

TEKNIK KULTUR HARA UNTUK SELEKSI DINI TOLERANSI CEKAMAN Fe PADA JAGUNG DI LAHAN SULFAT MASAM RAWA PASANG SURUT

NUTRIENT CULTURE TECHNIQUES FOR EARLY SELECTION OF Fe STRESS TOLERANCE IN MAIZE GROWN ON ACID SULFATE SOIL WITHIN TIDAL SWAMPS

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ABSTRAK

Teknik kultur hara merupakan salah satu metode skrining yang digunakan untuk menyeleksi tanaman toleran terhadap cekaman Fe, yang akan diuji lebih lanjut pada pemuliaan jagung untuk lahan pasang surut sulfat masam. Penelitian ini dilaksanakan pada bulan November tahun 2021 di Rumah Kaca Balai Besar Biogen Cimanggu Bogor untuk mengkonfirmasi keterkaitan antara parameter-parameter yang diamati pada uji kultur hara pada fase bibit (*seedling stage*) dengan tingkat toleransi-adaptasi jagung di lahan pasang surut berdasarkan produksinya untuk menentukan dosis ambang cekaman Fe yang efektif. Percobaan dilakukan menggunakan rancangan acak lengkap dua factor untuk menguji sepuluh genotipe jagung dengan berbagai variasi P27, Mpop₁₈₋₀₂₋₁, MPop₂₇₋₀₈₋₁, Sukmaraga, PAC_{2245-3-1-1-2-8B-B}, C_{31-1-B-2}, B11-209-3, G₃₀₁₃₆₃₁, G₁₀₂₆₁₂₋₁, and Advanta77 pada enam tingkat cekaman Fe; yaitu 0 mg/L, 100 mg/L, 200 mg/L, 400 mg/L, 600 mg/L, and 800 mg/L dengan tiga ulangan, sehingga diperoleh 180 satuan percobaan. Hasil percobaan menunjukkan konsentrasi Fe 400 mg/L merupakan dosis ambang yang optimal untuk skrining jagung terhadap level cekaman Fe. Tingkat bronzing daun merupakan parameter yang berkorelasi tinggi dengan parameter lainnya sehingga dapat digunakan dalam deteksi tanaman yang mengalami cekaman Fe. Teknik kultur hara pada tanaman di fase bibit menggunakan cekaman Fe dapat digunakan sebagai salah satu metode seleksi cepat untuk skrining tanaman jagung pada program pemuliaan jagung untuk rawa lahan pasang surut sulfat masam.

ABSTRACT

Nutrient culture is one of the screening methods used in the seedling stage of maize to select plants that are iron-tolerant to acid-sulfate soil in a tidal swamp land breeding program. This experiment was carried out at the Greenhouse of the Centre for Biotechnology and Genetics (BB Biogen) in Cimanggu, Bogor, in November 2021 to confirm the association between parameters seen in nutrient culture tests and the tolerance-adaptability level of maize in acid sulfate soil tidal swamps based on grain production and to estimate the effective threshold dose of Fe stress. The primary factor comprised six levels of Fe stress: 0 mg/L, 100 mg/L, 200 mg/L, 400 mg/L, 600 mg/L, and 800 mg/L. The secondary factor involved 10 maize genotypes, including P27, Mpop₁₈₋₀₂₋₁, MPop₂₇₋₀₈₋₁, Sukmaraga, PAC_{2245-3-1-1-2-8B-B}, C_{31-1-B-2}, B11-209-3, G₃₀₁₃₆₃₁, G₁₀₂₆₁₂₋₁, and Advanta77. The findings from research conducted using 180 experimental units demonstrated that a concentration of 400 mg/L was the optimal choice for selecting maize plants that exhibited tolerance to iron (Fe) stress in a nutrient culture experiment. The extent of leaf bronzing was found to be highly correlated with other observational characteristics, making it a reliable indicator of the tolerance level of maize plants to Fe stress. The nutrient culture method was deemed applicable as a rapid selection technique for maize plants that exhibited tolerance to environmental stress in acid-sulfate soil tidal swamps.

