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Research Article

Impact of high-temperature stress on anatomical adaptations in mungbean genotypes

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Abstract
Rising global temperatures present a major challenge to summer-season legumes like mungbean, affecting their growth and productivity. Understanding plant anatomical adaptations under high-temperature stress (HTS) is crucial for developing heat-resilient crop varieties. This study aimed to assess the anatomical modifications in three mungbean(*Vigna radiata* (L.) Wilczek) genotypes (MH 421, MH 1772, and IPM 312-19) under different sowing conditions. The experiment was conducted in a randomized block design (RBD) under field conditions, comparing normal-sown (March, <40°C) and late-sown (April, >40°C) crops. Root and stem transverse sections (T.S.) were analyzed to examine variations in xylem vessel characteristics. Results revealed a significant increase in xylem vessel size, thickness, and number under late-sown conditions, indicating structural modifications to cope with heat stress. IPM 312-19 exhibited the highest increase in vessel diameter, with a 32.8% expansion in root T.S. and 38.90% in stem T.S. compared to normal-sown plants. These anatomical changes suggest improved water transport efficiency and turgor maintenance under HTS. This study provides novel insights into xylem plasticity in mungbean, contributing to a better understanding of legume heat adaptation mechanisms. The findings can aid in breeding and selecting heat-tolerant mungbean genotypes, ensuring sustainable yields under rising temperatures.

Keywords: Anatomical, Climate change, Heat stress, Mungbean, Xylem

INTRODUCTION

Global warming significantly influences plant growth, development and agricultural yield. The primary consequence of global warming is the rise in surface temperatures which has increased by about 1 °C since 1906 (Mishra *et al.* 2021) If the current trend persists, global surface temperature will rise to approximately 1.5°C by 2052 (Allen *et al.,* 2018).The mungbean (*Vigna radiata* L.) Wilczek) is a short-duration, warm-season legume crop widely cultivated in northern India. It is characterized as an annual, semi-erect to erect plant with a highly branched, occasionally twining and deep-rooted structure. The plant's height typically ranges from 25 to 100 cm (Pratap *et al*., 2021) Mungbean is a crucial protein source in vegetarian diets and is highly versatile, functioning as an intercrop, green manure or cover crop. Due to its high market value, it is a favored pulse crop among farmers (Mansoor and Naqvi, 2013) .The optimal temperature range for mungbean growth is typically between 28-°30C (Bhardwaj *et al.,* 2023) .However, temperatures exceeding this range, particularly those surpassing 40° C during the reproductive growth stage, are expected to significantly reduce its potential yield, resulting in poor harvests (Sharma *et al.* , 2016).High-temperature stress (HTS) can impact various aspects of plant biology, including morphology, physiology, anatomy and genetic expression (Purnama *et al.* 2018) .It induces significant alterations in the anatomy of leaves, stems and roots (Shen *et al.,* 2017; Bano *et al.,* 2019).To survive HTS, plants adopt various strategies, including reducing cell size, increasing the diameter of xylem vessels and boosting stomatal density (Bañon *et al.,* 2004) .These adaptations improve water transport within the plant, aiding in its ability to withstand high temperatures (Chen *et al.,* 2014).Keeping this in view, the present study aimed to examine the effect of HTS on the root and stem anatomy of mungbean genotypes.

MATERIALS AND METHODS

Study area

The experiment was conducted in field conditions at the research area of pulses section, Department of Genetics and Plant Breeding at Chaudhary Charan Singh Haryana Agricultural University in Hisar, Haryana, India, during the summer of 2022-23. Hisar is located at 29º 10 ‘N latitude, 75º 46 ‘E longitude and an altitude of 215. 2m above mean sea level. The climate in Hisar is characterized as semi-arid and sub-tropical, with distinct seasons and significant temperature fluctuations throughout the year. Winters are marked by severe cold, while summers are hot and dry, often accompanied by desiccating winds. The monsoon season brings humid and warm conditions. Mean monthly maximum and minimum temperatures exhibit wide fluctuations annually. During the summer, temperatures can exceed 48°C, while winter temperatures can drop below freezing point, sometimes leading to frost. The region receives an average annual rainfall of about 425 mm, with considerable variability in total rainfall and distribution.

Experimental design

Experiments were performed using a randomized block design (RBD), with a minimum of three replicates per treatment.

