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Effect of thiourea on germination and seedling parameters of millet germplasms under drought stress

Zohaa Fatima[1*], Zain-Ul-Sajjad[2]

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Author Affiliation:
[1]Department of Agronomy, PMAS Arid Agriculture University, Rawalpindi, Pakistan
[2]Crop Science Institute (CSI), National agriculture research center, Islamabad, Pakistan

[*]**Corresponding author**
Department of Agronomy, PMAS Arid Agriculture University, Rawalpindi,
Pakistan
Email: zainulsajjad.edu@gmail.com

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ABSTRACT

Pearl millet plays an important role in livestock feeding. In our country, fodder or livestock feed production is less as compared to other countries. For getting quality yield, nitrogen element is necessary for fodder crops. This study aimed to judge the impact of various concentrations of Thiourea on the growth parameters of two distinct lines of pearl millet (Millet IABGR (7) 8810 and Millet IABGR (5) 8807) under drought stress. Thiourea is recognized as a significant bio-regulator, for stimulating the growth and development parameter of crop plants. Its exogenous application has been reported to enhance plant growth and productivity under both normal and adverse environmental conditions. A factorial experiment was conducted with three replications at Fodder and forage research laboratory, NARC, Islamabad to investigate the effect of three different concentrations of Thiourea (100 ppm, 300 ppm and 500 ppm) on various growth parameters of pearl millet. The study measured germination percentage, root and shoot length and root and shoot moisture content. Results showed that Line Millet IABGR (7) 8810 had the highest growth and performed the best in all measured parameters. The results showed that Line Millet IABGR (7) 8810 exhibited the best growth and maximum germination percentage (96.67%), while the lowest germination percentage was observed in Millet IABGR (5) 8807 (75%). Millet IABGR (7) 8810 was found to be the better hybrid for fodder production and can be recommended for future use in Millet breeding programs.

Keywords: Fodder and Forage, Germination parameter, Millet, Seedling, Thiourea

1. INTRODUCTION

Pearl millet (*Pennisetum americanum* L.) is a crop with many benefits that belongs to the Poaceae family. It has gained popularity in recent years as a food source for both humans and animals due to its versatility, high nutritional value and ability to grow in areas with either irrigation or rain-fed farming systems. Pearl millet is a multipurpose crop that originated in Africa and has been cultivated for centuries in various regions around the world, including Indo-Pak Subcontinent, China and Africa (Yusuf et al., 2012; Aswini et al., 2022). Pearl millet is typically cultivated for either grain or fodder purposes. Long and taller varieties are grown for fodder, while dwarf varieties are cultivated for grain. Due to its adaptability to both irrigated and barani areas, pearl millet is a common crop in Pakistan and is the third most important cereal used for livestock feed.

Pearl millet is a major contributor to the feeding of rural cattle and poultry in the country (Chughtai et al., 2004). Aside from being a valuable food source for livestock, pearl millet also has various health benefits for humans. It is a good source of dietary fiber, protein and essential minerals such as iron and calcium. In addition, pearl millet is gluten-free, making it an ideal grain for individuals with celiac disease or gluten intolerance.

Pearl millet is an annual C4 grass that has excellent tolerance to drought and is highly tillering. It is also cross-pollinated and very hardy. Its height ranges from 120 to 350 centimeters and its stems typically have a diameter of 1-3 cm or more. The number of culms it produces can vary depending on spacing, management and cultivar, with a potential range of one to twenty (FAO, 2020). One of the key characteristics of pearl millet is its high starch content, which can constitute up to 70% of the seed and is a crucial factor in determining the quality of millet products (Zhu et al., 2014). Pakistan has witnessed a notable increase in the usage of pearl millet as a feed for livestock and poultry. According to a study conducted by Nedumaran et al., (2013), it is anticipated that Pakistan will become the second-largest purchaser of pearl millet worldwide by the year 2030.

The study predicts that Pakistan will import 61,000 tons of pearl millet, following the example set by China. Pearl millet is also a crucial source of fodder in many countries worldwide, providing a promising crop for green fodder supply, particularly during the lean summer months from May to July. It is often combined with other fodder crops during the summer and kharif season. The dry fodder and straw of pearl millet are commonly used to feed livestock in areas with marginal agricultural production, particularly during the dry season when green fodder and grazing are scarce (Arya et al., 2009).

