



Exploring genetic variability for waterlogging tolerance related traits in early segregation generation in maize (*Zea mays*)

SANJAY KUMAR¹, RUMESH RANJAN^{1*}, TOSH GARG¹, GAGANDEEP SINGH¹, NIDA YOUSUF^{1,2}, ROHIT¹ and MAHESH KUMAR¹

Punjab Agricultural University, Ludhiana, Punjab 141 004, India

Received: 07 January 2025; Accepted: 22 December 2025

ABSTRACT

Climate change has increased the frequency of abiotic stresses, such as waterlogging (WL), caused by heavy, unpredictable rainfall in compacted soils that adversely affects the growth and yield of maize (*Zea mays* L.). To breed WL-tolerant maize hybrid, understanding genetic variability for WL tolerance traits in source germplasm is critical. The experiment was conducted during rainy (*khariif*) season 2023 at Punjab Agricultural University, Ludhiana, Punjab in F_{2,3} maize populations derived by crossing WL tolerant and susceptible parent for WL tolerance under pot and field conditions. Physiological, root, and yield-related traits were assessed after WL stress at the V₃₋₅ stage for six days. Experiment I identified root dry weight, shoot dry weight, root surface area, and root diameter as promising traits due to high heritability (h^2) and genetic advance, suggesting their utility in breeding WL-tolerant lines. Chlorophyll content before (CCBT-P) and after (CCAT-P) treatment showed low heritability, requiring further studies. In Experiment II, yield-related traits like ear height, plant height, and ear yield exhibited moderate to high heritability, making them suitable for selection. The findings highlight the importance of prioritising high-heritability traits for selection and fixing superior lines through continuous selfing. This approach can aid in developing WL tolerant maize hybrids, enhancing productivity in WL prone regions of northern India and supporting sustainable maize farming amid increasing climate-induced abiotic stresses.

Keywords: Early generation F_{2,3}, Maize, Root trait, Variability, Waterlogging

Maize (*Zea mays* L.) is a vital global crop but highly sensitive to waterlogging (WL), which reduces yield and production (Liang *et al.* 2020, Hu *et al.* 2022, Liang *et al.* 2025). While maize requires water but excess moisture (over 80%) slows development and decreases grain yields (Ren *et al.* 2014). In south-east Asia, 15% of maize-growing areas face WL causing 25–30% annual losses (Rathore *et al.* 2000). In India, it affects 8.5 million hectares, making it the second largest crop issue after drought (Ahsan *et al.* 2007). In North Western Plain Zone especially Punjab and Haryana, WL during *khariif* season limits maize yields to 3.62 t/ha, far below the potential 7–8 t/ha (Wajhat *et al.* 2023). Shifting from paddy to maize can save groundwater and reduce environmental issues in Haryana and Punjab, making bridging the productivity gap essential for viable, WL-tolerance maize farming. WL generally affects photosynthesis and transpiration, reducing them by 30–40% within a day and limiting root growth and nutrient uptake

(Hsaio and Bradford 1983, Najeeb *et al.* 2015, Sandhu *et al.* 2021, Kumar *et al.* 2025a). WL reduces yields by 20% and affects 12% of farmland each year (Bailey-Serres *et al.* 2012). Flooding is a major cause of crop losses in Asia (Yaduvanshi *et al.* 2012), expected to increase in frequency and intensity (Westra *et al.* 2014).

Studies such as Mano *et al.* (2009) emphasised root traits' role in WL tolerance, while Lopes *et al.* (2011) highlighted the combined influence of genetic and environmental factors under stress. Traits like root porosity, shoot elongation, and survival rates are analysed for genetic and phenotypic variability (Falconer and Mackay 1996). High genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) values indicate significant variability, while high heritability (h^2) and genetic advance (GA) suggest strong genetic control, making them ideal for breeding programmes (Singh 2015). Zaidi *et al.* (2012) identified traits with high GCV and PCV as crucial targets for breeding WL resilience. Breeding for WL tolerance requires enough genetic variability. The GCV, PCV, h^2 , and GA are key tools for quantifying genetic variability. Generally, root traits are labourious and time taken and are ignored by majority of researchers in their study. The variability of root traits are lesser study in segregating generation, therefore, our study

¹Punjab Agricultural University, Ludhiana, Punjab; ²Sher-e-Kashmir University of Agricultural Sciences and Technology-Kashmir, Jammu and Kashmir. *Corresponding author email: rumeshranjan@pau.edu



Fig. 1 Waterlogging (WL) treatment of $F_{2.3}$ population along with both parents in pit at $V_{3.5}$ stage and parent's I 185 and SE 565A after WL stress after a period of 6 days.

focused on accessing genetic variability for WL tolerance related traits especially root traits in early segregation generation in maize.

