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RESEARCH ARTICLE

Comparative assessment of screening techniques under pot and field conditions for waterlogging tolerance in maize (*Zea mays* L.) inbreds

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Abstract

Waterlogging (WL) stress is a major limitation to maize productivity and the development of efficient and reliable screening methods is essential for breeding WL-resilient hybrids. The present study compared pot and field-based screening approaches to evaluate their effectiveness and complementarity in assessing WL tolerance. For this, 154 $F_{2:3}$ lines derived from WL tolerance and WL susceptible crosses were evaluated in waterlogged pots and the field for six days. WL treatment was given at V_{3-5} stage in the pot experiment and the knee height stage in the field experiment. In the pot experiment, root and shoot parameters were recorded to capture early-stage physiological responses, while in the field; yield and yield-related traits were assessed. Results indicated that pot screening was more resource-efficient, less time-consuming, and effective in rapidly differentiating genotypes based on root and shoot traits. In contrast, field screening, though more labor-intensive, provided realistic insights into genotypic performance under actual stress environments. Both approaches showed consistent trends across genotypes, confirming the effectiveness of pot-based screening for field performance. The study highlights the complementary benefits of these methods and emphasizes the advantage of combining them to strengthen breeding strategies. Such approaches are vital for developing climate-resilient maize hybrids and sustaining productivity in waterlogged environments.

Keywords: Screening methods, Abiotic stress, Root traits, Yield, Maize inbreds.

Introduction

Waterlogging (WL) is a critical abiotic stress that significantly affects maize (*Zea mays* L.) growth and productivity. In tropical and subtropical areas, particularly in poor soil drainage and extended periods of seasonal rainfall consistently lead to significant crop losses. WL is one of the problems worldwide and it is estimated that about 16% of fertile land is affected by the WL problem. WL leading to excessive soil moisture (ESM) is a major constraint of maize production, mostly in the Tropical Asian regions, including India (Lone and Warsi 2009). India shares about 9.89 million hectares in area, which is about 4% of the total maize-grown area of the world. Maize is mostly grown during the *kharif* monsoon season in India, and most of the areas during *kharif* are lowland areas, which are prone to WL.

The maize plant is highly susceptible to WL conditions throughout its life cycle, but the most severe stages are the early V_{4-5} stage, Knee height and the flowering stage. The maize root is the first organ to suffer damage following WL at the seedling stage. In flooded soils, gas diffusion rates are approximately 100 times slower than in aerated soils, hindering root respiration (Ziadi et al. 2004). WL reduces oxygen concentration, which disrupts the balance of reactive oxygen species (ROS) and leads to their build-up. This accumulation initiates membrane lipid peroxidation and alters the antioxidant system in cells experiencing oxygen stress. Peroxidase (POD), a protective enzyme involved in membrane lipid peroxidation, plays a role in removing

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reactive oxygen species such as H_2O_2 and OH^- (Zhong et al. 2010). Diminished root activity under WL conditions significantly lowers plant nitrogen content, resulting in leaf yellowing. Nitrogen deficiency is a key factor in crop yield losses caused by WL during the post-anthesis stage, which significantly reduces root respiratory activity and leaf greenness (as indicated by SPAD readings) (Wu et al. 2018).

To initiate any breeding program, screening the germplasm is first and foremost an activity performed by breeders. Effective and efficient screening methods are needed to identify and select genotypes capable of withstanding WL conditions is a prime concern for researchers. For this, screening methodologies for WL tolerance in maize were conducted under pot experiments, as well as in natural field conditions, and correlating the data generated by both screening conditions. The root traits are mostly affected by WL, and generating root data is highly tedious and time-consuming (Ranjan et al. 2019; 2021). For this pot experiment was performed to get the root data, and a field experiment was performed to generate yield traits data. Both approaches aim to evaluate the response of maize plants to WL conditions, assessing root traits, physiological-morphological, and yield-related traits. Screening methods used by breeders decide the output of the breeding objectives. Therefore, the objective of this work was to correlate results of the pot experiment with the field experiment and establish an effective screening methodology that is more realistic, uses the resource efficiently, and speeds up the breeding work.