1. INTRODUCTION

Iron (Fe) is one of the crucial micronutrients for plant growth and is a cofactor for several enzymes involved in photosynthesis, mitochondrial respiration, nucleic acid synthesis, nucleic acid repair, metal homeostasis, and maintenance of the structural and functional integrity of proteins and chlorophyll (Kavita and Kumar, 2020). *Zea mays* L. access Fe through a specific mechanism commonly called chelation-based Strategy II (Bityutskii *et al.*, 2022). This procedure involved the proteins in Strategy II. Mugenic acid (MA) is synthesized in root cells by nicotianamine synthase (NAS), nicotianamine aminotransferase (NAAT), and DMAS (deoxymugineic acid synthase (DMAS)). MA is secreted into the rhizosphere by TOM1 (transporter of the mugineic acid family of phytosiderophores). Subsequently, Fe(III)-MA was transported into the cell by YSL (yellow strip-like) (Li *et al.*, 2018). Despite being an essential element, Fe toxicity in plants can occur if the availability of Fe is excessive in the soil, which generally occurs in acidic soils. Iron toxicity impairs the structural integrity of plant tissues, diminishes their capacity to absorb water and nutrients, and ultimately reduces crop yields (Rajonandraina *et al.*, 2023; Turhadi *et al.*, 2019). Iron, a heavy metal found in the environment, has raised concerns owing to its potential ecological, nutritional, evolutionary, and environmental impacts (Rout and Sahoo, 2015).

The optimization of acid sulfate soil tidal swamplands for maize cultivation is an alternative area expansion to increase national production (Sulaiman *et al.*, 2019). Land use is possible because acid sulfate tidal land in Indonesia covers an area of 6,70 million hectares (Suriadikarta, 2015). However, high levels of pyrite in acid sulfate soil tidal lands are a common problem that inhibits maize growth and production on such lands (Michael, 2013). Several previous studies have observed that maize yield increases through soil modification (Annisa and Djufry, 2020). However, few studies have been conducted on the potential of modifying maize through a breeding program under stress conditions in acid-sulfate soil in tidal swamps.

The development of a maize hybrid for tidal swamplands was initiated by selecting and combining some superior line genotypes that tolerate restrictions in tidal swamplands (Suwarti *et al.*, 2021). Supporting approaches are required to boost highly adaptive maize line selection efficiency and produce well in acid-sulfate soils in tidal swamp environments. Rapid selection during the early stages of plant growth in nutrient cultures can be applied. It is possible to identify tolerant genotypes by determining the specific characteristics of the markers of the plant reaction to Fe stress. Bronzing symptoms are markers of plant responses to Fe stress and can be easily observed visually (Nugraha *et al.*, 2016a). Due to the high Fe²⁺ concentration in the leaves, which increases polyphenol oxidase enzyme activity and the number of oxidized polyphenols in the leaves, bronzing is the formation of reddish-brown spots on the leaves that begin in the shoots and move toward the basal leaves (Harahap *et al.*, 2014; Nugraha *et al.*, 2016a).

The objectives of this study were to define the ideal threshold doses of Fe stress on maize plants in selection using the nutrient culture method and to determine the relationship between tolerance and adaptability of maize plants based on the grain yields of selection in acid sulfate soil in tidal swamplands by observing early growth phase characteristics at several doses of Fe stress.

2. MATERIALS AND METHOD

2.1 Study Area

This study was performed at the Greenhouse of the Centre for Biotechnology and Genetics (BB Biogen) in Cimanggu, Bogor, in November 2021.