MATERIALS AND METHODS

Plant materials: The experiment used $F_{2.3}$ populations, developed through selfing of F_1 and F_2 generations which was derived from a cross between I 185 (WL-tolerant) and SE 565A (WL-susceptible). These parental lines differ significantly in root traits and aerenchyma formation under waterlogged (WL) conditions (Thapa *et al.* 2025).

Experiment I, Phenotypic evaluation of $F_{2.3}$ populations in pots for WL stress tolerance for physiological and root traits: The experiment, conducted in rainy (*kharif*) season 2023, involved phenotyping 154 $F_{2.3}$ lines in pot filled with sterilised alluvial soil with two replications at artificial pit (Kumar *et al.* 2025b). Six seeds treated with bavistin were sown per pot and thinned to five seedlings, with both parent lines included. At the $V_{3.5}$ stage, seedlings underwent a six-day WL treatment in pits along with parent lines I 185 and SE 565A (Fig. 1) (Kumar *et al.* 2025b).

Data were collected on various traits, including Plant mortality percentage (PM%); Root dry weight (RDW) (g); Shoot dry weight (SDW) (g); Total dry weight (TDW) (g); Root surface area (RSA) (cm^2); Root fresh weight (RFW)

(g); Shoot fresh weight (SFW) (g), Root length (RL) (cm); Total root length (TRL) (cm); Root volume (RV) (cm^3), Root diameter (RD) (mm), and Chlorophyll content before (CCBT) and after treatment (CCAT). Chlorophyll content was measured using a SPAD MC-100, while fresh and dry weights were measured with an electronic balance (AMC 200). Root length was measured from the coleoptilar node to the longest root tip by measuring scale. Root traits (RSA, TRL, RV, RD) were analysed using Biovis PSM root scanner, with images captured by an EPSON Expression 12000XL scanner (Thapa *et al.* 2025). Total dry weight (TDW) (g) was calculated as the sum of SDW and RDW.

Experiment II, Phenotypic evaluation of $F_{2.3}$ populations in field for WL stress tolerance for yield and associated traits: The same 154 $F_{2.3}$ lines in experiment I were evaluated under waterlogged (WL) conditions in field along with control during rainy (*kharif*) season 2023 at the Maize Research Fields, Punjab Agricultural University, Ludhiana (30.90°N, 75.80°E; at an elevation of 224 m amsl), Punjab (Fig. 2). Each genotype or line was sown in two rows having 3 m length with 20 cm plant-to-plant and 60 cm row-to-row spacing on flat beds, with WL treatment applied at the knee-high for six days (Kumar *et al.* 2025c) in two replications in randomised block design (RBD). Control plants (non-waterlogged) were sown on ridge beds



A: Water logged field

B: Control (Non-water logged field)

Fig. 2 Maize plants subjected to waterlogging stress in field at knee-high stage (A), and control sowing on ridges (B).

in two replica in RBD fashion following recommended package of practices. Data on yield traits, including ear yield/plot (YLD), ear weight (using AMC 1 kg balance), plant height (PH), ear height (EH), days to tassel flowering (DT), and silk flowering (DS), were collected across two replications. WL tolerance coefficient (WTC) measured for each trait, viz. YLD, PH, EH, DT and DS was calculated using the formula:

$$\text{WTC} = \frac{\text{Waterlogging treatment of each trait}}{\text{Control of each trait}}$$

Statistical analysis: Variability parameters were measured by using the software RStudio 4.4.1 and all the variability parameters were cross checked by using the formula, Mean (\bar{X}). Genotypic and phenotypic coefficients of variation were estimated by formulae suggested by Burton and De Vane (1953), Heritability estimates were categorised as Low (0–30%), Moderate (30–60%), and High (>60%) (Robinson *et al.* 1949) and Genetic advance (GA) following Johanson *et al.* (1955).

