A chromosomal analysis confirmed the induction of polyploidy, which led to the successful duplication of the chromosome number from diploid (2n = 16) to tetraploid (4n = 32). The control recorded a survival rate of 91.57%, while 83.33%, 3.33%, and 0.00% survival rates were recorded for seedlings treated with 0.05%, 0.1%, and 0.2% (*w*/*v*) concentrations of colchicine, respectively. Furthermore, the tetraploids showed significant differences in morphology, producing the tallest seedlings (reaching up to 73.6 cm tall) and the greatest average bulb diameter (of 5.64 cm) after 14 weeks. The tetraploids also showed significant differences in antioxidant activity and the amount of dissolved sugars, recording the highest DPPH scavenging percentage of 72.58% and refractive index of 1.369. Successful induction of polyploidy was achieved with the application of 0.05% (*w*/*v*) colchicine, which produced tetraploids that are morphologically and biochemically superior to other treated and control plants at a significance level of $p < 0.05$.

Keywords: colchicine; diploid; tetraploid; spectrophotometry; refractive index; karyotyping

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1. Introduction

Allium cepa, commonly known as onion, is a vegetable that is the most widely cultivated species of the genus *Allium*. It has been valued as a food and a medicinal plant since ancient times and is a target vegetable crop worldwide. Its harvested area is about 4 million hectares, having yields greater than 85 million tons and productivity of 20,000 kg/ha in 2013, with China and India being the main producing countries [1]. It is also commonly known as the "queen of the kitchen" due to its highly valued flavor, aroma, and unique taste, as well as the medicinal properties of its flavor compounds [2]. Medicinally, onions

are used to cure many diseases, including cough, cold, and asthma [3]; cardiovascular diseases and heart attacks [4]; skin diseases and inflammatory swelling [5]; gastrointestinal infections; nausea; and constipation [5]. Onions are also used to reduce frequent urination problems [6] and to build and maintain collagen, which is of great help to give structure to hair and skin [7]. Onion is used in curries, in the form of spices; in salads, as a condiment; or cooked with other vegetables (e.g., boiled or baked). It is also used in different forms of processed food, such as pickles, powder, paste, and flakes. Vegetables are full of essential vitamins, minerals, and antioxidants that provide many important health benefits to the body [8].

Plant biotechnology is changing the agriculture industry [9]. Advances have produced crops that are resistant to certain diseases (thus resulting in higher yields), can grow in extreme soil conditions (e.g., arid or salty environments), and are infused with higher levels of nutrients [10]. Biotechnological innovation has the potential to increase agricultural productivity and quality, ultimately raising incomes for farmers across the world. Colchicine is a toxic chemical that is often used to induce polyploidy in plants [11]. Colchicine prevents microtubule formation during cell division; thus, the chromosomes do not pull apart, as they normally do [12,13]. In general, polyploid individuals have bigger cells that eventually develop larger organs, such as larger flowers, fruits, and seeds, and often have improved traits compared to their diploid relatives; therefore, they may offer greater advantages for immediate use in cultivation [14]. Polyploids may be induced through either sexual polyploidization, which generates 2n gametes, or somatic doubling, which is based on the mitotic doubling of somatic cells [15]. In the last century, somatic duplication using various mitotic inhibitors was the most widely used method for induction of polyploidy, and colchicine—an alkaloid extracted from meadow saffron—is a commonly used antimitotic agent for polyploidization [16]. Efficient polyploidy induction depends on various factors such as the type and concentration of antimitotic agent used, exposure time, and type of plant [17]. Polyploidy manipulation for improved production of secondary metabolites has been previously reported [18–20]. Antioxidants are vital in combating free radicals, which can damage human cells under 'oxidative stress' conditions and, as such, an imbalance in free radicals may cause grave disturbances in cell metabolism. Free radicals are unstable species as they have unpaired electrons and seek stability through electron pairing with biological macromolecules. The proteins, lipids, and DNA of healthy human cells are good sources for these pairing electrons. Thus, oxidative stress conditions can cause DNA and protein damage, lipid peroxidation, cancer, atherosclerosis, aging, and inflammatory diseases [21,22]. Sources of free radicals include metabolism by-products, neutrophiles, UV, radiation, air and water pollutants, fatty foods, hazardous chemicals, and cigarette smoke. Natural antioxidants such as dietary plant flavonoids are increasingly attracting attention. They are natural disease-preventing, health-promoting, and anti-aging substances. Flavonoids are essentially ingested through food rather than being metabolically synthesized. Flavonoids are plant secondary metabolites widely distributed in the plant kingdom, and more than 6000 flavonoids have been identified in plants [23]. Antioxidant capacity/activity assay methods in the existing literature based on measurement of the radical scavenging activity of antioxidant compounds suffer from the difficulties encountered in the formation and stability of colored radicals [24], such as ABTS (2,2_-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) [25], and DPPH (2,2_-diphenyl-1-picrylhydrazyl) [26], the former being more dependent on the type of reaction used for radical generation (i.e., enzymatic or chemical).

