

ASSESSMENT OF CHROMOSOMAL ABERRATIONS IN ONION ROOT CELLS UNDER POLYETHYLENE GLYCOL (PEG 6000) -INDUCED OSMOTIC STRESS

Uddipta Borthakur^{1,2*}, Mitali Roy¹, Nickolsova Handique¹, Kanishka Purkait¹, Sharmistha Gautam¹, Geetashree Sarma¹, Dikshita Choudhury¹, Nibedita Sarma²

¹Department of Botany, Handique Girls' College, Guwahati, Assam, India

² Department of Botany, Gauhati University, Guwahati, Assam, India

***Corresponding Author:** Uddipta Borthakur

Address: Department of Botany, Gauhati University, Guwahati, Assam, India

Email: borthakuruddipta@gmail.com

Abstract

This study explores the effects of osmotic stress induced by Polyethylene Glycol (PEG 6000) on chromosomal aberrations in *Allium cepa* L. roots. The roots were exposed to 10% and 20% PEG solutions, mimicking drought conditions. The control group exhibited healthy root growth with an average length of 12.6 ± 0.6 cm, while the 10% and 20% PEG treatments significantly reduced root lengths to 8.9 ± 1.0 cm and 6.6 ± 0.3 cm, respectively. The mitotic index decreased from 43.15% in the control group to 21.90% and 27.06% under 10% and 20% PEG treatments. Chromosomal aberrations were absent in the control group but increased with PEG concentration, showing frequencies of 0.26 in 10% PEG and 0.38 in 20% PEG treatments. Observed aberrations included chromosome breaks, anaphase bridges, disturbed metaphases, clumped chromosomes, and others. These results indicate that PEG-induced osmotic stress significantly impairs root growth, mitotic activity, and chromosomal integrity in *Allium cepa*. This study underscores the *Allium cepa* bioassay's utility in assessing environmental stress impacts on plants and provides insights into the mechanisms of stress-induced genetic damage, contributing to a better understanding of plant responses to drought conditions.

Keywords: *Allium cepa* L., Polyethylene Glycol (PEG 6000), Osmotic stress, Chromosomal aberrations, Mitotic index

Introduction

Gross chromosome aberrations or rearrangements have gained importance in the research of issues related to speciation and evolution. The common onion (*Allium cepa* L.) is an excellent plant for the assay of chromosomal aberrations once chemically treated (Grant, 1982). However, because these aberrations typically occur at very low frequencies, studying their spontaneous occurrence can be challenging. To get a deeper comprehension of the origins and characteristics of these reorganisations, numerous researchers have turned to examining analogous reorganisations that are synthetically generated through the application of certain external stimuli including water stress, heat, chemicals, radiation, and ageing. These investigations have provided a wealth of information about the many kinds of aberrations and their mechanisms (Nichols, 1941). With a broad range of genetic endpoints, the plant-

based bioassays are quick, affordable, and do not require complex laboratory infrastructure. It has been found to be an excellent phyto-indicator for evaluating DNA damage, including chromosomal abnormalities and disruptions in the mitotic cycle. The *Allium cepa* bioassay finds extensive application in cytotoxic and genotoxic investigations because of favourable chromosomal conditions, including large chromosome numbers and reduced numbers ($2n = 16$) (Hemachandra and Pathiratne, 2017; Kannangara & Pathiratne, 2015).

Around 98 million tonnes of bulb onions (*Allium cepa* L.) are produced worldwide, making them one of the major vegetable crops (FAOSTAT, 2018). China is the largest producer of bulb onions, with 23.2 million tons produced and 1.58 million tons exported. India is on the next. Onions are grown in a variety of weather conditions throughout India. Since a large portion of the land used for onion farming depends on monsoon rainfall to meet its water needs, the crop is susceptible to climatic aberrations like drought (Gedam et al., 2021). The frequency of drought events associated with climate change has resulted in a reduction of almost 30% in the worldwide production of bulbs. Drought stress causes changes at genetic level that leads to modified morphological, physiological, and metabolic processes (Diaz et al., 2010), destroys water relations, destroys cellular membranes, synthesizes new proteins and produces reactive oxygen species in plant tissues, resulting in significant harm to the plant (Sairam and Saxena, 2000). Extended periods of drought cause poor photosynthesis and plant growth, which ultimately leads to significant yield losses (Gedam et al., 2021). All of which can be further researched and understood by testing for aberrations induced by drought stress. Therefore, this study holds the motive of understanding chromosomal aberrations on common Onion (*Allium cepa* L.) by artificially induced water stress with Polyethylene Glycol (PEG) that mimics drought.