Millet refers to a group of small-seeded cereals that includes pearl (*Pennisetum americarum*), foxtail (*Setaria italica*), proso (*P. miliaceum* var. compactrm) and finger (Gramineae) millet among the major species (Marti and Tyl, 2021; Zhu et al., 2014). In China, millet has been traditionally used for making thin pancakes and porridge using a simple but time-consuming process (Kuo et al., 2018). However, there has been a growing interest in developing convenient millet-based food products. Currently, there is a wide range of research on millet products, including noodles, biscuits, bread, beverages, instant powder and more (Dissanayake and Jayawardena, 2016; Ajay and Pradyuman, 2019).

Thiourea (TU) is a type of synthetic plant growth regulator (PGR) that has been discovered to have a significant role in enhancing plant stress tolerance by providing nitrogen and sulfur (Waqas et al., 2019). PGRs are crucial in regulating the growth and development of plants and also in modulating their responses to various biotic and abiotic stresses (Garg et al., 2006). The use of TU in plant biology has gained much attention in recent years due to its positive impact on plant germination and breaking seed dormancy, as well as other beneficial effects (Jocelyn, 1972; Esashi et al., 1977).

Role of Thiourea in Plant Stress Tolerance

Thiourea has been identified as a key player in several important biochemical and physiological processes in plants that occur in stressful environments, particularly under metal toxicity conditions (Mansoora et al., 2021). It exerts a positive effect on plants by regulating their redox status, hormonal activity and calcium signaling. Furthermore, it mitigates the negative effects of oxidative stress on plant growth by enhancing the activity of antioxidant enzymes involved in ROS removal (Waqas et al., 2019).

Effects of Thiourea on Crop Growth and Productivity

Thiourea (TU) is a synthetic plant growth regulator that has been found to enhance plant growth and productivity in both normal and stressful conditions (Patade et al., 2020). A number of studies have reported the positive effects of TU as a seed pretreatment, foliar spray and medium supplement for various crop species (Garg et al., 2006; Waqas et al., 2019; Mansoora et al., 2021), suggesting its potential as a plant bioregulator. TU has been shown to play a crucial role in a range of biochemical and physiological processes in plants, particularly under stress conditions. Exogenous application of TU has been found to promote plant growth and productivity, but more research is needed to fully understand the potential of TU as a bio-regulator for crop plants under different environmental stress conditions.

The objective of the study is to investigate the impact of Thiourea on the germination and seedling growth of Millet Germplasm under drought stress. The study aims to determine the optimum concentration of thiourea for promoting germination and enhancing seedling growth, as well as to understand the mechanism by which thiourea affects these processes.

2. MATERIALS AND METHOD

The experiment was carried out in a completely randomized design with three replications at the Fodder and Forage Laboratory in the Crop Science Institute (CSI) of the National Agriculture Research Center in Islamabad. The location of the site was at latitude of 33.6982 and a longitude of 73.0393. On 22nd July 2022, two different hybrid lines of Millet, Millet IABGR (5) 8807 is line 1 hybrid, and Millet IABGR (7) 8810 is line 2 were sowed.

The experimental setup consisted of 24 experimental units (12 units for each hybrid line), where each experimental unit contained one pot with a height of 30 cm, top diameter of 33 cm and base diameter of 25 cm. The pot contained 4.5 kg dry sand and 10 seeds were sown in each pot. There were four different treatments (T0, T1, T2 and T3) and each treatment had three replications with one pot in each replication. T0 was a control test where no fertilizer application was done, while T1, T2 and T3 were treated with 500 ppm, 300 ppm and 100 ppm Thiourea solutions, respectively. Irrigation is done just twice a week to maintain stress. 250ml of water is applied per pot during each irrigation. The experiment intended to study numerous parameters, which included Germination Percentage (GP %), Shoot Length (SL), Root length (RL), Shoot Moisture Content (SMC %) and Root Moisture Content (RMC %). To analyze data collected on give higher, statistical software was used in the examiner study.

