Tolerance level of several hybrid maize genotypes to waterlogging stress

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Abstract. Waterlogging is one of the most important constraints for maize production and productivity in many parts of the world, caused by flooding, continuous rainfall coupled with inadequate drainage or a high water table. The objective of this study was to determine the tolerance level of several hybrid maize genotypes to waterlogging stress. Screening of 27 hybrid maize genotypes was carried out in waterlogged condition and not waterlogged condition (as control) in a green house using a plastic cup of 300 ml volume in a randomized block design, 3 replications. Each genotype was planted in 5 seeds, grown by watering to V1-V2 phase which is characterized by the appearance of 1-2 complete leaves. Screening on waterlogged conditions performed on plastic-coated wooden tub. The water level during flooding is kept the same for 5 days. The results showed that the estimation of tolerance of hybrid maize genotypes to waterlogging using a flooding sensitivity index (FSI) which was calculated based on certain variables showed different FSI values for each variable. Determination of variables that have a large influence on the diversity of tolerance of maize genotypes to waterlogging can be known from the results of Principal Component Analysis. The results of the analysis show that the first principle component, the variable which has a big influence on the diversity of maize genotype tolerance to waterlogging stress is the number of roots with a proportion of diversity of 37.3%. The second principle component shows that the root length variable has a big influence with a proportion of variance of 22.52%. The cumulative proportion of the first and second major components is quite large at 59.83%. Based on the FSI root length variable, there are 3 hybrid maize genotypes that are tolerant of standing water while in the root number variable there are 13 tolerant genotypes with average fresh weight of shoot character, root number and stomata density higher than that in normal conditions.

1. Introduction
Maize crop in Indonesia is the second food crop after rice and the government has been determined to achieve maize self-sufficiency. Therefore, efforts to increase production and productivity continue to be encouraged both through quality improvement and intensification area.

The obstacle faced in maize production is the increasingly limited optimal land as a result of the conversion of agricultural land for non-agricultural purposes. Consequently the need for land for agriculture can only be met through the use of sub-optimal land. In addition there are erratic climate changes such as changes in rainfall patterns, the extreme climate of La Nina and El Nino and an increase in sea surface air temperature, which can have an impact on the agricultural sector of food
crops, especially maize. Because the maize plant is an annual crop that is sensitive to the excess and water deficiency.

In Indonesia, maize plants are planted throughout the year and sometimes face extreme climatic conditions and biotic / abiotic pressures that limit plant growth and development, and ultimately limit potential yields. According to [1], among the abiotic stresses, excessive soil moisture caused by flooding, waterlogging or high water levels, is one of the most important constraints to maize production and productivity. Waterlogging has long been identified as a major abiotic stress and the constraints given to roots have a significant influence on plant growth and development. If this event occurs at the beginning of planting, then this waterlogging can reduce seed germination and seedling development. Thus, waterlogging is an important factor affecting the growth, development and survival of plant species, not only in natural ecosystems, but also in agricultural and horticultural systems [2].

To overcome this problem, selection of hybrid maize genotypes that are tolerant of standing water is needed to be used in areas where there is often excess water. Several studies have been conducted to look for water-tolerant genotypes, including [3, 4, 5, 6, 7, 8]. These genotypes can be used to assemble waterlogging-tolerant varieties. The objective of this study was to determine the tolerance level of several hybrid maize genotypes to waterlogging stress.

2. Materials and methods

The material used were 27 maize genotypes from advanced populations plus 3 check varieties (MGOLD, DKL, and NASA29). Screening was carried out under flooding conditions and without flooding in a green house using a 300 ml volume cup/plastic with a Complete Randomized Design (CRD), 3 replications. Each genotype was planted with 5 seeds, grown by splashing water until the V1-V2 phase which is characterized by the emergence of complete leaves of 1-2 sheet. Screening in flooding conditions is carried out in a plastic coated wooden tub. Treatment with waterlogging is carried out by flowing water carefully from the bottom of the tub flooding until the water level reaches 2 cm above the pot. The height of water during flooding is maintained to remain the same for 6 days.