Onions contain numerous phytomolecules, such as polyphenolic substances, phenolic acids, flavonoids (fisetin, quercetin) [27–30], ascorbic acid, and sulfur compounds [31,32], which are responsible for their color, flavor, and aroma, as well as their possible health

advantages. In addition to making a significant nutritional contribution to the human diet, onions also have medicinal and functional properties [33]. As with other vegetables, onions contribute to the intake of certain vitamins and minerals; however, carbohydrates are the major nutrient fraction, many of which are components of dietetic fiber. Non-structural and soluble carbohydrates form a substantial part of the dry matter of onions, mainly as fructo-oligosaccharides (FOS) and monosaccharides (glucose, fructose, and sucrose) [34].

This study aimed to induce polyploidy in onion (*Allium cepa*) seedlings through colchicine treatment, thus producing tetraploid varieties. These tetraploids were evaluated in terms of their improvements in morphological development, higher antioxidant activity, and increased dissolved sugar content.

2. Materials and Methods

2.1. Induction of Polyploidy

Onion Stuttgarter Riesen seeds were sourced from Siberian Varietal Seeds LLC, Novosibirsk, Russia. A stock solution of colchicine (tablets from AlPharma, Moscow, Russia) at a concentration of 0.2% (*w*/*v*) was prepared, followed by serial dilutions to achieve concentrations of 0.1% (*w*/*v*) and 0.05% (*w*/*v*). The seeds were individually immersed in these colchicine solutions (0.05%, 0.1%, and 0.2%) for 24 h. Following exposure, the seeds were thoroughly rinsed with tap water to eliminate any residual colchicine. Cocopeat was utilized to create seedbeds in which the germinated seedlings were nurtured for 4 months. Data collection commenced 1 week post-sprouting, focusing on the morphological parameters (height and number of shoots and bulb size) of the seedlings, measured at two-week intervals. The results were calculated as means and standard deviations, and the survival rate under each treatment was also assessed during this period.

2.2. Karyotyping

Carmine (Interhimmet LLC, Saint Petersburg, Russia) solution was prepared by dissolving 5 g of carmine in 45 mL of 70% ethanol. The root tips from both treated and control groups were placed in labeled vials containing cold water at varying concentrations (0.05%, 0.1%, 0.2%, and a control), and the vials were stored in a refrigerator at 8 °C for 24 h. To fixate the roots for easier examination, they were subsequently immersed in 1 N HCl for 20 min, which softened the tissue. After fixation, the roots were thoroughly rinsed in distilled water to remove any residual HCl. The tips were then sliced to discard the root caps, retaining only the apical meristem, and further symmetrically dissected using a sterilized razor blade. The prepared root samples were then placed in the carmine solution for 10 min to facilitate staining. Following this, the roots were positioned on a microscope slide, warmed briefly over a spirit lamp to enhance the uptake of the stain, and then covered with a coverslip. A gentle squash technique was applied to spread the root tissue evenly. Observations of the chromosomes were performed using the OMAX M834 Series microscope (OMAX Corporation, Washington, DC, USA) at 100× magnification with oil immersion, and images were captured for karyotyping analysis.

2.3. Extraction (Maceration)

Samples from the bulbs and shoots of treated onion seedlings (at concentrations of 0.05%, 0.1%, and 0.2%), as well as control samples, were dried separately in the shade and then ground into a powder using an electric blender. To facilitate the immersion of the samples and the diffusion of compounds into the solvent, 10 g of each sample was accurately weighed using a digital balance and transferred to a flask. Following this, 150 mL of 70% ethanol was added [35] to achieve a specific sample-to-solvent ratio of 1:15. The flasks were then sealed with foil paper and stored in a dark container for 24 h. After

this incubation period, the mixtures were filtered through the Whatman filter paper to obtain the respective extracts. The extracts were subsequently placed in 50 mL Eppendorf vials, labeled, and stored in a refrigerator for future use.

2.4. Antioxidant Capacity Determination

The extracts obtained from the samples (bulbs and shoots from both treated and control groups) underwent a free radical scavenging assay using DPPH (2,2-diphenyl-1-picrylhydrazyl). Initially, the spectrophotometer was calibrated by performing a serial dilution of ascorbic acid (the standard), resulting in five different concentrations that were measured for their absorbance at a wavelength of 517 nm. These absorbance values were used to calculate the percentage of inhibition, which facilitated the plotting of a calibration curve for ascorbic acid. A stock solution of DPPH was prepared by dissolving 8 mg of DPPH in 200 mL of 70% ethanol. From this stock solution, 3 mL was pipetted into a vial, to which 150 µL of distilled water was added, and the mixture was then stored in a dark container for 30 min. After this incubation period, absorbance was recorded at 517 nm using a spectrophotometer (Shimadzu Corporation, Tokyo, Japan). Additionally, a stock solution of ascorbic acid was prepared by dissolving 1.69 mg in 100 mL of distilled water, yielding a 1 mM concentration of ascorbic acid as the standard. Serial dilutions were conducted to create four concentrations ranging from 1 mM to 0.125 mM. The absorbance for each concentration of ascorbic acid was subsequently recorded at the same wavelength. For the extracts, 150 µL of each sample was placed in a vial, followed by the addition of 3 mL of DPPH solution. The mixture was thoroughly mixed and then stored in a dark container for 30 min, after which the absorbance for each sample was recorded at 517 nm using the spectrophotometer. Ethanol was used as the blank for these measurements, and absorbance readings were taken in triplicate for each sample and standard. The radical scavenging activities were calculated based on the percentage of DPPH scavenged using the following formula:

$$\%\ of\ Inhibition = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

2.5. Dissolved Sugars

The concentration of dissolved sugars (glucose, fructose, and sucrose) was determined using an Abbe refractometer (Carl Zeiss, Oberkochen, Germany). First, the device was calibrated with a standard mixture of these sugars. Dilutions were prepared to create five different concentrations. The refractive index for each concentration was recorded, and a graph was plotted to illustrate the resulting curve. To calibrate the refractometer, a few drops of distilled water were placed on the prism using a dropper, and the knob was adjusted until the scale read 0.000 for the refractive index. Next, the extracts were thoroughly shaken to ensure an even distribution of sugars from the bulbs and shoots of the samples. A few drops from each sample were then applied to the prism of the refractometer, followed by adjusting the knob until the dark field was aligned with the crosshairs. The refractive indices for the samples were recorded at a wavelength of 589 nm and a temperature of 20 °C. For improved accuracy, three measurements of the refractive index were taken from each sample to calculate the mean value.

2.6. Data Analyses

Data on morphology (shoot height and bulb size), karyotyping, antioxidant activities, and dissolved sugar content were collected from each replicate. The results are presented as mean values ± standard deviations (SDs). Analysis of variance (ANOVA) was used to compare the means, and statistical significance for all comparisons was determined

using Fisher's PLSD at $p < 0.05$. The analyses and graph plotting were performed using Microsoft Excel® 2019 (version 16.0, Microsoft Corporation, Washington, DC, USA), while karyotyping was conducted using the KaryoMeasure software (version 1.7.5.0).

3. Results

3.1. Survival Rate

Germination occurred 6 days after sowing, with the recorded survival rates being 83.33%, 33.33%, 0.00%, and 91.67% for the 0.05% (*w*/*v*), 0.1% (*w*/*v*), and 0.2% (*w*/*v*) concentrations of colchicine, along with the control group, respectively. Seeds treated with 0.2% colchicine did not germinate, while seedlings exposed to the 0.05% colchicine concentration demonstrated the highest survival rate among the treated groups. Furthermore, the control group exhibited the highest overall survival rate, as illustrated in Figure 1. Consequently, all further analyses in this study were conducted using the seedlings treated with 0.05% and 0.1% colchicine concentrations, as well as the control group.

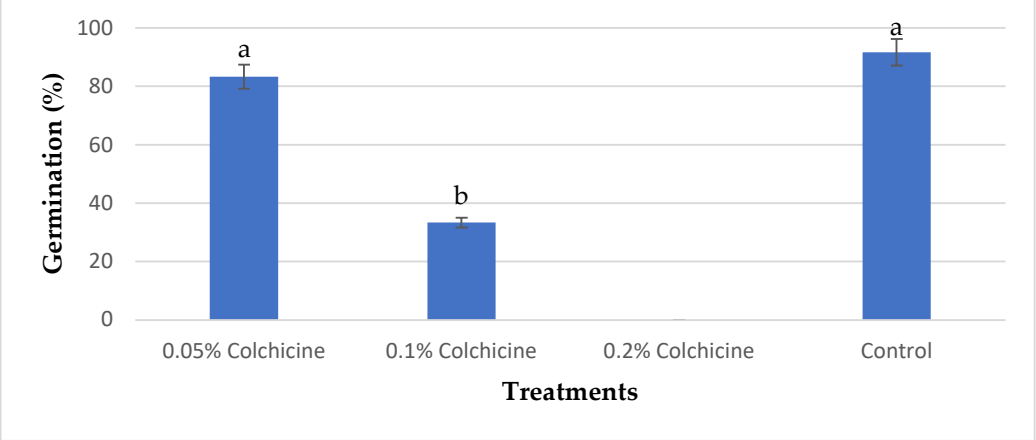

Figure 1. Survival rates (%) of seedlings treated with different concentrations of colchicine (0.05%, 0.1%, and 0.2%) compared to the control group (bars represent the standard deviation of the mean). Different superscripts above the bars indicate significant differences at $p < 0.05$, determined using Fisher's PLSD.

3.2. Morphological Parameters

After germination, morphological parameters (height of the shoots and the diameter of the bulbs) were derived from the seedlings and control, followed by analyses. Table 1 represents the analysis of variance for the height of seedlings at the significance level of $p < 0.05$, in which seedlings treated with 0.05% colchicine showed a significant difference in comparison to other treatments and control.

