

Evaluation of Wheat (*Triticum aestivum* L.) Genotypes for Heat Tolerance Using Physio-Biochemical Traits

Ramu Kumar, Shambhoo Prasad, Ahmer Rashid Khan,
Prabhat Singh, Adarsh Yadav, Nikhil Verma, Ajay Kumar

Received 18 May 2026, Accepted 22 June 2026, Published on 30 September 2026

ABSTRACT

A pot culture experiment was conducted with wheat genotypes during the *Rabi* season of 2023–24 at Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya. The temperature was maintained around 38 to 40°C in polyhouse condition so that reproductive stage of wheat genotypes could face heat stress environment. The wheat genotypes were evaluated for membrane stability index (MSI), chlorophyll stability index (CSI), catalase activity, test weight and grain yield performance under heat stress. The cell membrane stability highly affected due to electrolytes leakage under heat stress. The test

weigh and grain yield was less affected in high MSI genotypes. The chlorophyll stability genotypes stay green during terminal heat stress and maintained the metabolic activity during grain filling stage. High catalase activity genotypes prevent the lipid peroxidation and prevent plant from oxidative damage. Genotype NWL-102 had high MSI, CSI, high catalase activity and less reduction yield over NWL 101, NWL 103 and NWL 104. The heat stress significantly reduced test weight and grain yield under heat stress regimes. The tolerant wheat genotypes less affected as compare to susceptible ones. The high MSI, CSI, catalase activity and grain yield stability under heat stress environment may be use it as donor for further improvement of wheat genotypes for heat tolerant traits.

Keywords Heat stress, MSI, CSI, Yield stability and Genotype.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major global cereal crop and a vital component of food security, cultivated on over 220 million hectares globally and contributing significantly to both human nutrition and the livestock sector (Khalid *et al.* 2023). In India, it is grown on 32.76 million hectares with an annual production of 117.51 mt, with Uttar Pradesh being the largest contributor (IIWBR 2024, Devi *et al.* 2024). Rising global temperatures and climate variability pose serious threats to wheat productivity, with projections estimating a 6% yield loss per 1°C

Ramu Kumar¹, Shambhoo Prasad^{2*}, Ahmer Rashid Khan³,
Prabhat Singh⁴, Adarsh Yadav⁵, Nikhil Verma⁶

²Professor, ^{3,4}PhD Research Scholar

^{1,2,3,4,5,6}Department of Molecular Biology and Biotechnology,
Acharya Narendra Deva University of Agriculture and
Technology, Kumarganj, Ayodhya 224229, Uttar Pradesh, India

Ajay Kumar⁷

Lab Technician

Animal Genetics and Breeding, Acharya Narendra Deva
University of Agriculture and Technology, Kumarganj, Ayodhya
224229, Uttar Pradesh, India

Email: shambhoonduat@gmail.com

*Corresponding author

temperature increase, potentially causing billions in annual losses (Abasi *et al.* 2024, Chavan *et al.* 2019). Among abiotic stresses, drought and heat stress especially when combined—are the most harmful to wheat, disrupting physiological processes like photosynthesis and grain filling, leading to substantial yield reduction (Tyagi and Pandey 2022, Nyaupane *et al.* 2024, Khan *et al.* 2023). These stresses are particularly damaging during critical stages such as anthesis and grain filling, with potential yield losses up to 69% (Ali *et al.* 2023).

Physiological traits like cell membrane stability, chlorophyll stability, catalase activity and early maturity are key indicators of heat tolerance (Ahmad *et al.* 2023). Recent advances in genomics, including marker-assisted selection, SSR/SNP markers, and gene editing technologies such as CRISPR/Cas9, have enabled the identification of QTLs associated with drought and heat tolerance, accelerating the development of stress-resilient wheat varieties (Paudel *et al.* 2024). Despite this, large-scale screening using physiological traits remains limited due to high costs, though innovations in phenotyping technology offer promising solutions (Koltes *et al.* 2019, Dia *et al.* 2016b).