Materials and methods

The experimental material consisted of $F_{2:3}$ populations derived from a cross between two parental inbred lines, viz., I 185 (WL tolerant) and SE 565A (WL susceptible). The inbred lines I 185 and SE 565A differ from each other with respect to root traits and aerenchyma formation in the root after a week of WL treatment (Thapa et al. 2025; Rana et al. 2024).

Experiment I

Experiment I was conducted to make phenotypic evaluation of $F_{2:3}$ populations in pots for WL stress tolerance for root traits. A total of 154 $F_{2:3}$ genotypes from the F_2 population were maintained as $F_{2:3}$ seeds by selfing and raised for phenotyping in an artificial pit, Rice Research Area, Punjab Agricultural University, Ludhiana, during *kharif* 2023, as Fig. 1. A fine loamy soil was filled in pots and Bavistin was used for seed treatment. Six seeds were sown for each genotype in each pot with two replications and after germination, five seedlings were maintained in each pot by thinning. An artificial pit was sealed with a plastic sheet to avoid percolation and seepage. WL treatment was given at the V_{3-5} leaf stage over six days. The water was drained out immediately from the pit and then the pots were kept for a further seven days so that plants could recover from the shock. Plants were uprooted; shoot and root portions were separated and the root length was measured using by measuring scale. Data were collected for root traits, namely, root dry weight (RDW in g), shoot dry weight (SDW in g), root surface area (RSA) (cm^2), root length (RL in cm), and mean difference in chlorophyll content (CCDP) before and after WL treatment to potted plants. RDW and SDW were measured after oven drying of root and shoot for six days and the use of a weighing scale measured the weight. The average root and shoot weight for both replications was recorded separately. RSA has been measured by root scanner, for which root images were collected from the EPSON Expression 12000XL scanner and images were analyzed using Biovis PSM root software. The chlorophyll content of potted plants was measured by SPAD ApogeeMC-100 (Wajhat et al. 2023).

Experiment II

Phenotypic evaluation was also done of $F_{2:3}$ populations in the field for WL stress tolerance for yield and associated traits. $F_{2:3}$ plants were raised for phenotyping in the natural environment (field conditions) at Maize Research Fields, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, during *kharif* 2023 as Fig. 2.

The other set of the same 154 $F_{2:3}$ seeds was used for phenotyping in waterlogged conditions in the field. Each genotype was sown in two rows having 3 m row length, 20 cm plant-to-plant distance and 60 cm row-to-row distance with two replications on a flatbed. Trenches are made around the field to maintain submerged conditions. WL treatment was given at the knee-high stage over 6 days. The control condition was planted under a ridge bed in two replications, having paired rows of 3 m for each genotype, where the crop was raised according to recommended cultivation practices. Data were collected for yield and associated traits, including cob yield (YLD) per plot (gm), plant height (PH) (cm), ear height (EH) (cm) and Chlorophyll content. SPAD MC-100 measured the chlorophyll content of field-planted plants.

Waterlogging tolerance coefficient (WTC) is a quantitative measure used to assess a plant's ability to tolerate waterlogged conditions. It is typically calculated based on the reduction in growth, yield, or physiological performance of a plant under waterlogged conditions compared to normal (non-waterlogged) conditions (Zou et al. 2014). For this waterlogging tolerance coefficient for yield (WTC-YLD), Plant height (WTC-PH) and ear height (WTC-EH)

Waterlogging tolerance coefficient (WTC) for each trait, viz. PH, EH and yield were calculated using the formula:

$$WTC = \frac{\text{Performance of each trait under WL}}{Performance\ of\ each\ trait\ under\ Control}$$