Table 1. Analysis of variance (ANOVA) results comparing treatments and control groups at a significance level of $p < 0.05$. The results indicate significant differences between groups, with an F-value of 5.239 and a *p*-value of 0.0097, exceeding the critical F-value of 3.238.

Source of Variation	SS	df	MS	F	*p*-Value	F crit.
Between groups	2487.609	2	1243.805	5.239149	0.009653	3.238096
Within groups	9258.827	39	237.4058			
Total	11,746.44	41				

The growth was faster in the seedlings treated with 0.05% colchicine, with their shoots recording greater height (as presented in Figure 2); they even started producing flower buds toward the end of the twelfth week, while other seedlings (0.1% colchicine and control) showed no flower formation within the same period. The shoots from 0.05% colchicine

treatment were also broader and showed more physical vigor than the other seedlings. However, no significant differences were observed in the number of shoots between the treatments and the control over the 14-week period, with data collected at 2-week intervals (as illustrated in Figure 3).

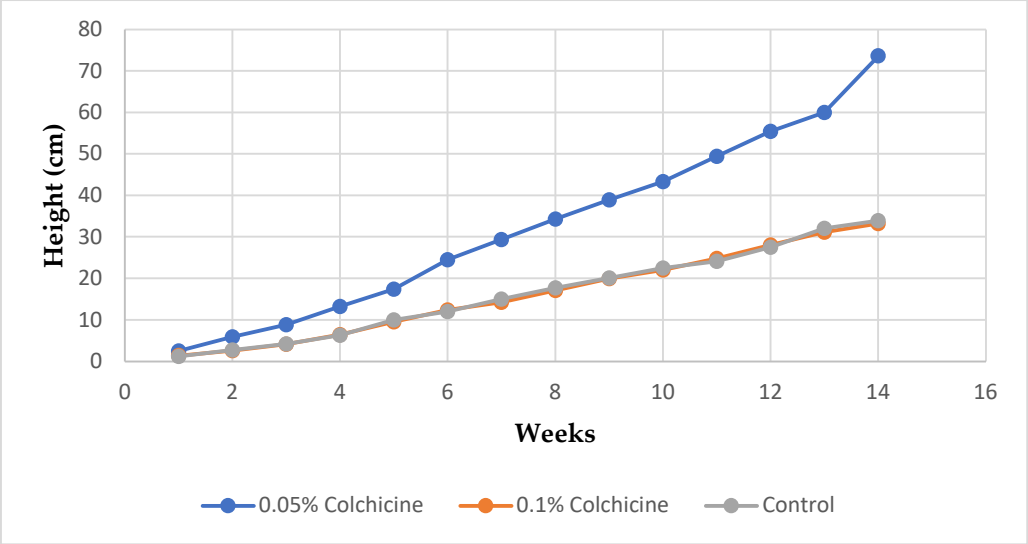

Figure 2. Shoot height over time for plants treated with 0.05% colchicine, 0.1% colchicine, and the control group. Means from seedlings treated with 0.05% colchicine showed significant difference at $p < 0.05$, determined using Fisher's PLSD.

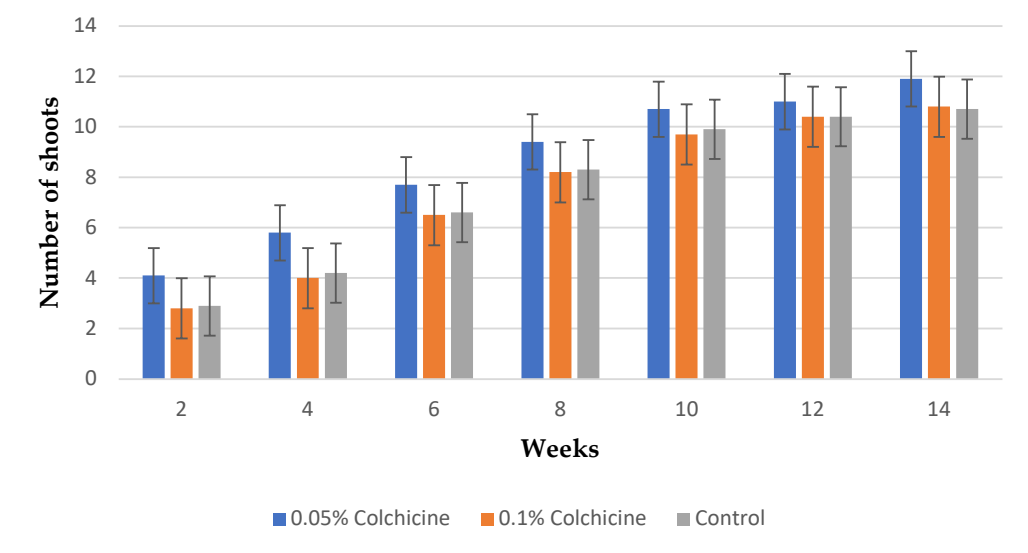

Figure 3. Number of shoots over time for plants treated with 0.05% colchicine, 0.1% colchicine, and the control group (bars represent the standard deviation of the mean). The means of bars with intersecting error bars for each sampling week are significantly similar at $p < 0.05$, determined using Fisher's PLSD.

