PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.6250

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REVIEW ARTICLE

Improved lodging resistance and altered gibberellic acid levels in Proso Millet (*Panicum miliaceum* L.) through anti-gibberellins and silicon applications

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OPEN ACCESS

ARTICLE HISTORY
Received: 22 November 2024
Accepted: 23 December 2024
Available online
Version 1.0 : 10 March 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Krishnan A, Chinniah R B, Balaiyan S, Sivaprakasam AP, Palanivelan J S, Kannan S, Muthukrishnan G, Venugopal A. Improved lodging resistance and altered gibberellic acid levels in Proso Millet (*Panicum miliaceum* L.) through anti-gibberellins and silicon applications. Plant Science Today (Early Access).https:/doi.org/10.14719/pst.6250

Abstract

This study examines the impact of foliar applications of growth regulators chlormequat chloride and Mepiquat Chloride (MC) combined with potassium silicate on the growth and productivity of Proso Millet (ATL 1). The field experiment was conducted at Tamil Nadu Agricultural University,Agricultural College and Research Institute, Vazhavachanur, India. A randomized block design with ten treatments, including control and varying concentrations of the growth regulators was used. The results showed that treatments involving 500 ppm chlormequat chloride + 1% potassium silicate and 500 ppm mepiquat chloride + 1% potassium silicate significantly reduced plant height and enhanced stem diameter, leaf area and specific leaf weight. The 500 ppm chlormequat chloride + 1% potassium silicate exhibited the highest chlorophyll content (3.898 mg g^{-1}) and crop growth rate (23.90 g m^{-2} day^{-1}), which correlated with increased grain yield (1548 kg ha^{-1}) and straw yield (2853 kg ha^{-1}). These treatments improved lodging resistance by increasing stem rigidity and overall structural integrity. These findings indicate that combining chlormequat chloride with potassium silicate enhances structural strength, minimizes the risk of lodging during adverse weather conditions and optimizes yield potential in Proso Millet, making it a viable strategy for enhancing productivity in climate-resilient crops.

Keywords

anti-gibberellins; chlormequat chloride; lodging resistance; mepiquat chloride; silicon application

Introduction

The growth and development of crops can be significantly enhanced by applying various growth hormones. The hormones function as signaling molecules and regulating plant growth, development and responses to different biotic and abiotic environmental stresses (1). Under unfavorable weather conditions, the production of two key plant hormones, ethylene and abscisic acid increases, leading to a decrease in vital hormones such as auxins for cell elongation and root development (2), cytokinins for promoting cell division and shoot growth (3) and gibberellic acid for stem elongation and seed germination (4). Crop growth and development can be severely affected when the levels of these growth-promoting hormones decline due to the increased presence of ethylene and abscisic acid. Porso Millet is highly climate-resilient but is characterized by low yields due to limited assimilate partitioning, minimal vegetative growth and hollow stems, which contribute to inefficient resource allocation. To address these challenges, it is essential to enhance source-sink efficiency. This can be achieved through the application of potassium and silicon, which improves stomatal activity, sugar transport efficiency, osmoregulation, stem strengthening, flowering and shoot development, yield, water-use efficiency and drought tolerance.

Crop lodging caused by strong wind and heavy rainfall, poses a significant challenge in physiological and biochemical problems during the vegetative and grain-filling stage. This phenomenon can lead to reduced grain yield (5, 6), decreased grain quality, increased harvest time, increased grain drying costs and increased mycotoxin levels in grain (7). The stress related to lodging may be alleviated through the strategic application of plant growth regulators (8), with gibberellic acid being commonly associated with stimulating internode growth.