The stress sensitivity index calculation is done by observing the growth character at the age of 13-16 days after planting (HST). Growth characters observed: number of stomata, SPAD value, shoot height, root length, number of roots, shoot and root fresh weight. Flooding stress sensitivity index (S) is calculated following the equation [9].

\[ S = \frac{(1 - Y_p/Y)}{(1 - X_p/X)} \]

\( S \) = Sensitivity index of flooding stress
\( Y_p \) = average value of a genotype that gets flooding stress
\( Y \) = average value of a genotype that does not get a flooding stress
\( X_p \) = the average of all genotypes that get flooding stress
\( X \) = the average of all genotypes without stress

The criteria for determining the level of inundation tolerance are if the value of \( S < 0.5 \) is categorized as tolerant genotype, \( 0.5 \leq S \leq 0.1 \) category of moderately tolerant genotype, and \( S > 1.0 \) for sensitive genotype. The higher the sensitivity index (S) value, the more sensitive to inundation stress and the the higher percentage of yield reduction. To choose growth characters that have a large diversity and can classify the tolerance of maize genotypes to waterlogging stress is done by Principle Component Analysis (PCA).
3. Results and Discussion

Estimation of tolerance of hybrid maize genotypes to waterlogging using a flooding sensitivity index (FSI) calculated based on certain variables shows different FSI values for each variable, so it is difficult to determine the FSI of variables that can be used for grouping the tolerance of maize genotypes to waterlogging stress.

Based on the sensitivity index of waterlogging (Table 1) in the root length variable shows that there were 3 lines classified as tolerant (G48, G53, and G64), 13 lines classified as moderate, and 11 lines classified as sensitive. There is no consistent tolerant genotype on the variable root length and number of roots, but there is 1 genotype that is consistently sensitive namely G28.

| Table 1. Sensitivity index of some hybrid maize lines against waterlogging |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Genotype | SH | SFW | RFW | RL | NR | SPAD | SD | Average |
| G5 | 2.85 | 12.59 | 1.24 | 1.35 | 0.93 | 1.08 | 0.81 | 2.98 |
| G8 | 3.30 | 5.59 | 1.50 | 0.82 | -1.28 | 1.41 | 1.86 | 1.89 |
| G12 | 1.29 | 19.50 | 1.02 | 1.24 | -2.00 | 1.68 | 1.01 | 3.39 |
| G16 | 1.42 | 12.49 | 1.30 | 0.97 | -1.02 | 0.53 | 2.68 | 2.64 |
| G19 | 1.07 | 10.98 | 1.22 | 1.50 | -3.22 | 0.78 | 0.57 | 1.84 |
| G22 | 0.67 | 10.83 | 0.83 | 0.86 | 1.38 | 1.45 | 1.52 | 2.51 |
| G27 | 0.85 | 12.84 | 1.24 | 0.66 | -0.30 | 1.10 | 1.89 | 2.61 |
| G28 | 0.08 | 4.14 | 1.20 | 1.35 | 1.76 | 1.00 | 1.40 | 1.56 |
| G29 | 0.97 | 18.00 | 1.16 | 0.91 | 1.43 | 2.04 | 1.65 | 3.74 |
| G31 | 1.13 | 3.86 | 1.31 | 0.95 | -0.53 | 1.40 | 0.73 | 1.26 |
| G43 | 0.52 | -4.78 | 0.86 | 0.95 | 0.38 | 0.66 | 0.73 | -0.10 |
| G47 | 0.49 | 0.23 | 0.82 | 0.62 | 5.46 | 0.49 | 1.58 | 1.38 |
| G48 | -0.89 | 5.58 | 0.49 | 0.20 | 3.01 | 1.16 | 0.25 | 1.40 |
| G49 | 0.94 | -4.89 | 0.15 | 1.20 | 0.85 | 1.62 | -0.03 | -0.02 |
| G50 | 0.82 | -4.14 | 0.43 | 0.74 | 0.59 | -0.19 | 0.70 | -0.15 |
| G51 | 1.15 | -9.17 | 1.16 | 1.38 | -1.57 | 0.37 | 0.59 | -0.87 |
| G52 | 1.10 | -10.55 | 1.25 | 1.46 | -1.57 | 1.02 | 1.40 | -0.84 |
| G53 | 0.63 | -0.22 | 0.69 | 0.41 | 3.46 | 0.86 | 1.17 | 1.00 |
| G57 | -0.19 | -0.07 | 0.98 | 1.33 | 0.47 | 1.31 | 0.95 | 0.68 |
| G59 | 1.25 | -9.72 | 0.56 | 1.04 | -0.60 | 0.73 | 0.26 | -0.92 |
| G60 | 0.13 | 5.71 | -0.09 | 0.74 | 4.96 | 0.85 | 0.42 | 1.82 |
| G62 | 0.47 | 7.74 | 0.84 | 0.69 | 2.97 | 1.13 | 0.65 | 2.07 |
| G64 | 0.32 | 0.29 | 0.85 | 0.25 | 3.44 | 1.20 | 1.40 | 1.11 |
| G65 | 0.59 | 2.22 | 0.57 | 0.85 | 4.36 | 1.17 | 1.05 | 1.54 |
| G67 | 0.97 | -3.10 | 0.48 | 0.79 | 10.31 | 0.82 | 0.54 | 1.54 |
| G76 | 0.53 | -1.75 | 0.77 | 0.77 | 3.41 | 1.09 | 1.03 | 0.83 |
| G78 | 0.71 | -12.27 | 0.83 | 1.52 | -1.75 | 0.93 | 1.81 | -1.17 |
| MGOLD | 1.98 | -1.51 | 1.39 | 1.14 | -1.12 | 0.63 | 0.75 | 0.46 |
| DKL | 1.30 | 9.27 | 0.97 | 1.37 | 1.87 | 0.79 | 0.84 | 2.35 |
| NASA29 | 1.27 | 6.67 | 1.36 | 1.11 | -0.30 | 0.82 | 0.69 | 1.66 |
| Average | 0.92 | 2.88 | 0.91 | 0.98 | 1.19 | 1.10 | 1.03 | 1.27 |