Further growth superiority was observed in the bulbs of the seedlings treated with 0.05% colchicine, which recorded the highest mean and showed a significant difference (Figure 4). In comparison, seedlings treated with 0.1% colchicine and the control showed no significant differences after the 14-week growth period.

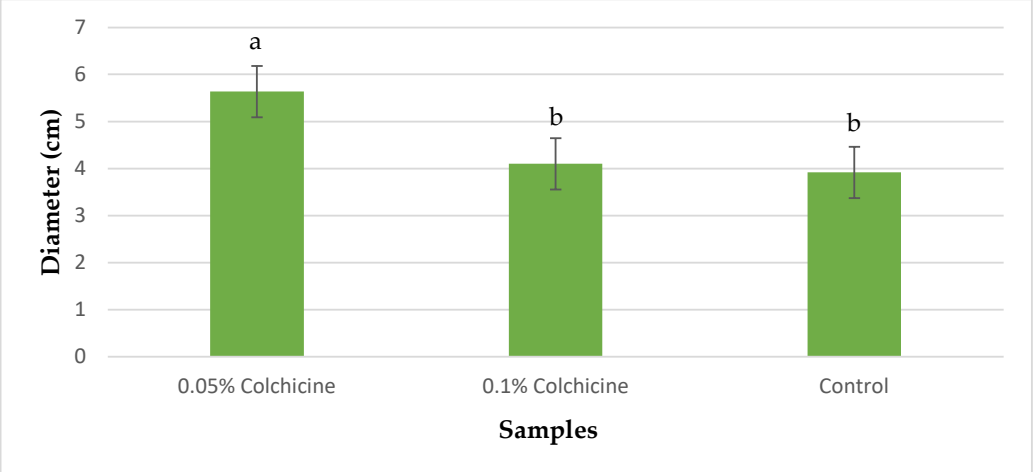

Figure 4. Bulb size over time for plants treated with 0.05% colchicine, 0.1% colchicine, and the control group (bars represent the standard deviation of the mean). Different superscripts above the bars indicate significant differences at $p < 0.05$, determined using Fisher's PLSD.

3.3. Chromosome Counting

Karyotyping was performed using the root tips from the 0.05%, 0.1%, and control seedlings. Mean values were recorded from the haploid chromosome numbers of the seedlings. It was clear that seedlings treated with the 0.05% concentration of colchicine showed twice the number of chromosomes (2 × 2n = 4n) when compared to the 0.1% concentration and control (with chromosome number 2n). During the karyotyping, the software (KaryoMeasure) was calibrated at 10 µm to accurately read the chromosomes' parameters. Chromosome pictures were represented using an idiogram to give a clear picture of the chromosomes from treated seedlings and control. Pictures from seedlings treated with a 0.05% concentration of colchicine showed 16 haploid chromosome numbers (n = 16) during the karyotyping, while the 0.1% colchicine concentration and control seedlings showed 8 haploid numbers of chromosomes (n = 8). After karyotyping, means for chromosome length were generated from the cells of the samples, which showed variations in chromosome length with a noticeable difference in chromosome haploid number for seedlings treated with 0.05% colchicine, as presented in Figure 5.

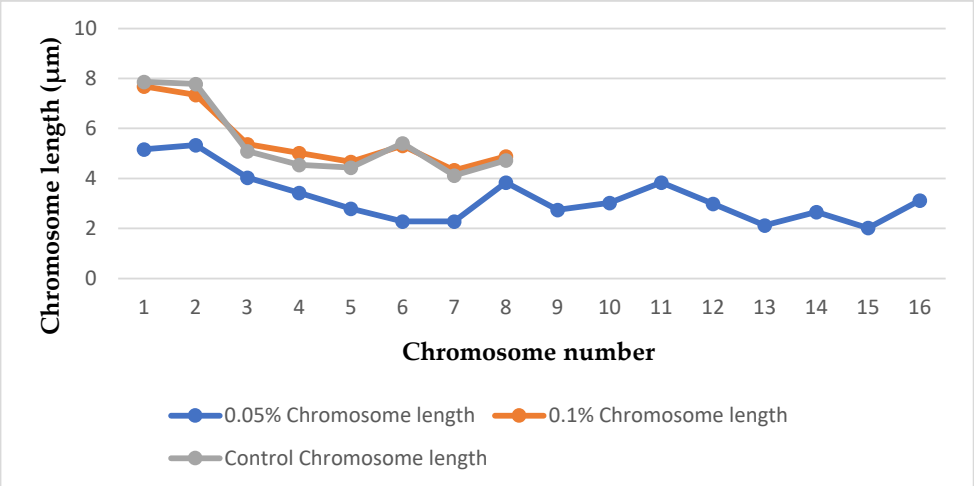

Figure 5. Mean chromosome length (µm) for the haploid chromosome set across 16 chromosomes in plants treated with 0.05% colchicine, 0.1% colchicine, and the control group. Means from seedlings treated with 0.05% colchicine showed significant differences in the number of chromosomes at $p < 0.05$, as determined by Fisher's PLSD.