Methodology

The experiment was conducted in field conditions at the research area of pulses section, Department of Genetics and Plant Breeding at Chaudhary Charan Singh Haryana Agricultural University in Hisar, Haryana, India, during the summer of 2022-23 (located at 29º 10 ‘N latitude, 75º 46 ‘E longitude and an altitude of 215. 2m above mean sea level). Three mungbean genotypes (MH 421, MH 1772 and IPM 312-19) were cultivated under field conditions at two different sowing dates: (1 normal sown (NS) in March (Date of sowing- March 18, 2023), when the maximum daytime temperature was below 40°C, and (2) late sown (LS) in April (Date of

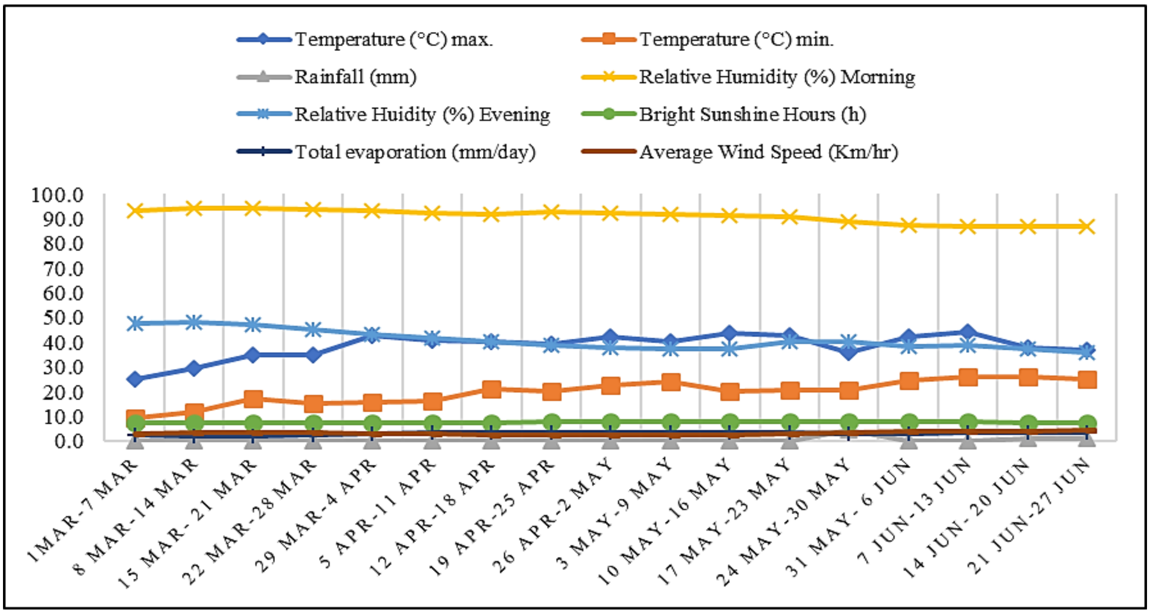

Fig. 1. *Graphical representation of weather parameters during the summer crop season under field conditions at Chaudhary Charan Singh Haryana Agricultural University in Hisar, Haryana*

