



Effects and Recovery of Maize (*Zea mays* Linn) to Waterlogging Imposed at Early Seedling Stage

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10.18805/ag.DF-500

ABSTRACT

Background: This study aimed to determine the effects of different waterlogging duration imposed at the V2 leaf stage and assess the impact of waterlogging on maize growth during the recovery period, identify susceptible and tolerant lines and traits conferring tolerance to waterlogging at the early seedling stage.

Methods: The study was arranged in a Split-plot RCBD and replicated three times. Intercharacter and waterlogging durations to maize growth parameters were correlated to determine the degree of the linear relationship using Pearson's product moments correlation and simple linear regression analysis.

Result: The result shows that the different waterlogging durations negatively influenced the maize growth parameters regarding plant height and root length. These parameters became shorter and did not recover after waterlogging stress was removed. Therefore, plant height and root length are traits sensitive to waterlogging stress. The USM Var 10, BRK and T. Monkayo obtained a high degree of leaf greenness, heaviest shoot and root dry matter compared to other evaluated maize lines. Maize with greener leaves, taller plant height, longer root length and high total dry matter accumulation could be a good criterion in selecting a parent material in the maize waterlogging breeding program.

Key words: Corn, Recovery period, V2 leaf stage, Waterlogging tolerance, Waterlogging.

INTRODUCTION

Maize (*Zea mays* Linn.) is an essential component of agricultural food, livestock feed and many essential industrial products (Gazal *et al.*, 2017). In Asia, this crop is considered one of the most important food and feed plants that provide a source of income and energy for millions of farmers (Shiferaw *et al.*, 2011). In the Philippines, the crop is the second most productive and essential crop after rice, reaching 759,578 million tonnes in November 2020 on 245,271 million hectares of harvest area, a continuous increase in productivity since 2003 (PSA, 2020). Thus, the demand for maize is still very high and continuously increasing through the years for human and livestock consumption. However, excess soil moisture (ESM) stress caused by temporary waterlogging, heavy rains, high groundwater table, or dense soil texture can significantly affect crop productivity.

Globally, 12% of cropping areas are affected by waterlogging (Li *et al.*, 2006). In Southeast Asia alone, about 15% of the total maize-growing area is affected by floods and waterlogging (Rathore *et al.*, 1998). In the Philippines, maize is cultivated in 245,271 ha (PSA, 2020) and 500,000 ha of this is affected by waterlogging (Rathore *et al.*, 1998). Furthermore, this factor in cornfields is common, especially in low-lying areas and gives a significant yield reduction of over 40% for more than three of waterlogging, which will increase as waterlogging continues (Li *et al.*, 2011). Thus, this undesirable effect of waterlogging causes yield reduction, significant financial losses to the farmers, a reduced supply of food for people and feed as an ingredient for the livestock and poultry industry.

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How to cite this article: Esteban, J.A.C. and Baldo, N.B. (2023). Effects and Recovery of Maize (*Zea mays* Linn) to Waterlogging Imposed at Early Seedling Stage. Agricultural Science Digest. doi: 10.18805/ag.DF-500.

Submitted: 15-07-2022 **Accepted:** 12-01-2023 **Online:** 06-02-2023

So far, most studies have investigated the effects on maize growth responses during waterlogging (Esteban and Solilap, 2016; Lone and Warsi., 2009; Liu *et al.*, 2010; Bin *et al.*, 2010; Zaidi *et al.*, 2012, 2007, 2004, 2003) while plant recovery after waterlogging have been overlooked (Striker *et al.* 2011, 2012a). Striker (2012b) accurately emphasized the real estimate tolerance to waterlogging stress that plant performance should consider during flooding and recovery periods. This information must be considered to be equally important. Therefore, the physiological mechanisms for waterlogging tolerance must not only study the effects of waterlogging on maize growth but also consider the ability of the maize to recover from it. That is why the findings are

meager and the progress of the maize waterlogging research is moderately sluggish.

