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Research article

Screening of waterlogging-tolerant maize (*Zea mays* Linn.) at early seedling stage

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Abstract

Importance of the work: Water is a crucial factor for maize growth and development; however, excessive water in the root system can have negative effects.

Objectives: 1) To investigate the impact of waterlogging on various maize lines; 2) to identify the duration of waterlogging adversely affecting maize growth; 3) to identify tolerant and susceptible varieties; and 4) to determine waterlogging tolerance traits during vegetative growth stage 2 for waterlogged conditions.

Materials & Methods: The experiment was laid-out in a split-plot randomized complete block design, with the main plot representing the waterlogging duration and the subplot being the maize line. Regression and correlation analyses were used to determine the relationships between waterlogging duration and maize growth parameters.

Results: After 10 d of waterlogging at the V2 leaf stage, there were significant reductions in plant height, root length and dry matter accumulation, with induced leaf chlorosis and the promotion of nodal root formation. As the duration of waterlogging increased, leaf chlorosis increased. Hybrid 1, Hybrid 2 and BRK were more tolerant to waterlogging stress than the other evaluated maize lines. In addition, taller plant height, longer root length and higher dry matter accumulation, along with nodal root formation were identified as suitable criteria for selecting tolerant maize in a waterlogging maize breeding program.

Main finding: The study identified Hybrid 1, Hybrid 2 and BRK as the most tolerant maize lines to waterlogging stress. These lines had longer root length, taller plant height, heavier dry matter weight and clear nodal root formation for waterlogged conditions, which are adaptive traits for plants to withstand soil waterlogging stress.

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Introduction

Maize (*Zea mays* Linn) is commonly referred to as corn which and is one of the most important cereal crops in the world, serving as a staple food for millions of people globally. For example, in the Philippines, it is the second most productive and essential crop after rice, with 2.44 million t harvested during January–March 2022 on 671,190 ha in 2022 after a nearly constant increase in productivity since 2003 (Philippine Statistic Authority, 2022). In addition, according to the Food and Agriculture Organization and the US Department of Agriculture, the Philippines is one of the seven major maize-producing countries in Asia, along with China, India, Indonesia, Nepal, Thailand and Vietnam (Gerpacio and Pingali, 2007; Wada et al., 2008). Thus, the demand for corn has remained very high and has been continuously increasing through the years, as a food source for both humans and livestock (Cañete and Baldo, 2019). However, the productivity of corn is still vulnerable to unfavorable climatic conditions due to climate change, which can cause significant financial losses to farmers (Esteban and Edwin, 2016).

One of the detrimental effects of climate change is excessive and erratic rain patterns, which is now experienced in the Philippines, which result in temporary waterlogging in the cornfields, especially in low-lying areas (Cañete and Baldo, 2019). When corn is subjected to waterlogging for more than 3 d, the yield is reduced by more than 40%, with this amount increasing with increasing waterlogging duration (Li et al., 2011). Thus, this undesirable effect of waterlogging in addition to causing yield reductions and significant financial losses to the farmers, results in reduced feed supply as it is an ingredient in demand by the livestock and poultry industries (Cañete and Baldo, 2019).

To date, most research has concentrated on how waterlogging affects corn growth (Zaidi et al., 2003, 2004, 2007, 2010, 2012; Lone and Warsi, 2009; Tang et al., 2010; Liu et al., 2010; Esteban and Edwin, 2016). New varieties with excellent adaptation are essential to increase corn production during temporary waterlogging. However, there are still no approved varieties that are resistant to waterlogging. Researchers are still screening and validating potential traits of maize on waterlogging. Furthermore, this problem has gone unseen by researchers because most research interest is on drought and insect resistance development in maize. Consequently, the reported findings are meager and the progress of maize waterlogging research is moderately sluggish. Hence, to achieve a stable agronomic yield, is paramount to achieve steady corn production, screening and evaluation of the different potential lines of maize subjected to the waterlogging breeding program.