3.4. Antioxidant Capacity

Extracts were made from the dried bulbs and shoots of both the treated and control groups. These extracts underwent a DPPH scavenging assay following established protocols, and their activities were compared to that of ascorbic acid, which served as the standard. The standard curve was achieved with an r^2 value of 0.9929, revealing that the 1 mM concentration exhibited the highest antioxidant activity, while the 0.125 mM concentration displayed the lowest.

To calculate the DPPH scavenging activity, the means were utilized along with the inhibition percentage formula. Notably, the antioxidant capacity showed significant variation across different treatments and plant parts. The highest values were found in the bulbs treated with 0.05% colchicine, whereas the lowest antioxidant activities were noted in the bulbs from seedlings subjected to 0.1% treatment, followed closely by the shoots of the control group (as illustrated in Figure 6).

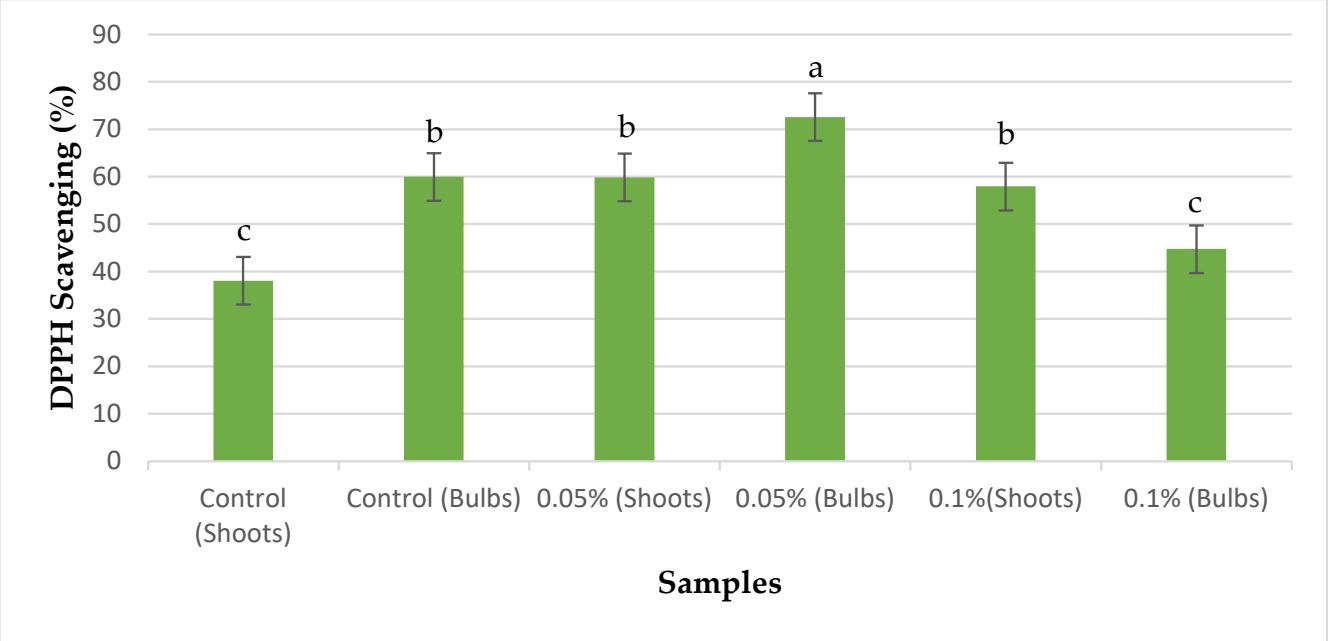

Figure 6. Antioxidant capacity (% DPPH scavenging activity) of shoots and bulbs from control and colchicine-treated samples (bars represent the percentage of inhibition). Different superscripts above the bars indicate significant differences at $p < 0.05$, determined using Fisher's PLSD.

3.5. Water-Soluble Carbohydrates

From the extract of each sample, dissolved sugars were examined. The refractive indices were recorded, and the bulbs from seedlings treated with the 0.05% concentration of colchicine recorded an average refractive index of 1.369, followed by other concentrations and control that recorded lower mean refractive indices. The sample with the highest dissolved sugar content was observed in the bulbs treated with 0.05% colchicine, while shoots from the control recorded the lowest refractive index (Figure 7).

