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Tolerance response of varied tomato genotypes grown at excess manganese (Mn)

SITI ZAHARA[1,2,♥♥], NONO CARSONO[3,♥], RIJA SUDIRJA[3], MOCHAMAD ARIEF SOLEH[3]

[1]Agricultural Sciences, Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung Sumedang KM 21, Jatinangor 45363, Jawa Barat, Indonesia
[2] Faculty of Agriculture, Universitas Borneo Tarakan. Jl. Amal Lama No.Kel, Pantai Amal, Kec. Tarakan Tim., Kota Tarakan, Kalimantan Utara. ♥♥ email: szahara72@borneo.ac.id
[3]Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung Sumedang KM 21, Jatinangor 45363, Jawa Barat, Indonesia. Tel./Fax.: +62-813-9556-7514, ♥email: n.carsono@unpad.ac.id

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Abstract. *Zahara S, Carsono N, Sudirja R, Soleh MA. 2022. Tolerance response of varied tomato genotypes grown at excess manganese (Mn). Biodiversitas 23: 3209-3218.* High Mn concentration in acidic soil has been known as a limiting factor of sustainable tomato production. In addition, the number of tomato cultivars available to be grown under such conditions is still limited. Therefore, this study tested the Mn tolerance response among four tomato genotypes, Opal, Mutiara, Ratna, and Mirah, grown hydroponically. Nutrient solutions were added to Mn for concentrations of 0, 50, 100, and 200 ppm. The increase of Mn concentration ([Mn]) significantly decreased chlorophyll fluorescence and stomatal conductance. Shoot Tolerance Index (STI) and Root Tolerance Index (RTI) among genotypes were significant differences in response to Mn concentration. Genotype Mirah was the lowest Mn accumulation in the shoot and root compared to other genotypes. Most of the Mn absorbed by Mirah was accumulated in the roots. The tolerance ranks of the tomato genotype tested for Mn toxicity were Mirah > Opal > Ratna > Mutiara. This indicates that genetic composition plays an essential role in Mn tolerance. The tolerance response of Mirah was due to its ability to prevent Mn from being absorbed by the shoot, which led to the effect of shoot toxicity. This response might help improve tomato genotypes tolerance under high Mn soil.

Keywords: Chlorophyll fluorescence, Mn toxicity, stomatal conductance, tolerance index, tomato

INTRODUCTION

Mn toxicity often occurs in acidic soil and reduces plant yield due to low soil fertility (Alejandro et al. 2020). The increase of Mn stress in tomato plants caused a reduction in biomass production of about 17.6% and a decrease in total yield of approximately 25.7% (Kleiber and Grajek 2015). According to Gallagher (1972), Mn toxicity in tomatoes occurs when Mn concentration in the soil is more than 80 $mg.kg^{-1}$ and or in plants more than 1000 $mg.kg^{-1}$. In the previous study, treatment of Mn concentrations of more than 50 ppm in nutrient solution could decrease the total dry weight of tomatoes (Le Bot et al. 1990). In contrast, the optimal concentration of Mn in nutrient solution was reported to be between 0.3 and 0.6 $mg.dm^{-3}$ for tomatoes (Kleiber 2014).

Mn toxicity is well known for damaging photosynthetic apparatus and reducing chlorophyll content (Ribera et al. 2013; Millaleo et al. 2013), reducing stomatal conductance and CO_2 assimilation (Santos et al. 2017) as a result of reducing shoot biomass due to over uptake of O_2 and can affect root growth. According to Inostroza-Blancheteau et al. (2017), root growth is directly affected by high Mn concentration by changing metabolic activity, which tends to accumulate in the root rather than in the shoot. For example, Mn toxicity in Arabidopsis inhibits primary root elongation coincides with decreasing auxin formation in the root tip (Zhao et al. 2017). Meanwhile, Gong et al. (2019) reported that Mn toxicity represses primary root growth through secondary toxicity arising from disturbed nutrient accumulation in plants.

Plant species and genotypes have different stress tolerances, such as drought (Nawiri et al. 2017) and Mn tolerances, as was the case for Al (Rengel 2015; Pradeep et al. 2020). The determinants of tolerance were plant resistance to the progress of Mn toxicity symptoms and decreased growth compared to non-stressed plants (Tang et al. 2021). The extensive genetic variation in Mn tolerance is used to develop and improve crops in Mn tolerance (Fernando and Lynch 2015). Pradeep et al. (2020) reported that the tolerance traits of crops under high Mn were associated with greater Mn absorption and its translocation from root to shoots on chickpeas, both local cultivars, and wild genotypes. Thus, selecting plant genotypes with greater Mn excess tolerance may be an alternative approach to improving crop yields in acidic soils.