MATERIALS AND METHODS

A pot experiment was conducted during the *Rabi*

season of 2023–24 at Acharya Narendra Deva University of Agriculture & Technology, Ayodhya, to evaluate 4 wheat genotypes (NWL, NWL 102, NWL 103-101 and NW-104) for terminal heat tolerance. The seven days terminal heat stress (38–40°C) was applied under polyhouse condition against control. The membrane stability index (MSI) (Saadalla *et al.* 1990), and chlorophyll stability index (CSI) (Dhopte and Livera 1989) were used to assess plant responses under stress. Chlorophyll content was measured spectrophotometrically using 80% acetone, following Arnon (1949), Catalase enzyme activity, an indicator of oxidative stress tolerance, was quantified calorimetrically based on H₂O₂ decomposition, as described by Sinha (1972). These standard biochemical and physiological protocols helped in identifying tolerant genotypes for future stress-resilient wheat breeding programs.

RESULTS AND DISCUSSION

Heat stress reduced the membrane stability due to ionic leakage from the cell membrane (Fig.1). The maximum membrane stability under stress condition was recorded in NWL-102 (71.7%) while minimum in NWL 104 (60.2%).

Membrane Stability Index (MSI) significantly declined under heat and dual stress conditions, indicating increased membrane damage and electrolyte

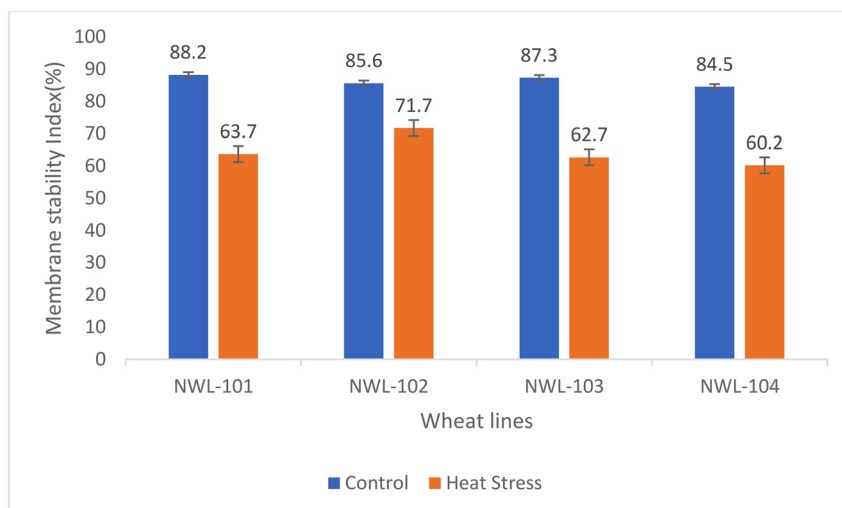


Fig. 1. Effect of heat stress on MSI (%) of wheat genotypes at anthesis stage.