Proso Millet, also known as broomcorn Millet, common Millet, hog Millet and Russian Millet, is an ancient crop well suited for dry lands, hill and tribal agriculture. It contributes to regional food security (9). It is majorly cultivated in northern China, Mongolia, the Republic of Korea, Southeastern Russia, Afghanistan, Pakistan, India and southern Europe. The cultivation area spans 0.82 m ha in Russia, 0.32 m ha in China (10), 0.20 m ha in the USA (11) and 0.03 m ha in India (12). Proso Millet is one of the short-duration crops, completing its life cycle within 75 days. It requires less water, matures quickly and the resilience unveiled by these crops is helpful in their alteration to different ecological situations and makes them ideal crops for climate change and contingency planning. It is also called the "poor man's crop" due to its low incidence of pest and disease attacks during the cropping season. Despite its progressive cultivation in hilly and plain regions, the yield is still insufficient and mechanical harvesting is hindered by the plant's thin, hollow structure, which breaks easily. Thus, harvesting by hand is the only option which makes it more costly.

Due to its climate resilience and high nutritional value, Proso Millet is a critical focus for lodging studies. The research on lodging in Proso Millet is far less advanced compared to other staple crops presenting an opportunity to fill a critical knowledge gap and improve its structural integrity. As a low-input crop requiring minimal water and fertilizers, enhancing lodging resistance in Proso Millet supports sustainable agricultural practices and food security, especially in the face of climate change. Utilizing low-cost plant growth promoters may improve the stem's ability to repair itself. Lodging significantly reduces grain yield and grain quality and increases harvest expenses (13) and it adversely affects photosynthetic efficiency, leading to substantial losses.

A novel approach to improve lodging resistance in Proso Millet involves developing a product based on anti-gibberellins (Chlormequat Chloride or Cycocel (CCC) and Mepiquat Chloride) combined with potassium silicate (plant growth promoters). This method aims to increase the rigidity of the basal stem and its compounds of carbohydrates, cellulose, lignin and hemicelluloses which decreases the plant height. The application of anti-gibberellins with potassium silicate increased culm diameter, internode filling and wall thickness which collectively enhanced lodging resistance. This combination is particularly effective because anti-gibberellins limit excessive stem elongation, promoting vigorous growth, while potassium silicate strengthens the cell walls and enhances structural integrity through silica deposition.

Materials and Methods

Study area and physicochemical properties

The field experiment was conducted at Tamil Nadu Agricultural University, Agricultural College and Research Institute, Vazhavachanur, Tiruvannamalai, Tamil Nadu, India during 2023-2024. The physicochemical properties of the topsoil were as follows when it was sampled at a depth of 0-20 cm. The soil of the experimental plot had a sandy loam texture with a pH of 7.4, the amount of organic carbon is 1.75 %, available nitrogen of 109 kg ha^{-1}, available phosphorous of 15.9 kg ha^{-1} and available potassium of 150 kg ha^{-1}.

Experimental design

A randomized block design was used in the experiment and each of the ten treatments was replicated three times (Table 1). Applying silicon, chlormequat chloride and mepiquat chloride to Proso Millet during the vegetative stage and then again 15 days after the initial spraying.

Growth Parameters

The good quality Proso Millet (ATL 1) seeds were collected from Tamil Nadu Agricultural University. The experimental field was prepared by ploughing with a tractor-drawn disc plough followed by harrowing, levelling and manual formation of field bunds around the experiment plots. A basal application of 44:22:0 NPK kg ha^{-1} was applied evenly across each plot at the sowing time. Germination was noted from the third day after sowing and five randomly selected plants from each plot were tagged for growth trait measurement. The plant height was determined by measuring the distance in centimetres from the ground to the tip of the main shoot at

Table 1. Treatment combinations of growth regulators and potassium silicate for Porso Millets