Materials and methods

Preparation of Stain

45% of glacial acetic acid is boiled and 2g of carmine powder is added to the conical flask pinch by pinch and mix it thoroughly with the help of glass rod. The mixture is then kept boiling until it gets mixed properly. After that this mixture is kept in the dark for cooling. After this the mixture is kept overnight and after 24 hours this mixture is filtered using filter paper and this filtrate is the stain aceto-carmine.

Test Material

Onion (*Allium cepa* L.: $2n=16$) bulbs with healthy, uniform size and shape were taken for this study. A series of bulbs were grown in the test formulation. For this experiment infected and dried bulbs were not used.

Test Method

The outer loose scaly layers of bulbs and old dry roots are removed with the help of sharp forceps, it helps the roots primordia to come in contact with the test solution. The onion bulbs were then set on the distilled water-filled test tubes at room temperature for 3 days. After that, onion bulbs were exposed to PEG (6000) 10% and PEG (6000) 20% concentration for 48 hours and some bulbs of onions were also exposed to sterile distilled water as a standard control under laboratory conditions.

Fixation

Roots were collected separately maintaining proper exposure time during the morning hours between 6 and 7 a.m. Immediately after collection, roots were fixed in chilled Carnoy's solution (ethanol: glacial

acetic acid=3:1) for 24 h keeping at 4 °C overnight. After fixation the thoroughly washed root tips were preserved in 70% alcohol. Then for preparation of squash, the root tips were hydrolyzed in 1N HCl and incubated for 12 minutes at 60 °C to prepare them for chromosomal analysis. They were then moved to watch glass filled with aceto-carmin and 1N HCl (9:1) with gentle warming for 5 min without boiling. After being sharply chopped, the root's tips was put on a glass slide, and the root tips were kept in the stain for 4-5 minutes and the excess stain was removed using blotting paper after that the coverslip was placed on the root tip pressed down by applying uniform pressure with the thumb through a piece of blotting paper so as to prepare the root squash which was followed by heat fixing and finally sealed with paraffin or DPX. The cells were then viewed under the light microscope using 40x objective lens. For the cytotoxicity test, the mitotic index was calculated by dividing the number of cells undergoing mitosis by the total number of cells examined with each treatment (Balog, 1982).

$$\text{Mitotic Index (MI)} = \frac{\text{No. of cells divided}}{\text{Total No. of cells}} \times 100$$

The Chromosomal Aberration Frequency was calculated as the number of abnormal cells over the total number of dividing cells.

$$\text{Chromosomal Aberration Frequency (CAF)} = \frac{\text{No. of abnormal cells}}{\text{Total No. of dividing cells}}$$

Statistical Analysis

The data were analyzed by two-way analysis of variance (ANOVA) and sample means were compared by Tukey's test. Three significant P-values were considered. Significant code $p \leq 0.05$ “*”, $p \leq 0.01$ “**”, $p \leq 0.001$ “***”.

Results

Effect of different concentrations of PEG (6000) on root length

The effect of different concentrations of polyethylene glycol (PEG 6000) on the root length of the studied plants was significant. Under control conditions, the average root length was 12.6 ± 0.6 cm, with individual measurements of 12.1, 13.4, and 14.3 cm. When exposed to a 10% PEG solution, the root length showed a marked decrease, with an average length of 8.9 ± 1.0 cm, and individual measurements of 8.7, 7.2, and 10.7 cm, indicating a notable reduction in growth due to osmotic stress. The highest PEG concentration (20%) further inhibited root growth, resulting in an average root length of 6.6 ± 0.3 cm, with individual measurements of 7.3, 6.4, and 6.0 cm.

Table 1. Effect of different concentrations of PEG (6000) on root length (Data presented as mean \pm SE, $n = 3$). The significant level represented were with respective their individual control calculated by performing two-way ANOVA with post hoc Tukey analysis. Significant codes $p \leq 0.05$ “*”, $p \leq 0.01$ “**”, $p \leq 0.001$ “***”.