The difference between crop tolerance to Mn toxicity, especially in tomato genotypes, is not well known due to a lack of information compared to Mn tolerance in cereal crops such as rice (Rout et al. 2001; Chen et al. 2013), corn (Silva et al. 2017) and wheat (Sieprawska et al. 2016; Dimkpa et al. 2018) and legumes such as soybeans (Kuswantoro 2015) and mung beans (Rout et al. 2001). Specific studies on Mn tolerance in tomato genotypes have not been carried out. Moreover, tomato cultivars to Mn toxicity tolerance in acidic soils have not explicitly been assembled. This study aimed to determine tolerance levels among four lowland commercial tomato genotypes to Mn

toxicity. The tolerance information of these tomato genotypes tested is crucial to developing Mn tolerance which is adaptable in acidic soils in the future.

MATERIALS AND METHODS

Study area

The study was conducted in the greenhouse of the Faculty of Agriculture, Universitas Padjadjaran, Bandung, Jatinangor Campus, Indonesia, which is located at 6º 55' 20" S and 107º 46' 27" E.

Plant materials and pre-culture conditions

This study used four genotypes of lowland superior tomato seeds with different genetic backgrounds, i.e., Opal, Mutiara, Ratna, and Mirah were derived from the Vegetable Research Institute of Lembang, Bandung, Indonesia. These genotypes were used due to their adaptability in the lowlands, resistance to several diseases, and being introduced from different countries (Balitsa 2018). As many as 192 seeds of four tomato genotypes were sown in plastic trays with Rockwool seedling media for 30 days. In pre-culture, the tomato seed was irrigated using distilled water and kept grown media moist for 23 days.

The basic nutrient solution was composed of the chemical composition adopted from Horiguchi (1987), consisting of NH_4NO_3, $Ca(NO_3)_2$. KH_2PO_4, KCI, $MgSO_4$, Fe-EDTA, $MnCI_2$, $ZnS0_4$, $CuS0_4$, $(NH_4)_6Mo_7O_{24}$, and H_3BO_3, equivalent to 40 ppm N (10 ppm NH_4-N and 30 ppm NO_3-N), 20 ppm P_205, 40 ppm K_20, 40 ppm CaO, 40 ppm MgO, 3 ppm Fe, 0.3 ppm Mn, 0.01 ppm Zn, 0.01 ppm Cu, 0.01 ppm Mo, and 0.1 ppm B. Distilled water was used to dissolve the chemicals and the addition of HCl 0.1 N or NaOH 0.1 N was carried out to maintain the pH solution at around 5.5 (Horiguchi 1987). After 23 days of seedlings, irrigation was carried out with half concentration of the basic solution until 30 days old of the seedlings.

Mn treatments

After 30 days of pre-culture treatment, the seedlings of four tomato genotypes were transplanted into washed and dried burnt rice hull media and then placed in plastic pots. The pots to grow seedlings hydroponically were placed in a plastic box that contained 100% of the basic nutrient solution (Horiguchi 1987), as much as 1.5 liters. Seedlings were grown in a basic nutrient solution given by Mn treatment, i.e., 0 ppm, 50 ppm, 100 ppm, and 200 ppm for 19 days. The solutions were renewed every four days, and the solution pH was arranged to 5.5 with the adjunct of 0.1 N HCl or 0.1 N NaOH (Horiguchi 1987).

Experimental design

The experiment was arranged in a completely randomized block design with 16 treatments repeated three times, consisting of four plants in each treatment, so there were 192 plants. The treatments consisted of four tomato genotypes and four Mn concentrations of 0 ppm (control), 50 ppm, 100 ppm, and 200 ppm.

Shoot and root elongation

Determined by measuring the difference between the length of the primary shoot and root seedlings before Mn treatment (at 30th days pre-culture) and after Mn treatment (at 19th day) (Rout et al. 2001) for each treatment in each replication.

Shoot and root dry weight

Determined on the 19th day after Mn treatment. The measurement was conducted using laboratory balance after plant samples were separated to shoot and root, thus drying at 70 ºC for 24 hours.

The Mn contents in shoot and root

After the plant samples were measured dry weight of shoots and roots, the same plant samples in each replication were composited based on the treatment for measured Mn content. Mn analysis was carried out according to Horiguchi (1987). The Mn content in shoot and root was determined by Spectrophotometer UV mini 1240 Shimadzu.

Chlorophyll fluorescence (f_v/f_m)

Fluorescence initiation was determined using a Handy PEA fluorometer (Hansatech Instruments ltd.) against leaf samples on all tomato genotypes. The maximum quantum yield of photosystem II (PSII) symbolizes by f_v/f_m, where the subtracting among the maximum (f_m) and minimum (f_0) fluorescence emission in dark-adapted leaves is the variable fluorescence (f_v) (Murchie and Lawson 2013) The measurements were carried out every four days when the plant was subjected to Mn treatment starting from day four until the 16th day on the third leaf from the top of the plant. Leaf samples were exposed highlight of 1500 $\mu mol.m^{-2}.s^{-1}$ after exposed dark adaptation for 5-10 min during measurement (Soleh et al. 2018).

Stomatal conductance

The measurement was conducted using a Leaf-Porometer (Decagon Devices Inc., USA), before midday on a bright day on the third leaf from the top of the plant. Stomatal conductance was measured on the 19th day after treatment.