	Treatments
T_1	Control
T_2	250 ppm chlormequat chloride
T_3	Foliar spray of 500 ppm chlormequat chloride
T_4	Foliar spray of 250 ppm mepiquat chloride
T_5	Foliar spray of 500 ppm mepiquat chloride
T_6	1.0 % Potassium silicate foliar spray
T_7	Foliar spray of 250 ppm chlormequat chloride + 1.0 % potassium silicate
T_8	Foliar spray of 250 ppm mepiquat chloride + 1.0 % potassium silicate
T_9	Foliar spray of 500 ppm chlormequat chloride + 1.0 % potassium silicate
T_{10}	Foliar spray of 500 ppm mepiquat chloride + 1% potassium silicate

30 and 60 days after sowing (DAS) and at the harvest stage. Using a leaf area metre (Li-Cor Model 3100) the leaf area of the entire sampling unit was calculated and expressed as cm^2 $plant^{-1}$. The stem diameter was measured using a digital Venire calliper at the third internode of the stem after stripping off leaves and leaf sheaths.

Non-destructive SPAD meter

A non-destructive SPAD meter was used to measure the amount of chlorophyll on the fully developed third leaf from the top. This leaf was selected because it tends to exhibit constant physiological and morphological characteristics compared to the topmost leaf, making it a dependable optimal for assessing plant health. The third leaf is typically mature and fully expanded, ensuring consistent and representative measurements of chlorophyll content. The measurements were recorded between 09:00 and 11:30 on a clear, sunny day and were reported as mg g^{-1} of fresh weight. The data was recorded from 25-30 DAS, 45-55 DAS and 65-75 DAS. The crops were harvested at ground level when they reached physiological maturity. The maturity stage is characterized by the grains reaching their maximum dry weight and the loss of green coloration in the canopy. At this point, the seeds at the tip of the upper heads are ripe and may shatter before the seeds in the lower parts and later panicles have fully matured. The tagged plants were manually threshed, cleaned and dried to a moisture level of 12-14 %, the productive tillers were gathered one by one to calculate the grain yield. To calculate dry matter production in kilograms per hectare, the remaining plant sample was dried at 65±5°C. The crop growth rate (CGR) was calculated at 30 and 60 days after sowing (DAS) and at harvest, using the formula suggested by (14) and expressed in g/m^2/day.

Specific Leaf Weight (SLW)

Specific Leaf Weight (SLW), which is represented in mg cm^{-2}, was computed using the formula (15).

$$SLW = \frac{\text{S Leaf dry weight per plant (mg)}}{\text{Leaf area per plant (cm}^2\text{)}} \qquad \text{(Eqn. 1)}$$

Crop Growth Rate (CGR)

The Crop Growth Rate (CGR) was calculated in g m^{-2} day^{-1} using the formula.

$$CGR = \frac{W_2 - W_1}{P\,(t_2 - t_1)} \qquad \text{(Eqn. 2)}$$

Where,

W_1 and W_2 = Whole plant dry weights (g) at time t_1 and t_2 respectively.

t_2 and t_1 = Time of sampling (days)

P = Ground area occupied by the plant (m^2)

Lodging percentage

Randomly select a set number of plants (consistent number of plants from representative plots) from representative plots, visually assess the orientation of each plant to determine if they are lodged or upright (Fig. 1), record the number of lodged plants and calculate the lodging percentage using the given formulae

$$\text{Lodging Percentage} = \frac{\text{Total Number of Plants}}{\text{Number of Lodged Plants}} \times 100 \qquad \text{(Eqn. 3)}$$

Quantitative estimation of gibberellins by HPLC

High-performance liquid chromatography (HPLC) using a NEXERA X2 apparatus was employed to quantify gibberellic acid. The apparatus has a C18 Eclipse Plus c 18 column and a PDA (190-800 nm) detector. The sample was extracted using methanol. The flow rate is generally set to 1.0 mL/min and a 20 µL sample from each gibberellin-containing solution was injected into the HPLC at a wavelength range of 206 nm, ascertained by utilizing a photodiode array to identify absorption maxima (16). Each run was performed three times, the filtered plant extract and the standard solutions are then injected into the HPLC system, allowing for the measurement of retention times and peak areas for each compound. Data analysis involves constructing a calibration curve by plotting the peak areas of the standards against their concentrations, which facilitates the quantification of gibberellins in the plant extracts. The results are expressed in µg/g of fresh weight. Finally, the method is validated by assessing parameters such as accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). This HPLC methodology provides a precise and reliable means to quantify gibberellins, offering valuable insights into their roles in plant growth and development.