On the way to determine the germination percentage (GP %), the number of germinated seeds was divided by the total number of seeds, and the quotient was multiplied by 100. The fresh and dried weights of the samples were calculated and formulas were used to compute the percentages to determine the shoot and root moisture content. The formula for shoot moisture content percentage (SMC %) was (Fw of the shoot - Dw of the shoot) / Fw of shoot × 100 and for root moisture content percentage (RMC %) was (Fw of root - Dw of the root) / Fw of root × 100, where Fw represents the fresh weight and Dw represents dry weight. Additionally, shoot length (SL) and root length (RL) were measured in millimeters. The means were compared using the Least Significant Difference (LSD) at a significance level of $p \leq 0.05$.

Table 1 Stages, Dates and Doses of Thioure Application

Stage	Date	T_0	T_1	T_2	T_3
Sowing	22- July-2022	No Thiourea solution	250ppm Thiourea solution	150ppm Thiourea solution	50ppm Thiourea solution
Seedling	26-July-2022	No Thiourea solution	250ppm Thiourea solution	150ppm Thiourea solution	50ppm Thiourea solution

3. RESULTS & DISCUSSIONS

The results of an experiment in which different treatments (T0, T1, T2 and T3) were applied to test the germination percentage, root length, shoot length, shoot moisture content and root moisture content of Millet. The results for each treatment are presented in Table 2, with different letters next to the values indicating statistically significant differences ($P<0.05$) between the treatments.

Germination Percentage

The statistical analysis of the germination percentage data revealed significant differences ($P<0.005$) between the two Millet Lines treated with Thiourea under drought stress. However, no significant differences ($P>0.005$) were observed among the various levels of Thiourea applied to the Millet Lines. This suggests that the influence of different Thiourea levels on germination percentage was not statistically significant, but the two Millet Lines showed different responses to Thiourea application.

The observed germination percentage mean values ranged from 73.3% to 96.6%. The highest mean value was in treatment T1 where 500ppm thiourea was applied, followed by T2 (300ppm) and T3 (100ppm). The lowest mean value was observed in T0. These findings suggest that the application of Thiourea had a positive impact on the germination percentage of Millet under drought stress. Treatments T1, T2 and T3 exhibited higher germination percentages compared to the control treatment (T0). Moreover, Line 2 of T1 displayed the highest germination percentage (96.6%), while Line 1 of T3 showed the lowest germination percentage (86.67%), as demonstrated (Tables 1, 3). Wahid et al., (2017) reported in their study that thiourea aids in improving seed germination under stress conditions and the efficacy of this effect was remarkable. Thiourea was found to enhance the germination of seeds under salt stress. Several studies have demonstrated that thiourea is effective in breaking seed dormancy that is caused by environmental factors in plants. Our study yielded similar results, and the study conducted by Wahid et al., (2017) supports the validity of our findings.

Table 2 "Comparative Analysis of Different Parameters under Varied Thiourea Doses"

Treatment	Germination %age	Root length (cm)	Shoot length (cm)	Shoot moisture content %age	Root moisture content %age
T0	75.000 b	2.5833 d	15.267 d	61.167 d	55.000 d
T1	96.667 a	9.6333 a	21.217 a	88.667 a	85.167 a
T2	90.000 a	8.7167 b	18.900 b	79.000 b	75.333 b
T3	88.333 a	8.0500 c	16.383 c	69.500 c	67.500 c

Root length

In a scientific study conducted on two lines of Millet, the application of Thiourea was found to significantly influence root length. The results showed that there was a significant difference between the Millet Lines and various levels of Thiourea applied, with P-values less than 0.005. The root length observations revealed that the highest root length was observed in treatment T1, where 500ppm thiourea was applied, followed by T2 (300ppm) and T3 (100 ppm). The mean root length ranged from 2-10.4 cm, while the control treatment or without thiourea treatment (T0) had the lowest root length. These findings suggest that Thiourea had a positive impact on the root length of Millet under drought stress, with longer roots observed in treatments T1, T2 and T3 than in the control treatment (T0).

Among all experimental units, Line 2 of T1 had the longest root length (10.4 cm), while Line 1 of T3 had the smallest root length (7.4 cm). A study by Asthir et al., (2013) revealed that the height of all plant genotypes decreased in the late-sown crop, but thiourea pretreatment resulted in a noteworthy increase in height in both normal and late-sown crops. Meanwhile, Yadav et al., (2017) investigated the effects of salinity and drought stress on pearl millet-wheat in 2020 and found that pearl millet displayed significantly greater height, with a difference of 16.52 cm between 2017 and 2016. Additionally, it's worth noting that an increase in shoot length is typically accompanied by an increase in root length, demonstrating a relationship between plant height and root length. These results align with the findings of the current study.