Tolerance Index (TI)

TI calculation was according to Rout et al. (2001):

$$TI\ (\%) = \frac{\textit{Mean root or shoot elongation in solution with Mn}}{\textit{Mean root or shoot elongation in solution with optimal Mn (control)}} \times 100$$

Data analysis

The effect of genotype and Mn concentration on the observed characters and the differences were determined by Analysis of Variance (ANOVA). Duncan's multiple range test (DMRT) was used for the post-hoc test. Normality test was done using Shapiro-Wilk, and abnormal data was corrected with Replace Missing Value. All tests were performed by using SPSS 22.0 (IBM Corp.).

RESULTS AND DISCUSSION

Shoot elongation (cm)

The interaction between genotype and Mn concentration significantly affected shoot elongation on the 19th day after treatment (Table 1).

The increase of [Mn] in the nutrient solution suppressed shoot growth of tomato genotypes, especially at the highest [Mn] of 200 ppm had more suppression on the shoot elongation (Figure1 and Figure 2).

When [Mn] was increased to 50 and 100 ppm, shoot elongation of the Mirah genotypes showed significantly different from the other genotypes. On [Mn] 200 ppm, shoot elongation of Mirah was not significantly different to Opal and Ratna but significantly different to Mutiara. Shoot elongation of Mutiara tended to be the lowest with increasing [Mn] up to 200 ppm, and Mirah tended to be the highest. However, shoot elongation of Mirah at 200 ppm Mn was not significantly different from Opal and Ratna.

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Table 1. Analysis of Variance (ANOVA) of Shoot elongation (SE), Root elongation (RE), Chlorophyll fluorescence (CF) on 4, 8, 12, 16 days, Stomatal conductance (SC), Shoot dry weight (SDW), root dry weight (RDW), Shoot Tolerance Index (STI) and Root Tolerance Index (RTI)

Source of Variance	df	SE (cm)	RE (cm)	CF 4	CF 8	CF 12	CF 16	SC (μM $H_2O.m^{-2}.s^{-1}$)	SDW (g)	RDW (g)	STI (%)	RTI (%)
Genotype (G)	3	70.595*	30.112*	0.006*	0.009*	0.023*	0.006^{ns}	316886.033*	0.149*	0.044*	1934.129*	3220.533*
Concentration(C)	3	224.624*	61.359*	0.002^{ns}	0.03*	0.048*	0.048*	2131600.905*	0.813*	0.233*	11666.911*	11444.551*
G X C	9	6.965*	11.021^{ns}	0.001^{ns}	0.002^{ns}	0.004^{ns}	0.003^{ns}	47310.709^{ns}	0.033*	0.013*	387.656*	1812.686*
Block	2	0.319	21.335	0.004	0.038	0.004	0.002	30498.960	0.021	0.006	159.279	308.809
Error	30	1.084	5.966	0.001	0.002	0.003	0.003	37161.080	0.013	0.005	46.781	768.323
Corrected Total	47											

Note: Data show mean square value: SE = Shoot elongation; RE = Root elongation; CF 4 = Chlorophyll fluorescence day-4; CF 8 = Chlorophyll fluorescence day 8; CF 12 = Chlorophyll fluorescence day 12; CF 16 = Chlorophyll fluorescence day 16; SC = Stomatal conductance; SDW = Shoot dry weight; STI = Shoot tolerance index; RTI = Root tolerance index; ns = non-significant, * = significant at $\alpha = 0.05$

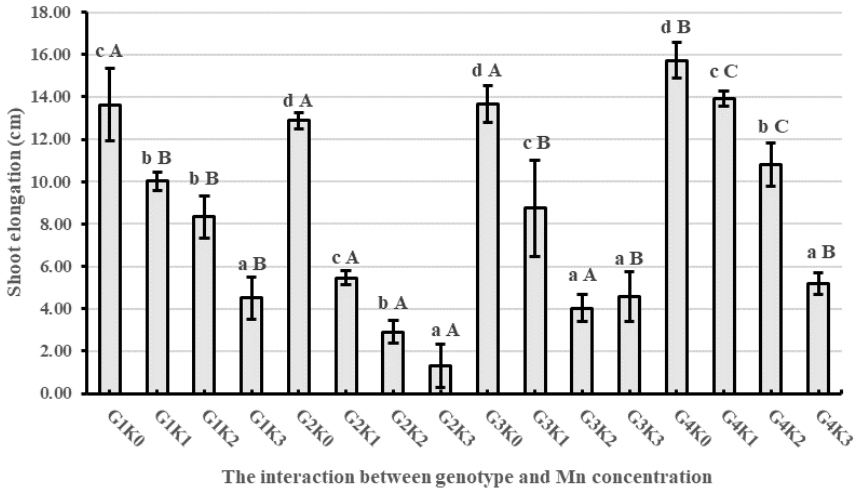

Figure 1. The interaction effect between genotype and Mn concentration on shoot elongation (cm). Note: G1 = Opal, G2 = Mutiara, G3 = Ratna, G4 = Mirah: K0 = Mn 0 ppm, K1 = Mn 50 ppm, K2 = Mn 100 ppm, K3 = Mn 200 ppm. Values with the same letters were not significantly different according to Duncan's Multiple Range Test at $\alpha = 0.05$. Lowercase letters are comparisons among Mn concentrations within the same genotype (G), and capital letters are comparisons among genotypes within the same Mn concentration (K)