To achieve stable agronomic and yield; hence, steady maize production is essential to screen and evaluate different potential lines of maize subjected to temporary waterlogging for the breeding programs. Therefore, the study was conducted to (i) determine the effects of different waterlogging duration on maize lines, (ii) assess the impact of waterlogging on maize during the recovery period, (iii) identify susceptible and tolerant lines and (iv) identify traits that conferring tolerance to waterlogging at V2 leaf stage.

MATERIALS AND METHODS

The study was conducted in the Davao de Oro State College research area at Maparat, Compostela, Davao de Oro, Philippines, from January 2019 to May 2019. The experiment was arranged in the Split-plot in a Randomized Complete Block Design and replicated three times. The main plot of the study was the waterlogging duration and the subplot was the maize lines. Each treatment combination has 15 sample plants and 10 data plants.

Before seed sowing, a germination test was performed using the tissue and petri dish method to ensure the seeds had a 100% germination rate. The screening was done using the cup screening method by Zaidi *et al.* (2003) but was modified to fit the experimentation requirements.

Growth parameters were gathered during the waterlogging and on the 10th day of the recovery period. A standard data-gathering procedure of growth parameters was practiced during waterlogging and recovery period in all data plants. For the degree of leaf greenness, it was determined using a grade criterion presented in Table 1 and it was used in scoring the degree of leaf greenness using the formula:

$$\frac{DLG = 9(n) + 7(n) + 5(n) + 3(n) + 1(n)}{\text{Total number of sample (Maximum rating)}} \times 100$$

Where,

n = Number of samples and the maximum rating is 9.

The data gathered were analyzed using Analysis of Variance in the RCBD Split plot arrangement using Statistical Tool for Agricultural Research software version 2.0.1. At a 5% significance level, differences among treatment means were determined using Tukey's HSD. Intercharacter and waterlogging durations to maize growth parameters were correlated using Pearson's Product Moments Correlation. The correlation strength of correlated parameters was

described using the Rumsey (2009) scale. The degree of the linear relationship of the associated traits and waterlogging durations to maize traits was calculated using Simple Linear Regression Analysis.

RESULTS AND DISCUSSION

Effects of waterlogging duration on maize growth

The result shows that different waterlogging durations significantly affected the growth parameters (Table 2). It shows that maize can survive up to nine days of waterlogging, but a decline in percentage survival was observed on the 12 days. This result implies that maize can withstand up to nine days of waterlogging; however, 12 days can significantly decrease the percentage of survival. This catch the researcher's attention to further research on increasing the duration of waterlogging on maize to have substantial evidence on the limitation of the maize to survival.

Interestingly, the degree of leaf greenness decreases as the waterlogging duration is prolonged. This result could be a contributory factor to the survival rate. It was observed that as the leaf greenness decreases, the survival rate also decreases. Moreover, it was observed that leaf chlorosis was noticed at the six-day of waterlogging and severe leaf chlorosis was observed as the waterlogging duration was prolonged. This result is similar to the findings of Zaidi *et al.* (2004) that waterlogging can cause severe leaf chlorosis. Leaf chlorosis significantly affects the photosynthetic performance of the plant and, as explained by Zaidi *et al.* (2004) and Lizaso and Ritchie (1997) waterlogging reduced leaf chlorophyll and causes severe leaf chlorosis.

On the other hand, a significant reduction in plant height, root length, shoot and root dry weight and total dry matter were noticed when maize experienced waterlogging. These findings were supported by the findings of Zaidi *et al.* (2004) and Liu *et al.* (2010) that waterlogging can reduce plant growth. The finding of Li *et al.* (2018) is similar to the result of this study, which showed that waterlogging significantly decreased the root length at the early growth stage. This root length reduction during waterlogging is due to oxygen deficiency and phytotoxins, which reduce root growth and formation and promote root decay (Kaur *et al.*, 2018).

Effects of waterlogging duration on maize lines

A remarkable variation in growth parameters was observed on the different maize lines when experienced different waterlogging duration (Table 3). All lines show a reduction of leaf greenness due to waterlogging. The decline of leaf greenness of maize is due to leaf chlorosis induced by

Table 1: The rating scale for leaf greenness of maize as affected by waterlogging.

