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Histochemical Observations of *Eleusine coracana* Stem Tissues Under Multiple Abiotic Stresses

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***Abstract*— *This study investigates the histochemical changes of lignin deposition in the stems of seven finger millet genotypes under Drought, Salinity, Heat, Cold, and Heavy metal stress using the Phloroglucinol-HCl staining method. Well-grown plants were subjected to individual stresses at the pre-reproductive stage, and stem cross-sections were analyzed for lignin accumulation and structural differences under light microscope. This study revealed significant genotypic variation among genotypes and also in lignin deposition under stress conditions compared to respective controls. Among different stresses given, heat is showing maximum effect on vascular bundles and stem structural stability of plant. Based on lignin, stress tolerant and susceptible varieties were identified. Results suggested few genotypes which can be used as animal feed, fodder, are agronomically important and industrially applicable genotypes.*

Keywords*— *Abiotic stress, Finger millet (Eleusine coracana), Lignin, Vascular tissue.

I. INTRODUCTION

Finger millet (*Eleusine coracana (L.) Gaertn*), a C_4 crop cultivated in semi-arid tropics of Africa and South Asia. It plays a vital role in supporting livelihoods and nutritional security of millions (Kheya *et al.,* 2023). It offers an outstanding nutritional profile, rich in calcium, dietary fibre, and essential amino acids, making it especially valuable for daily dietary consumption (Gaikwad *et al.,* 2024). It also has capacity to tolerate various abiotic stresses such as drought, salinity, heavy metal toxicity, and temperature extremes but ongoing unpredictable rapid climate change affecting its cultivation (Binodh *et al.,* 2025)

Plants respond via different physiological and structural adaptations to these environmental constrains (Kanathala *et al.,* 2026). Among these, the immediate effect of stress includes dehydration (lack of water availability) and changes osmotic potential resulting in overall disturbances in cell homeostasis, leads to structural rearrangements in water transport system i.e. vascular tissue (Hussain *et al.,* 2019). Drought makes development of thicker xylem with reduced vessel diameter and increased vessel number in stems and roots (Yang *et al.,* 2021). In salinity and heavy metal stress, vascular bundles often thicken with increased parenchyma cells, phloem and xylem to facilitate ion compartmentalization (Yao *et al.,* 2023). Along with this cell wall lignification provides mechanical support against turgor pressure (Barros *et al.,* 2015).

Lignification also stands out as a key mechanism aiding tissue integrity, regulating water and ion movement, thereby protects cell from damage under stress (Barros et al 2015). Lignin is a complex phenolic polymer synthesized from p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) monolignols via the phenylpropanoid pathway (Vanholme el., 2019). Stress responsive alterations in lignin content and composition enhances cell wall rigidity, hydrophobicity, and mechanical strength to withstand stress providing partial tolerance. Lignification in xylem vessels, sclerenchyma, endodermis, and bundle sheath cells which directly influences water transport, ion exclusion, and mechanical stability (Choi *et al.,* 2023; Han *et al.,* 2022). There are several studies supporting the modification of lignin as a stress responsive adaptation such as, Drought increases lignification in xylem with higher proportions of

G and S units helping hydraulic safety (Liu *et al.,* 2021; Choi *et al.,* 2023); Salinity and Heavy metal stress promotes lignin accumulation that limits ion movement through the apoplast and safeguards vascular tissues (Han *et al.,* 2022); Heat accelerates lignin depositions to strengthen cell wall while, Cold modifies its composition and vascular patterning (Zhao *et al.,* 2019). Usually, tolerant genotypes across many species show greater lignin accumulation with higher expression of lignin biosynthetic genes. Biochemical and transcriptomic studies of Finger millet also suggests altered lignin content and involvement of lignin specific genes with abiotic stress (Puranik *et al.,* 2011; Satish *et al.,* 2016). Histochemical staining and microscopic studies of transverse stem sections for lignin depositions and structural changes in Finger millet under different abiotic stresses provides actual visible changes occurred (Satish *et al.,* 2016). This anatomical marker can also be used for partial screening of various cultivars to identify tolerant varieties.