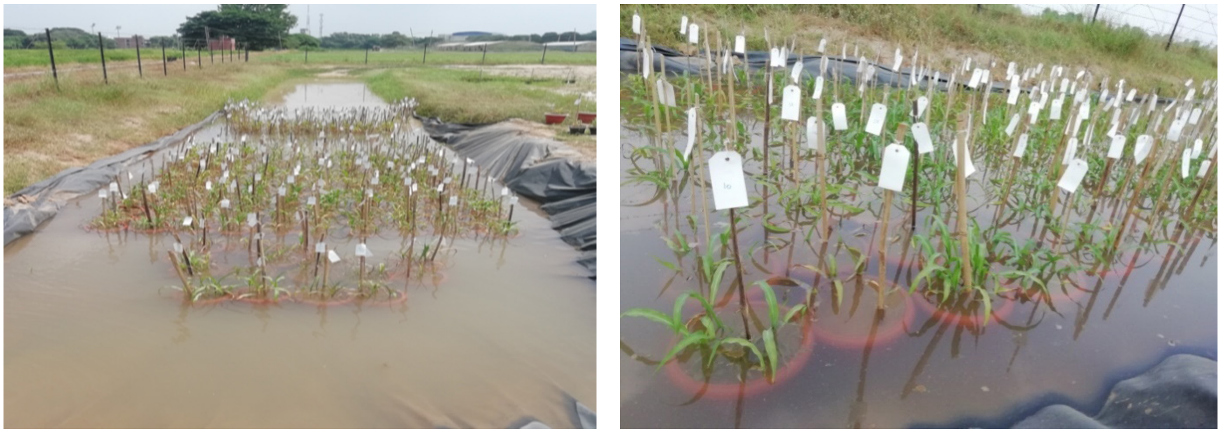


Fig. 1. Potted maize plants subjected to WL stress at the most susceptible V_{3-5} leaf stage

Results

The results of both the experiments conducted under pot and field conditions are presented below with suitable Figures and Tables.

Phenotypic evaluation of $F_{2:3}$ populations for variability in root and shoot traits under WL stress in pots

The mean performance of various genotypes and analysis of variance (ANOVA) showed that all the traits under consideration were highly significant under waterlogged conditions. For root dry weight (RDW), the mean was recorded at 0.54 ± 0.03, with a range from 0.08 to 0.93 g. For shoot dry weight (SDW), the mean was 0.71 ± 0.06, ranging from 0.07 to 2.34 g. Root surface area (RSA) showed a mean of 22.66 ± 0.99, with values ranging from 2.43 to 40.71 cm^2. Root length (RL) averaged 20.67 ± 1.23, spanning from 6.00–38.00 cm.

Correlation between different root traits under WL stress in pots

The correlation analysis of the phenotypic traits revealed a very strong positive and significant correlation of RDW with RSA (0.932**) and CCDP (0.261**), while showing a very strong significant negative correlation with SDW (-0.584**) and RL (-0.905**), as Figure 3. SDW displayed a positive correlation with RL (0.527**), but a very strong, significant negative correlation with RSA (-0.563**) and CCDP (-0.199*). RSA was positively correlated with CCDP (0.211**) but showed a very strong, significant negative correlation with RL (-0.890**). RL exhibited a significant negative correlation with CCDP (-0.175*).

Phenotypic evaluation of $F_{2:3}$ populations for yield and associated traits under WL stress in field

The variability parameters viz., ANOVA and mean of various genotypes under stress conditions, as shown in Table 2, revealed significant variation across selected yield and related traits, 1942.16 ± 215.13, ranging from 654.09 to 3375.39 g. Similarly, plant height (PH) averaged 90.53 ± 5.33, with a range from 25.57 to 179.29 cm, and ear height (EH) showed a mean of 39.21 ± 1.21, with a range from 12.34 to 75.25 cm.