Figure 2. The damaged difference among four tomato genotypes at the four Mn concentrations (ppm) (G1 = Opal; G2 = Mutiara; G3 = Ratna; G4 = Mirah)

Root elongation (cm)

The root elongation was influenced by Mn concentration in the nutrient solution (Table 1). Among four tomato genotypes, Mirah showed higher root elongation than Opal and Mutiara, even if there was no significant difference with Ratna (Figure 3). The increase of [Mn] decreased root growth and root elongation by 343% at 200 ppm [Mn] compared to [Mn] 0 ppm.

Chlorophyll fluorescence (f_v/f_m)

Mn concentration affected the chlorophyll fluorescence of tomatoes on the 8th and 12th days after Mn treatment (Table 1). There were significant differences in chlorophyll fluorescence among tomato genotypes on the 4th, 8th, and 12th days but not on the 16th (Table 2). On the fourth day, chlorophyll fluorescence of Ratna to Opal, Mutiara, and Mirah showed significant difference but no significant difference among Opal, Mutiara, and Mirah. Mutiara had the lowest chlorophyll fluorescence values among the four genotypes compared to others on the 8th and 12th days after Mn treatment on the seedling. The decrease in chlorophyll fluorescence began to occur when [Mn] in the solution increased to 100 ppm. The lowest chlorophyll fluorescence of seedlings occurred when [Mn] was increased to 200 ppm on the 8th, 12th, and 16th days (Table 2).

Stomatal conductance (mmol $H_2O.m^{-2}.s^{-1}$)

Mn concentration affected the stomatal conductance of tomatoes (Table 1), but the stomatal conductance of Opal, Mutiara, Ratna, and Mirah was not significantly different (Figure 4). In addition, [Mn] of 0, 50, and 100 ppm also showed no significantly different stomatal conductance, but the stomatal conductance decreased significantly compared to Mn 0 ppm when Mn increased to 200 ppm.

Shoot and root dry weight (g)

The increase of Mn concentration in the nutrient solution decreased the dry weight of shoots (Figure 5).

Table 2. The differences in chlorophyll fluorescence on various tomato genotypes and Mn concentrations (f_v/f_m)

Treatment	Chlorophyll fluorescence (f_v/f_m)			
	day-4	day-8	day-12	day-16
Opal	0.669 a	0.693 ab	0.684 b	0.655 ns
Mutiara	0.667 a	0.661 a	0.632 a	0.655 ns
Ratna	0.712 b	0.715 b	0.737 c	0.693 ns
Mirah	0.669 a	0.724 b	0.698 bc	0.693 ns
Mn 0 ppm	0.695 ns	0.733 c	0.737 c	0.734 c
Mn 50 ppm	0.685 ns	0.741 c	0.736 c	0.699 bc
Mn 100 ppm	0.671 ns	0.689 b	0.672 b	0.676 b
Mn 200 ppm	0.666 ns	0.631 a	0.605 a	0.586 a

Note: The numbers followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test at $\alpha = 0.05$, ns = No significant.

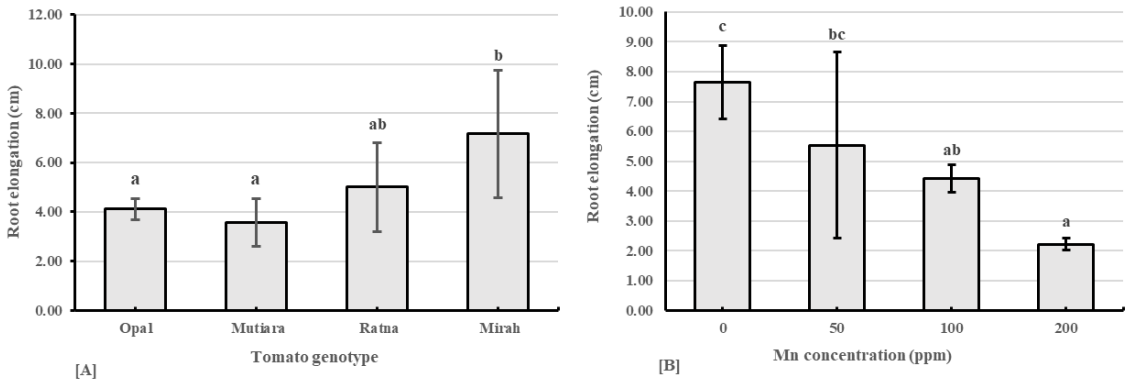

Figure 3. The difference in root elongation (cm) on various tomato genotypes [A] and Mn concentration (ppm) [B]. Note: Values with the same letters were not significantly different according to Duncan's Multiple Range Test at $\alpha = 0.05$