In the present study, Seven Finger millet germplasms were subjected to various abiotic stresses. The staining of cross-sectional samples from second internodal region of stems was done using Phloroglucinol-HCl stain and visualised under light microscope. We aim to reveal structural changes and lignin patterns in cell walls occurred as a result of stress exposures aids understanding cell wall-mediated stress tolerance in millets. Hence, identifying these visible changes helps in selecting specific germplasms of interest to specific industrial usage and also further targeted modifications enhances their agronomical and economical value.

II. MATERIALS AND METHODS

Place of Work

The present study was conducted in the laboratory and net house facilities at the Center for Plant Molecular Biology (CPMB) and the Department of Genetics & Biotechnology, Osmania University, Hyderabad, India, in the year 2025.

Plant Material

The present study utilized seven finger millet germplasms cultivated in South India ie., Karnataka, Andhra Pradesh and Telangana. Four cultivars Uduru Mallige, CFMV1, GPU28, and GE4449 were procured from the Indian Council of Agricultural Research (ICAR)-Indian Institute of Millets Research (IIMR), Hyderabad. The remaining three cultivars-Gowthami, Vakula, and Godavari were obtained from local farmers of Vizianagaram District, Andhra Pradesh, India.

Pot Experiment

Plants were grown in 26 cm diameter pots filled with a potting mixture of soil: sand: vermicompost at a 3:2:1 ratio (v/v). The experiment was conducted in a completely randomized design (CRD) under controlled net and glass house conditions.

Abiotic Stress Treatments

Drought stress was imposed by Mannitol (250 mM), salinity by NaCl (250 mM) and Heavy metal by $CuSO_4$ (500 µM) solutions. Heat stress (40°C) and cold stress (4°C) were imposed by adjusting temperature in plant growth chambers. Well-watered plants were taken as controls. All plants were maintained under optimal glass house conditions. These treatments were applied to 45 days old plants at pre flowering stage for about 36 hours.

Histochemical Staining of Stem Internodes

Second Internode region of each stem sample of control and stress treated plants were collected. Thin transverse cross section was manual cut using number eight blade from the middle portion of internode region and sections were mounted on glass slides. Samples were stained immediately by 3% phloroglucinol (0.3g in 10ml Absolute Ethanol) followed by 37% fuming HCl solution in 2:1 ration for lignin visualization (Clifford, 1974). Examined under an Olympus light microscope with images captured using a mobile camera.

III. RESULTS

The changes observed under 10x magnification were captured and modifications in lignin content, stem structure were compared among the samples under stress conditions.

In Uduru Mallige

Control plants showed high lignin across vascular bundles including metaxylem, protoxylem, phloem and epidermal regions. Under Heavy metal stress, decrease in lignin was observed with depositions found only around xylem and lower number of vascular bundles are present. Under drought stress, lignin was decreased. Under Salinity stress, lignin content decreased with depositions only around protoxylem walls but protoxylems were more damaged. Under low temperatures, lignin was highly decreased and size of protoxylem increased a bit whereas under heat stress, lignin was decreased which is found only around protoxylem along with decrease in number of vascular bundles.

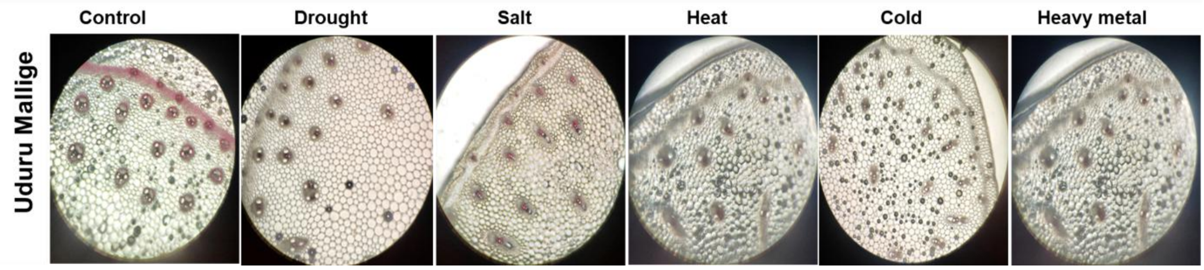

Fig.1: Microscopic images of Uduru Mallige stem cross sections under multiple stress conditions showing lignin content and vascular bundles pattern.