For Waterlogging tolerance Coefficient based on yield (WTC-YLD), the mean was 0.54 ± 0.04, with range values 0.45 to 0.85, (WTC -PH) had a mean of 0.61 ± 0.03, ranging from 0.16 to 1.19 and for ear Height (WTC-EH) had a mean of 0.51 ± 0.01, with a range of 0.14 to 1.05.

A: Water logged field

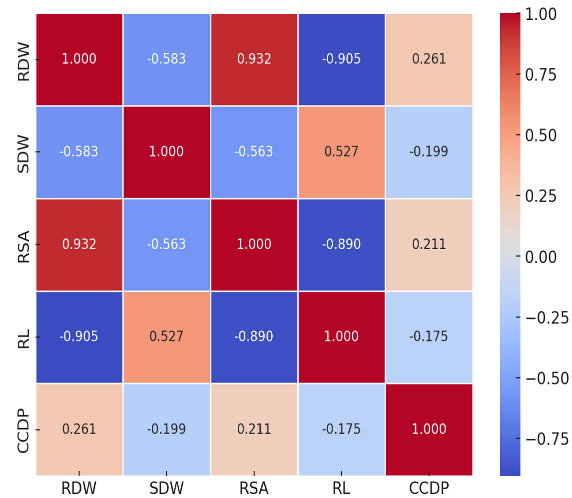
B: Control (Non water logged field)

Fig. 2. Maize plants subjected to WL stress in a field at the knee-high stage

Table 1/ Variability analysis in $F_{2:3}$ populations in pots under WL conditions

Traits	Populations $F_{2:3}$			
	Mean ± SE	Range		Mean sum of square
		Min	Max	
RDW	0.54 ± 0.03	0.08	0.93	0.06***
SDW	0.71 ± 0.06	0.07	2.34	0.02***
RSA	22.66 ± 0.99	2.43	40.71	145.73***
RL	20.67 ± 1.23	6.00	38.00	98.61***

*, **, *** - significant at the 5, 1 and 0.01 % level
RDW: Root dry weight(gm), SDW: Shoot dry weight (gm), RSA: Root surface area (cm^2) and RL: Root length (cm)



RDW = Root dry weight (g), SDW = Shoot dry weight (g), RSA = Root surface area (cm^2) RL = Root length (cm), CCDP = difference in chlorophyll content before and after WL treatment in pot condetions. Red colour for positive correlations, blue for negative correlations, neutral colour for non-significant (NS) correlations and intensity of colour represents the strength of correlation

Fig. 3. Correlation between different root traits under WL stress in pots

Correlation between different yield and associated traits under WL stress in field

The correlation analysis of yield and associated traits (Fig. 4) revealed a positive and significant correlation among YLD and CCDF (0.338**), followed by PH (0.243), EH (0.344**), and WTC (YLD) (0.670**). There was also a positive, non-significant correlation with WTC (PH) (0.021^{NS}) and WTC (EH) (0.047^{NS}). PH showed a strong positive and significant correlation with EH (0.981**), followed by WTC (PH) (0.369**), WTC (EH) (0.248**), and CCDF (0.308**), and a positive non-significant correlation with WTC (YLD) (0.174^{NS}). EH (1.00) exhibited a positive and significant correlation with WTC (PH) (0.312**), WTC (EH) (0.248**), and CCDF (0.265**), along with a positive non-significant correlation with WTC (YLD) (0.167^{NS}). WTC (YLD) showed a non-significant correlation with WTC (PH) (0.052^{NS}) and WTC (EH) (0.090^{NS}). WTC (PH) had a strong correlation with WTC (EH) (0.936**) and a positive non-significant correlation with CCDF ($0.0.152^{NS}$). WTC (EH) (1.00) also showed a positive non-significant correlation with CCDF (0.060^{NS}).