Shoot length

The application of Thiourea to two different Millet Lines showed significant variations in shoot length (P <0. 005) between the lines and different levels of Thiourea. This indicates that both Millet Lines and Thiourea levels had a significant effect on the shoot length of Millet. The mean values for shoot length varied from 9.7 to 21.7 cm, with treatment T1 showing the highest value, followed by T2 and T3 and T0 shows the lowest value. The results indicate that the application of Thiourea enhanced the length of Millet shoots under drought stress. Treatments T1, T2 and T3 demonstrated longer shoots than the control treatment (T0). Among all testing units, Line 2 of T1 (500ppm thiourea) had the longest shoot length (22.767 cm), whereas Line 1 of T3, where 100 ppm thiourea was applied, showed the smallest shoot length (14.0 cm).

According to a study conducted by Asthir et al., (2013), the height of all genotypes of plants decreased in the late-sown crop. However, a noteworthy increase in height was observed in both the normal and late-sown crops when they were pretreated with thiourea. In a study conducted by Yadav et al., (2020), the effects of salinity and drought stress on physiological traits governing yield in pearl millet-wheat were investigated. The researchers observed that pearl millet exhibited a significantly greater height of 202.08 cm in 2017 compared to 185.56 cm in 2016. However, no significant differences were observed due to various deficit irrigation. These findings are consistent with the results of the present study.

Shoot moisture content

Shoot moisture content was measured across different treatments and lines and the results showed a significant variation in the outcomes. The highest shoot moisture content (88.667%) was observed in treatment T1 where 500ppm thiourea was applied, followed by treatments T2 (79%) and T3 (69.500%) where 300ppm thiourea was applied, while treatment T0 had the lowest (61.167%). Line 2 exhibited higher shoot moisture content than Line 1 under drought stress and the application of thiourea improved the moisture content of the plant. In the study conducted by Yadav et al., (2020), the effects of salinity and drought stress on the physiological traits that govern yield in pearl millet and wheat were investigated.

The researchers found that the application of Salicylic acid and thiourea mitigated the negative effects of these stressors. Specifically, the application of Thiourea resulted in significantly higher relative water content (RWC) levels in both pearl millet (80.79% and 81.08%) and wheat (72.08% and 73.91%). These findings are consistent with the results of my own research.

Root moisture content

The results from the study indicate significant variability in the root moisture content among the different treatments and lines tested. The highest root moisture content (85.167%) was demonstrated by Treatment T1, which applied 500ppm of thiourea, followed by T2 (75.333%), which applied 300ppm of thiourea, T3 (67.500%), which also applied 300ppm of thiourea and finally T0, which had the lowest root moisture content (55.000%). These findings are presented (Table 2). Under drought stress, Line 2 exhibited more root moisture content than Line 1 and showed an increase in moisture content with the application of thiourea to the plant (Kaya et al., 2013).

The leaves of maize seedlings were sprayed with two levels of mannitol (15 and 30 mM) or Thiourea (3.5 and 7.0 mM) ten days after germination. The application of either mannitol or thiourea resulted in a decreased root: Shoot ratio. In comparison to control plants, salinity (S) treatment significantly reduced leaf RWC. However, foliar application of mannitol or thiourea significantly improved RWC in salt-stressed maize plants, with mannitol showing more improvement. When RWC improved under stress conditions due to thiourea application, it directly related to root shoot moisture content, which also increased. Our study showed similar results, indicating that the application of urea increases RWC. According to a study conducted by Saleem et al., (2022), the application of thiourea (TU) was found to mitigate the reduction in relative water content (RWC) in potato cultivars under drought stress.

The most effective concentration of TU was found to be 0.75 mM, although differences were observed between cultivars in terms of the reduction in membrane stability. In essence, the use of thiourea was shown to increase the moisture content of both the root and shoot under drought stress conditions.