$$\text{GA} = k. \sigma_p . H_{bs}$$

RESULTS AND DISCUSSION

Experiment I: The $F_{2,3}$ populations exhibited significant variability for root and shoot traits under WL stress in the pot experiment (Table 1), attributed to allele segregation and near-normal distribution patterns. This indicated polygenic control of traits, where multiple alleles contribute to the expression of WL tolerance traits. The choice of genetically contrasting parents WL-tolerant I 185 (Thapa *et al.* 2025) and WL-susceptible SE 565A minimised background bias and enhanced the detection of genetic variability. Genetic parameters like GCV, PCV, heritability (h^2), and genetic advance (GA) revealed traits genetic potential, while the difference between GCV and PCV reflected environmental influences (Zaidi *et al.* 2012). Genetic parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad-sense heritability (h^2), and genetic advance (GA) provided insights into the genetic potential and environmental influence on trait expression. A narrow gap between GCV and PCV suggested minimal environmental influence, while high heritability coupled with substantial GA indicated potential for selection (Zaidi *et al.* 2012).

For RDW (Table 1), GCV and PCV were 31.44 and 32.84, respectively, with a high heritability of 0.91 and GA of 0.33 (62.04% of the mean), indicating strong genetic control. Similarly, SDW showed GCV of 47.50 and PCV of 49.21, heritability 0.93, and GA 0.68 (94.44% of the mean), supporting its use in selection (Sarkar 2020). Total dry weight (TDW) showed lower GCV (20.17) and PCV (21.89), but high heritability at 0.84. The GA of 0.48, or 38.30% of the mean, suggests moderate potential for improvement, consistent with studies on TDW under stress (Mano *et al.* 2009). For root traits, viz. Root surface area (RSA), GCV and PCV were 37.51 and 38.03, respectively

Table 1 Variability analysis of pot sown $F_{2,3}$ populations for physiological and root traits

Traits	GCV	PCV	Heritability (Broad sense)	Genetic advance	Genetic advance as percentage of mean (GAM %)
RDW	31.44	32.84	0.91	0.33	62.04
SDW	47.50	49.21	0.93	0.68	94.44
TDW	20.17	21.89	0.84	0.48	38.30
RSA	37.51	38.03	0.97	17.22	76.23
RFW	39.21	39.77	0.97	2.59	79.63
SFW	56.87	57.93	0.96	10.47	115.02
RD	47.32	48.20	0.96	0.57	95.72
RV	34.94	35.51	0.96	13.94	70.82
RL	33.44	34.50	0.93	13.80	66.78
TRL	34.90	35.40	0.97	81.61	71.06
PM%	47.05	49.42	0.90	52.85	92.28
CCBT-P	11.51	13.18	0.76	3.59	20.72
CCAT-P	8.25	10.72	0.59	1.10	13.07

GCV, Genotypic coefficient of variation; PCV, Phenotypic coefficient of variation; RDW, Root dry weight; SDW, Shoot dry weight; TDW, Total dry weight; RSA, Root surface area; RFW, Root fresh weight; SFW, Shoot fresh weight; RL, Root length; TRL, Total root length; RV, Root volume; RD, Root diameter; PM%, Plant mortality %; CCBT, Chlorophyll content before treatment; CCAT, Chlorophyll content after treatment.