Collinearity of results between the screening methods under pot vs field conditions

Under waterlogged conditions, the correlation between pot experiment for root traits *viz.,* RDW, RL, RSA and shoot traits *viz.,* SDW and CCDP and field experiment for yield and associated traits *viz.,* YLD, PH, EH, WTC(YLD), WTC(PH), WTC(EH), and CCDF highlights several key relationships as Figure 5. RDW shows significant negative correlations with yield traits such as YLD (-0.235**) and CCDF (-0.682**), indicating that higher RDW may negatively impact yield in waterlogged environments. Conversely, SDW has positive correlations with yield traits, including YLD (0.376**) and CCDF (0.895**), suggesting that higher SDW is associated with improved yield performance under these conditions. Root length (RL) is postively correlated with YLD (0.216**) and CCDF (0.624**), implying that longer roots may be favourable for yield under waterlogged conditions. CCDP exhibit weaker correlations, with a small positive correlation with RDW (0.261**) but minimal influence on yield traits like YLD (-0.177**) and CCDF (-0.180*). Overall, shoot traits SDW tend to show a stronger positive association with yield traits than root traits under waterlogged conditions.

The slope of the line for RDW-P *Vs* YLD(WL)-F indicates that slope of -942.56 indicates a strong negative relationship between RDW and ear yield (YLD), meaning as RDW increases, YLD decreases significantly (Fig 6). while slope of the line for SDW-P *Vs* YLD (WL)-F which indicates that for every 1-unit increase in SDW, YLD increases by 748.05 units (Fig 6).

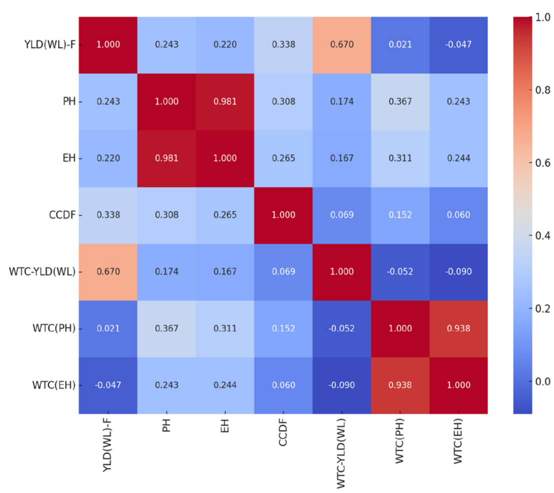
Discussion

The results of both the experiments (Experiments 1 and 2) are discussed separately to differentiate the findings. WL stress significantly affects maize crops throughout its life from germination to harvest. Recent research has shown that the early third leaf stage (V_3) to the seventh leaf stage (V_7) is the most critical period for root growth and development. Under WL conditions, root decomposition begins, preventing the plant from absorbing nutrients, leading to deficiency. WL conditions also hinder the diffusion of soil and atmospheric air, causing hypoxia or anoxia in the root zone, which negatively impacts crop yield. Plants are evaluated under field conditions for yield and morphological traits such as plant height (PH) and Ear height (EH), which are crucial indicators of WL tolerance. Yield under stress reflects the plant's overall productivity, while PH and EH offer insights into the plant's structural adaptability and reproductive success in a waterlogged environment.

Table 2. Variability analysis in $F_{2:3}$ populationsin field under WL conditions