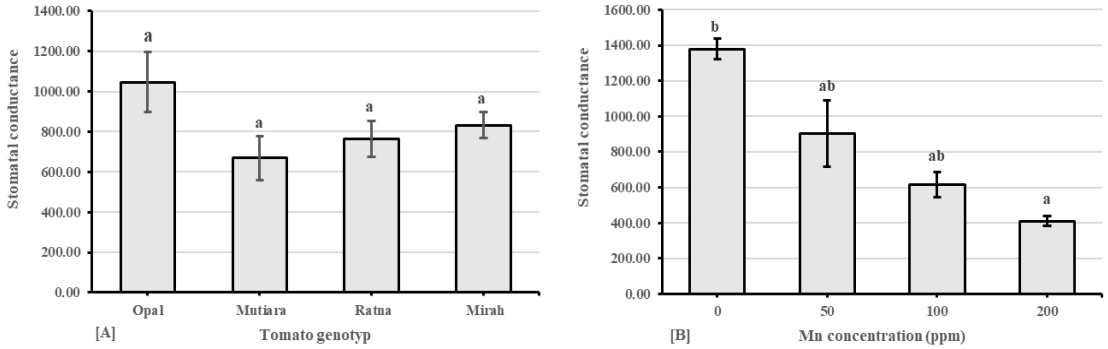

Figure 4. The differences in stomatal conductance (mmol H_2O.m.s) of four tomato genotypes [A] and four Mn concentrations (ppm) [B]. Note: Values with the same letters were not significantly different according to Duncan's Multiple Range Test at $\alpha = 0.05$

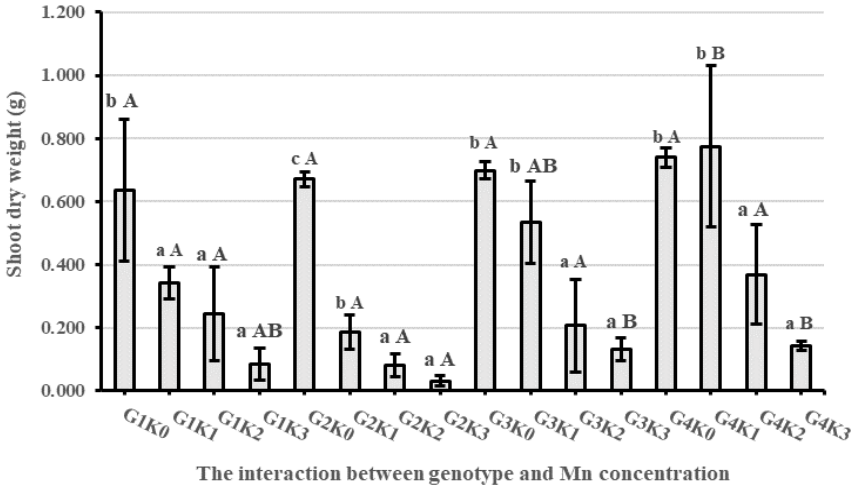

Figure 5. The interaction between genotype and Mn concentration on the shoot dry weight (g). Note: G1 = Opal, G2 = Mutiara, G3 = Ratna, G4 = Mirah: K0 = Mn 0 ppm, K1 = Mn 50 ppm, K2 = Mn 100 ppm, K3 = Mn 200 ppm. Values with the same letters were not significantly different according to Duncan's Multiple Range Test at $\alpha = 0.05$. Lowercase letters are comparisons among Mn concentrations within the same genotype (G), and capital letters are comparisons among genotypes within the same Mn concentration (K)

The decrease in shoot dry weight of Opal and Mutiara was beginning to occur at the [Mn] of 50 ppm while Ratna and Mirah were begun at the [Mn] of 100 ppm. The shoot dry weight of Mirah was significantly greater than Mutiara but not significantly different from Opal and Ratna when the nutrient solution contained Mn 200 ppm.

The root dry weight was also influenced by the interaction between genotype and Mn concentration, as shown in Table 1. The root dry weight of Opal, Mutiara, and Ratna seedlings began to decrease when Mn increased to 50 ppm, while Mirah on Mn 100 ppm (Figure 6). In addition, the root dry weight of Opal and Mutiara was very low compared to Ratna and Mirah when the Mn concentration increased to 200 ppm.

Shoot Tolerance Index (STI) and Root Tolerance Index (RTI) (%)

The interaction between genotype and Mn concentration affected the shoot and root tolerance index after 19 days of seedlings (Table 1). The shoot tolerance index for all genotypes decreased by increasing Mn concentration. The lowest index was recognized at the [Mn] of 200 ppm (Figure 7). In addition, Mutiara is the lowest tolerance genotype among the four genotypes at [Mn] of 50, 100, and 200 ppm.

It also happened to the root tolerance index of Opal, Mutiara, and Ratna seedlings. However, when Mn increased to 50 and 100 ppm, the root tolerance index of Mirah was not significantly different from those that did not experience stress (Mn 0 ppm), and a decrease occurred on Mn 200 ppm (Figure 8).