Treatment	Root length (cm)	Mean \pm SE
Control	12.1	12.6 ± 0.6
	13.4	
	14.3	

10 % PEG	8.7	8.9 ± 1.0*
	7.2	
	10.7	
20 % PEG	7.3	6.6 ± 0.3***
	6.3	
	6.3	

Effect of different concentrations of PEG 6000 on mitotic activity

The mitotic index of *Allium cepa* roots in the control group was found to be 43.15%. But the treatment of the roots with different concentrations of PEG 6000 showed significant reduction in the mitotic index as compared to the control. The highest reduction in the mitotic index was seen in the treatment with 10% PEG 6000 solution that was 21.90%. And the treatment with 20% PEG 6000 solution showed 27.06% mitotic index.

Table 2. Mitotic index and chromosomal aberration frequency of *Allium cepa* root tips at distilled water and treatment with 10% and 20% PEG 6000. (Data presented as mean ± S, n = 3). The significant level represented were with respective their individual control calculated by performing two-way ANOVA with post hoc Tukey analysis. Significant codes $p \leq 0.05$ “*”, $p \leq 0.01$ “**”, $p \leq 0.001$ “***”

Treatment	Total no. of cells analysed	No. of dividing cells	No. of aberrant cells	Mitotic index % (MI %)	MI Mean ± SE	Chromosomal aberration frequency (CF)	CF Mean ± SE
Control	270	116	0	42.96	43.15 ± 0.24	0	0
	280	120	0	42.86		0	
	282	123	0	43.62		0	
10% PEG	342	73	15	21.35	21.90 ± 0.28**	0.21	0.26 ± 0.05**
	344	76	16	22.09		0.21	
	310	69	25	22.26		0.36	
20% PEG	291	80	26	27.49	27.06 ± 0.54***	0.33	0.38 ± 0.04***
	267	74	33	27.72		0.45	
	254	66	24	25.98		0.36	

Various chromosomal aberrations induced due to treatment with different concentrations of PEG 6000

Since no chromosomal aberrations were observed in the *Allium cepa* roots kept in distilled water that is control, the chromosomal aberration frequency for the control group was found to be 0. But chromosomal aberration frequency significantly increased in roots under treatments with different concentration of PEG 6000 as compared to control. Treatment with 20% PEG 6000 solution showed the highest chromosomal aberration frequency that was 0.38. And treatment with 10% PEG 6000 solution showed chromosomal aberration frequency of 0.26.

As a result of application of 10% and 20% PEG 6000 various chromosomal aberrations were seen to

occur in the roots of *Allium cepa*. The different aberrations observed in the root tips were: chromosome break, scattered chromosome, anaphase bridge, disturbed metaphase, clumped chromosome, pole shift anaphase, sticky chromosome, sticky anaphase, chromosome bridge and laggards, ringed chromosome, micronucleus, diagonal anaphase, chained chromosome, telophase with vagrant chromosome, metaphase chromosome degradation, grouped chromosome and bridge formation, spindle attachment disturbance, disturbance assembly of chromosome (Figure 1 and Table 3) .