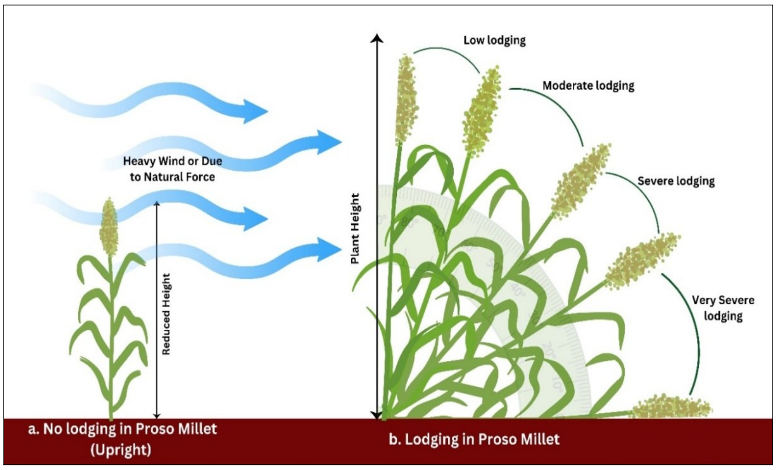

Fig. 1. Differences between upright and lodged Proso Millet.

Statistical analysis

Statistical tests were made using SPSS 17.0 (IBM, Chicago, IL, USA). Significant differences between treatment means were determined using non-parametric tests and a p-value of ≤ 0.05.

Result and Discussion

The experimental results on plant height, culm girth, leaf area, specific leaf weight and crop growth rate are detailed in Table 2. The tallest plants were recorded in T_1 (56.4 cm), while the shortest plants were observed in T_9 (46.4 cm). The consistently shorter heights observed in treatments T_2 to T_8 (47.4 to 54.2 cm) indicate that the foliar application of mepiquat chloride and chlormequat chloride effectively reduces plant height by preventing the conversion of geranyl pyrophosphate to coponyl pyrophosphate, the initial step in gibberellin production and these substances function as anti-gibberellin dwarfing agents (17). When comparing the two growth retardants, chlormequat chloride was more successful than mepiquat chloride at reducing plant height in porso Millet. Another study (18) also observed a relationship between stem shortening and the application of mepiquat chloride and chlormequat chloride in Proso Millet. The morpho-physiological effects of cell division and cell enlargement were reduced with the use of mepiquat chloride, indicating a reduction in the plant height (19). Reductions in gibberellin can have an impact on intercellular transport by reducing cell wall thickness and stiffness, which can prevent cell division, cell elongation and duplication (20).

In terms of lodging resistance, the control treatment (T_1) showed the highest lodging percentage at 5.3%, while T_9 exhibited the lowest (2.9%) (Fig. 2) and T_4, T_6 and T_8 tended to cluster around 3.5% to 3.6%. In this study the application of chlormequat chloride, mepiquat chloride combined with potassium silicate resulted in increased stem diameter, particularly in treatment T_9 (1.83 cm) and T_{10} (1.73 cm), indicating stronger structural support and contributing to improved lodging resistance. Crops with thicker stems are more mechanically stable, enabling them to tolerate the grain weight and abiotic factors (21). The results indicate that T_9 and T_{10} could potentially enhance the overall resilience of the plants and lead to better performance in terms of yield and stability. According to the previous study (22), mepiquat chloride not only increases stem physical strength but also boosts lignin production and lodging resistance in maize. The current research findings suggest that the combination of 500 ppm chlormequat chloride + 1 % potassium silicate may be more effective at enhancing stem thickness specifically. Potassium silicate plays a dual role by physically reinforcing cell walls through silica deposition and metabolically enhancing lignin synthesis, nutrient transport and photosynthetic efficiency. These effects collectively reduce lodging risk and improve crop stability.