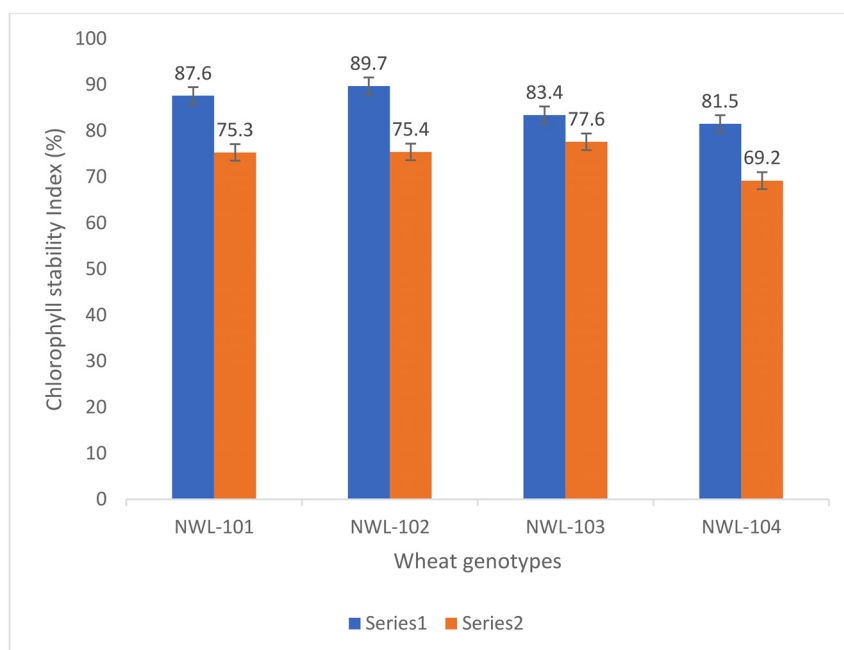


Fig. 2. Effect of heat stress on CSI (%) of wheat genotypes at anthesis stage.

leakage in sensitive wheat genotypes (Poudel *et al.* 2021). NW-119 consistently maintained higher MSI values across stress conditions, suggesting better membrane integrity and stress tolerance, whereas NW-110 showed the lowest MSI, reflecting high susceptibility. MSI is a reliable physiological indicator

for identifying heat and drought-tolerant wheat lines (Lamba *et al.* 2023).

A significant reduction in chlorophyll stability index (CSI) was observed in wheat genotypes under heat stress at the anthesis stage, indicating chloro-

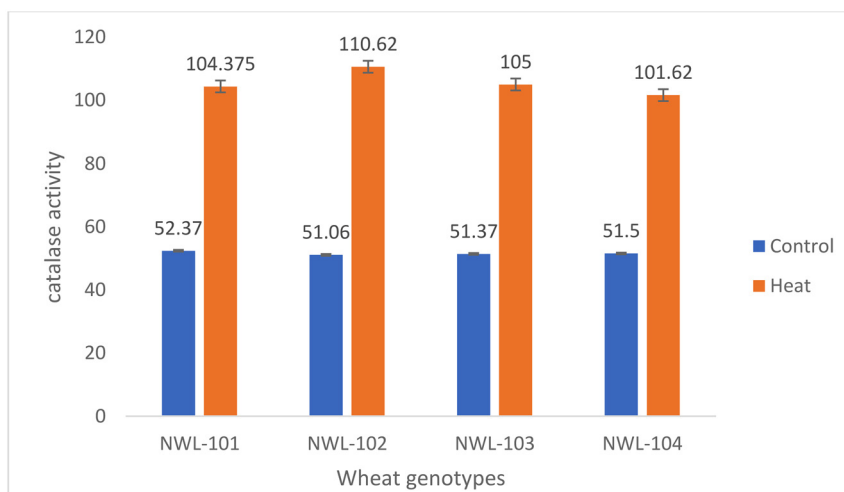


Fig. 3. Effect heat stress on catalase activity (μmole/min/ml) of wheat genotypes.

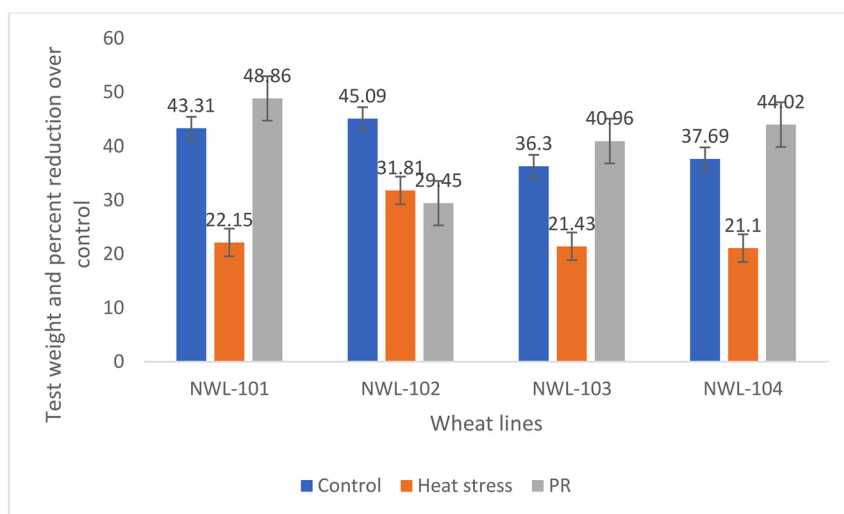


Fig. 4. Effect of heat stress on test weight(g) of wheat genotypes.