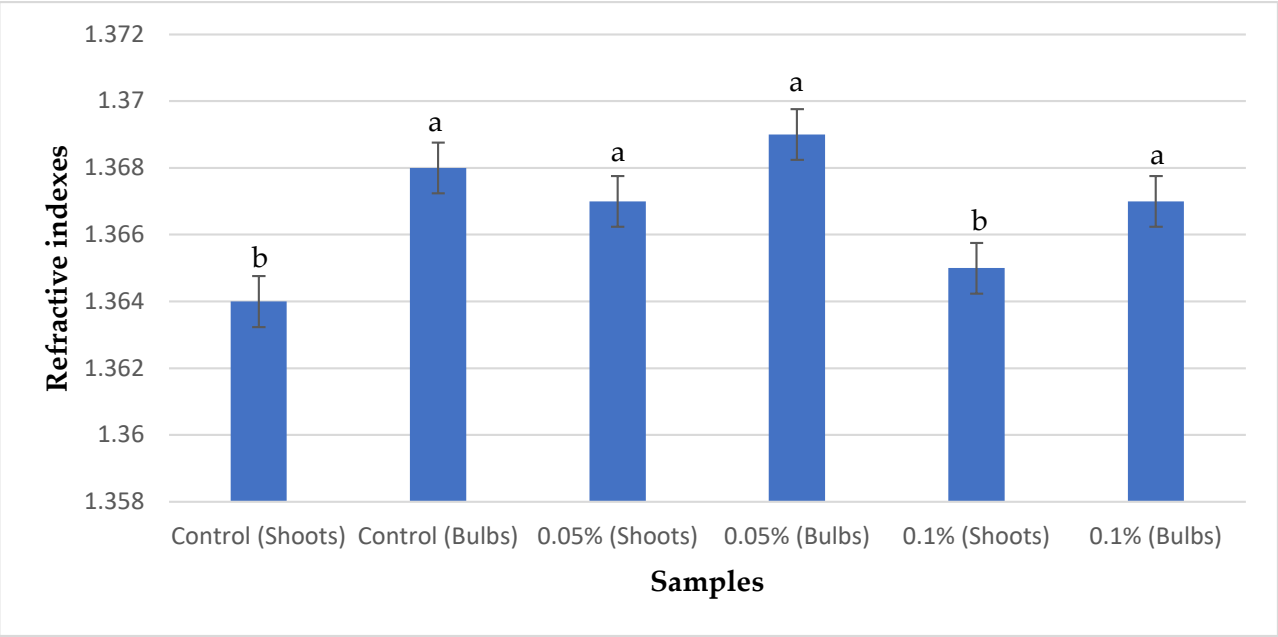

Figure 7. Dissolved sugar content, measured as refractive indices for shoots and bulbs of control and colchicine-treated samples (bars represent the standard deviation of the mean). Different superscripts above the bars indicate significant differences at $p < 0.05$, determined using Fisher's PLSD.

4. Discussion

The effects of colchicine application were evident in the mortality and germination rates of the various treatments, with the severity and failure of germination being dependent upon the concentration of colchicine. This is in agreement with a previous report [10], indicating that the fatality of colchicine differs for different plants depending upon its concentration, where high mortality rates can occur with an increase in its concentration. In this study, the seeds that were treated with a 0.2% concentration of colchicine failed to germinate, possibly due to the toxicity of colchicine at this high concentration; the adverse effects of higher doses of colchicine treatment on the survival rate of treated plants have also been reported in other studies on polyploidy induction [19,20,36]. These studies support our findings, as a 0% germination rate was recorded in the group treated with a higher concentration of colchicine (0.2%) followed by the 0.1%, 0.05%, and control groups, recording 33.33%, 83.33%, and 91.67% germination rates, respectively. In [37], it was reported that a high concentration of colchicine significantly decreased seed germination. Similarly, in our study, it was observed that the higher the concentration of colchicine, the higher the mortality in the seedlings. Another previous study also demonstrated that colchicine at high concentrations had a negative effect on the germination of seeds [38]; furthermore, this inverse relationship between colchicine concentration and survival of seedlings has also been reported in other plants [39], with chemical mutagens having been reported to have inhibitory effects on seeds, thus leading to a low percentage of germination [40].

On the other hand, polyploidy was confirmed by karyotyping, in which the chromosome number was doubled after the application of 0.05% (w/v) colchicine, leading to the formation of tetraploids (4n = 32). This is because colchicine, as an antimitotic substance, stops cell division at the metaphase after the chromosomes have been doubled, leading to polyploidy [41]. Consequently, polyploid plants possess larger and more vigorous vegetative organs, compared with their diploid counterparts, and can thus be used for the screening and discrimination of plants with different diploid levels [42]. In this study, morphological superiority was observed in the tetraploids, which showed significant dif-

ferences ($p < 0.05$) in the height of shoots and diameter of bulbs (recording 73.6 cm and 5.64 cm, respectively), in contrast to the diploid and control groups after 14 weeks. It is well-recognized that the increase in ploidy level enhances the general vigor of plants, as polyploid organisms have more than two sets of chromosomes in their cell nuclei [14,17]. Therefore, the cells of polyploid plants are usually larger than those of their corresponding diploid plants, resulting in bigger organs (e.g., leaves and flowers), as well as thicker and stronger foliage, stems, and branches [43].