Note: SH = Shoot height; SFW = Shoot fresh weight; RFW = Root fresh weight; RL = Root length; NR = Number of roots; SPAD = SPAD Value; SD = Stomata density

Determination of variables that have a large influence on the diversity of tolerance of maize genotypes to waterlogging can be known from the results of Principal Component Analysis. Principal component analysis is used to reduce the number of origin variables (p) that are correlated to q new variables that are not correlated (q <p) without greatly reducing the originator information, so that the selection of genotypes can be done by using fewer variables that can describe the diversity of a phenotype. The number of main components used is the main component which has the eigenvalue ≥ 1, because it has a great diversity contribution. The results of the analysis show that the first principle component, the variable which has a big influence on the diversity of maize genotype tolerance to
waterlogging stress is the number of roots with a proportion of diversity of 37.3%. The second principle component shows that the root length variable has a big influence on the tolerance of maize genotype tolerance to waterlogging with a proportion of variance of 22.52%. The cumulative proportion of the first and second principle components is quite large at 59.83% (Table 2).

Table 2. Value of the main components of several variables

| Variables            | PC1     | PC2     | PC3     | PC4     |
|----------------------|---------|---------|---------|---------|
| Shoots height        | -0.4326 | 0.0988  | -0.0289 | 0.7887  |
| Shoot fresh weight   | -0.218  | -0.6041 | 0.1602  | 0.2175  |
| Root fresh weight    | -0.5421 | -0.0563 | -0.2005 | -0.0319 |
| Root length          | -0.3774 | 0.4126  | 0.3612  | -0.2116 |
| Number of roots      | 0.4718  | -0.2568 | -0.1774 | 0.3332  |
| SPAD Value           | -0.113  | -0.5342 | 0.5869  | -0.2047 |
| Stomata density      | -0.3062 | -0.3172 | -0.6534 | -0.3631 |
| Proportion of Variance| 0.373  | 0.2252  | 0.1387  | 0.0963  |
| Cumulative Proportion| 0.373  | 0.5983  | 0.737   | 0.8333  |
| Eigenvalues          | 2.6113  | 1.5765  | 0.971   | 0.6739  |

The effect of waterlogging on agronomic character of shoot height, root fresh weight, root length and SPAD value of the lines tested under normal conditions is better than under waterlogged conditions (Figure 1), conversely shoot fresh weight, number of roots, and stomata density are better at flooded conditions compared to normal conditions (Figure 2).

The shoot height under normal conditions ranges from 26.12 - 42.37 cm while in the flooded condition the height of the crown ranges from 15.10 to 37.59 cm. The biggest decrease in shoot height is in the G8 strain (Figure 1a). Plant height affects the solar radiation distribution in the maize canopy. The taller plants may have greater canopy light distribution and the construction of high-yield groups [10]. Li et al. [11] concluded that waterlogging did not significantly affect plant height. In this study, plant height decreased significantly after lines received inundation at V1-V2, and [12] plant height was significantly decreased after waterlogging at V3 and V6, which was inconsistent with the results of Li et al [11].
Average root fresh weight and root length under waterlogging conditions decreased compared to normal conditions (Figure 1b and c). In the study of Zubairi et al [13], it was found that the root fresh weight and the root length of germination seed which were 12 days old, decreased after inundation. The average fresh weight of roots under flooded conditions is lower than in normal conditions. The fresh weight of the roots in waterlogging conditions ranged from 1.07 to 2.3 g while in normal conditions it ranged from 1.78 to 4.38. The reduction in root wet weight was found in the G52 strain.