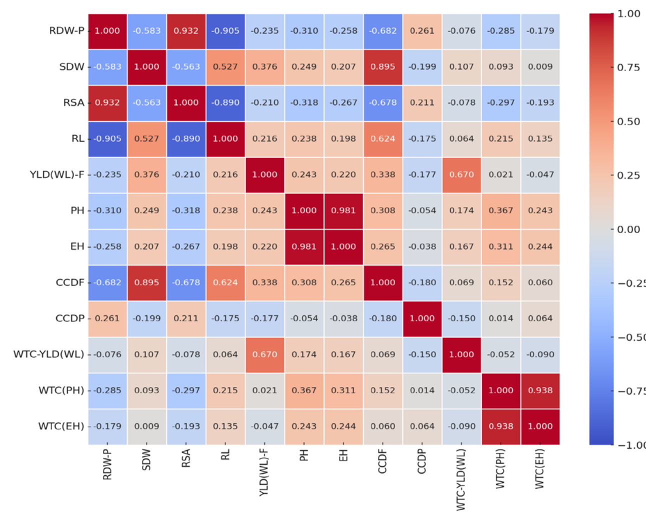
Traits	Populations $F_{2:3}$			
	Mean ± SE	Range		Mean sum of square
		Min	Max	
YLD(WL)-gm	1942.16 ± 215.13	654.09	3375.39	1418046***
PH-cm	90.53 ± 5.33	25.57	179.29	2357.7 ***
EH-cm	39.21 ± 1.21	12.34	75.25	414.00**
WTC-YLD	0.54 ± 0.04	0.45	0.85	0.01***
WTC-PH	0.61 ± 0.03	0.16	1.19	0.033**
WTC-EH	0.51 ± 0.01	0.14	1.05	0.023**

*, **, *** - significant at the 5, 1 and 0.01 % level



YLD(WL)-F = Ear yield (g), PH = Plant height (cm), EH = Ear height (cm) and CCDF = Chlorophyll content different in field sown before and after waterlogging stress in $F_{2\text{-}3}$, WTC = Waterlogging tolerant coefficient. Red colour depict positive correlations, blue negative correlations, neutral colour indicates non-significant (NS) correlations, whereas intensity of colour represents the strength of correlation

Fig. 4. Correlation between different yield and associated traits under WL stress in field



Scale represents the strength of correlation with red and blue colours.
YLD (WL)-F = yield under waterlogged field, RDW-P = Root dry weight in pot, SDW = Shoot dry weight, RSA = Root surface area, RL = Root length, PH = Plant height, EH = Ear height, CCDF and CCDP = Diiference in cholorophyll content before and after treatment in field and pot, WTC = Waterlogging tolerance coefficient

Fig. 5. Correlation between the root, shoot, yield and associated traits in pots and fields under WL conditions

The phenotypic evaluation of root and shoot traits under WL stress in pots in $F_{2:3}$ populations at $V_{3\text{-}5}$ stage as $V_{3\text{-}5}$ stage is most critical stages for WL tolerance screening (Zaidhi et al. 2004). The significant variation shown in (Table 1) for RDW and SDW across genotypes is consistent with the role of genetic variability in determining plant resilience to WL. Previous studies have emphasized that RDW is a critical trait, as a robust root system under WL helps mitigate oxygen deficit stress by improving metabolic activities and nutrient uptake efficiency a direct measure of the plant's shoot response to stress, reflecting its ability to sustain above-ground biomass, which is essential for long-term survival and productivity (Ren et al. 2014).The wide range differences observed in RSA and RL under WL stress further validate the importance of these traits in plant tolerance. RSA, responsible for maximizing nutrient and oxygen absorption in hypoxic conditions, has been highlighted in several studies as a key determinant of WL tolerance. Likewise, RL plays a role in penetrating deeper soil layers where oxygen availability may be higher. These findings are supported by literature that emphasizes the genetic variability of root traits in maize under waterlogged conditions, which are important for breeding efforts aimed at improving stress tolerance (Mano et al. 2005; Ziadi et al. 2007).

The correlation between root traits under WL stress (Fig. 3) reveals critical insights into the trade-offs between roots and shoot growth. A strong positive correlation between RDW and RSA) suggests that genotypes with more root mass also tend to have larger root surface areas, which is important for improving water and nutrient absorption under stressful conditions like WL. This finding aligns with research suggesting that increased root mass can enhance a plant's ability to adapt to adverse environments by improving nutrient uptake efficiency (Rich and Watt 2013). The negative correlations of RDW with SDW (-0.584**), and RL (-0.905**) indicates that plants allocating more biomass to roots may exhibit reduced shoot growth and root elongation, this is because root and shoot compete for resources. Similarly, waterlogged conditions favour increased shoot biomass as root growth is inhibited in the WL situation due to lack of oxygen (respiration). This trade-off is commonly observed in stress conditions where plants prioritize either above or below-ground biomass depending