2.2 Procedures

2.2.1 Design of Experiment Field

The method applied in this study is based on the procedure described by Turhadi *et al.*, (2018). The chemical composition used as a nutrient solution in this experiment refers to Ohki (1987), were 0,24 mM NH_4NO_3 ; 0,03 mM $(\text{NH}_4)_2\text{SO}_4$; 0,088 mM 1,5 mM K_2SO_4 ; 0,38 mM KNO_3 ; 1,27 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 0,27 mM $\text{Mg}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 0,14 mM NaCl ; 6,6 μM H_3BO_3 ; 5,1 μM $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$; 0,61 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 0,16 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 0,1 μM $\text{Na}_2\text{Mo}_7 \cdot 7\text{H}_2\text{O}$; 45 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ -EDTA. Other materials used were distilled water, 1 M NaOH , 1 M HCl , Styrofoam, and foam. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ -EDTA was applied to determine the Fe concentration. As much as 0.2% of the medium volume, technical agar was added to the nutrient culture media as a layer to prevent Fe oxidation. The equipment consisted of seedling trays, media pots, a ruler, a vernier, a hose, an $\frac{1}{4}$ inch PVC pipe, an aerator, a pH meter, a measuring device, a stirrer, a balance, and a SPAD/chlorophyll meter. The experiment used a two-factor, completely randomized design (CRD) with three replications. The first factor was the six concentrations of Fe solution: 0, 100, 200, 400, 600, and 800 mg/L. The second factor was ten maize genotypes obtained from previous field selection in the acid sulfate soil tidal swampland of East Tanjung Jabung, Jambi Province in 2019 (Table 1).

Genetic grouping was performed by summing the adaptability and tolerance characteristic scores of acid sulfate soil in tidal swamp environmental selection. Adaptive (A) and tolerant (T) received scores of 3, whereas medium (M) and sensitive (S) received scores of 2 and 1, respectively. Grouping was arranged with the following conditions: group 1 was considered as a group that had a high level of adaptability-tolerance that has a value of $X > \bar{x} + StDev(x_1, \dots, X_n)$; group 2 is a group that has a low level of adaptability-tolerance $X < \bar{x}$; group 3 is between the two groups is the one that has a medium level of adaptability-tolerance.

The selected maize seeds were germinated for seven days without stress using styrofoam trays containing nutrient culture media. In the next step, maize seeds that had grown to become seedlings were selected based on size uniformity in each genotype and then measured using a ruler to obtain preliminary data on plant height, root length, and stem diameter characters. Selected seedlings were carefully planted in pots containing the chemical solutions for 15 days. Each pot contained 1 liter of solution, which was then covered with styrofoam perforated with a diameter of 1.5 cm. The base was wrapped with foam rolls to hold the seedlings upright, so that the seedling shoots were in free air and the roots were in the nutrient solution.

Observations were made to obtain data on plant height or shoot length, measured from the surface of the stem to the tip of the longest leaf, the number of leaves that opened completely, stem diameter measured on the main stem using a caliper at 2 cm from the base of the stem every three days (age 0, 3, 6, 9, 12, and 15 days after transplanting (DAT), and leaf bronzing symptoms every three days starting at age three days based on the leaf bronzing index scale developed by (Shimizu *et al.*, 2005). Primary root length (cm) was measured from the base of the stem to the tip of the longest root, number of primary roots, number of dry leaves, shoot fresh weight, root fresh weight, root dry weight, and root dry weight.

2.2.2 Data Analysis

Statistical analysis of collected data was performed using MS Excell for ANOVA analysis, and PBStat that could be accessed openly at <https://apps.pbstat.com/reports/> for clustering analysis. Means were compared by the Duncan's multiple range test (DMRT) at Prob $F > 5\%$. To test whether investigated parameters were correlated, Pearson's coefficient (r) was determined.

Table 1. Grouping of Maize Genotypes Based on Yield Response to High Fe Acid Sulfate Soil Tidal Swamp Stress Based on the Field Selection, East Tanjung Jabung, Jambi 2019.

Genotype	Yield response group					Code	General Field Group	Genetic Description
	Adaptability Test			Tolerance Test				
	SSC	SSC+ TF	DL	SSC+ TF	DL			
P27	A	A	A	T	T	AAA-TT	1	Commercial Hybrid
Mpop_18-02-1	S	A	A	T	T	SAA-TT	1	Line
MPOP27-08-1	A	S	M	T	T	ASM-TT	3	Line
Sukmaraga	A	M	M	T	T	AMM-TT	3	Composite Variety
PAC2245-3-1-1-2-8B-B	M	M	A	M	M	MMA-MM	3	Line
C_31-1-B-2	S	S	A	M	M	SSA-MM	2	Line
B11-209-3	S	S	M	M	M	SSM-MM	3	Line
G_3013631	S	S	S	M	S	SSS-MP	2	Line
G_102612-1	S	S	S	M	M	SSS-MM	2	Line
Advanta77	0	0	0	0	0	000-000	3	Commercial Hybrid

Note: A= adaptive; M= moderate; S= sensitive; T= tolerant; 0= not tested during field selection.