Rate	Reactions of the individuals to waterlogging	Mean percentage score	Description
9	Only a few leaf tips became yellow. Normal plant growth	81%-100%	Highly tolerant
7	Less than 50% of the first leaves are chlorotic	61%- 80%	Tolerant
5	The first leaves are chlorotic and the second leaves are taking off the green	41%-60%	Moderately tolerant
3	Most of the leaves became dead or taking off the green	21%-40%	Susceptible
1	Plant growth is completely inhibited. Most of the leaves died	1%-20%	Highly susceptible

Table 2: Effects of and recovery of maize to different waterlogging durations on the growth parameters.

DW	PS (%)		DLG (%)		PH (cm)		RL (cm)		SDW (g)		RDW (g)		RSR		TDM (g)	
	DW	RP	DW	RP	DW	RP	DW	RP	DW	RP	DW	RP	DW	RP	DW	RP
No W	100.00 ^a	100.00	100.00 ^a	100.0	36.11 ^a	54.3 ^a	19.84 ^a	29.62 ^a	0.81 ^a	1.12 ^a	0.26 ^a	0.31	0.34	0.26 ^{ab}	1.07 ^a	1.42 ^{ab}
3 DW	100.00 ^a	97.41	100.00 ^a	97.80	15.43 ^e	33.60 ^c	12.58 ^{ab}	16.43 ^d	0.14 ^c	0.44 ^b	0.09 ^b	0.14	0.60	0.34 ^{ab}	0.22 ^c	0.58 ^b
6 DW	100.00 ^a	100.00	97.14 ^a	100.0	21.40 ^d	41.72 ^b	11.68 ^{ab}	17.76 ^{cd}	0.26 ^b	0.74 ^{ab}	0.08 ^b	0.37	0.42	0.53 ^a	0.34 ^{bc}	1.11 ^{ab}
9 DW	100.00 ^a	100.00	84.46 ^b	100.0	25.12 ^c	46.17 ^{ab}	11.28 ^b	23.14 ^b	0.44 ^b	1.04 ^{ab}	0.11 ^b	0.26	0.40	0.26 ^b	0.55 ^b	1.30 ^{ab}
12 DW	98.02 ^b	98.02	60.62 ^c	100.00	27.65 ^b	50.20 ^{ab}	11.75 ^{ab}	20.72 ^{bc}	0.49 ^b	1.17 ^a	0.11 ^b	0.28	0.22	0.40 ^{ab}	0.61 ^b	1.45 ^a
F-Test	**	ns	**	ns	**	**	*	*	**	**	**	ns	ns	*	**	*
CV (%)	0.29	6.06	15.22	5.12	12.08	20.72	14.70	27.37	13.19	22.86	14.54	24.73	16.23	20.69	15.31	28.76

PS: Percentage survival; DLG: Degree of leaf greenish; PH: Plant height; RL: Root length; SDW: Shoot dry matter; RSR: Root and shoot ratio; RDW: Root dry weight; TDM: Total dry matter; DW: Duration of waterlogging; W; Waterlogging; RP: Recovery period; ns: Not significant; *, ** indicates statistical significance at $P \leq 0.05$ and $P \leq 0.01$, respectively. Means with the same letter in a column are not significantly different.

Table 3: Maize lines growth response during waterlogging and recovery period.