The photosynthetically active leaves (3rd and 4th) were used to measure the leaf area, specific leaf weight and crop growth rate in Proso Millet. Silicon coupled with anti-gibberellins increases the length and breadth of the leaves by depositing in cell walls, making them more robust and resistant to environmental stressors. The foliar spray of 500 ppm chlormequat chloride + 1% potassium silicate and foliar spray of 500 ppm mepiquat chloride + 1% potassium silicate resulted in increased leaf areas of T_9 (202.3 cm^2 $plant^{-1}$) and T_{10} (196.9 cm^2 $plant^{-1}$) respectively. Anti-gibberellins cause the plant to grow shorter, but they increase the leaf area by cell proliferation and elongation. This balanced growth ensures that while the plant remains compact, the

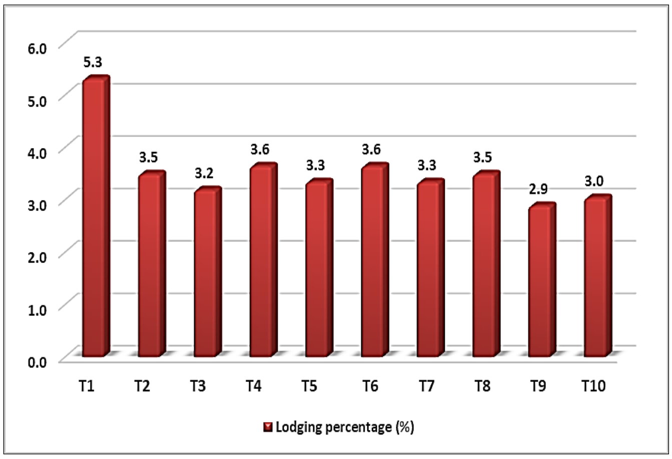

Fig. 2. Influence of anti-gibberellins on lodging percentage in various treatments.

Table 2. Effect of plant growth regulators and chemicals to prevent lodging in Proso Millet

Treatments	Plant Height (cm)	Culm Girth (cm)	Leaf Area (cm^2 $plant^{-1}$)	Specific Leaf Weight ($mg\ cm^{-2}$)	Crop Growth Rate ($g\ m^{-2}\ day^{-1}$)
Control (T_1)	56.4^{a}	1.50^{e}	173.7^{f}	1.017^{f}	10.40^{e}
Foliar spray of 250 ppm Chlormequat Chloride (T_2)	48.6^{b}	1.52^{e}	185.4^{de}	1.133^{e}	13.45^{de}
Foliar spray of 500 ppm Chlormequat Chloride (T_3)	47.5^{b}	1.61^{c}	181.5^{ef}	1.185^{e}	16.70^{cd}
Foliar spray of 250 ppm Mepiquat Chloride (T_4)	49.3^{b}	1.50^{e}	189.7^{cd}	1.128^{e}	12.55^{de}
Foliar spray of 500 ppm Mepiquat Chloride (T_5)	47.7^{b}	1.57^{d}	183.2^{ef}	1.159^{e}	14.30^{cde}
1.0 % Potassium Silicate foliar spray (T_6)	54.2^{ab}	1.55^{d}	185.1^{de}	1.678^{c}	16.95^{cd}
Foliar spray of 250 ppm Chlormequat Chloride + 1% Potassium Silicate (T_7)	47.4^{b}	1.58^{d}	192.2^{bc}	1.447^{d}	18.90^{cd}
Foliar spray of 250 ppm Mepiquat Chloride + 1% Potassium Silicate (T_8)	49.3^{b}	1.60^{cd}	186.5^{e}	1.794^{b}	18.04^{c}
Foliar spray of 500 ppm Chlormequat Chloride + 1% Potassium silicate (T_9)	46.4^{b}	1.83^{a}	202.3^{a}	1.956^{a}	23.90^{a}
Foliar spray of 500 ppm Mepiquat Chloride + 1% Potassium Silicate (T_{10})	48.5^{b}	1.73^{b}	196.9^{ab}	1.860^{a}	22.75^{b}
SEm	6.00	0.16	39.86	0.50	0.27
CD (P=0.05)	17.81	0.48	118.41	1.49	0.82