3. RESULT AND DISCUSSION

3.1 Environmental conditions

Temperature and humidity data in the greenhouse during the experiment were measured using the Elitech RC-HC tool. The average temperature during the experiment was 29.4°C, with a temperature range between 23.3°C in the early morning and 44.9°C in the afternoon. The average humidity measured using the Elitech tool was 61.1%, with the highest and lowest values being 76.4% and 29.8 %, respectively.

3.2 Symptoms of Leaf Bronzing

3.2.1 Interaction of Fe Dosage with Leaf Bronzing Symptom

The results of the data analysis in Table 2 show that the interaction between Fe stress treatment and maize genotype had a highly significant effect on leaf bronzing three DAT (days after transplanting). At 9 and 12 DAT, bronzing symptoms were only affected by the Fe stress single factor, and the genotype factor did not affect the bronzing score. At 15-day observation, every single factor of Fe stress or genotype had a significant effect on leaf bronzing symptoms. It is possible that during the early stages of growth when the plants were moved from seedling trays into agar media in pots, they underwent an adaptation process. Maize plants were stressed and exhibited symptoms of leaf senescence on their leaves. Additional research has demonstrated that transplanting techniques on maize plants can cause a reduction in agronomic growth components, especially in the leaves (Andonova, 2014; Kumar *et al.*, 2014; Patra *et al.*, 2020).

The data displayed in Table 3 show the order of bronzing index scores. The highest bronzing score was achieved at 800 mg/L Fe stress, with a bronzing index score of 6.87. This score then decreased in a sequential manner based on the extent of Fe stress impairment. At 600 mg/L, the bronzing score was 5.92; at 400 mg/L, it was 5.20; at 200 mg/L, it was 4.65; and the lowest bronzing score was obtained at 100 mg/L, with a score of 2.92. These results imply that 9 days after transplanting is the optimal time to collect data on leaf bronzing as an indicator of Fe abundance stress in maize genotypes using nutrient culture. This is because the data can be used to distinguish bronzing symptoms at each level of Fe stress. Different conditions were obtained at 15 DAT, which

showed that high doses of stress (400, 600, and 800 mg/L) had no different bronzing scores, whereas 100 mg/L, 200 mg/L, and 300 mg/L stress showed lower bronzing scores. Visual symptoms include chlorophyll oxidation and photosynthetic impairment caused by reactive oxygen species (ROS) production, which cause leaf bronzing (Nugraha *et al.*, 2016c; Rajonandraina *et al.*, 2023).

MPop27-08-1 is a genotype with ASM-TT code (adaptive SSC, sensitive SSC + TS, moderate DL-based adaptation to field test tolerance to SSC + TF, and DL based on tolerance test) in the field selection of maize lines in an acid sulfate soil tidal swamp. Based on the genotype response at 15 DAT, the MPop27-08-1 line had the lowest bronzing index value among the lines. In contrast, the genotype G_3013631, which had an SSS-MS coded line (sensitive to SSC, sensitive to SSC + TF, and sensitive to DL based on adaptation field test moderately at field tolerance test to SSC + TF trial and sensitive to DL based on tolerance test) in the selection of line in acid sulfate soil tidal land had the highest bronzing index value. The 15-DAT bronzing score data in rapid selection in nutrient culture experiments are less relevant for separating all strains based on adaptability and tolerance in tidal land. However, the bronzing score during rapid selection can distinguish strains with low adaptation-tolerance levels. In contrast, genotypes with a high level of adaptation tolerance and genotypes with a moderate level of adaptation have similar bronzing levels.

Table 2. Variance Analysis of Fe Stress Treatment on Ten Maize Genotypes on Bronzing Symptoms on Leaves.