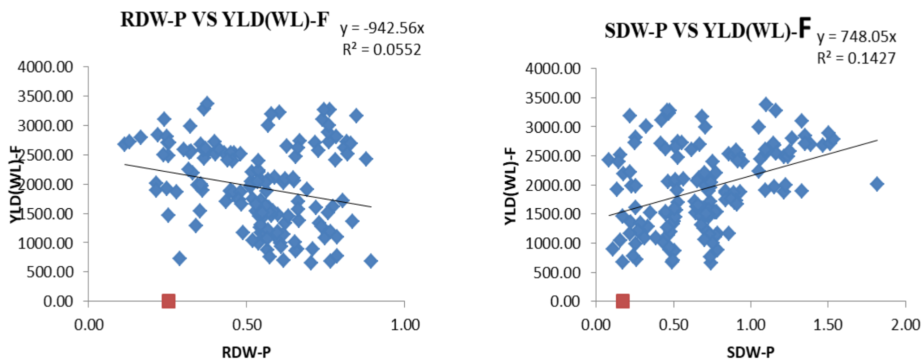


Fig. 6. Correlation of traits between pot experiment denoted by (P) and field experiments denoted by (F) for RDW and SDW with YLD

on the environmental demand (Kramer and Boyer 1995). A similar relationship has been documented where root-heavy plants may compromise shoot growth under stress as a survival mechanism (Wu and Cosgrove 2000). The SDW shows positive correlation with RL indicates that plants with larger shoots tend to have longer roots, reflecting a balance between shoot vigour and root exploration, this is because,under waterlogged conditions, plants often develop larger shoots to increase oxygen transport from the shoots to the roots, which helps the roots survive in low-oxygen environments. This process, known as *aerenchyma formation at root tip*, allows for better internal oxygen diffusion and supports root growth despite stressful conditions. However, the negative correlation between SDW and RSA suggests that increased shoot biomass may coincide with a reduction in root surface area. It is likely that a shift in resource allocation favouring shoot development at the expense of root surface area under waterlogged conditions, a phenomenon also observed by (Bouranis et al. 2003) in studies of root architecture under abiotic stress.

The phenotypic evaluation of the $F_{2:3}$ populations under WL stress in field revealed significant genetic variation among genotypes in yield and related traits, as shown in (Table 2) essential for breeding efforts focused on improving stress tolerance. A high significant mean yield indicates high genetic variability, suggesting that some genotypes maintain higher yield potential under adverse conditions (Kumar et al. 2021). Plant height and ear height showing significant variation with wide range of variation reflecting differential genotype responses to WL (Guo et al. 2018) and also indicating variability in reproductive traits that may impact grain production under stress (Zhao et al. 2020). The waterlogging tolerance coefficients for yield, plant height, and ear height (WTC (YLD), WTC (PH), and WTC (EH)) showed significant genetic variation, underlining its importance for understanding adaptive responses under WL (Liu et al, 2019). These findings highlight key traits for potential selection in breeding programs aimed at enhancing resilience to environmental stress (Acquaah 2007).

The correlation analysis between yield and associated traits under WL stress as shown in (Fig. 4) provides valuable insights into the relationships between these traits and their contributions to plant performance in adverse conditions. The positive correlation of yield (YLD) with chlorophyll content difference in field sown (CCDF) emphasizes the critical role of photosynthetic capacity in determining yield under stress, photosynthesis produces the energy needed for growth and development. The correlation of yield with plant height (PH) aligns with findings that taller plants often have an advantage in competitive environments, potentially accessing more light and resources (Zhao et al. 2020).