In GE4449

Control plants had very little lignin depositions in protoxylem walls. Under Heavy metal stress, overall lignin content was highly increased in Phloem, xylem and epidermal regions. Under drought stress also lignin content was highly increased in Phloem, xylem and epidermal regions. Under salinity stress, lignin around xylem tissue both metaxylem and protoxylem but damage was occurred to vessels of protoxylem. In low temperatures, presence of lignin in both metaxylem and protoxylem with increase in size of bundle sheath regions was observed. In high temperature stress, the stems were rigid with increase in lignin around phloem, xylem, and also increase in number of bundles.

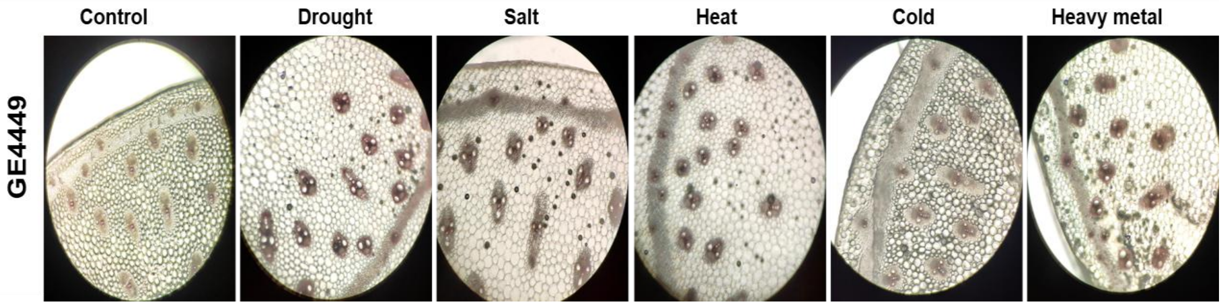

Fig.2: Transverse stem sections of GE4449 variety under multiple abiotic stresses showing different lignification patterns and anatomical differences.

In Gowthami

Control plants showed healthy vascular bundles with lignin in walls of protoxylem regions. Under heavy metal stress, the stems are similar to control ones. Under drought stress, the number of protoxylems were increased and surrounded by more lignin depositions. Under salinity stress, minor increase in lignin content with thickened xylem walls were observed. Under low temperatures, lignin was found only in protoxylem regions but number of protoxylems were increased up to five in number. Under heat, the vascular bundles were damaged but lignin can be observed around protoxylems.

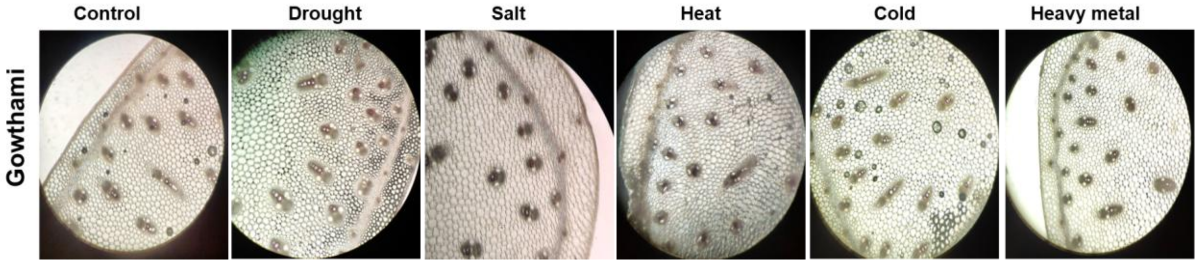

Fig.3: Microscopic images of Gowthami stem cross-sectional area under multiple stress conditions showing lignin content and vascular bundles pattern.