Source of Variance	Genotype (V)	Stress Level (Fe)	VxFe	Average	CV
Degree of Freedom	9	5	45		
Bronzing Score 3 DAT	2,90 **	45,21 **	0,62 **	3,84	15,00
Bronzing Score 6 DAT	1,23 *	21,32 **	0,83 ns	4,23	18,18
Bronzing Score 9 DAT	0,82 ns	69,48 **	0,53 ns	4,80	13,55
Bronzing Score 12 DAT	1,06 ns	100,27 **	0,45 ns	4,94	19,62
Bronzing Score 15 DAT	3,73 **	30,08 **	1,36 ns	5,89	19,91

Note: DAT= days after transplanting; CV= Coefficient of variance; *Significant at $P \leq 0.05$ level of significance, **Significant at $P \leq 0.01$ level of significance; ns = Non significant.

Table 3. Bronzing Index of Maize Leaves at Each Observation Period Under Fe Stress Treatment Using the Nutrient Culture Method.

Treatment	3 DAT	6 DAT	9 DAT	12 DAT	15 DAT
<i>Fe Concentration</i>					
0 mg/L	2,33 d	2,83 d	2,92 f	2,47 e	4,63 c
100 mg/L	2,72 c	3,68 c	3,27 e	3,33 d	4,82 c
200 mg/L	3,20 b	4,18 b	4,65 d	4,53 c	5,67 b
400 mg/L	4,90 a	4,82 a	5,20 c	5,53 b	6,62 a
600 mg/L	4,90 a	5,00 a	5,92 b	6,82 a	6,68 a
800 mg/L	5,00 a	4,83 a	6,87 a	6,93 a	6,93 a
<i>Genotypes</i>					
P27	3,67 bc	4,17 ab	4,40	4,56	6,33 ab
G_102612-1	3,72 bc	4,44 ab	4,83	5,00	6,14 ab
Sukmaraga	4,06 ab	4,28 ab	4,83	5,06	6,33 ab
B11-209-3	4,17 a	4,50 a	4,94	5,11	5,61 abc
Adv77	4,00 abc	4,67 a	4,89	5,33	6,11 ab
G_3013631	4,33 a	4,28 ab	4,94	5,00	6,47 a
Mpop_18-02-1	2,97 d	3,86 b	4,53	4,61	5,71 abc
C_31-1-B-2	3,61 c	3,89 b	4,67	5,00	5,50 bc
MPop27-08-1	3,67 bc	4,08 ab	4,89	5,00	5,06 c
PAC224-5	4,22 a	4,08 ab	5,11	4,69	5,64 abc

Note: Different letters indicate that, within the same observation day, treatments are significantly different ($P < 0.05$) in terms of bronzing index.

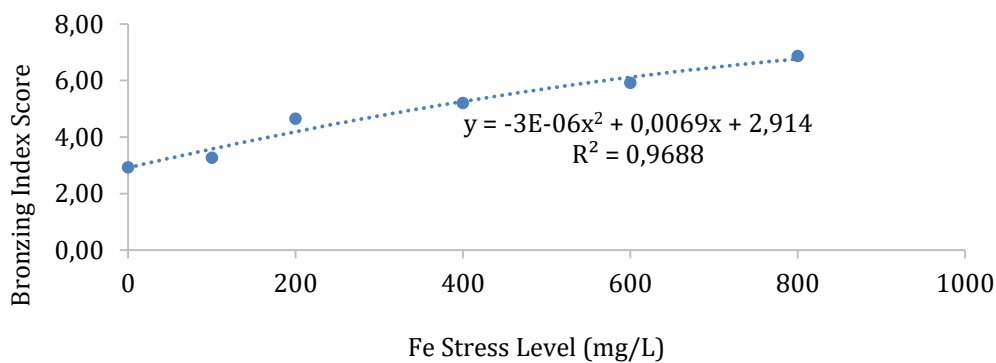


Figure 1. Relationship between Fe stress level and leaf bronzing index nine days after transplanting.

At 3 and 6 DAT, the bronzing score of maize leaves at the 400 mg/L level and higher doses showed significantly higher bronzing score values than lower doses of stress. Treatment with low-dose Fe stress at 100 mg/L, 200 mg/L, 300 mg/L, and 400 mg/L showed significantly different results at each treatment dose at 3 and 6 DAT. The higher dose of Fe application resulted in an increase in bronzing symptoms in the leaves. Based on these results, a dose of 400 mg/L was the appropriate dose for maize screening under Fe abundance stress in this study. An iron stress dose of 400 mg/L can be used as the threshold dose in the selection and screening of Fe stress-tolerant maize strains. Previous research has stated that 400 mg/L Fe dose was effectively used for screening Fe stress-tolerant on rice genotypes (Nugraha *et al.*, 2016b). However, at 9 DAT, all genotypes were perfectly separated and affected by the stress level (Table 3).