Maize lines	PS (%)		DLG (%)		PH (cm)		RL (cm)		SDW (g)		RDW (g)		RSR		TDM (g)	
	DW	RP	DW	RP	DW	RP	DW	RP	DW	RP	DW	RP	DW	RP	DW	RP
SS (Milako)	100.00	100.00	88.79	100.00	26.01 ^{ab}	43.09 ^b	15.23 ^a	21.71 ^{ab}	0.45 ^{ab}	0.93	0.18 ^a	0.28	0.44	0.30	0.63 ^{ab}	1.21
B. White	100.00	96.97	89.40	98.52	25.00 ^{abc}	45.22 ^{ab}	13.94 ^{ab}	23.33 ^a	0.41 ^{ab}	0.86	0.17 ^{ab}	0.30	0.58	0.34	0.58 ^{ab}	1.16
Katorsa	100.00	100.00	88.20	100.00	24.67 ^{abc}	46.91 ^{ab}	12.30 ^{ab}	18.19 ^b	0.38 ^b	0.78	0.12 ^{ab}	0.25	0.42	0.43	0.50 ^{ab}	1.02
T. Bohol	98.89	100.00	87.89	100.00	23.62 ^{bc}	42.93 ^b	12.98 ^{ab}	20.76 ^{ab}	0.32 ^b	0.78	0.08 ^b	0.26	0.31	0.40	0.40 ^b	1.04
SS Kuyas	97.56	100.00	86.68	100.00	21.66 ^c	43.57 ^b	13.34 ^{ab}	23.62 ^a	0.38 ^b	0.91	0.14 ^{ab}	0.24	0.45	0.27	0.52 ^{ab}	0.91
USM Var. 10	100.00	98.67	87.95	97.53	27.62 ^{ab}	49.09 ^a	13.68 ^{ab}	22.80 ^a	0.51 ^{ab}	1.02	0.12 ^{ab}	0.29	0.34	0.33	0.63 ^{ab}	1.31
B. Red	100.00	100.00	86.97	100.00	24.42 ^{bc}	43.94 ^b	12.09 ^b	19.97 ^{ab}	0.38 ^b	0.85	0.10 ^{ab}	0.24	0.29	0.31	0.48 ^{ab}	1.10
BRK	100.00	100.00	91.71	100.00	28.60 ^a	49.22 ^a	15.20 ^a	21.78 ^{ab}	0.64 ^a	0.80	0.12 ^{ab}	0.28	0.26	0.49	0.76 ^a	1.08
T. Monkayo	100.00	100.00	88.40	100.00	24.68 ^{abc}	42.90 ^b	12.08 ^b	21.66 ^{ab}	0.40 ^{ab}	1.15	0.11 ^{ab}	0.33	0.49	0.35	0.52 ^{ab}	1.48
F-Test	ns	ns	ns	ns	**	**	**	**	**	ns	*	ns	ns	ns	**	ns
CV (%)	2.28	4.45	6.47	3.52	14.26	14.26	19.63	18.24	16.80	11.77	21.80	14.66	11.44	10.14	13.63	13.63

PS: Percentage survival; DLG: Degree of leaf greenish; PH: Plant height; RL: Root length; SDW: Shoot dry matter; RSR: Root and shoot ratio; RDW: Root dry weight; TDM: Total dry matter; DW: Duration of waterlogging; W; Waterlogging; RP: Recovery period; ns: Not significant; *, ** indicates statistical significance at $P \leq 0.05$ and $P \leq 0.01$, respectively. Means with the same letter in a column are not significantly different.

waterlogging. It was observed that the yellowing of leaves would start at the younger leaf of all lines going to older leaves, from green to yellowish-green and to chlorotic leaf. However, even though a reduction of leaf greenness was observed in maize, the tolerance description of maize under waterlogging is still highly tolerant (Tables 1 and 2). It was noted that the yellowing of leaves will start on the older leaf and the leaf tips will start to yellow, followed by the leaf blade. This observation is also similar to Shin *et al.* (2016) observation that waterlogging at the early growth stage of maize, leaves turned yellow and the lower ones started senescing. This yellowing of leaves is an indication of chlorophyll reduction due to waterlogging. Kaur *et al.* (2018) reported that a reduction in chlorophyll reading was observed in the second leaf stage exposed to waterlogging. Furthermore, the decrease in leaf chlorophyll content because of the destruction of chlorophyll mediated by superoxide radicals formed under waterlogging stress may have caused lower chlorophyll meter readings in waterlogged pots (de Souza *et al.*, 2011; Wang *et al.*, 2012).

On the other hand, all lines have a 100% survival rate during waterlogging except for T. Bohol and SS Kuyas. BRK had the tallest plant height, longest root length, heaviest shoot dry matter and total dry matter during waterlogging. Interestingly, during the experimentation, there was upward growth of lateral roots of maize during the waterlogging period. This upward movement of maize's lateral roots could provide oxygen gas for the root system. Also, it was observed that there was an increased number of lateral roots during waterlogging. These upward movement roots or surface rooting and increased number of lateral roots were not fully documented during the experiment. However, these root traits will be considered for the next set-up of the experiment. This observation confirms the suggestion of Zaidi *et al.* (2007) that surface rooting might have some temporary role in coping with excess moisture stress because the visible root tips and shallow roots are placed under hypoxic rather than the anoxic condition and, therefore, might sustain partial aerobic respiration, leading to a higher chance of survival under waterlogging stress.