In Vakula

Control plants are well developed stem architecture and presence of lignin in only protoxylem walls. Under heavy metal stress, xylems vessels were increased along with lignin depositions. Under drought stress, amount of lignin was similar to controls but more thinning of vessels was observed. Under salinity stress, a very slight decrease in red pigment intensity thereby decrease in lignin; size of bundle sheath region was enlarged and xylem was reduced. Under low temperatures, no change in lignin level but vascular bundles size was narrowed. Interestingly, under heat the stem tissue got damaged except protoxylems which are increased in number and highly deposited by lignin in their walls.

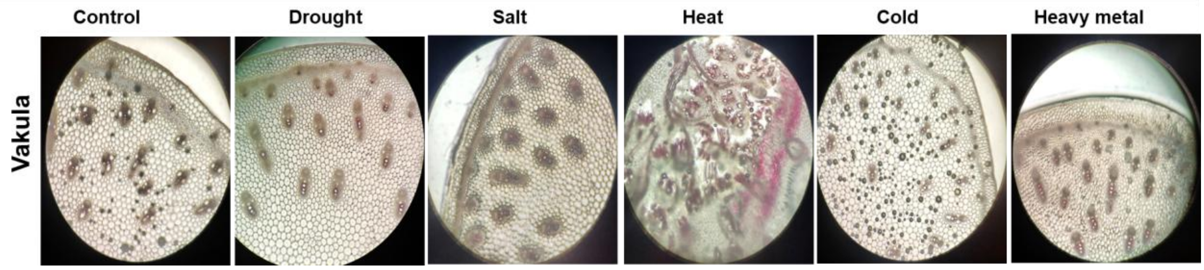

Fig.4: Transverse stem sections of Vakula variety under multiple abiotic stresses showing different lignification patterns and anatomical differences.

In GPU28

Control plants showed well developed vascular bundles with no lignin depositions. Under heavy metal stress, there is no change compared in lignin content was observed. Under drought conditions, a drastic increase of lignin deposition was observed around xylem vessels, Phloem and in epidermal cells of bundle sheaths region. Drought also caused a decrease in number of protoxylems compared to control. Under salinity stress, lignin depositions occurred walls of protoxylems and also thinning of vascular bundles was observed. In low temperatures, the lignin increased in phloem, xylem vessels and epidermal cells of bundle sheaths region similar to drought stress whereas high temperatures led to complete damage to stem regions.

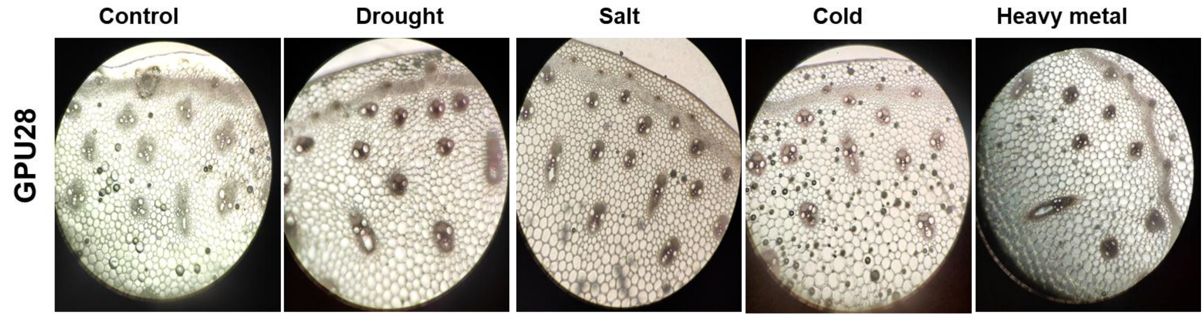

Fig.5: The microscopic image of transverse stem sections of GPU28 under drought, salt, heavy metal and cold along with controls, complete stem damaged under heat.

In Godavari

Control plant shows presence of lignin around xylem tissues. Under heavy metal stress, decrease in overall lignin content and found in only protoxylem regions. Under drought stress, heavy damage to vascular bundles were observed and lignin content was similar to heavy metal stress ones. Under salinity stress, lignin content was highly increased in Phloem, xylem and epidermal regions. In low temperatures, lignin is found only around protoxylems whereas in high temperatures, the stem is totally damaged.