leaves expand to capture more sunlight (Fig. 3). The chlormequat chloride together with other chemical combinations boosted the leaf area in sorghum (23).

The application of chlormequat chloride combined with potassium silicate significantly increased specific leaf weight and crop growth rate during the vegetative stage and 15 days after the initial spray. The foliar spray of 500 ppm chlormequat chloride + 1% potassium silicate recorded a specific leaf weight of 1.956 mg cm^{-2} and a crop growth rate of 23.90 g m^{-2} day^{-1}, outperforming the control which showed values of 1.017 mg cm^{-2} and 10.40 g m^{-2} day^{-1} (Table 3). As a C4 plant, Proso Millet benefits from increased leaf thickness, likely due to enhanced photosynthetic efficiency and greater stacking of mesophyll and bundle sheath cells, allowing for better recapture of CO_2 released during photorespiration. The photosynthetic efficiency of C4 crops like Millets is superior to that of C3 crops, contributing to higher chlorophyll content during the grain-filling stage. The foliar spray of 500 ppm chlormequat chloride + 1% potassium silicate exhibited a chlorophyll content of 3.898 mg g^{-1} which is superior to other treatments. The mepiquat chloride applications increase the leaf thickness resulting in longer palisade and more spongy parenchyma cells within the leaf mesophyll, which further enhances chlorophyll content per unit area (24).

The higher chlorophyll levels observed in T_9 and T_{10} lead to improved photosynthetic efficiency, providing more energy for growth and development, ultimately resulting in increased yields. The photosynthetic efficiency increases with the application of mepiquat chloride, which also raises the total chlorophyll content in chili (25). Gibberellic acid relative abundance is highest in the control (Fig. 4), suggesting that a larger concentration of gibberellic acid is needed to enhance cell elongation (26).

The yield and yield components increased with the foliar application of 500 ppm chlormequat chloride + 1% potassium silicate. At the harvest stage, the foliar application of 500 ppm chlormequat chloride + 1% potassium silicate show a statistically significant improvement in yield and yield components, viz., total dry matter production of 26.21 mg cm^{-2}, grain yield of 1548 kg ha^{-1} and straw yield of 2853 kg ha^{-1} compared to the control. This was on par with the foliar spray of 500 ppm mepiquat chloride + 1% potassium silicate.

The foliar spray of 1 % potassium silicate was one of the reasons for the increase in grain yield (27) and there are several other factors, including the reduction in sterility rates, increase in the rate of photosynthesis, higher number of tillers and decrease in pest and disease incidence. These findings are consistent with the work of (28). Potassium silicate enhances plant resistance by stimulating defence mechanisms and reducing damage from insects and pests. This fortification is achieved through improved uptake of essential nutrients and helps crops to develop greater resistance to pest infestations and lodging. Research (29) indicates that silicon can suppress insect and non-insect pests. Furthermore, the observed improvement in straw yield can be attributed to silicon's role in regulating stomatal activity, enhancing photosynthesis and improving water use efficiency, contribute to better vegetative growth and increased straw yield (30).