Figure 1 displays a regression graph illustrating how the dose of Fe stress increases with an increase in the leaf bronzing index. The regression coefficient value was 96.88%, which means that there was a high correlation between the Fe dose and the increase in maize leaf bronzing symptoms. Consistently increasing the Fe stress dose to 800 mg/L increased maize leaf bronzing in ex. The leaf bronzing index ranged from 1 to 7, based on Shimizu *et al.*, (2005). Previous studies have also found that an increase in the Bronzing Severity Score indicates an increase in iron content in the rice shoot (Onaga *et al.*, 2012).

The results of this experiment showed an increase in the bronzing index graph with an increase in the Fe stress dosage. The bronzing index at an Fe stress of 400 mg/L was 5.2, which is equivalent to 50% of the leaves of maize plants experiencing bronzing. This indicates that 400 mg/L can be used as a threshold dose in the selection of maize plants against Fe stress in the early growth phase using the nutrient culture method. Iron or Fe is one of the heavy metals that is essential for plants at optimal concentrations and is toxic at supra-optimal concentrations (Rai *et al.*, 2021). The threshold dosage of metals could indicate a maximum level of potential toxicity in plant tissues (Xu *et al.*, 2022). However, heavy metals are non-degradable and persist in soils, and above-threshold levels alter soil-plant systems (Rizvi *et al.*, 2022).

The appearance of maize plants in the experiment to determine the threshold dose in Figure 2 shows that the low-dose treatment of Fe0 and Fe100 generated nearly the same length of shoots and roots of maize plants. According to Marschner (1986), iron is an essential element for plants at low doses. The average Fe sufficiency limit required by plants is 100 mg/L; therefore, at the Fe stress level of 100 mg/L, maize plants have almost the same root and shoot lengths as the control.

According to plant appearance, growth suppression began to occur at the Fe200 stress level, whereas severe suppression appeared at the Fe400 stress level. Meanwhile, the Fe600 and Fe800 dose treatments resulted in almost all maize leaves becoming bronzing to dry; thus, that dosage could no longer be used to separate tolerance levels between genotypes. High doses of Fe stress impose

oxidative damage on plants, thus reducing their ability to optimally perform metabolic functions. A previous study reported a decrease in growth components due to Fe abundance stress, characterized by shorter plant shoots, shorter roots, and an increase in the bronzing index compared to plants that were not exposed to Fe stress (Turhadi *et al.*, 2018).

The interaction between genotype and Fe stress treatment had a significant effect on the number of leaves, leaf length, root length, fresh shoot weight, and medium pH. The treatment interaction was also significantly affected by plant height, fresh root weight, and media residue. In contrast, stem diameter, leaf width at 9 DAT, number of roots, dry root weight, and dry shoot weight were not affected by the interaction between treatments. The single factor of maize genotype and the single factor of Fe stress showed a significant effect on all observation parameters (Table 4). The traits affected by treatment interactions indicated that both Fe and genotype treatments affected the maize plant response simultaneously. Furthermore, the data affected by the interaction of treatment could be analysed with correlation analysis to understand how each trait interacts with the other.



Figure 2. Shoot and root appearance of maize plants under several levels of Fe abundance stress in tolerant plants.

Table 4. Variance Analysis of Maize Plant Growth in Determining the Threshold Dose of Fe Stress Using Nutrient Culture Method.