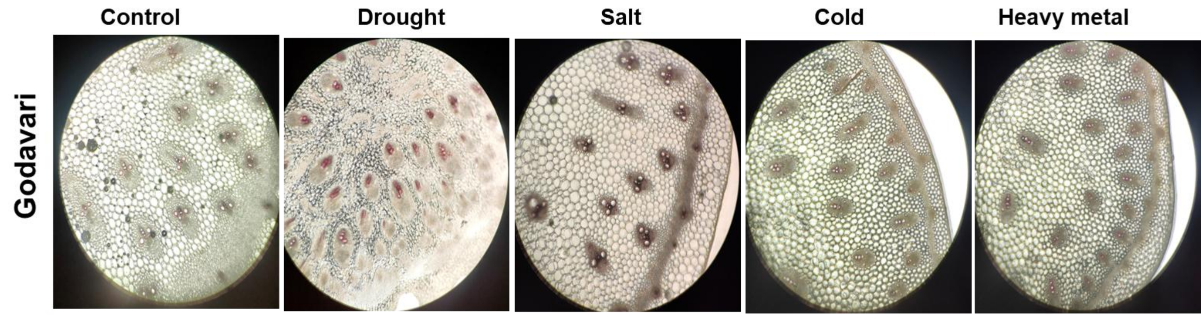

Fig.6: The microscopic image of transverse stem sections of Godavari under drought, salt, heavy metal and cold along with controls, complete stem damaged under heat.

In CFMV1

Control plant shows presence of lignin around xylem vessels. Under heavy metal stress, increased lignin content was found in Phloem and xylem regions. Under drought stress, widening in vessels was observed but overall bundle size was reduced and lignin content was present at only protoxylem regions. Under salinity stress, lignin content was highly increased in protoxylem region. In low temperatures, good amount of lignin deposition around xylem tissue, uneven distribution in epidermal tissues with increase in number of vessels whereas in high temperatures, the stem is totally damaged which cannot be sectioned at all.

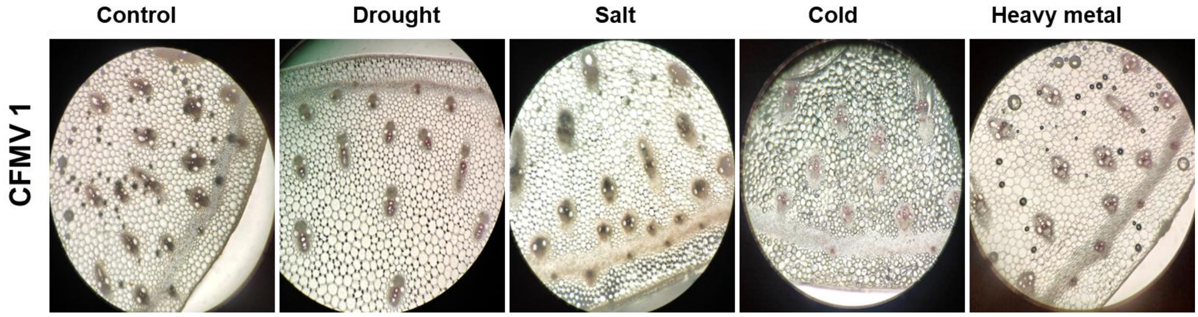

Fig.7: The microscopic image of transverse stem sections of CFMV1 under drought, salt, heavy metal and cold along with controls, complete stem damaged under heat.

IV. DISCUSSION

Lignin is mostly deposited in the walls of xylem and phloem tissue of vascular bundle in plants, which aids in providing mechanical support and structural stability (Nakamura *et al.,* 2020). The main role of vascular bundles is aiding water transport and providing mechanical support to plants. Lignin accumulates more around these vascular bundles as a positive stress adaptation mechanism making plant withstand under unfavourable conditions (Han *et al.,* 2022). On the other hand, higher accumulation of lignin poses a significant challenge for domestic feed and manufacturing industries related to pulp, paper and lignocellulosic biofuel ones (Bachir *et al.,* 2022). Thus, observing lignin qualitatively by Histochemical staining method provides information regarding the intensity and deposition patterns of lignin in vascular tissues and also the stress related alterations in vascular bundles compared to controls.