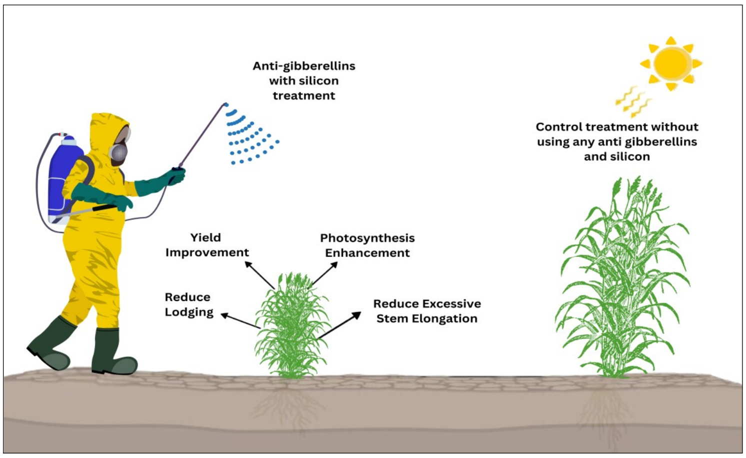

Fig. 3. Visual comparison of treatment (Anti-gibberellins 500 ppm Chlormequat Chloride + 1% Potassium Silicate) outcomes on Proso Millet.

Table 3. Effect of plant growth regulators and chemicals to prevent lodging in Proso Millet

Treatments	Total Chlorophyll Content (mg g^{-1})	Total Dry Matter Production	Grain Yield (kg ha-^{1})	Straw Yield (kg ha^{-1})
Control (T_1)	2.805^{c}	16.78^{f}	1321^{f}	2562^{f}
Foliar spray of 250 ppm Chlormequat Chloride (T_2)	3.006^{bc}	17.69^{e}	1429^{bcd}	2666^{d}
Foliar spray of 500 ppm Chlormequat Chloride (T_3)	3.261^{b}	21.20^{bc}	1435^{bcd}	2716^{c}
Foliar spray of 250 ppm Mepiquat Chloride (T_4)	2.956^{bc}	19.32^{d}	1362^{e}	2620^{e}
Foliar spray of 500 ppm Mepiquat Chloride (T_5)	3.023^{bc}	18.34^{d}	1402^{d}	2671^{d}
1.0 % Potassium Silicate foliar spray (T_6)	3.105^{bc}	20.12^{c}	1418^{cd}	2727^{c}
Foliar spray of 250 ppm Chlormequat Chloride + 1% Potassium Silicate (T_7)	3.360^{b}	20.57^{c}	1467^{bc}	2784^{b}
Foliar spray of 250 ppm Mepiquat Chloride + 1% Potassium Silicate (T_8)	3.223^{bc}	21.84^{bc}	1457^{b}	2725^{c}
Foliar spray of 500 ppm Chlormequat Chloride + 1% Potassium Silicate (T_9)	3.898^{a}	26.21^{a}	1548^{a}	2853^{a}
Foliar spray of 500 ppm Mepiquat Chloride + 1% Potassium Silicate (T_{10})	3.256^{b}	22.31^{b}	1470^{ab}	2791^{b}
SEm	0.15	0.54	69	87.68
CD (P=0.05)	0.44	1.61	204.98	260.46