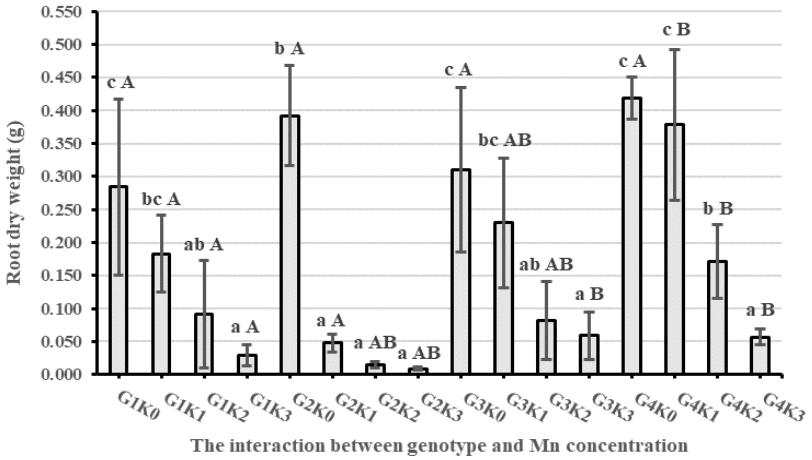

Figure 6. The interaction effect between genotype and Mn concentration on the root dry weight (g). Note: G1 = Opal, G2 = Mutiara, G3 = Ratna, G4 = Mirah: K0 = Mn 0 ppm, K1 = Mn 50 ppm, K2 = Mn 100 ppm, K3 = Mn 200 ppm. Values with the same letters were not significantly different according to Duncan's Multiple Range Test at α = 0.05. Lowercase letters are comparisons among Mn concentrations within the same genotype (G), and capital letters are comparisons among genotypes within the same Mn concentration (K)

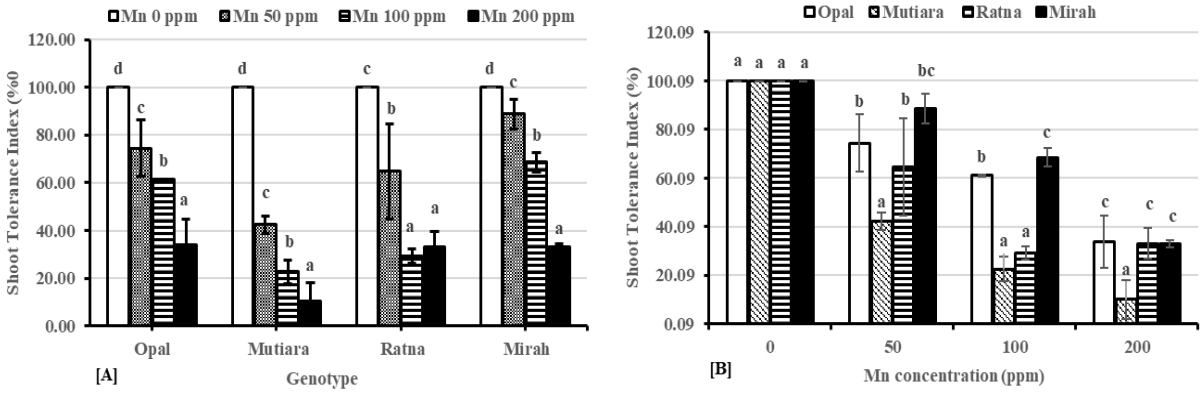

Figure 7. The interaction effect between genotype and Mn concentration on Shoot Tolerance Index (STI) (%). Note: Values with the same letters were not significantly different according to Duncan's Multiple Range Test at α = 0.05. [A] is a comparison among Mn concentrations within the same genotype, and [B] is a comparison among genotypes within the same Mn concentration

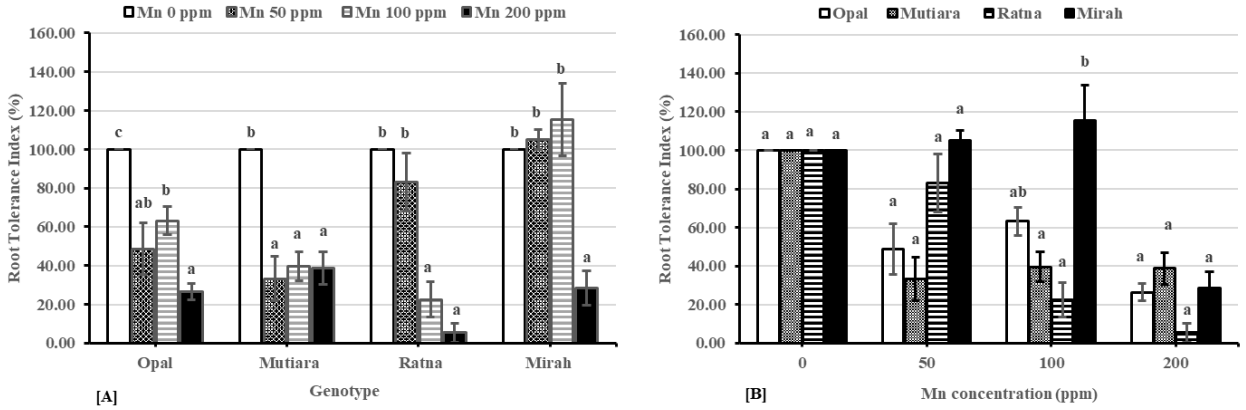

Figure 8. The interaction effect between genotype and Mn concentration on Root Tolerance Index (STI) (%). Note: Values with the same letters were not significantly different according to Duncan's Multiple Range Test at α = 0.05. [A] is a comparison among Mn concentrations within the same genotype, and [B] is a comparison among genotypes within the same Mn concentration