Moreover, adventitious root formation was also noted during the waterlogging period. It was reported that adventitious root formation indicates waterlogging tolerance in maize (Mano *et al.* (2006). Adventitious root formation was also suggested to provide an alternative for some teosinte to address soil flooding or waterlogging (Bird, 2000). Furthermore, Kaur *et al.* (2020) indicate that nodal root development is an adaptive trait to withstand soil waterlogging stress.

Maize growth response to waterlogging during the recovery period

Table 3 presents the maize growth response on the 10th day of the recovery period from waterlogging. It was observed that the leaf greenness showed an improvement in the recovery period after waterlogging was removed. This result

implies that the leaf greenness can be improved and the leaf chlorosis will be reduced after waterlogging stress was removed. It further means that leaf greenness can recover from the waterlogging stress. However, the plant height, root length, shoot dry weight, root and shoot ratio and total dry matter still shows variability in the recovery period. This result indicates that adverse effects of waterlogging on the plant height, root length, shoot dry weight, root shoot ratio and total dry matter can still be observed on the recovery period. This result further indicates that these traits cannot recover after ten days of recovery period from waterlogging stress regardless of waterlogging duration.

It was observed that during the recovery period, a remarkable increase in plant height was noted at normal conditions. This result shows that the second leaf stage that experienced waterlogging retarded plant height increment after waterlogging stress is removed imposed at the V2 leaf stage. This finding is congruent to Kaur *et al.* (2018), who emphasize that maize second leaf stage experienced waterlogging has slower growth than the nonwaterlogged treatments.

Maize lines response to waterlogging during the recovery period

Table 3 presents the maize growth response on the 10th day of the recovery period from waterlogging. The result shows an improvement in the maize leaf greenness, shoot and root dry matter, root and shoot ratio and the total dry matter. However, maize plant height and root length show variability on the 10th day of the recovery period. It means that these maize traits are sensitive to waterlogging. It implies that maize can recover to waterlogging, however, maize plant height and root length become shorter when experiencing waterlogging stress. Meanwhile, it was consistent that USM Var 10 obtained the tallest plant height and longest root length but statistically comparable to B. White and BRK.

Regression and pearson correlation analysis between waterlogging duration and growth parameters

Simple linear regression analysis was used to determine the magnitude effects of waterlogging duration on the growth parameters. Table 4 shows the impact of waterlogging duration as a predictor of the growth parameters. The results indicate that the waterlogging duration was positively and significantly contributed to the leaf chlorosis of maize. It implies that about 78.50% of the degree of greenness variation is due to waterlogging. It further means that the 21.5% differences may be due to other factors or variables other than the waterlogging duration. Moreover, no significant relationship was observed in percentage survival, plant height, root length, shoot and root dry matter, root and shoot ratio and the total dry to waterlogging duration.

The degree of association between the waterlogging duration and growth parameters is presented in Table 5. The result shows that waterlogging duration has a negative

and significant correlation to the degree of leaf greenness, implying that when waterlogging duration increases, leaf greenness decreases. Thus, leaf greenness is a waterlogging tolerance trait and a good criterion for selecting waterlogging-tolerant maize. On the other hand, survival rate, root length, plant height and shoot dry weight has a strong relationship to waterlogging duration, indicating that these traits could also be a good criterion in selecting maize tolerant to waterlogging.

Intercharacter correlation of maize traits

A Pearson correlation was calculated between the growth parameters of all maize lines during the waterlogging period. Table 6 presents the correlation analysis between the maize growth parameter and waterlogging duration. The result shows that the maize plant height was positively correlated

to leaf greenness, which indicates that greener leaf during waterlogging has a taller plant height. Meanwhile, the shoot dry weight is significantly and positively correlated to leaf greenness, plant height and root length during the waterlogging period. This result implies that maize shoot dry weight increases as the leaf greenness, plant height and root length increase during the waterlogging period.