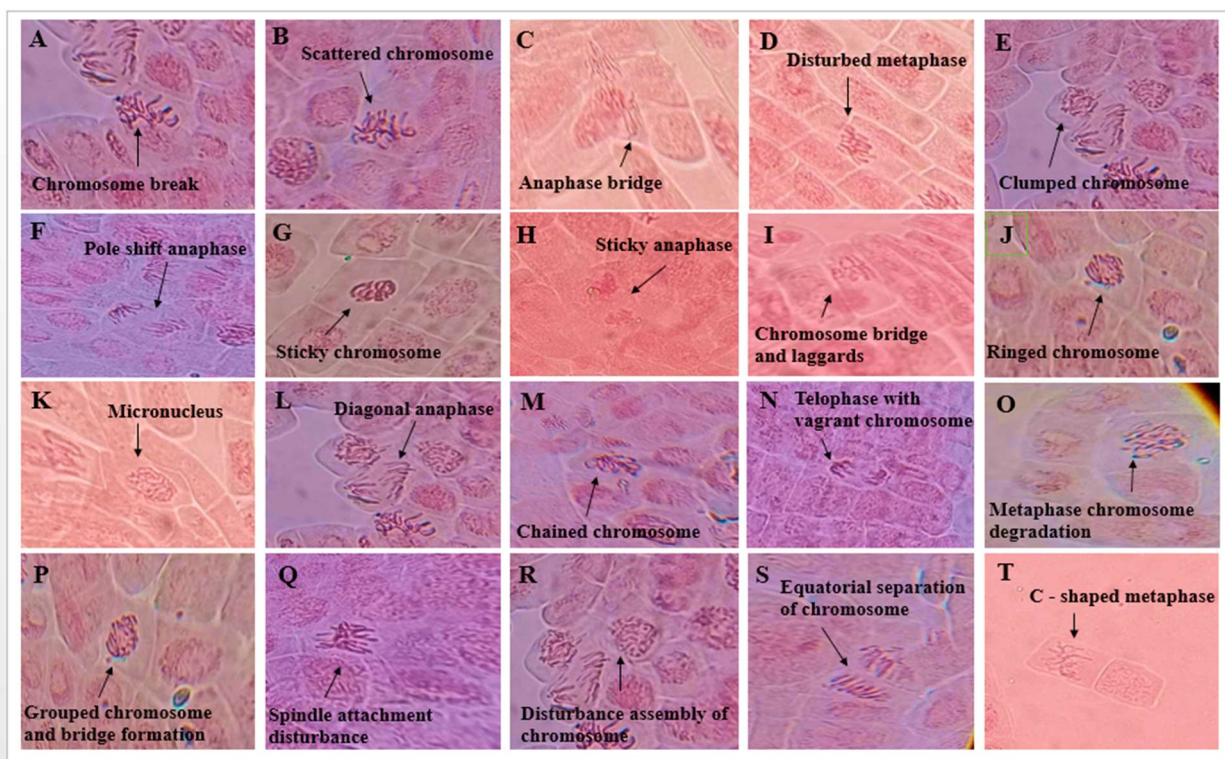


Fig 1. Chromosomal aberrations in mitotic phases in roots of *Allium cepa* under treatment with 10% and 20% PEG 6000. A) Chromosome break, B) Scattered chromosome, C) Anaphase bridge, D) Disturbed metaphase, E) Clumped chromosome, F) Pole shift anaphase, G) Sticky chromosome, H) Sticky anaphase, I) Chromosome bridge and laggards, J) Ringed chromosome, K) Micronucleus, L) Diagonal anaphase, M) Chained chromosome, N) Telophase with vagrant chromosome, O) Metaphase chromosome degradation, P) Grouped chromosome and bridge formation, Q) Spindle attachment disturbance, R) Disturbance assembly of chromosome, S) Equatorial separation of chromosome and T) C- shaped metaphase.

Table 3. Various chromosomal aberrations with their descriptions observed in the root tips of *Allium cepa* under treatment with 10% and 20% PEG 6000.

S no.	Types of chromosomal aberrations	Phases of cell division	Descriptions
A	Chromosome break	Anaphase	The chromosome is fractured in several places, compromising its structure.
B	Scattered chromosome	Metaphase	The chromosomes are broken apart and its pieces are scattered.

C	Anaphase bridge	Anaphase	Cells with abnormally joined chromosomes or sister chromatids experience specific problems during cell division, leading to incorrect distribution of genetic material.
D	Disturbed metaphase	Metaphase	The cell's nucleus breaks down, and its chromosomes condense and gather together. However, these chromosomes fail to arrange themselves in the middle of the dividing cell as they normally would.
E	Clumped chromosome	Metaphase	Chromosomes clump together due to incorrect packaging of their genetic material.
F	Pole shift anaphase	Anaphase	The sister chromatids shift slightly apart from each other at both the poles of the cell.
G	Sticky chromosome	Metaphase	The chromosomes clump together abnormally and become tangled during cell division.
H	Sticky anaphase	Anaphase	During the anaphase stage, the chromosomes don't separate normally and move apart. Instead, they stay stuck together.
I	Chromosome bridge and laggards	Anaphase	During cell division, chromosome bridges happen when pairs of chromosomes get stuck together. Laggards are chromosomes that get left behind and don't move to where they're supposed to go.
J	Ringed chromosome	Anaphase	A ring chromosome is an abnormal chromosome that forms a circle when its ends join together.
K	Micronucleus	Anaphase	Micronuclei are small, extra pieces of chromosome that break off and form outside the nucleus. They are often used to determine if a substance can damage DNA.
L	Diagonal anaphase	Anaphase	The chromosomes break in the middle and the two halves don't move apart