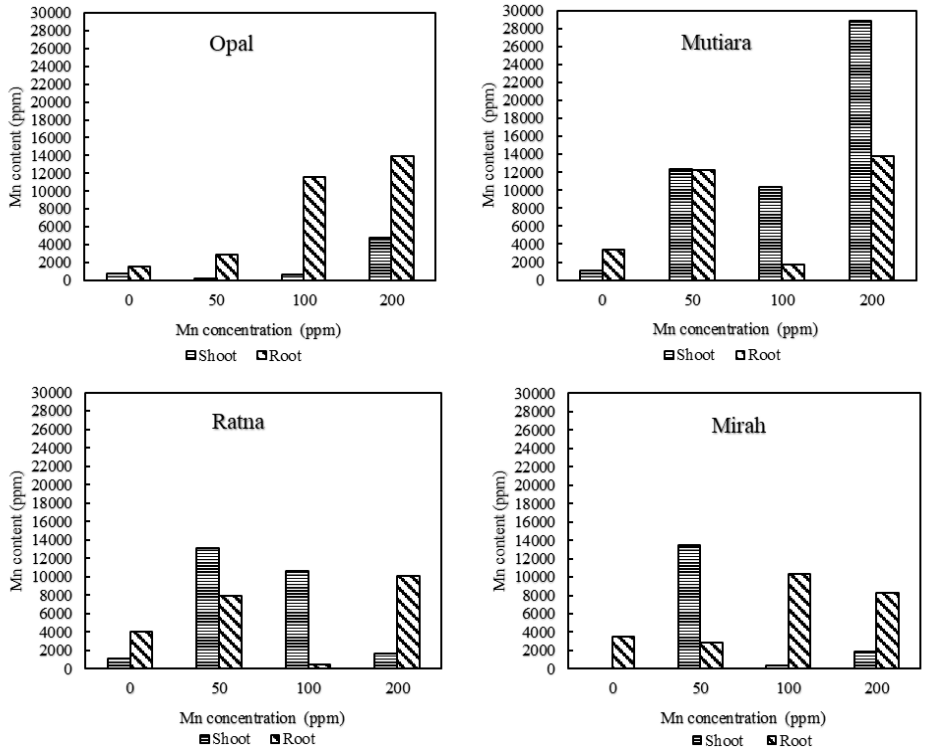

Figure 9. The Mn contents in shoot and root of four tomato genotypes (Opal, Mutiara, Ratna, and Mirah) at four [Mn] in nutrient solutions

The Mn contents in shoots and roots (ppm)

The Mn accumulation in the shoots and roots of four tomato genotypes at the four levels [Mn] in nutrient solution can be seen in Figure 9.

There are differences in Mn accumulation in the root and shoot among the four tomato genotypes. Mirah accumulated more Mn in the roots, while Mutiara in the shoots (Figure 9). Increasing [Mn] to 200 ppm raises the accumulation of Mn in plant tissue (shoots and roots). In addition, the amount of Mn accumulated in the shoots and roots of Opal, Mutiara, Ratna, and Mirah also showed a difference. At four [Mn] (0, 50, 100, and 200 ppm), Mutiara accumulated more Mn in its shoots than Opal in the roots. Meanwhile, the Mn accumulation absorbed by Ratna was more in the shoot when 50 and 100 ppm Mn concentrations, but on [Mn] was 0 and 200 ppm, more Mn was accumulated in the roots. Mirah accumulated more Mn in the roots at the Mn level of 0, 100, and 200 ppm, but at the Mn level of 50 ppm, more Mn accumulated in the shoot. In addition, at [Mn] 0 ppm, there was no Mn accumulation in the Mirah's shoots (Figure 9).

Discussion

Tomato genotypes of Opal, Mutiara, Ratna, and Mirah showed a difference in tolerance response at four levels of [Mn] treatments. This will can be seen by the damage tomato seedling tested. Chlorosis and browning occurred in the shoot of seedlings coinciding with the increase of [Mn], the highest was at 200 ppm. According to González-Villagra et al. (2021), the Mn toxicity in plants is complex, and its toxicity involves many physiological and biochemical mechanisms and a variety of genes. Meanwhile, it has been indicated that genetics control Mn tolerance (Rout et al. 2001). Tolerance traits require many characters, and no specific genotype has all of them (Pradeep et al. 2020). These cause the difference in growth, and physiological responses showed the tolerance differences among Opal, Mutiara, Ratna, and Mirah to 4 levels of Mn concentration tested, such as shoot and root elongation and chlorophyll fluorescence response. According to Li et al. (2019), the diverse expressions of Mn toxicity showed different tolerance abilities between cultivars and plant species.