phyll degradation due to thermal damage (Fig. 2). NWL-103 maintained the highest CSI (77.6%) under stress, suggesting better heat tolerance, while NW-104 showed the lowest (69.2%). CSI has been widely recognized as a useful physiological indicator for screening heat-tolerant genotypes (Gao *et al.* 2021, Lamba *et al.* 2023).

The Catalase activity abruptly increased in all wheat genotypes under stress condition (Fig. 3). The maximum catalase activity was observed in NW-102

(110.62 $\mu\text{mole}/\text{min}/\text{ml}$) and NW-103 (113.75 $\mu\text{mole}/\text{min}/\text{ml}$) while minimum in NW-104 (101.6 $\mu\text{mole}/\text{min}/\text{ml}$) under heat stress condition.

Catalase activity increased significantly in all wheat genotypes under heat and dual stress, suggesting its vital role in combating oxidative stress (Gill and Tuteja 2010). Genotypes NW-120 and NW-119 showed high enzyme activity under stress, while NW-105 had the highest percentage increase under heat. This variation among genotypes highlights catalase

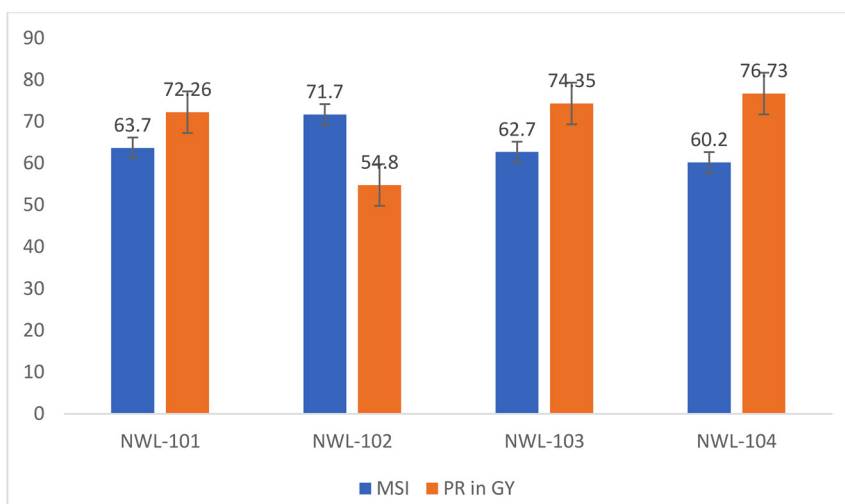


Fig. 5. MSI and its relation to percent reduction in grain yield.

as a potential biochemical marker for stress tolerance (Hasanuzzaman *et al.* 2012, Karki *et al.* 2021).

The genotypes that showed maximum test weight under control condition were NW-102 (45.09g) while minimum in NW-103 (36.3g) (Fig. 4). The maximum reduction in test weight under heat stress was recorded in NW-101 (48.86g) and minimum in NW-102 (31.81.64g).

Significant variation in grain test weight was observed among wheat genotypes under control and stress conditions, reflecting differential effects of heat and combined stresses on grain filling (Farooq *et al.* 2011). NW-117 and NW-105 maintained high test weights under control and heat stress, respectively, indicating better grain development. Severe reductions in test weight under dual stress in genotypes like NW-106 highlight the sensitivity of grain filling to combined abiotic stresses (Poudel *et al.* 2021, Lamba *et al.* 2023).