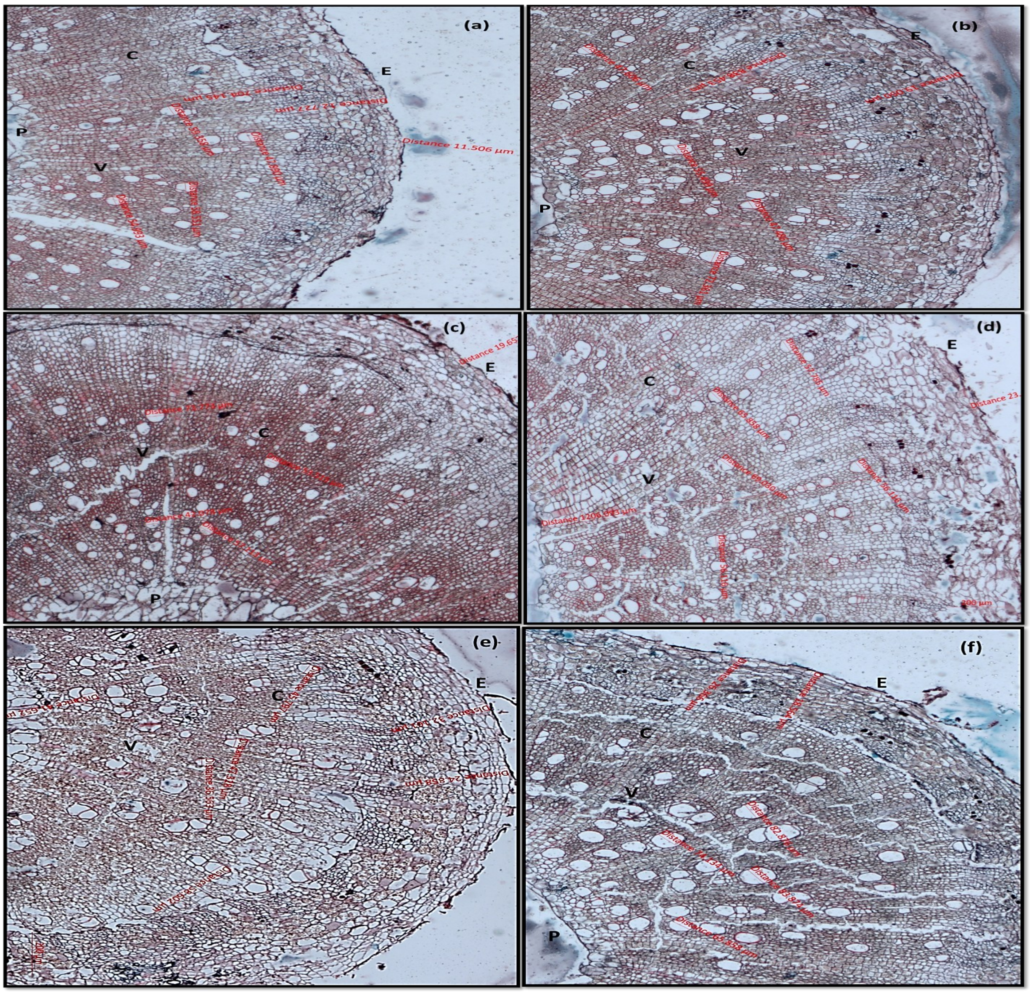

Fig. 2. *Effect of high-temperature stress on root anatomy in mungbean genotypes. Images were taken at the same magnification. (10X); scale bar- 200 µm; P- Pith, C- Cortex, V- Vessel, E- Epidermis; (a) normal sown, MH 421 genotype (b) late sown, MH 421 genotype (c) normal sown, MH 1772 genotype (d) late sown, MH 1772 genotype (e) normal sown, IPM 312-19 genotype (f) late sown, IPM 312- 19genotype. Showing increase in vessel size and number during late sown conditions in all three genotypes as shown in Fig. (b),)d) and (f) as compared to normal shown conditions in Fig.(a),)c) and (e). Showing maximum difference in IPM 312-19 genotype during late sown condition (Fig. F) as compared to normal sown condition (Fig.e)*

sowing- April 6, 2023) when the maximum daytime temperature exceeded 40°C. During experimentation, variations in minimum and maximum temperatures, sunshine duration, rainfall, evaporation, wind speed and relative humidity were recorded)Figure 1). Agrometeorological data was obtained from Department of Agricultural Meteorology, CCS HAU, Hisar (http://hau.ernet.in/coa/agromet.htm).

Root and stem samples were collected in both NS and LS plants during the reproductive stages. Samples were preserved in Formalin-Acetic Acid-Alcohol (FAA) solution- a solution consisting of formalin 10) ml) ,glacial acetic acid 5)ml) and ethyl alcohol 70% (85 ml)). The specimens were then washed in 50% ethyl alcohol, dehydrated using a normal butyl alcohol series, embedded in paraffin wax with a melting point of 56°C and sliced into 20-micrometer (µm) thick sections. These sections were double-stained with safranine-fast green, cleared in xylene and mounted in Canada balsam. The prepared sections were examined to observe histological changes and were observed through photography (Nassar and El-Sahhar, 1998) .Photographs were taken using the Phase contrast microscope (Axio-imager M2 ZEISS). It offers magnifications ranging from 5X to 100X, with high-resolution objective lenses for detailed imaging.

RESULTS AND DISCUSSION

Anatomical studies of the transverse sections (T.S.) of mungbean roots and stems showed distinct structural features, including the epidermis, cortex, vascular bundles and pith. The noteworthy changes in the size, thickness and number of xylem vessels were observed in LS conditions, impacting both stem and root tissues.