with a heritability of 0.97 and a GA of 17.22 (76.23% of the mean). Root fresh weight (RFW) showed GCV of 39.21 and PCV of 39.77, with a heritability of 0.97 and a GA of 2.59 (79.63% of the mean). Both traits showed strong genetic control, supporting their potential for WL tolerance (Kadam *et al.* 2017). Root diameter (RD) showed GCV of 47.32 and PCV of 48.20, with a heritability of 0.96 and a GA of 0.57 (95.72% of the mean), indicating the importance of these traits under WL (Sarkar *et al.* 2023). For root length (RL), the GCV and PCV were 34.94 and 35.51, respectively with heritability of 0.96 for root volume (RV) and 0.93 for RL. The GA was 13.94 for RV (70.82% of the mean) and 13.80 for RL (66.78% of the mean), supporting the role of these traits in WL tolerance (Zaidi *et al.* 2012). Total root length (TRL) had GCV of 34.90 and PCV of 35.40, with heritability of 0.97 and a GA of 81.61 (71.06% of the mean), indicating good selection potential for WL resilience (Saenchai *et al.* 2016). Shoot fresh weight (SFW) had GCV of 56.87 and PCV of 57.93, with a heritability of 0.96 and a GA of 10.47 (115.02% of the mean). Plant mortality (PM%) had GCV of 47.05 and PCV of 49.42, with a heritability of 0.90 and a GA of 52.85 (92.28% of the mean). Reduced PM% under WL is crucial for tolerance, and the high heritability and GA support its

Table 2 Variability analysis of field sown F_{2:3} populations for yield and associated traits

Traits	GCV	PCV	Heritability (Broad Sense)	Genetic Advance (GA)	Genetic Advance as percentage of mean (GAM %)
YLD	29.93	31.95	0.87	1570.89	57.76
PH	37.46	38.38	0.95	68.20	75.34
EH	36.56	36.56	0.98	29.32	74.77
DT	3.92	4.18	0.88	4.27	7.59
DS	4.13	4.34	0.90	4.67	8.10
WTC (YLD)	11.67	15.79	0.54	0.10	17.78
WTC (PH)	20.60	21.87	0.88	0.24	39.97
WTC (EH)	21.11	21.38	0.97	0.21	42.95
WTC (DT)	5.70	5.8343	0.95	0.09	11.46
WTC (DS)	5.77	5.90	0.95	0.09	11.63

GCV, Genotypic coefficient of variation; PCV, Phenotypic coefficient of variation; YLD, Yield; PH, Plant height; EH, Ear height; DT, Days to tasseling; DS, Days to silking; WTC, Waterlogging tolerance coefficients.

improvement (Kadam *et al.* 2017). Chlorophyll content before (CCBT-P) and after treatment (CCAT-P) showed lower variability, with CCBT-P having GCV of 11.51 and PCV of 13.18, and heritability of 0.76. CCAT-P had GCV of 8.25 and PCV of 10.72, with heritability of 0.59. These traits showed moderate genetic control, influenced by environmental stress (Bhargava and Singh 2019).

Collectively, traits such as SFW, RSA, RFW, TRL, RDW, and SDW demonstrated high heritability and genetic advance, rendering them prime candidates for selection in WL stress breeding programmes. In contrast, traits with low heritability, like CCAT-P, require cautious selection and possibly indirect improvement.

Experiment II: Field evaluation further confirmed significant genetic variability among F_{2:3} families under WL stress (Table 2). Grain yield (YLD) showed a GCV of 29.93 and PCV of 31.95, with heritability of 0.87 and GA of 1570.89 (57.76%). The near-normal frequency distribution of yield revealed the quantitative nature of WL tolerance. Similar trends have been observed in maize under abiotic stress (Zhang *et al.* 2023). Plant height (PH) and ear height (EH) demonstrated higher heritability (0.95 and 0.98) with GCV/PCV values of 37.46/38.38 and 36.56/36.92, and GA values of 68.20 (75.34%) and 29.32 (74.77%), respectively, indicating strong genetic regulation (Singh *et al.* 2016). These are key agronomic indicators and are often correlated with biomass and sink strength under WL. Days to tasseling (DT) and days to silking (DS) showed moderate variability. DT had GCV of 3.92 and PCV of 4.18, with heritability of 88% and GA of 4.27 (7.59% of the mean). DS had GCV of

4.13 and PCV of 4.34, with heritability of 0.90 and GA of 4.67 (8.10% of the mean), suggesting moderate improvement potential (Kadam *et al.* 2017).

Waterlogging tolerance coefficients (WTC) varied considerably. WTC-Yield had low heritability (0.54), GCV of 11.67, and GA of 0.10 (17.78%), suggesting substantial environmental effects and need for careful multi-environment selection. On the contrary, WTC-PH and WTC-EH showed higher heritability (0.88 and 0.97, respectively) with GA of 0.24 (39.97%) and 0.21 (42.95%), indicating suitability for use in selection indices (Rathore *et al.* 2020).