Zaidi et al. (2004) reported that vegetative growth stages 2 (V2) and 7 (V7) are the most susceptible to waterlogging stress. Hence, the current study imposed waterlogging at the V2 leaf stage of maize. Resources can be used efficiently by imposing waterlogging stress during this stage while retaining the effectiveness of selection. Thus, this experiment was performed to determine the effects of waterlogging on different corn varieties, to identify number of days of waterlogging that negatively affected the growth of maize lines, to identify tolerant and susceptible lines and to identify waterlogging tolerance traits during this V2 stage.

Materials and Methods

The experiment was conducted in the research area of Davao de Oro State College at Maparat, Compostela, Davao de Oro, the Philippines, from August 2018 to January 2019. The experiment was arranged in a split plot in a randomized complete block design and replicated three times. The main plot was the waterlogging duration (WD), while the subplot was the maize line. Each treatment combination had 10 sample plants, resulting in 1,500 plants in the study.

Screening was done using the cup screening method (CSM) reported by Zaidi et al. (2003), with modification to fit the current experimental requirements. The modified CSM used 340 g plastic cups perforated at the base at four points with an orifice of 5.0 mm. The plastic cups were rinsed with running water before they were filled with growing media. After rinsing with the water, the plastic cups were air-dried for 1 hr. Plastic cups used to ensure no defects that could cause biased experimental results. Initially, ordinary garden soil was sieved through a fine mesh (2 cm ×2 cm) to remove stones, pebbles, sticks and other materials that resulted in a uniform soil fineness to avoid hindering the proper growth of maize. The ordinary garden soil was thoroughly mixed with fine sand at a 1:1 ratio and then used as the soil medium. Before filling the plastic cups with the growing medium, it was sundried for

1 d to kill harmful microorganisms. After sun-drying the soil medium, it was placed in a large pan, cooked and subsequently thoroughly mixed for 2 hr under high heat. After cooking, the soil was removed from the cooking pan and transferred onto a plain aluminum sheet to cool before being used to fill the plastic cups. Each cup was weighed after filling to maintain the same quantity of soil (300 g) and retain the same moisture level in each container. Next, 5 g of complete inorganic fertilizer (14-14-14, N-P-K) were applied to each maize plant as a basal fertilizer, after which the inorganic fertilizers were covered with the soil, followed by sowing of two seeds per cup. The sown seed had no defects and a high germination percentage. Soil watering was done after seed sowing to ensure there was sufficient moisture for the germination process. After 7 d of seed germination, seedling that had defects or were unhealthy were thinned out, leaving one healthy and vigorous seedling per pot as the data plant.

All cups were filled with water to about 2 cm above the soil surface for the waterlogging treatment of all maize lines, except for the untreated control treatment. The water level in the cups was maintained continuously at the same level using a marker line placed above the soil surface throughout the experiment. Another 1,500 unperforated cups were used to cover the perforated cups to avoid water leakage and maintain the water level. The water level was monitored daily, with water being added if the water level dropped below the demarcation line due to evaporation. After being subjected to the waterlogging treatment, any ponded water was drained from the cups. The seedlings were carefully thinned to avoid root damage and then immediately measured for the degree of leaf chlorosis, percentage survival, plant height, root length, total dry matter weight and other observations on the maize lines that were properly documented during the experiment.

Statistical analyses

The data were analyzed using analysis of variance. Then differences among treatment means were evaluated using Tukey's honest significant difference (HSD). Simple linear regression and Pearson correlation analyses of all experimental parameters were computed using a statistical software package developed by the International Rice Research Institute (International Rice Research Institute, 2014). The statistical significant was set at $p < 0.05$.

Result and discussion

Effects of waterlogging duration

The results indicated that waterlogging significantly reduced the growth parameters of the maize (Table 1). Despite surviving for up to 10 d of waterlogging at the V2 leaf stage, all maize lines experienced leaf chlorosis. Notably, the degree of leaf chlorosis increased as the duration of waterlogging was prolonged, with the day 10 showing the highest degree of chlorosis. These findings were consistent with Zaidi et al. (2004) and Malik et al. (2001) who reported severe chlorosis due to waterlogging. Chlorosis, which indicates a reduction in chlorophyll, can affect photosynthesis performance and lead to lower growth and yield. For example, similar studies by Esteban and Edwin (2016), and Drew and Saker (1986) showed that leaf chlorosis significantly reduced the chlorophyll content and affected the photosynthetic capacity of leaves, leading to lower growth and yield in maize. Furthermore, studies by Subha (2008), Gowri (2009), and Leghari et al. (2016) suggested that waterlogging reduced chlorophyll content, resulting in leaf yellowing and