The relation between membrane stability index and percent reduction (PR) grain is positive type under heat stress condition (Fig. 5). The high membrane stable genotype NWL 102 less reduction (54%) in grain yield over other genotypes while high reduction was recorded in Less MSI genotypes NWL 104 (76.73%).

The high membrane stable genotypes maintained cell integrity, enzymes activity and cell metabolisms under stress regimes. The grain filling phenomena comparatively maintain up to some extent in MSI stable genotypes and less reduction in grain yield comparatively heat sensitive genotypes Hütsch *et al.* (2019), Djanaguiraman *et al.* (2020).

CONCLUSION

The heat stress significantly reduced MSI, CSI, test weight and grain yield in wheat genotypes. The tolerant wheat genotypes had high membrane stability, stay green, high catalase activity under heat stress environment. The genotypes NWL-102 showed better performance at terminal stage of heat stress showed less reduction in test weight and grain yield over other genotypes.

REFERENCES

- Abasi, F., Raja, N. I., Mashwani, Z. U., Ehsan, M., Ali, H., & Shahbaz, M. (2024). Heat and Wheat: Adaptation strategies with respect to heat shock proteins and antioxidant potential; an era of climate change. *International Journal of Biological Macromolecules*, 256(Pt 1), 128379. <https://doi.org/10.1016/j.ijbiomac.2023.128379>
- Ahmad, M. A., Prakash, P., Srivastava, J. P., Dujeshwer, K., Singh, A. K., & Jatav, H. S. (2023). Physiological and Biochemical Effect of Elevated Night Temperature Stress on Bread Wheat (*Triticum aestivum* L.). *Emirates Journal of Food and Agriculture*, 35(12) 1-8. <https://doi.org/10.9755/ejfa.2023.3186>
- Ali, I., Anwar, S., Ali, A., Ullah, Z., Binjawhar, D. N., Sher, H., & Jaremko, M. (2023). Biochemical and phenological characterization of diverse wheats and their association with drought tolerance genes. *BMC Plant Biology*, 23(1): 326.
- Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, 24 (1), 1–15. <https://doi.org/10.1104/pp.24.1.1>
- Chavan, S. G., Duursma, R. A., Tausz, M., & Ghannoum, O. (2019). Elevated CO₂ alleviates the negative impact of heat stress on wheat physiology but not on grain yield. *Journal of Experimental Botany*, Volume 70, Issue 21, 1 November 2019, Pages 6447–6459, <https://doi.org/10.1093/jxb/erz386>
- Devi, S., Singh, V., Kumar, M., Yashveer, S., Kumar, R., Sharma, S., Chawla, R., Sapna, Rani, K., & Redhu, M. (2024). Assessing wheat genotype performance under heat, drought and combined stresses using multiple stress indices. *Euphytica*, 220(11). <https://doi.org/10.1007/s10681-024-03424-0>
- Dhopte, A. M., & Livera, M. M. (1989). Interpretation of plant test analysis. Publication of Agro Botanical Publishers.
- Dia, M., Wehner, T. C., & Arellano, C. (2016b). Analysis of genotype × environment interaction (G×E) using SAS programming. *Agronomy Journal*, 108(5), 1838–1852. <https://doi.org/10.2134/agronj2016.02.0085>
- Djanaguiraman, M., Narayanan, S., Erdayani, E., & Prasad, P. V. (2020). Effects of high temperature stress during anthesis and grain filling periods on photosynthesis, lipids and grain yield in wheat. *BMC Plant Biology*, 20, 1–12.
- Farooq, M., Bramley, H., Palta, J. A., & Siddique, K. H. (2011). Heat Stress in Wheat during Reproductive and Grain-Filling Phases. *Critical Reviews in Plant Sciences*, 30(6), 491–507. <https://doi.org/10.1080/07352689.2011.615687>
- Gao, C., Sun, M., Anwar, S., Feng, B., Ren, A., Lin, W., & Gao, Z. (2021). Response of physiological characteristics and grain yield of winter wheat varieties to long-term heat stress at anthesis. *Photosynthetica*, 59(4), 640–651. <https://doi.org/10.32615/ps.2021.060>
- Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48(12): 909–930.
- Hasanuzzaman, M., Hossain, M. A., & Fujita, M. (2012). Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmad, P. & Prasad, M. N. V. (Eds.), *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change*. Springer. pp. 25–87.