Flavonoids and anthocyanins, which are secondary metabolites commonly found in many fruits and vegetables (including onions), are responsible for the attractive color of leaves, fruits, and flowers. Over the past few decades, these phytochemicals have attracted the attention of health professionals, mainly because of their antioxidant properties [44]. The best-described property of these compounds is the antioxidant capability toward free radicals, which are produced by cell metabolism or in response to exogenous environmental factors [44]. In this study, extracts from the bulbs of tetraploids showed promising antioxidant activity, recording the highest DPPH scavenging percentage (approximately 72.58%) when compared to the other samples. This might be a result of the presence of flavonoids, which improve the suppression of free radicals [45], as polyploidy influences the evolution of both the primary and secondary metabolism of plants [46]. A previous report [47] has claimed a significantly enhanced level of the total phenolic and chlorogenic acid contents in the leaves of tetraploid *Cichorium intybus*. In *Scutellaria baicalensis*, baicalin levels in a tetraploid line were increased by 4.6% [48]. From this study, we can report that these phenolic constituents might be responsible for the observed antioxidant activity. This result might suggest that the antioxidant effects of several polyphenols that act as inhibitors of hydroxyl radical formation are correlated with DPPH chelating properties. The second-best performing sample came from the bulbs of control plants (approximately 59.97%), followed by the least-performing sample (approximately 38.04%) from the bulb of the diploids. It can be said that the bulbs of the *Allium cepa* plants showed greater antioxidant activities than the shoots of the plant; this claim regarding the bulbs of onions has been supported by a report [49] stating that onions—especially the outer layers—are major sources of various biologically active phytochemicals, such as phenolic acids, flavonoids, cepaenes, thiosulfinates, and anthocyanins. Studies on the genes involved in the synthesis of metabolites such as vindoline, artemisinin, and morphine have confirmed that the over-expression of these genes was accompanied by a positive regulation of metabolite synthesis, which could be attributed to the differential modulation of expression of genes involved in the biosynthetic pathway due to polyploidization [50].

As anticipated, the tetraploids showed higher levels of dissolved sugars when compared to the diploids. The tetraploids recorded the highest refractive index, which was within the range of conventional refractive indices of dissolved sugars (1.3357 to 1.4117); this is in line with a previous report [51], which observed higher total sugar content in polyploid than diploid watermelon fruit. The control was the second-best performing sample with regard to dissolved sugar content, followed by the diploids, which recorded the lowest refractive index (below the conventional range for dissolved sugars). Similar results showing a wide variation in reducing as well as non-reducing sugars in some Indian cultivars have also been reported previously [52]. Likewise, as observed from our results, the enhancement of polyploidy plant quality has also been reported in previous studies. For instance, [53] claimed that the contents of soluble solids and soluble sugars in tetraploid apple fruits were greatly higher than those in corresponding diploids. In addition, it has also been reported that the contents of soluble sugars, Vitamin C, and titratable acids in tetraploid *Ziziphus jujuba* were significantly higher than those in diploid fruits [54,55].

All the value-added characteristics of induced tetraploids of *A. cepa* serve to enhance their commercial and agricultural significance, as the tetraploids presented a greater bulb size, antioxidant capacity, and dissolved sugars. These qualities are some of the defining figures when a farmer selects an onion variety, as profitable farming will more likely be achieved. Furthermore, the improved qualities will also benefit the consumers. Overall, these improvements can be considered to prioritize tetraploids of *Allium cepa* over diploids as a vital addition to functional foods and health-conscious consumers.

5. Conclusions

Chromosomal analysis demonstrated the induction of polyploidy in onion seedlings treated with the 0.05% concentration of colchicine, which led to the duplication of their chromosome number (producing tetraploids with chromosome numbers of 4n = 32). Polyploidy was achieved via the application of a 0.05% concentration of colchicine for 24 h, which showed significant differences in morphology, antioxidant activities, and dissolved sugars at the level of $p \leq 0.05$. It should be noted that a high concentration of colchicine may lead to the destruction of seeds due to its toxic nature; therefore, the higher the concentration of colchicine, the less chance for polyploidy to be induced. For farmers that wish to induce polyploidy in onions, it is possible to anticipate tetraploids that have higher antioxidant capacity and dissolved sugars, compared to their diploid counterparts, as well as morphological enhancements in the edible parts of the plant (bulbs and shoots). At the same time, failure of seeds to germinate is possible, considering the high toxicity of colchicine, leading to high mortality rates across the treatments. Some of the limitations that can be addressed in the future include optimization of the concentrations of antimitotic agents and the exposure period of the treatment; in this way, an appropriate concentration of colchicine that is suitable for the induction of polyploidy may be determined.

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