Table 1 Effects of number of days of waterlogging on different growth parameters in 10 maize lines

Waterlogging duration	DLC	PS (%)	PH (cm)	RL (cm)	DM (g)
No W	$1.00±0.50^{b}$	100.00	$41.64±0.57^{a}$	$31.78±0.54^{a}$	$1.75±0.58^{a}$
4 DW	$1.25±0.53^{b}$	100.00	$24.50±0.31^{c}$	$21.03±0.55^{cb}$	$0.58±0.67^{c}$
6 DW	$1.31±0.57^{b}$	100.00	$24.39±0.99^{c}$	$24.39±0.62^{bc}$	$0.53±0.57^{c}$
8 DW	$1.71±0.60^{b}$	100.00	$25.72±0.30^{c}$	$26.05±0.52^{b}$	$0.92±0.50^{bc}$
10 DW	$2.54±0.55^{a}$	100.00	$32.71±0.23^{b}$	$17.21±0.57^{c}$	$1.29±0.51^{ab}$
F test	**	ns	**	**	**
CV	54.17%	0.00%	9.24%	33.44%	69.79%

DLC: degree of leaf chlorosis; PS: percentage survival; PH: plant height; RL: root length; DM: dry matter; W: waterlogging; DW = days of waterlogging; CV = coefficient of variation; ns = non-significant; * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$).
Values (mean ± SD) in each row superscripted with different lowercase letters are significantly ($p < 0.05$) different.

nitrogen deficiency. The decrease in leaf chlorophyll content due to chlorophyll degradation mediated by superoxide radicals formed during waterlogging stress could result in lower chlorophyll meter readings in waterlogged pots (Yan et al., 1995; de Souza et al., 2011; Wang et al., 2012)

In addition to leaf chlorosis, waterlogging reduced plant height, root length and dry matter content. While plant height increased slightly on day 10 of waterlogging duration, its growth remained significantly slower than for the non-waterlogged treatments. Similar findings by Kaur et al. (2019) highlighted that maize growth is slower when experiencing waterlogging. Moreover, the negative effects of waterlogging on corn are similar to those on water deficiency, as observed in the findings of Molla et al. (2014) and Udomprasert et al. (2005). Studies by Bragina et al. (2003) and Bragina et al. (2001) indicated that prolonged waterlogging significantly reduced plant height. Furthermore, waterlogging significantly reduced root length, with the longest duration producing the shortest length, suggesting that roots are more sensitive to waterlogging stress than the shoot. Studies by Ding et al. (2017) and Malik et al. (2001) supported this finding, indicating a higher decrease in the relative growth rate of roots compared to shoots during waterlogging. Reduced root length could result from reduced oxygen concentration in the soil solution due to waterlogging, leading to root cell death and decay. This, in turn, reduces the root's ability to absorb water and nutrients, leading to nutrient deficiencies and poor plant growth, as was suggested by Steffens et al. (2005). Therefore, growth stages that experience waterlogging have poor plant growth, as was observed in the current study.

Notably, while dry matter weight decreased during days 4–8 of waterlogging, subsequently it increased as waterlogging was prolonged. This suggested that despite experiencing waterlogging, maize still undergoes growth and development. However, the overall effect of waterlogging on maize growth was negative, as seen in the reduced growth parameters and chlorosis.

Effects of waterlogging on maize lines

The results from the current study revealed that there was notable variation among the maize lines subjected to waterlogging (Table 2). Hybrid 2 and BRK had the greatest plant heights among the maize lines, which were still comparable to Hybrid 1. Additionally, Hybrid 1 and Hybrid 2 had the longest root length and BRK had the greatest weight of dry matter during the waterlogging period.