- Hütsch, B. W., Jahn, D., & Schubert, S. (2019). Grain yield of wheat (*Triticum aestivum* L.) under long-term heat stress is sink-limited with stronger inhibition of kernel setting than grain filling. *Journal of Agronomy and Crop Science*, 205(1) 22–32.
- IIWBR. (2024). Project Coordinated Wheat Improvement Report. *Indian Institute of Wheat and Barley Research*, Karnal. www.aicrpwheatbarleyicar.in > 2024-25.
- Karki, P., Subedi, E., Acharya, G., Bashyal, M., Dawadee, N., & Bhattarai, S. (2021). A review on the effect of heat stress in wheat (*Triticum aestivum* L.). *Archives of Agriculture and Environmental Science*, 6(3), 381–384. <https://doi.org/10.26832/24566632.2021.0603018>
- Khalid, A., Hameed, A., & Tahir, M. F. (2023). Wheat quality: A review on chemical composition, nutritional attributes grain anatomy, types, classification, and function of seed storage proteins in bread making quality. *Frontiers in Nutrition*, 10:1053196. doi: 10.3389/fnut.2023.1053196
- Khan, M. A., Akram, M. W., Iqbal, M., Ahmed, H. G. M., Rehman, A., Iqbal, H. S. M. A., & Alam, B. (2023). Multivariate and association analyses of quantitative attributes reveal drought tolerance potential of wheat (*Triticum aestivum* L.) genotypes. *Communications in Soil Science and Plant Analysis*, 54(2): 178–195.
- Koltes, J. E., Cole, J. B., Clemmens, R., Dilger, R. N., Kramer, L. M., Lunney, J. K., McCue, M. E., McKay, S. D., Mateescu, R. G., Murdoch, B. M., Reuter, R., Rexroad, C. E., Rosa, G. J. M., Serão, N. V. L., White, S. N., Woodward-Greene, M. J., Worku, M., Zhang, H., & Reecy, J. M. (2019). A vision for development and utilization of High-Throughput Phenotyping and Big Data Analytics in livestock. *Frontiers in Genetics*, 10, 1197. <https://doi.org/10.3389/fgene.2019.01197>
- Lamba, K., Kumar, M., Singh, V. *et al.* (2023). Heat stress tolerance indices for identification of the heat tolerant wheat genotypes. *Scientific Reports*, 13, 10842 (2023). <https://doi.org/10.1038/s41598-023-37634-8>
- Nyaupane, S., Poudel, M. R., Panthi, B., Dhakal, A., Paudel, H., & Bhandari, R. (2024). Drought stress effect, tolerance, and management in wheat – a review. *Cogent Food & Agriculture*, 10(1): 2296094.
- Paudel, P., Pandey, Manoj., Subedi, M., Paudel, P., Kumar, R. (2024). Genomic approaches for improving drought tolerance in wheat (*Triticum aestivum* L.): A comprehensive review. *Plant Archives*. 24. 1289-1300.
- Poudel, P. B., Poudel, M. R., & Puri, R. R. (2021). Evaluation of heat stress tolerance in spring wheat (*Triticum aestivum* L.) genotypes using stress tolerance indices in western region of Nepal. *Journal of Agriculture and Food Research*, 5, 100179. <https://doi.org/10.1016/j.jafr.2021.100179>
- Saadalla, M. M., Quick, J. S., & Shanahan, J. F. (1990). Heat tolerance in winter wheat: II. Membrane thermostability and field performance. *Crop Science*, 30, 1248–1251.
- Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*, 47, 389–394.
- Tyagi, M., & Pandey, G. C. (2022). Physiology of heat and drought tolerance in wheat: An overview. *Journal of Cereal Research*, 14(1): 13-25. <https://doi.org/10.25174/2582-2675/2022/122868>