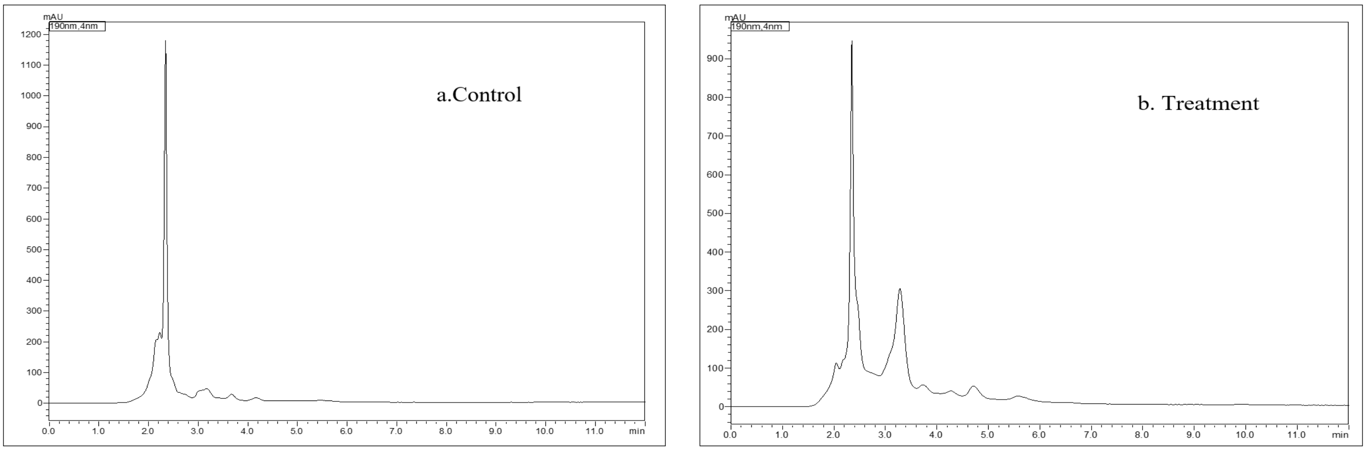

Fig. 4. HPLC Chromatograms: a. Control b. low GA_3 Proso Millet treated with anti-gibberellins 500 ppm Chlormequat Chloride + 1% Potassium Silicate.

The T_9 shows the most effective method and have a clear correlation between higher total chlorophyll content and improved yields. The elevated chlorophyll levels likely contributed to increased photosynthetic efficiency, which is essential for biomass accumulation and ultimately leads to higher grain and straw yields. The silicon application improves sorghum leaf erectness, enhancing solar radiation penetration and leading to increased dry matter production (31). The anti-gibberellins foliar spray alters the morphogenesis of plants, resulting in reduced plant height, increased leaf area, crop growth rate, grain yield and straw yield in finger Millet (32). The chlormequat chloride may have increased the yield up to 16.3% in finger Millet, while an 8.2% increase in yield was recorded in wheat (33, 34). Foliar application of 500 ppm chlormequat chloride + 1% potassium silicate reduces the lodging percentage compared to other treatments.

Conclusion

The growth and productivity of Proso Millet are improved by the foliar application of chlormequat chloride + potassium silicate. These treatments enable farmers to produce more and enhance the quality of their crops, leading to higher market value. The foliar spray of 500 ppm chlormequat chloride + 1% potassium silicate and foliar spray of 500 ppm mepiquat chloride + 1% potassium silicate shows good results, with decreased plant height, increased stem diameter, leaf area, specific leaf weight and crop growth rate. The increased chlorophyll content in these treatments correlates with improved photosynthetic efficiency which leads to higher grain and straw yields. Overall, the findings suggest that combining chlormequat chloride and mepiquat chloride with potassium silicate helps enhance structural strength, minimize the risk of lodging during adverse weather and optimize yield potential in Proso Millet. It also promotes sustainable agricultural practices by improving crop resilience, reducing the risk of lodging and increasing grain yield. This synergistic approach minimizes the excessive chemical input, thereby supporting environmental health. It offers a strategy for farmers aiming to improve both the quality and market value of their crops in a changing climate.

Acknowledgements

The author would like to express their sincere gratitude to Tamil Nadu Agricultural University, Tamil Nadu, India, for providing the facilities throughout the research studies.

Authors' contributions

AK conceptualized the study and writing the original draft of MS, RBC framed methodology, SB data curation, APS helped to revise the manuscript and visualization, JSP formal analysis, SK carried out the manuscript alignment, GM carried out the reference check, AV analysed data in software. All co-authors reviewed the final version and approved the manuscript before submission. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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