The correlation analysis between pot and field conditions under WL insights into the relationships between root traits observed in pots and yield and assessed traits observed in the field as shown in Fig. 5. The significant negative correlations of RDW with SDW and (YLD) (Fig. 3) suggested that genotypes allocating more resources to root biomass may experience reduced shoot dry matter and yield performance due to oxygen deficiency, which hampers root respiration and nutrient uptake. Changes in root morphology, increased susceptibility to diseases, and nutrient imbalances, lead to lower plant health and yield. This aligns with previous studies indicating that excessive root growth can divert essential resources away from shoot development, ultimately impairing yield potential in stressful environments (Fischer et al. 2020). The strong positive correlations of SDW with YLD (Fig. 3) and CCDF (0.895**), highlight the importance of shoot biomass for achieving higher yields under WL, this is due to enhanced photosynthesis and energy production, as larger shoots increase leaf area for sunlight capture. Also, its revealed that higher the shoot growth, higher is the yield as the cereals crop have high capacity to translocate reserve from shoot to grain yield (Ranjan et al. 2019). Additionally, maize plants may allocate resources to above-ground growth as a survival strategy, supporting reproductive structures and compensatory growth to maximize light and nutrient access. This robust shoot biomass improves stress tolerance and reproductive development, leading to higher yields in WL conditions. This finding is supported by research that emphasizes the significance of shoot growth in enhancing photosynthetic capacity and overall plant health, which are critical for yield stability during stress (Zhang et al. 2019). Additionally, the postive correlation of root length (RL) with yield traits (YLD) (Fig. 3) suggests that longer roots may necessarily confer benefits in terms of yield under waterlogged conditions. This could be attributed to the fact that in waterlogged environments, excessive root elongation might improve access to water or nutrients but could instead lead to physiological stress or root dysfunction (Silva et al. 2021). The complex interactions between root and shoot traits under WLstress, highlight the need for a balanced investment in biomass allocation to optimize yield performance.

The linear regression slope (Fig. 6) for RDW *Vs* YLD (WL)-F of (-942.56) between RDW and YLD indicates a strong negative relationship, suggesting that as RDW increases, YLD decreases significantly. This negative association may be due to excessive resource allocation to root growth under WL stress, leading to reduced energy and nutrient availability for reproductive development and yield formation. Such findings are consistent with earlier studies, such as (Shabala et al. 2014), which highlighted that increased root biomass under waterlogged conditions often reflects a stress-response mechanism rather than a productivity-enhancing trait. Furthermore, the low R^2 value (0.052) indicates that only 5.2% of the variation in YLD is explained by RDW, suggesting that other physiological or environmental factors play a larger role in determining yield under these conditions. The slope in Fig. 6 for SDW *Vs* YLD (WL)-F (748.05) between SDW and YLD indicates a significant positive relationship, where higher SDW leads to an increase in YLD. This suggests that shoot dry weight is an important contributor to yield formation under WL stress, likely due to its role in carbohydrate storage and remobilization during grain filling (Lopes et al. 2011) and (Setter et al. 2009), which emphasized the importance of above-ground biomass, particularly shoot reserves, in supporting grain yield under stress conditions making it a relatively better predictor under waterlogged conditions.

Thus to initiate any breeding program, breeders must screen a large number of genotypes, which generally demands considerable resources. Therefore, breeding programs should prioritize efficient and effective screening techniques that minimize resource use and time (Ranjan et al. 2019). Our results suggest that pot screening provides an excellent option, as it is less labor, space, and time-intensive compared to conventional field screening. Subsequently, field evaluation can be carried out on a selected subset of promising genotypes identified from pot screening to validate their performance across multiple locations and seasons.

Authors' contributions

Conceptualization of research (RR, YB, G); Designing of the experiments (RR, TG, YB); Contribution of experimental materials (RR, TG); Execution of field/lab experiments and data collection (SK, R, GC); Analysis of data and interpretation (SK, RR); Preparation of the manuscript (SK, RR).

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