The root length of the maize was decreases with the increase in the water level. In this study, maximum growth was demonstrated under normal conditions of the G78 strain and a minimum decrease was observed in G19 strains under waterlogged conditions from root length (Figure 1c). Zubairi et al [13] the analysis of variance also shows the significant result due to the reduction in the length of root. The roots has direct interaction with the water and by the increasing the level of water the root systems was destroy which ultimately results in the reduction of shoot length similar results were obtained by Savita et al [14].

SPAD values under normal conditions was vary from 27.83 to 45.30 and when under flooded conditions was vary from 23.03 to 34.13. A much lower decrease in SPAD (25.12%) was observed in the G29 strain under standing water at the seedling stage (Figure 1d). Chlorophyll levels are generally inversely proportional to the level of waterlogging stress. Waterlogging at the seedling stage produces significant results in reduced levels of chlorophyll [15]. Chlorophyll is one of the important pigments in photosynthesis so pigment reduction is an appropriate indicator in observing plant responses to stress conditions such as waterlogging stress [6]. Zaidi et al. [8] used a chlorophyll meter, which was observed that maize genotypes of more tolerant had lower chlorophyll degradation. SPAD values of maize leaves was significantly lower in plants grown in waterlogged soil by contrast in Z. nicaraguensis, it was unaffected [16]. Significant decrease in leaf chlorophyll contents was identified as one of the first stress symptoms [17, 18, 19].

![Figure 2](image_url)  
**Figure 2.** Effect of waterlogging on character (a). Wet weight of shoot, (b). Root number, (c). Stomata density

Some of the lines tested showed a higher shoot fresh weight under waterlogging conditions compared to normal conditions. There are 17 strains with higher fresh weight in flooded conditions. The range of shoot fresh weight under waterlogging conditions 1.84 - 3.19 g while under normal conditions 1.62 - 3.83 g (Figure 2a). The height of the shoot fresh weight under waterlogging condition is likely caused by the amount of water that has been absorbed by the roots to be expelled through the leaves. Hu et al. [20] showed that waterlogging in wheat significantly reduced the accumulation of dry matter in each organ and caused an increase in the proportion of stem and leaf distribution of dry matter [12]. Hu et al. [20] showed that standing water in wheat significantly
reduced the accumulation of dry matter in each organ and caused an increase in the proportion of dry matter distribution in stems and leaves. Shoot growth in maize started to slow down after 8 d of waterlogging, resulting in significantly shorter plants after 19 d of treatment compared to plants in drained soil, by contrast, *Z. nicaraguensis* kept growing similarly in both treatments and after 15 d even marginally faster [12].

In this study the average number of roots in flooded conditions was higher than in normal conditions (Figure 2b). This is due to the formation of adventitious roots to be used to take oxygen from the surface of the water. The formation of aerenchyma and adventitious roots is an indicator of the presence of adaptive mechanisms in many flood-tolerant plants. Increased root development is one of the avoidance mechanisms for waterlogging stress through adventitious root development [13]. Increase in the development of brace root in tolerant genotypes under water logged conditions was identified as one of the stress adaptive traits in maize [21], [22], [23]. In the genus *Zea*, some flooding tolerant maize lines formed adventitious roots at the soil surface during experimental flooding conditions [24]. The stomata density under waterlogged conditions on average was higher than under normal conditions (Figure 2c). In contrast to research conducted by Rahayuningsih et al, [25] that inundation effect causes stomata closure but does not affect stomata density. Similar to sugarcane stress does not affect stomata density. The density of stomata is determined by plant age and leaf area increase. Although inundation affects stomata, photosynthesis does not correlate with stomata in all plant phases.

4. Conclusions
The results of the analysis of the principle components showed that the number of roots and root length significantly affected the tolerance of maize genotypes to waterlogging stress. Based on the sensitivity index of the root length variable, there are 3 hybrid maize genotypes that are tolerant of waterlogging while in the variable number of roots there are 13 tolerant genotypes with average shoot fresh weight character, root number and stomata density higher than in normal conditions.

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