Moreover, the maize total dry matter has shown a significant and positive correlation to plant height, root length and shoot dry weight during the waterlogging period. The result indicates that as the plant height, root length and shoot dry weight increase during the waterlogging period, total dry matter also increases.

These results imply that the greener leaf, taller plant height, longer root length and high total dry matter accumulation are maize traits that confer tolerance to

Table 4: Summary table of significantly correlated of maize growth parameters during waterlogging period.

Correlated parameters		Correlation coefficient (r)	Pr>f	Strength of correlation
Plant height	Percentage survival	0.7234	0.0276*	Strong relationship
	Degree of leaf chlorosis	0.7501	0.0199*	Strong relationship
Shoot dry weight	Degree of leaf chlorosis	0.7744	0.0143*	Strong relationship
	Plant height	0.8668	0.0025**	Strong relationship
	Root length	0.6904	0.0395*	Moderate relationship
Total dry matter	Plant height	0.8078	0.0085**	Strong relationship
	Root length	0.7927	0.0108*	Strong relationship
	Shoot dry weight	0.9560	0.0001**	Strong relationship

p<0.01 or ** - Highly significant; p<0.05 or * - Significant.

Table 5: Regression analysis of waterlogging duration on the growth parameters.

Growth parameters	Root MSE	Mean	CV (%)	R-Square	Adj R-sq
PS	0.72	99.60	0.89	0.6613 ^{ns}	0.5767
DLG	9.01	88.44	19.03	0.7850*	0.7134
PH	8.75	25.14	10.48	0.0223 ^{ns}	-0.3037
RL	2.69	13.43	16.94	0.5840 ^{ns}	0.4454
SDW	0.2284	0.4280	19.69	0.0443 ^{ns}	-0.2743
RDW	0.0682	0.1300	16.79	0.3596 ^{ns}	0.1462
RSR	0.1378	0.3960	14.88	0.2537 ^{ns}	0.0049
TDM	0.3614	0.5580	18.53	0.0816 ^{ns}	-0.2246

PS: Percentage survival; DLG: Degree of leaf chlorosis; PH: Plant height; RL: Root length; SDW: Shoot dry weight; RDW: Root dry weight; RSR: Root and shoot ratio; TDM: Total dry matter; ns: Not significant; *, ** indicates statistical significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 6: Correlation analysis between days of waterlogging to growth parameters.

Correlated parameters		Correlation Coefficient (r)	Pr>f	Strength of correlation
Water logging duration	Percentage survival	-0.7844	0.1817 ^{ns}	Strong relationship
	Degree of leaf chlorosis	-0.8860	0.0454*	Strong relationship
	Plant height	-0.1492	0.8108 ^{ns}	Strong relationship
	Root length	-0.7642	0.1325 ^{ns}	Strong relationship
	Shoot dry weight	-0.2104	0.7341 ^{ns}	Strong relationship
	Root dry weight	0.5997	0.2851 ^{ns}	Very weak relationship
	Root shoot ratio	0.5037	0.3870 ^{ns}	Moderate relationship
	Total dry matter	0.2856	0.6413 ^{ns}	Moderate relationship

p<0.01 or ** - Highly significant; p<0.05 or * - Significant.

waterlogging and could be a good criterion in selecting parent materials in the maize waterlogging breeding program.

CONCLUSION

The different waterlogging durations negatively influenced the maize growth parameters. Plant height and root length are traits sensitive to waterlogging stress. However, during the recovery period, the leaf greenness has noticeably improved. The shoot and root dry weight, root and shoot ratio and total dry matter also improved during the recovery period. On the other hand, USM Var 10, BRK and T. Monkayo obtained a high degree of leaf greenness and heaviest shoot and root dry matter, indicating that these maize lines are tolerant to waterlogging imposed at the V2 leaf stage.

ACKNOWLEDGEMENT

The researcher is grateful to Dr. Nenita B. Baldo for sharing her maize germplasm collection, CHED for the graduate scholarship program and DDOSC for the undying support.

Conflict of interest: None.

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