The given Figure 1 shows the germination percentage, root and shoots length and moisture content of shoot and root of plants under different treatments. Treatment T1 showed the highest germination percentage (a), longest root and shoot length (b and c), and the highest shoot and root moisture content compared to the other treatments (d and e), Treatment T0 showed the lowest values for all parameters.

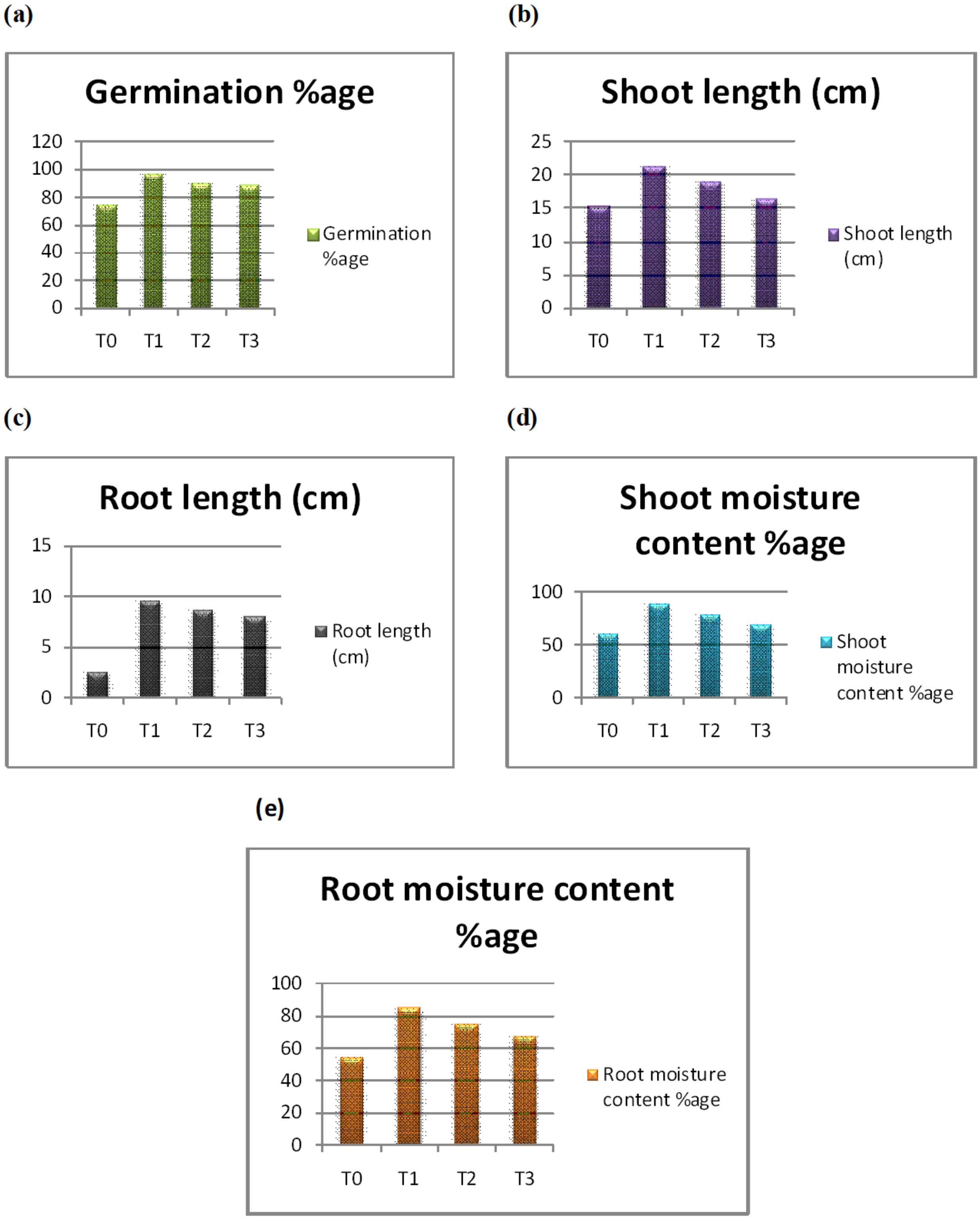

Figure 1 "Assessment of Various Parameters under Different Thiourea Doses"

Thiourea levels (500ppm, 300ppm and 100ppm) significantly affected millet plant parameters like germination, root & shoot length and moisture content under drought stress. Treatment T1 performed best in terms of germination, root and shoot length and moisture content. Line 2 outperformed Line 1 across parameters. These findings could benefit millet growth and yield in agriculture.