Source of Variance Degree of Freedom	Genotypes (V)		Stress Level (Fe)		V*Fe		Mean	CV
	9		5		45			
Plant height (cm)	325,42	**	207,21	**	30,39	*	28,26	15,18
Stem diameter (mm)	2,19	**	18,51	**	0,25	ns	2,58	0,50
Number of leaves	1,94	**	15,54	**	0,29	**	1,70	23,51
Leaf length at 9 DAT (cm)	225,44	**	232,10	**	24,77	**	21,50	17,58
Leaf width at 9 DAT (cm)	0,53	**	1,79	**	0,06	ns	15,63	15,63
Roots length (cm)	0,33	**	0,50	**	0,04	*	1,12	13,64 ^{t1}
Number of roots	34,76	**	62,06	**	3,49	ns	8,88	18,91 ^{t2}
Fresh roots weight (g)	0,42	**	1,21	**	0,09	*	1,23	19,50 ^{t2}
Shoot fresh weight (g)	0,48	**	3,97	**	0,06	**	1,38	13,56 ^{t2}
Dry roots weight (g)	0,02	**	0,10	**	0,01	ns	0,84	8,96 ^{t2}
Dry shoot weight (g)	0,01	**	0,03	**	0,00	ns	0,80	6,89 ^{t2}
pH media	1,12	**	60,16	**	0,76	**	4,76	13,27
Residue media (ml)	23.980,07	**	120.344,86	**	9976,21	*	780,45	10,23

Note: **significant effect on 1% level; *significant effect on 5% level; ns non-significant; ^{t1} transformed at log (1+x); ^{t2} transformed at $\sqrt{0.5 + x}$; CV=Coefficient of Variance; MS=mean square of genotypes.

3.3 Correlation of Leaf Bronzing Symptoms with Other Agronomic Traits

The relationship between the observed characteristics and bronzing appearance is presented in Table 5. The bronzing index had a significant negative correlation with plant height, number of leaves, leaf length, root length, root fresh weight, shoot fresh weight, and media pH. The negative effect of excess Fe on agronomic traits also appears in rice (Turhadi *et al.*, 2019). Furthermore, excessive iron in the soil can harm the root system and negatively impact the uptake of other minerals, such as phosphorus, zinc, and magnesium, which can cause plants to grow less, lose their yield, or even die (Li *et al.*, 2019).

In the media residue data, the bronzing index showed a positive correlation and was highly correlated with the bronzing index nine at DAT. The higher the bronzing index, the greater the volume of the media residue in the pot. Plants with a high bronzing index have a low ability to absorb water. This is caused by plant organ damage in the leaves, associated with plant metabolism, particularly the ability to transport and photosynthesize (Nikolic and Pavlovic, 2018).

Table 5. Correlation Between Observation Characters in the Interaction of Genotype Treatment and Fe Stress with Bronzing Index at 9 DAT Observation.

	PH		LN		LL		SBz		SBzHv		RL		FRW		FShW		pH
PH	1																
LN	0,47	**	1														
LL	0,88	**	0,51	**	1												
SBz	-0,32	**	-0,77	**	-0,42	**	1										
SBzHv	-0,10	ns	-0,49	**	-0,14	ns	0,51	**	1								
RL	0,67	**	0,54	**	0,67	**	-0,54	**	-0,25	**	1						
FRW	0,53	**	0,56	**	0,57	**	-0,41	**	-0,40	**	0,60	**	1				
FShW	0,63	**	0,79	**	0,65	**	-0,68	**	-0,52	**	0,76	**	0,84	**	1		
pH	0,29	**	0,66	**	0,39	**	-0,76	**	-0,54	**	0,50	**	0,43	**	0,66	**	1
MR	-0,47	**	-0,64	**	-0,53	**	0,54	**	0,37	**	-0,47	**	-0,59	**	-0,73	**	-0,47

Notes: **= highly significantly different, *= significantly different, ns= not significantly different, PH=plant height, LN=total leaf number, LL=leaf length, SBz= bronzing index 9 DAT, SbzHv= bronzing index at harvest, RL=root length, FRW=root fresh weight, FShW=shoots fresh weight, pH=media pH, MR=media residue.