In present study, Lignin present in cross section of stem internode region was assessed qualitatively by phloroglucinol staining method. Seven Finger millet cultivars were taken to compare lignin accumulation under various abiotic stresses. The genotype Uduru mallige showed naturally more lignin content without any stress treatment given which makes it useful under lodging conditions. Upon imposition of stress, it showed reduction in lignin content and damage to vascular bundles suggests that it is susceptible variety which failed to induce lignin biosynthesis under stress and unable to maintain xylem against osmotic damage. In cold conditions, the enlargement of vessels suggests elasticity to maintain water transport (Ren *et al.,* 2025). Under heavy metal stress plants usually thicken the walls of xylem with lignin to avoid the transport of heavy metals via apoplast (ShangGuan *et al.,* 2023). CFMV1 had lignin surrounding the xylem in controlled conditions showing secondary wall development for providing mechanical support. Under all stress treatments lignin has highly increased conferring to stress tolerance mechanism (Moura *et al.,* 2010). Compared to other stresses, drought has greatly affected forming more widen vessels, helps higher uptake of water by plant (Agustí *et al.,* 2026;2020). Protoxylems are small, less efficient compared to metaxylems but plants maintain minimum water supply by increasing the number of protoxylems as a stress adaptation approach (Augstein *et al.,* 2022). Hence, CFMV1 shows tolerant behaviour under cold, drought, salt and heavy metal stresses. In GPU28 and Gowthami varieties there is no change in lignin, vascular bundles arrangement due to heavy metal treatment recommends that they have adopted a different detoxification mechanism other than lignin. GPU28 showed no lignin in control stems. Under drought, salinity and cold stress conditions, there is a huge lignification observed implicating stress responsive but contrastingly thinning of vessels occurred to withstand stress as a primary priority than water transport efficiency (Barros *et al.,* 2015). High temperatures have completely damaged the stems of GPU28, CFMV1 and Godavari as they couldn't maintain cellular homeostasis and heavy dehydration. GE4449 is the only cultivar showed increased lignification under heat stress with a compact structure of vascular bundles therefore having a lignin induced thermal tolerance property and stronger capacity to maintain structural stability (Han *et al.,* 2024). In Vakula, the structural rearrangements of vascular bundles have dominated the lignin depositional changes comparatively. Change in xylem number, thinning of vessels and presence of more protoxylems were observed under stress conditions to survive multiple stresses by using minimum metabolism energy rather than uniformly increasing lignin (Qaderi *et al.,* 2019).

Based on anatomical studies of seven cultivars studied here, GE4449, CFMV1 showed good tolerance mechanisms under multiple abiotic stresses. These are nutrient rich crops along with tolerance suggesting best suitable for agronomic purposes and for further studies. Uduru mallige variety had naturally more lignin content making it hard to use as animal feed due to its low digestibility and also not suitable for paper, pulp and Ligno-cellulosic biofuel industries contrastingly GPU28 had no lignin suggesting for biomass usage for these industries by decreasing the upstream cost of lignin degradation process (Yoo *et al.,* 2020). Uduru mallige as a Lignin-rich variety can be economically utilized for thermal bioenergy and bioplastic production (Laurichesse *et al.,* 2014). Additionally, helps reduce biowaste accumulation, as generally less preferred for animal feed. Further, quantification of monolignols and changes in composition of monolignols can be studied to make effective use of finger millet biomass industrially.

V. CONCLUSION

Current study links variations in lignin content along with structural rearrangements to multiple abiotic stress adaptations in seven finger millet germplasms. Finger millet consumption has increased recently as malnutrition awareness among population increased. Finger millet is nutritionally rich crop and its increase in recent cultivation, studying lignin helps in better usage of biomass industrially and economically. Additionally, it also helps partial identification of abiotic stress tolerant germplasms. Thus, our study provides fundamental insights into lignin-mediated stress adaptation mechanisms, thereby paves the way for targeted strategic modification of genes which are lignin related in stress tolerant crops with high agronomic value enhances industrial applications.

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