			evenly. Instead, they slide apart at an angle, forming a diagonal line.
M	Chained chromosome	Prophase	Chain chromosomes happen when several chromosomes become linked together in a line.
N	Telophase with vagrant chromosome	Telophase	A vagrant chromosome is one that moves ahead of the other chromosomes to one end of the cell.
O	Metaphase chromosome degradation	Metaphase	Chromosomes can break or get damaged while they are lined up in the middle of the cell during cell division.
P	Grouped chromosome and bridge formation	Anaphase	Chromatin bridges are strands of genetic material that connect the dividing chromosomes during cell division or the newly formed cells after division.
Q	Spindle attachment disturbance	Metaphase	The sister chromatids don't separate because the spindle fibers don't attach correctly to them due to a problem in their formation.
R	Disturbance assembly of chromosome	Metaphase	Disturbance assembly of chromosome happen when chromosomes are being formed. This can be caused by mistakes when DNA is copied, damage to DNA, or issues with the proteins that make up chromosomes.
S	Equatorial separation of chromosome	Anaphase	Equatorial separation of chromosome happened because the parts of the cell that help chromosomes divide formed incorrectly and didn't work right.
T	C - shaped metaphase	Metaphase	Homologous chromosomes come together and form pairs that often resemble a C-shape due to a specialized protein structure.

Discussion

The effect of polyethylene glycol (PEG 6000) on root length demonstrates a significant inhibition of growth, consistent with the findings of earlier studies on osmotic stress. Under control conditions, the average root length was 12.6 ± 0.6 cm, reflecting healthy growth. However, with a 10% PEG solution, root length decreased significantly to an average of 8.9 ± 1.0 cm, indicating that osmotic stress reduced

the plant's ability to elongate roots. This trend was even more pronounced at a 20% PEG concentration, where the average root length was further reduced to 6.6 ± 0.3 cm. These results align with previous research that has shown PEG-induced osmotic stress adversely affects root growth by limiting water uptake and cell elongation (Yamaguchi-Shinozaki and Shinozaki, 2006; Verslues et al., 2006). Recent studies have also corroborated that higher PEG concentrations exacerbate the stress, resulting in more severe growth inhibition (Zhang et al., 2020; Li et al., 2022).

In this study we observed that the application of various concentration of PEG 6000 showed reduction in mitotic index. Application of 10% PEG 6000 showed 21.90% reduction in mitotic index and application of 20% PEG 6000 showed inhibitory effect on mitotic index value up to 27.06%. Similarly, when Ozmen et al (2022) treated barley seeds with 24% PEG 6000 they also observed decreased in the mitotic index value by 40% compared to the control. Schuppler et al. (1998) stated that mitotic index decreased in the wheat plant because of drought stress while Heckenberger et al. (1998), found that drought stress had the same effect in *Ricinus communis* species. Aydin et al. (2016) stated that mitotic index decreased because of drought stress in their study on wheat plants. In the light of previous studies, it can be stated that the reduction of mitotic index may be due to the inhibition of DNA synthesis or because of the cell cycle blocking during G2 phase of cell cycle.

The result of cytological analysis of the current study conducted on *Allium cepa* showed no significant chromosomal abnormalities in the roots grown as control while different concentrations of PEG 6000 treated roots showed various types of chromosomal aberrations. The roots which were treated with 10% PEG 6000 solution showed chromosomal aberration frequency of 0.26 and the roots which were treated with 20% PEG 6000 solution showed chromosomal aberration frequency of 0.38. The different chromosomal aberrations found in the present study were: scattered chromosome, anaphase bridge, disturbed metaphase, clumped chromosome, pole shift anaphase, sticky anaphase, sticky chromosome, chromosome bridge and laggards, ringed chromosome, micronucleus, metaphase chromosome, chromosome degradation, diagonal anaphase, telophase with vagrant chromosome, grouped chromosome and bridge formation, spindle attachment disturbance, disturbed assembly of chromosome, chained chromosome, chromosome break etc. A similar study conducted by Shrivastava and Kumar (2016), on *Sesbania cannabina* revealed presence of same type of chromosomal aberrations. Similar genotoxic events in *Allium cepa*, *Vicia faba*, *Zea mays*, *Tradescantia*, *Nicotiana tabacum*, *Crepis capillaries* and *Hordeum vulgare* were reviewed by Khanna and Sharma (2013). Khanna and Sharma (2013) grouped chromosomal aberrations into two categories: 1. Clastogenic, which includes chromatin bridges, chromosomal breaks and ring chromosomes etc. and 2. Physiological aberration which includes c-mitosis, vagrants, stickiness, delayed anaphase and laggards etc.