Table 3 "Comparative Study of the Effects of Different Thiourea Parameters on Multiple Lines"

Treatment Of Thiourea levels	Lines	Germination %age	Root length (cm)	Shoot length (cm)	Shoot moisture content %age	Root moisture content %age
T1 500ppm	Line 2	100.00 a	10.40 a	22.767 a	91.000 a	88.667 a
T1 500ppm	Line 1	93.33 ab	8.867 c	19.667 c	86.333 b	81.667 b
T2 300ppm	Line 2	90.00 ab	9.400 b	20.800 b	80.667 c	77.667 c
T2 300ppm	Line 1	90.00 ab	8.033 d	17.000 e	77.333 d	73.000 d
T3 100ppm	Line 2	90.00 ab	8.700 c	18.367 d	71.000 e	69.000 e
T3 100ppm	Line 1	86.67 bc	7.400 e	14.400 f	68.000 f	66.000 f
T0 control	Line 2	76.67 cd	2.833 f	16.533 e	63.000 g	58.667 g
T0 control	Line 1	73.33 d	2.333 g	14.000 f	59.333 h	51.333 h

Figure 2 could be showing the effects of different Thiourea treatments on multiple lines. The x-axis represents treatments (T1, T2, T3 and T0) and the y-axis represents lines (Line 1 and Line 2). Separate lines or bars represent each measured parameter. T1 had the highest germination percentage, longest shoot and root lengths and highest shoot and root moisture content percentages. T2 had lower germination percentages than T1 but higher values than T3 and T0. T3 and T0 had the lowest germination percentages and shortest shoot and root lengths. T0 had the lowest shoot and root moisture content percentages.

Drawing definitive conclusions about the specific effects of Thiourea on Millet germination and growth based on the information provided in tables 2 and 3 and the experiment's description is challenging. However, previous research has investigated the potential benefits of using Thiourea as a plant growth regulator. Thiourea is a nitrogen-containing substance that has been shown to enhance plant growth and development in various plant species. For example, low concentrations of Thiourea have been found to increase plant height, leaf area, root length and biomass production (Yadav et al., 2017). Similarly, Thiourea spraying on maize plants has been found to promote root and shoot growth and increase leaf chlorophyll content (Granaz et al., 2022).

Studies have shown that Thiourea can enhance a plant's ability to cope with abiotic stresses such as drought and salt. In wheat, Thiourea spraying has been found to absolutely impact plant development under stress conditions, leading to increased plant height, root length, biomass output and water consumption efficiency Wahid et al., (2017), Yadav et al., (2017), Kaya et al., (2013). Another study on pea plants found that Thiourea improved plant growth, photosynthetic pigments and antioxidant enzyme activity under salt stress (Waqas et al., 2019). These findings suggest that Thiourea has the potential to be a valuable tool for promoting plant growth and production under testing environmental conditions. Multiple research studies have exposed that the use of Thiourea on maize plants experiencing drought conditions can lead to an increase in both crop yield and nutrient absorption. Thiourea has generally shown to be effective in controlling plant growth and development across different plant species. The outcomes demonstrated in the tables indicate that the utilization of thiourea has a positive impact on the germination and growth of millet. However, further research is necessary to fully comprehend the potential advantages and disadvantages of incorporating this substance in millet cultivation.