In anatomical assessment of roots under LS condi-

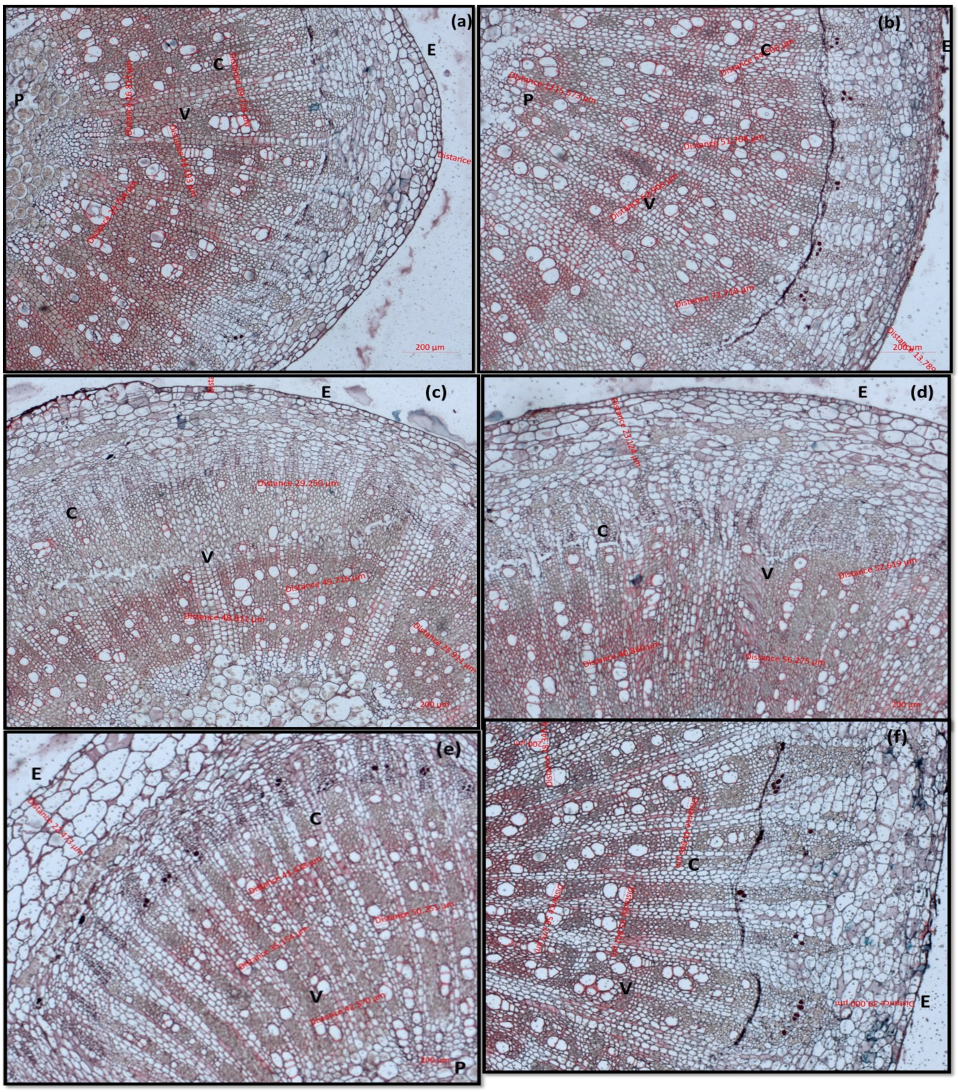

Fig. 3. *Anatomical changes in the mungbean stem at normal sown and late sown conditions. Images were taken at same magnification 10)X). Scale bar- 200 µm; P- Pith, C- Cortex, V- Vessel, E- Epidermis; (a) normal sown, MH 421 genotype (b) late sown, MH 421 genotype (c) normal sown, MH 1772 genotype (d) late sown, MH 1772 genotype (e) normal sown, IPM 312-19 genotype (f) late sown, IPM 312- 19genotype ; Increase in vessel size and number was observed during late sown conditions in all three genotypes, as shown in Fig. (b), (d) and (f) as compared to normal shown conditions in Fig. (a), (c) and (e). However maximum increase in vessel size observed in IPM 312-19 genotype, as shown in Fig. (f) as compared to Fig. (e) and minimum change in vessel size was observed in MH 1772 genotype (Fig.d) during late sown condition as compared to normal sown condition (Fig. C).*