Appearance of morphological abnormalities and precocious movement of chromosomes may be due to improper functioning of spindle fiber (Shrivastava and Kumar; 2014). Evans (1986) reported that stickiness of chromosomes can be resulted from partial dissociation of nucleoproteins and changes in pattern organization. Scattering of chromosomes resulted from inhibition of spindle formation or due to destruction of spindle fibers formed in presence of stress condition while chromosome laggards form due to failure of spindle fiber attachment. Above mentioned abnormalities were also reported by Turkoglu (2007). Bridges are formed when two chromosomes of two separate poles remain attached. Pole shift of anaphase chromosome occurs due to spindle attachment disturbance.

According to Khanna and Sharma (2013), stress induced micronuclei are formed when fragmented chromosomes are not incorporated into the main nucleus during cell cycle progression. Chromosome clump formation and condensation appears because of increased chromosomal contraction or might be from the depolymerization of DNA and partial dissolution of nucleoproteins. Vagrant chromosome appears when a chromosome moves toward pole earlier than its group. This may be a result of dysfunctional spindle assembly checkpoint. Improper attachment of kinetochore to spindle fibres leads to diagonal anaphase formation. Similar cases were also reported by Sondhi et al. (2008) in *Allium cepa*. The chromosome rings appeared when telomere is lost, and chromosome becomes instable. Chromatin bridges could happen during the translocation of the unequal chromatid exchange and cause structural chromosome mutation. This type of anomaly was also reported by Turkoglu (2007), in the mitosis of *Allium cepa* after treatments with food additives. Similar findings were also reported by Gomurgen (2005), in *Vicia faba*. Chromosomes appear in chain form when chromatin thread fails to undergo proper coiling mechanism to become shorter and thicker. Chained chromosomes were also observed by Prajitha and Thoppil (2016), in *Allium cepa* roots when treated with *Amaranthus spinosus* leaf extracts.

Conclusion

This study highlights the utility of the *Allium cepa* bioassay as a reliable and sensitive method for assessing cytotoxic and genotoxic effects of environmental stressors. The observed chromosomal aberrations provide valuable insights into the mechanisms of stress-induced genetic damage, with implications for understanding plant responses to drought conditions. Future research could explore the molecular pathways involved in these aberrations, enhancing our ability to mitigate the adverse effects of environmental stress on crop productivity. The artificial induction of osmotic stress in *Allium cepa* using PEG 6000 effectively mimics drought conditions, revealing significant disruptions in root growth, mitotic activity, and chromosomal integrity. These findings contribute to a deeper understanding of plant responses to water stress, offering potential avenues for improving crop resilience in the face of increasing climate variability.

References

1. Aydın, M., Pour, A. H., Tosun, M., and Haliloğlu, K. (2016). Effect of application of putrescine on seedling growth and cell division of wheat (*Triticum aestivum* L.) under drought stress. *Yuzuncu Yıl University Journal of Agricultural Sciences*, 26(3), 319-332.
2. Balog, C. (1982). The mitotic index in diploid and triploid *Allium* roots. *Cytologia*, 47(3-4), 689-697.
3. Díaz, P., Betti, M., Sánchez, D. H., Udvardi, M. K., Monza, J., and Márquez, A. J. (2010). Deficiency in plastidic glutamine synthetase alters proline metabolism and transcriptomic response in *Lotus japonicus* under drought stress. *New Phytologist*, 188(4), 1001-1013.
4. Evans, D. O. (1986). *Sesbania* research in Hawaii: Summary of a project. *Nitrogen fixing tree research reports*.
5. Fao, F. A. O. S. T. A. T. (2018). Food and agriculture organization of the United Nations. Rome, URL: <http://faostat.fao.org>, 403-403.