Evaluation of F_{2:3} populations under pot and field WL conditions revealed significant genetic variability, heritability, and genetic advance for multiple root, shoot, and yield-related traits. Root traits like RDW, RSA, RD, and TRL, along with SDW and SFW, exhibited strong potential for improving WL tolerance. Field performance confirmed YLD, PH, and EH as key selection traits, while WTC traits, although variable, offer insights into genotype performance under differential stress levels.

The integration of these traits into breeding pipelines through recurrent selection and line fixing can facilitate the development of resilient hybrids. This is particularly critical for mitigating yield losses in WL-prone regions of Punjab and other northern Indian states, thereby enhancing the sustainability and profitability of maize cultivation.

REFERENCES

- Ahsan N, Lee D G, Lee S H, Lee K W, Bahk J D and Lee B H. 2007. A proteomic screen and identification of waterlogging-regulated proteins in tomato roots. *Plant and Soil* **295**(1–2): 37–51.
- Bailey-Serres J, Fukao T, Gibbs D J, Holdsworth M J, Lee S C, Licausi F, Perata P, Voesenek L A and van Dongen J T. 2012. Making sense of low oxygen sensing. *Trends in Plant Science* **17**(3): 129–38.
- Bhargava A and Singh M. 2019. Plant breeding for chlorophyll content: Environmental and genetic variability. *Plant Science Research* **55**: 61–69.
- Burton G N and De Vane E M. 1953. Estimating heritability in fall fescue (*Festuca arundinacea* L.) from replicated clonal materials. *Agronomy Journal* **45**: 478–81.
- Falconer D S and Mackay T F C. 1996. *Introduction to Quantitative Genetics*, 4th edn. Addison Wesley Longman, Harlow.
- Hsiao T C and Bradford K J. 1983. Physiological consequences of cellular water deficits. (In) *Limitations to Efficient Water Use in Crop Production*, pp. 227–65. Taylor H M, Jordan W R and Sinclair T R (Eds). American Society of Agronomy, Madison, WI, USA.
- Hu J, Ren B, Dong S, Liu P, Zhao B and Zhang J. 2022. Poor development of spike differentiation triggered by lower photosynthesis and carbon partitioning reduces summer maize yield after waterlogging. *The Crop Journal* **10**(2): 478–89.
- Johanson H W, Robinson H F and Comstock R E. 1955. Estimates of genetic and environmental variability in soybeans. *Agronomy Journal* **47**: 314–18.
- Kadam N N, Tamilselvan A, Lawas L M, Quinones C, Bahuguna R N, Thomson M J and Jagadish S K. 2017. Genetic control of plasticity in root morphology and anatomy of rice in response to water deficit. *Plant Physiology* **174**(4): 2302–15.