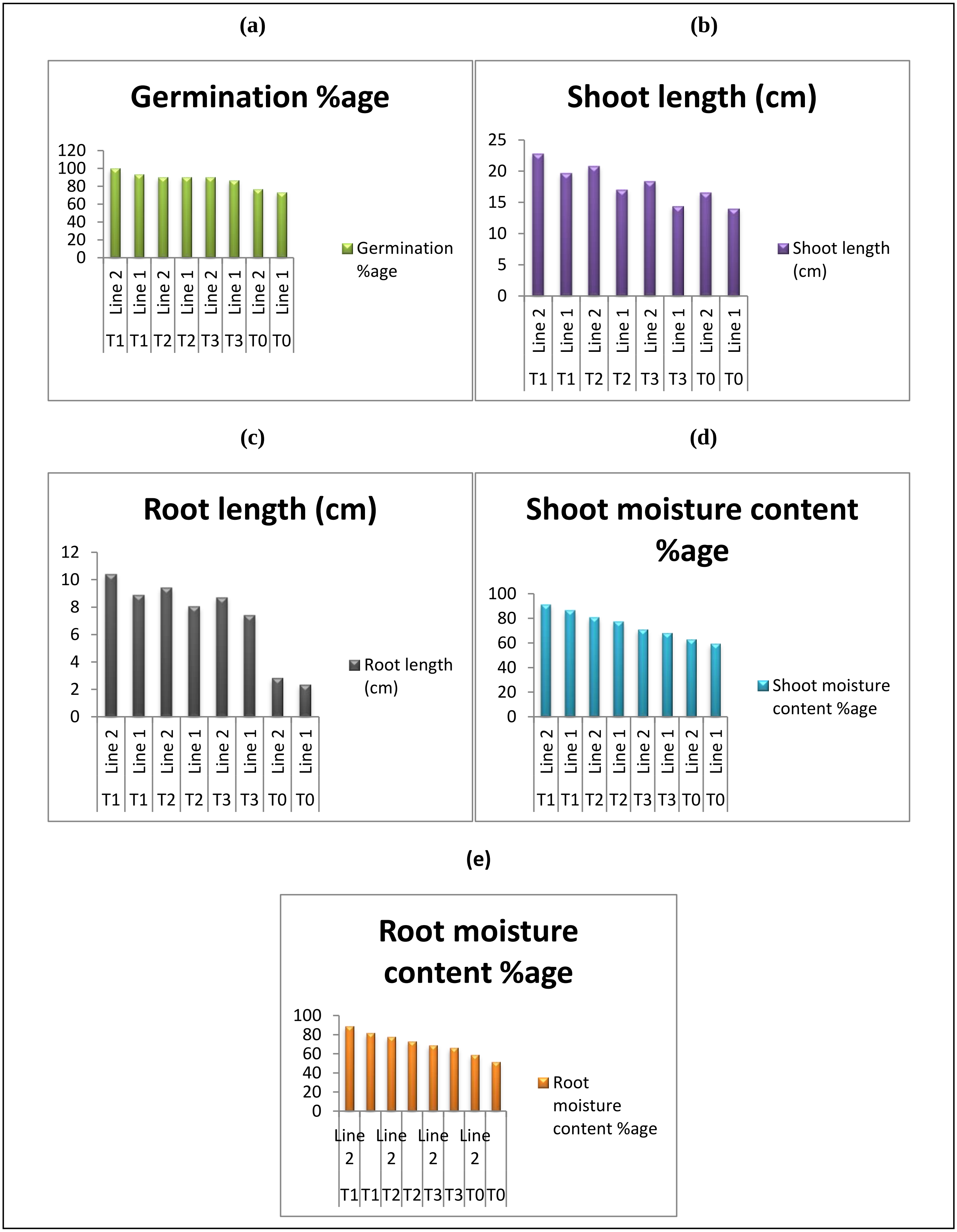

Figure 2 "Effects of Various Thiourea Parameters on Multiple Lines: A Comparative Study," a suitable title for a graph could be "Comparison of Thiourea Parameters on Multiple Lines"

4. CONCLUSION

The application of Thiourea at concentrations of 500ppm, 300ppm and 100ppm had a positive effect on the growth and development of Millet under drought stress. The two Millet lines responded differently to the application of Thiourea, but Treatment T1 (500ppm) had the most significant effect on the growth of Millet, with the highest germination percentage, longest root and shoot length and highest shoot and root moisture content. Line 2 generally had higher shoot and root moisture content than Line 1, indicating different water use efficiency between the two Millet lines. Therefore, the findings suggest that Thiourea can improve the growth and yield of Millet under drought stress and it should be applied at a moderate concentration of 500ppm for maximum benefit. The hybrid Line Millet IABGR (7) 8810 is proposed for optimal results. Further research should explore the mechanisms underlying the positive effects of Thiourea and its potential for improving the growth and yield of other crops under drought stress.

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Informed consent

Not applicable.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for sample collection & experimentation.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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