Notably, waterlogging promoted nodal root development (Fig. 1). This nodal root development was likely a contributing factor to the survival of the maize lines, enabling them to increase plant height and dry matter and withstand up to 10 d of waterlogging imposed at the V2 leaf stage. Other studies have reported that newly emerged nodal roots due to waterlogging in maize had large air spaces in their cortical regions, which is pronounced under anoxic conditions (Jackson and Armstrong, 1999; Rathore et al., 1997; Gibberd et al., 2001; Rubinigg et al., 2002)

Table 2 Effects of waterlogging on different parameters of maize lines subjected to waterlogging

Variety	DLC	PS (%)	PH (cm)	RL (cm)	DM (g)
Sige-Sige	1.40±0.56	100.00	28.45±0.57^{c}	22.68±0.67^{bcde}	0.93±0.30^{bcd}
NSIC CN 2008-222	1.56±0.60	100.00	25.27±0.61^{c}	20.39±0.66^{de}	0.78±0.32^{cb}
PSB CN 93-27	1.74±0.67	100.00	24.78±0.57^{c}	18.51±0.59^{e}	0.61±0.29^{d}
Hybrid 1	1.40±0.68	100.00	34.02±0.60^{ab}	28.55±0.61^{a}	1.42±0.24^{ab}
Hybrid 2	1.55±0.65	100.00	36.01±0.57^{a}	28.13±0.63^{a}	1.16±0.25^{abc}
T. Compostela	1.50±0.48	100.00	29.09±0.62^{c}	22.27±0.57^{cde}	0.93±0.23^{bcd}
T. Bohol	1.54±0.50	100.00	29.02±0.58^{c}	22.09±0.63^{cde}	1.03±0.25^{bcd}
BRK	1.73±0.47	100.00	34.48±0.61^{a}	27.15±0.67^{ab}	1.57±0.33^{a}
Hybrid 3	1.68±0.51	100.00	29.23±0.59^{bc}	24.87±0.68^{abcd}	0.80±0.35^{cd}
Hybrid 4	1.48±0.48	100.00	27.58±0.58^{c}	26.32±0.71^{abc}	0.90±0.29^{bcd}
F Test	ns	ns	**	**	**
CV	26.79%	0.00%	13.94%	16.52%	44.57%

DLC = degree of leaf chlorosis; PS = percentage survival; PH = plant height; RL = root length; DM = dry matter; CV = coefficient of variation; ns = non-significant; ** = highly significant ($p < 0.01$)

Values (mean ± SD) in each row superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Fig. 1 Nodal root formation (white arrows) on maize for waterlogged conditions

Zhu et al. (2016) found that adventitious root formation increased significantly during waterlogging compared to normal conditions as the formation of nodal roots in the early growth stage during waterlogging helped the maize breathe to avoid anaerobic respiration and continue metabolic processes. This finding was consistent with the discovery by Cañete and Baldo (2019) that nodal roots helped the plant access atmospheric gases to prevent anaerobic respiration. Additionally, Malik et al. (2001) reported that the formation of adventitious roots to potentially replace the basal roots was one possible morphological adaptation that plants exhibit with waterlogging stress.

The current findings aligned with Kaur et al. (2020), who suggested that nodal root development is an adaptive trait for plants to withstand soil waterlogging stress. In summary, the current results provided valuable insights into the mechanisms underlying maize adaptation to waterlogging stress, which can be used to develop strategies to enhance crop productivity and resilience under such conditions.

Regression and correlation analysis

A simple linear regression analysis was conducted to evaluate the effect of waterlogging duration on maize growth parameters. The results of the analysis are presented in Table 3, which shows that the waterlogging duration had a significant positive effect on leaf chlorosis. The coefficient of determination (R^2) indicated that 78.13% of the variation in leaf chlorosis was explained by the waterlogging duration, while the remaining 21.87% could be attributed to other factors. However, there was no significant relationship observed between waterlogging duration and other growth parameters, such as plant height, percentage survival, root length and dry matter weight, indicating that these growth parameters were influenced by factors other than waterlogging duration.

The degree of association between waterlogging duration and growth parameters was determined by calculating Pearson correlation coefficients, which are presented in Table 4. The results showed that leaf chlorosis had a high positive correlation with waterlogging duration, indicating that leaf chlorosis increased as the waterlogging duration was prolonged. Therefore, leaf chlorosis can be considered a good criterion for selecting maize varieties with waterlogging tolerance traits.