- Kumar S, Ranjan R, Garg T, Vikal Y and Chhabra G. 2025c. Comparative assessment of screening techniques under pot and field conditions for waterlogging tolerance in maize (*Zea mays* L.) inbreds. *Indian Journal of Genetics and Plant Breeding* **85**(03): 437–44.
- Kumar S, Ranjan R, Garg T, Vikal Y and Das A. 2025b. Breeding resilience: Decoding waterlogging tolerance traits in maize. *Cereal Research Communications* 1–16.
- Kumar S, Ranjan R, Garg T, Vikal Y, Das A, Sandhu S K and Singh S. 2025a. Stress indices: Exploring the selection tool for waterlogging-tolerant maize inbred lines in early segregating generations. *Maydica* **67**(3): 1–9.
- Liang K, Tang K, Fang T and Qiu F. 2020. Waterlogging tolerance in maize: Genetic and molecular basis. *Molecular Breeding* **40**(12): 111.
- Liang K, Zhao C, Wang J, Zheng X, Yu F and Qiu F. 2025. Genetic variations in ZmERE179 are associated with waterlogging tolerance in maize. *Journal of Genetics and Genomics* **52**(3): 367–78.
- Lopes M S, Araus J L, Van Heerden P D and Foyer C H. 2011. Enhancing drought tolerance in C4 crops. *Journal of Experimental Botany* **62**(9): 3135–53.
- Mano Y, Omori F, Loaisiga C H and Bird R M. 2009. QTL mapping of above ground adventitious roots during flooding in maize × teosinte “*Zea nicaraguensis*” backcross population. *Plant Root* **3**: 3–9.
- Najeeb U, Bange M P, Tan D K and Atwell B J. 2015. Consequences of waterlogging in cotton and opportunities for mitigation of yield losses. *AoB Plants* **7**: plv080.
- Rathore T R, Warsi M Z, Singh N N and Vasal S K. 2000. Production of maize under excess soil moisture (waterlogging) conditions. (In) *Proceedings of the 4th Asian Regional Maize Workshop: Strengthening Hybrid Maize Technology and Public-Private Partnership to Accelerate Maize Production in the Asian Region*, Los Banos, Philippines, 23–27 Feb 1998, pp. 232–39.
- Rathore M, Singh A and Sharma S. 2020. Genetic variability studies for waterlogging tolerance in maize. *Journal of Plant Stress Physiology* **16**(2): 80–89.
- Ren B, Zhang J, Li X, Fan X, Dong S, Liu P and Zhao B. 2014. Effects of waterlogging on the yield and growth of summer maize under field conditions. *Canadian Journal of Plant Science* **94**: 23–31.
- Robinson H F, Comstock R E and Harvey P H. 1949. Estimation of heritability and the degree of dominance in corn. *Agronomy Journal* **41**: 353–59.
- Saenchai C, Bouain N, Kisko M, Prom-U-Thai C, Doumas P and Rouached H. 2016. The involvement of OsPHO1; 1 in the regulation of iron transport through integration of phosphate and zinc deficiency signaling. *Frontiers in Plant Science* **7**: 396.
- Sandhu S K, Wajhat-Un-Nisaand, Ranjan R. 2021. Breeding for waterlogging tolerance in tropical maize. *Agricultural Research Journal* **58**: 759–67.
- Sarkar B, Varalaxmi Y, Vanaja M, RaviKumar N, Prabhakar M, Yadav S K and Singh V K. 2023. Mapping of QTLs for morphophysiological and yield traits under water-deficit stress and well-watered conditions in maize. *Frontiers in Plant Science* **14**: 1124619.
- Sarker U. 2020. Variability, heritability, character association, and path coefficient analysis in advanced breeding lines of rice (*Oryza sativa* L.). *Genetika* **52**(2): 711–26.
- Singh B D. 2015. *Plant Breeding: Principles and Methods*, 10th edn. Kalyani Publishers, New Delhi.
- Singh R K, Kumar M and Pandey M. 2016. Genetic improvement of waterlogging tolerance in maize. *Plant Breeding Reviews* **40**: 141–76.
- Thapa S, Garg T, Ranjan R, Singh G and Vikal Y. 2025. Efficient and rapid identification of tropical maize inbred lines tolerant to waterlogging stress. *Scientific Reports* **15**(1): 2600.
- Wajhat-Un-Nisa, Sandhu S and Ranjan R. 2023. Root plasticity: An effective selection technique for identification of drought tolerant maize (*Zea mays* L.) inbred lines. *Scientific Reports* **13**: 5501.
- Westra S, Fowler H J, Evans J P, Alexander L V, Berg P, Johnson F, Kendon E J, Lenderink G and Roberts N M. 2014. Future changes to the intensity and frequency of short-duration extreme rainfall. *Reviews of Geophysics* **52**(3): 522–55.
- Yaduvanshi N P, Setter T L, Sharma S K, Singh K N and Kulshreshtha N. 2012. Influence of waterlogging on yield of wheat (*Triticum aestivum*), redox potentials and concentrations of microelements in different soils in India and Australia. *Soil Research* **50**(6): 489–99.
- Zaidi P H, Yadav P and Singh R P. 2012. Genetic analysis of waterlogging tolerance in maize. *Plant Breeding* **131**(2): 111–23.
- Zhang Y, Chen X, Geng S and Zhang X. 2025. A review of soil waterlogging impacts, mechanisms, and adaptive strategies. *Frontiers in Plant Science* **16**: 1545912.