tions, xylem vessel enlargement with thickened walls was observed (Figure 2).Compared to normal sown conditions, late sowing resulted in a noticeable increase in vessel diameter in the transverse sections (T.S.) of roots, with an expansion of 15.44% in MH 421, 13.18% in MH 1772, and 32.8% in IPM 312-19. Similarly, in the T.S. of stems, vessel diameter increased by 18.07% in MH 421, 16.65% in MH 1772, and 38.90% in IPM 312- 19 under late-sown conditions. Also number of xylem vessels was found to be higher in both T.S. of root and stem anatomy during late sown conditions compared to normal sown conditions (Fig. 2 and 3) These anatomical modifications suggest an adaptive response to environmental variations associated with late sowing.

Plants undergo diverse cellular and metabolic modifications to adapt to adverse environmental conditions, potentially enhancing their survival capabilities (Teixeira *et al.* , 2013) .Chen *et al.* , 2014 and Purnama *et al.* (2018) have reported significant anatomical alterations in leaves, stems and roots in Raphanus sativus and *Thalassia hemprichii* under HTS. The present observations revealed an increase in vessel size in mungbean genotypes, which could be advantageous for additional water storage to counteract dehydration during HTS. Additionally, an increase in vessel wall thickening in roots suggests the maintenance of turgor under high temperature, potentially facilitating increased transpiration to reduce the temperature effect. Notably, the vessel size in heat-stressed plants increased, possibly enhancing the efficiency of water transport within the plant and regulating the turgidity of heat-stressed plants. Previous research has demonstrated that rising temperatures lead to an increase in xylem hydraulic conductance. This enhancement in conductance helps maintain turgor pressure within guard cells (Buckley *et al.* , 2015).An increase in hydraulic demand can result in either larger channels, a higher density of channels or an expanded xylem size within the stem's cross-sectional area. This adaptive response boosts the plant's capacity to transport water, increasing water transport required during elevated temperatures (Medeiros and Ward, 2013) .The increased diameter of tracheids or xylem vessels enhances the plant's efficiency in transporting water (Ocheltree and Gleason, 2024). Limited data on anatomical changes in plants due to HTS are available. The literature has documented increased vessel size and pith area in mungbean (Iqbal *et al.* , 2023)and potato (Paul *et al.* , 2017), suggesting that increased vessel size may play a role in regulating water flow.The observed increase in xylem vessel diameter and wall thickness under LS conditions suggests that mungbean genotypes, employ vascular plasticity as a heat adaptation strategy. These modifications enhance water transport capacity, maintain turgor pressure and regulate transpiration, ultimately contributing to drought and heat resilience.Unlike previous studies, this research quantitatively assesses anatomical adaptations in mungbean genotypes under field conditions, offering valuable insights for breeding heat-tolerant cultivars. Further investigations on the physiological and molecular mechanisms underlying these structural changes could help optimize mungbean cultivation under extreme climatic conditions.

Conclusion

Plants exhibit diverse cellular and metabolic modifications to counteract adverse environmental conditions. The present study provides compelling evidence that mungbean genotypes respond to HTS through significant anatomical adaptations, particularly in vascular tissues. The observed increase in vessel size suggests an enhanced water storage and transport capacity, which are crucial for maintaining plant hydration under HTS. Furthermore, thickened vessel walls in roots may contribute to sustaining turgor pressure and facilitating transpiration, thus helping plants to regulate temperature. These findings align with previous studies, reinforcing the role of xylem modifications in mitigating heat stress effects. This study provides novel insights into the anatomical modifications in mungbean genotypes under HTS, particularly on xylem vessel dynamics. The findings contribute valuable information to the limited literature on anatomical changes in mungbean under heat stress, offering a deeper understanding of vascular plasticity in legumes.Future research should explore the physiological and molecular mechanisms underlying these anatomical modifications, particularly the regulatory pathways governing xylem differentiation under HTS. Additionally, a comparative analysis of multiple legume species could help determine whether these structural adaptations are conserved across different plant taxa, thereby aiding in the development of heat-resilient crop varieties.

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Conflicts of interest
The authors declare that they have no conflict of interest.

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