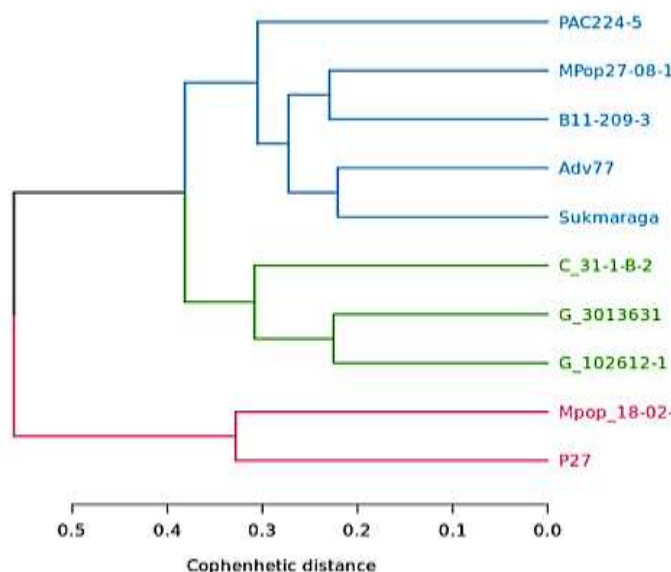


Figure 3. Dendrogram of maize genotypes grouping based on the agronomic trait in Fe excess stressing nutrient culture method.

Table 6. Comparison of Adaptation and Tolerance Characters in Tidal Land with Resistance Groups in Nutrient Culture Tests.

Genotypes	Code	Adaptability			Tolerance		Sum	Field Group	NC Group
		SSC	SSC+TFS	DL	SSC+TF	DL			
P27	AAA-TT	5	5	5	5	5	25.00	1	1
Mpop_18-02-1	SAA-TT	1	5	5	5	5	21.00	1	1
C_31-1-B-2	SSA-MM	1	1	5	3	3	13.00	2	2
G_3013631	SSS-MS	1	1	1	3	1	7.00	2	2
G_102612-1	SSS-MM	1	1	1	3	3	9.00	2	2
MPop27-08-1	ASM-TT	5	1	3	5	5	19.00	3	3
Sukmaraga	AMM-TT	1	3	3	5	5	17.00	3	3
PAC224-5#	MMA-MM	3	3	5	3	3	17.00	3	3
B11-209-3	SSM-MM	1	1	3	3	3	11.00	2	3
Adv77	000-000	3	3	3	3	3	15.00	3	3
Mean (\bar{x})							15.40		
StDev							5.56		
Mean+StDev							20.96		

Note: A=adaptive; M=moderate; S=sensitive; T=tolerant; 0=not tested in field selection; NC=Nutrient Culture. SSC=saturated soil culture; SSC + TFS = saturated soil culture + temporary flooding; DL= dry land.

The second cluster consisted of genotypes C_31-1-B-2, G_3013631, and G_102612-1, which were sensitive to the adaptation-tolerance test on acid sulfate soil tidal land using the SSC technique and medium tolerance in the SSC+TF treatment. These genotypes represent the sensitive group to acid sulfate soil tidal land stress. In rapid selection using the nutrient culture method, these genotypes are precisely grouped into one cluster and have characteristics classified as sensitive.

The third cluster contained MPop27-08-1, Sukmaraga, PAC224-5, B11-209-3, and Ad 77 genotypes. These genotypes were categorized as mediums based on nutrient culture experiments. Based on the grouping of adaptability and tolerance in acid sulfate soil tidal lands, genotype B11-209-3 is a sensitive genotype, which is different from that found in the nutrient culture experiment. Nutrient culture experiments specifically test the plant's tolerance to one stress, whereas in the field, other environmental conditions also affect crop yield. However, in general, rapid selection of maize plant tolerance to Fe stress using nutrient culture techniques can reflect conditions in the field; therefore, it can be an option to select strains that are tolerant to abiotic stresses in acid sulfate soil tidal land. A comparison between adaptability and tolerance characteristics in acid sulfate soil tidal stress, and clustering characteristics using nutrient culture selection, is shown in Table 6.

4. CONCLUSION

This experiment showed that 400 mg/L Fe was the appropriate dose for selecting maize genotypes in the nutrient culture method and was consistent with maize adaptability-tolerance in field acid sulfate soil tidal swamp results. Leaf bronzing symptoms in nutrient culture experiments have a negative and high correlation with root length, shoot fresh weight, and media pH, and have a positive correlation with the rest of the media, so it be used as the main characteristic to mark the level of tolerance to Fe stress. Rapid selection using the nutrient culture method is effective in selecting maize genotypes tolerant to Fe abundance stress in acid sulfate soil tidal swamp land.

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