Table 3 Regression analysis of effects of days of waterlogging on growth parameters

Growth Parameter	Root MSE	Mean	CV (%)	R^2	Adjusted R^2
DLC	0.32	1.56	38.61	0.78*	0.71
PS	0.00	100.00	0.00	0.00ns	-0.33
PH	7.48	29.79	25.04	0.25ns	-0.01
RL	4.14	24.09	22.71	0.57ns	0.43
DM	0.56	1.01	50.51	0.11ns	-0.18

DLC = degree of leaf chlorosis; PS = percentage survival; PH = plant height; RL = root length; DM = dry matter; RSE = mean square error; R^2 = coefficient of determination; CV = coefficient of variation; ns = non-significant; * = significant ($p < 0.05$)

Table 4 Correlation analysis between days of waterlogging and growth parameters

	Degree of leaf chlorosis	Percentage survival	Plant height	Root length	Dry matter
WD					
Coefficient	0.8839*	NA	-0.4963ns	-0.7555ns	-0.3339ns
p value	0.0466		0.3951	0.1397	0.5829

WD = waterlogging duration; NA = not applicable; ns = non-significant; * = significant ($p < 0.05$)

Furthermore, a significant positive correlation was observed between plant height and root length (Table 5) indicating that as the plant height increased, the root length also increased. This correlation between plant height and root length also had a significant positive correlation with dry matter weight, indicating that as the plant height and root length increased, the dry matter weight also increased and suggesting that dry matter weight is another good criterion for selecting maize varieties with waterlogging tolerance traits.

Other studies have indicated that plant dry matter or biomass is the primary variable in determining flooding stress tolerance (Esteban and Edwin, 2016). Therefore, lines with longer root length, taller plant height and a heavier dry matter weight, along with nodal root formation, were more tolerant to waterlogging stress at the V2 leaf stage. This finding was consistent with the performance of Hybrid 1, Hybrid 2 and BRK regarding these characteristics among the maize lines with waterlogging stress. It can be concluded that Hybrid 1, Hybrid 2 and BRK have a high tolerance to waterlogging stress.

Conclusion

The results showed that waterlogging significantly affected maize growth parameters, such as leaf chlorosis, plant height, percentage survival, root length and dry matter weight. However, the degree of association between waterlogging duration and growth parameters varied, with some parameters not being significantly related to waterlogging duration.

Leaf chlorosis was a good criterion for selecting maize waterlogging tolerance traits, as it increased with the prolongation of waterlogging duration. In addition, plant height, root length and dry matter weight were good criteria for selecting waterlogging tolerant maize, as they showed a high positive correlation with each other for waterlogged conditions.

Overall, the study identified Hybrid 1, Hybrid 2 and BRK as the most tolerant maize lines to waterlogging stress. These lines exhibited longer root length, taller plant height, heavier dry matter weight and good nodal root formation for waterlogged conditions, which are adaptive traits for plants to withstand soil waterlogging stress.

The findings of this study provided valuable information for the selection of maize varieties with higher waterlogging tolerance, which can help to improve maize production in areas prone to waterlogging stress. Further research can be carried out to investigate the mechanisms behind the adaptive traits identified in the current study and to explore the potential of other maize lines for waterlogging tolerance.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Table 5 Correlation matrix of growth parameters of maize lines for waterlogging conditions

	Degree of leaf chlorosis (DLC)	Percentage survival (PS)	Plant height (PH)	Root length (RL)	Dry matter (DM)
DLC					
Coefficient	1.0000				
p value					
PS					
Coefficient	NA	1.0000			
p value					
PH					
Coefficient	-0.1084	NA	1.0000		
p value	0.7656				
RL					
Coefficient	-0.2493	NA	0.8677**	1.0000	
p value	0.4873		0.0006		
DM					
Coefficient	-0.1310	NA	0.8676**	0.7737**	1.0000
p value	0.6408		0.0006	0.0043	

NA = not applicable; ** = highly significantly ($p < 0.01$)

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