6. Gedam, P. A., Thangasamy, A., Shirsat, D. V., Ghosh, S., Bhagat, K. P., Sogam, O. A., ... and Singh, M. (2021). Screening of onion (*Allium cepa* L.) genotypes for drought tolerance using physiological and yield based indices through multivariate analysis. *Frontiers in Plant Science*, 12, 600371.
7. Gömürçen, A. N. (2005). Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L. *Cytologia*, 70(2), 119-128.
8. Grant, W. F. (1982). Chromosome aberration assays in *Allium*: A report of the US Environmental Protection Agency gene-tox program. *Mutation Research/Reviews in genetic toxicology*, 99(3), 273-291.
9. Heckenberger, U., Roggatz, U., and Schurr, U. (1998). Effect of drought stress on the cytological status in *Ricinus communis*. *Journal of Experimental Botany*, 49(319), 181-189.
10. Hemachandra, C. K., and Pathiratne, A. (2017). Cytogenotoxicity screening of source water, wastewater and treated water of drinking water treatment plants using two in vivo test systems: *Allium cepa* root based and Nile tilapia erythrocyte-based tests. *Water Research*, 108, 320-329.
11. Kannangara, D. N. M., and Pathiratne, A. (2015). Toxicity assessment of industrial wastewaters reaching Dandugan Oya, Sri Lanka using a plant-based bioassay. *Journal of the National Science Foundation of Sri Lanka*, 43(2).
12. Khanna, N., and Sharma, S. (2013). *Allium cepa* root chromosomal aberration assay: a review. *Indian journal of pharmaceutical and biological research*, 1(03), 105-119.
13. Li, Y., Tan, B., Wang, D., Mu, Y., Li, G., Zhang, Z., ... and Zhu, L. (2022). Proteomic analysis revealed different molecular mechanisms of response to PEG stress in drought-sensitive and drought-resistant sorghums. *International Journal of Molecular Sciences*, 23(21), 13297.
14. Nichols, C. (1941). Spontaneous chromosome aberrations in *Allium*. *Genetics*, 26(1), 89.
15. Özmen, S., Tabur, S., Öney-Birol, S., and Özmen, S. (2022). Molecular responses of exogenous polyamines under drought stress in the barley plants. *Cytologia*, 87(1), 7-15.
16. Prajitha, V., and Thoppil, J. E. (2016). Genotoxic and antigenotoxic potential of the aqueous leaf extracts of *Amaranthus spinosus* Linn. using *Allium cepa* assay. *South African Journal of Botany*, 102, 18-25.
17. Sairam, R. K., and Saxena, D. C. (2000). Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. *Journal of Agronomy and Crop Science*, 184(1), 55-61.
18. Schuppler, U., He, P. H., John, P. C., and Munns, R. (1998). Effect of water stress on cell division and Cdc2-like cell cycle kinase activity in wheat leaves. *Plant physiology*, 117(2), 667-678.
19. Sondhi, N., Bhardwaj, R., Kaur, S., Kumar, N., and Singh, B. (2008). Isolation of 24-epibrassinolide from leaves of *Aegle marmelos* and evaluation of its antigenotoxicity employing *Allium cepa* chromosomal aberration assay. *Plant Growth Regulation*, 54, 217-224.
20. Srivastava, N., and Kumar, G. (2014). Influence of Drought Stress on Cytological Behavior of Green Manure Crop *Sesbania cannabina* Poir. *Cytologia*, 79(3), 325-329.

21. Srivastava, N., and Kumar, G. (2016). Effect of waterlogging stress on meiotic course, tetrad formation and pollen fertility of *Sesbania* pea. *Cytology and Genetics*, 50, 28-31.
22. Türkoğlu, Ş. (2007). Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 626(1-2), 4-14.
23. Verslues, P. E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J., and Zhu, J. K. (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *The Plant Journal*, 45(4), 523-539.
24. Yamaguchi-Shinozaki, K., and Shinozaki, K. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.*, 57(1), 781-803.
25. Zhang, X., Yang, Z., Li, Z., Zhang, F., and Hao, L. (2020). Effects of drought stress on physiology and antioxidative activity in two varieties of *Cynanchum thesioides*. *Brazilian Journal of Botany*, 43, 1-10.

Authors' Contributions

In this research, the experiment was planned by the author UB. The authors MR, NH, KP, SG, GS and DC conducted the experimental work. The draft manuscript was prepared by all the authors. The final manuscript was edited by the authors UB and NS.

Conflict of Interest

There is no conflict of interest among the authors.