According to Zhao et al. (2017), leaves (shoots) and roots of plants are the main targets of toxicity of Mn. Inhibition of shoot and root growth began at a concentration of Mn 50 ppm and continued to get worse with increasing Mn concentration. Furthermore, the research results by Zhao et al. (2017) also showed that the toxicity of Mn inhibited primary root elongation of Arabidopsis because meristematic cell division decreased due to decreased IAA biosynthesis, so that are reducing auxin levels at the root tips.

Chlorophyll fluorescence is one of the indicators to determine the impact of the environment on the growth and development of plants. Chlorophyll fluorescence has been used to assess the PSII (fv/fm) function of photosynthesis (Guidi et al. 2019) on deficiency conditions and stress of Mn toxicity (Messant et al. 2022; Millaleo et al. 2013) in several plant species.

Inline, as reported by Alejandro et al. (2020), the chlorophyll fluorescence (fv/fm) of olive plants decreased under Mn stress conditions, and the decrease was possibly due to damage to leaf structure caused by the Mn-oxide accumulation or the starch accumulation in chloroplasts. The increase in Mn concentration affected chlorophyll fluorescence on the 8th, 12th, and 16th days after treatment. The lowest chlorophyll fluorescence values were found in Mutiara, whereas Opal, Ratna, and Mirah had chlorophyll fluorescence values that were not significantly different on the 8th and 12th days. Meanwhile, the chlorophyll fluorescence decreased significantly on Mn 200 ppm. The decrease was due to high Mn (200 ppm) damaging the leaf structure (shoots) on the four tomato genotypes seedlings that were chlorosis and the leaves browning. Damage to this leaf structure affects the photosynthetic activity of the leaves, especially the function of PSII, which will reduce the production of dry weight (shoots and roots) of tomato seedlings.

The increase in Mn in the nutrient solution causes a decrease in stomatal conductance, indicating that stomata will close once Mn toxicity increases. In line with Santos et al. (2017) research, the value of the stomatal conductance of soybean that grows on soil with Mn treatment decreased with increasing concentration of Mn in the soil. Santos et al. (2017) also reported decreased stomatal conductance and finally decreased shoot biomass on soybean plants exposed to excess Mn. The same as what we found in this study, when the stomatal conductance of tomato seedlings decreased, which in turn decreased the dry weight of shoot seedlings.

Increasing the Mn concentration reduced the dry weight of shoots and roots. In line with the research results by Kleiber and Grajek (2015), which showed that Mn stress reduced the biomass production of Alboney F1 cultivar tomatoes by 17.6%. The research results by Wang et al. (2015) also showed that Mn caused a decrease in the shoot and root dry weight of rice. In addition, Akinci et al. (2010), from the results of their research, reported that an increase in Mn from 100-200 $mg.L^{-1}$ limited the growth and absorption of micro and macronutrients by tomato plant parts in the seedling phase. The dry weight of shoots and roots of Mutiara from this study significantly has started to decrease at the Mn level of 50 ppm, unlike the other three genotypes, indicating that Mutiara was more sensitive (low tolerance) to increasing Mn concentrations in the nutrient solution.

The tolerance index (TI) is the ratio between treatment and control data to characterize the tolerance of individual populations to metals (Chen et al. 2013). In addition, Awasthi et al. (2017) also added that the tolerance index of root (RTI) is one of the most important markers for screening metal tolerance genotypes and varieties. The results showed variations in the RTI and STI of the four tomato genotypes with increasing Mn concentration.

The difference in tolerance among tomato genotypes to Mn toxicity is connected with Mn accumulation in their tissues. According to Socha and Guerinot (2014), many plant species have accumulated sufficient amounts of Mn in the root systems or the shoots while planted in sufficient Mn conditions, and Mn will be mobilized to leaves to meet Mn nutritional needs if Mn deficiency conditions occur. Among the four tomato genotypes tested, the Mn content in the shoots and roots of Mirah seedlings was the lowest, and the Mn content was greater in the root tissue even though at Mn level 50 ppm retention (accumulation) of absorbed Mn more in the shoots. On the other hand, the shoot and root Mn content of the Mutiara was greater than that of other genotypes, and more Mn was absorbed in the shoots. It shows that the Mn uptake rate of Mirah is lower than other genotypes, and retention of Mn, which was absorbed, is greater in the roots. These also caused the shoot and root dry weight of Mutiara genotypes to be the lowest among Opal, Ratna, and Mirah. According to Takagi et al. (2021), photosynthesis is reduced, and thus growth decreases because of the high content of Mn in leaves.

Several plant species with a large tolerance of high Mn are caused by the low Mn uptake levels and Mn retention in the roots (Tsunemitsu et al. 2017). These two things are thought to be the mechanisms for tolerance of Mirah in dealing with the excess Mn or the Mn toxicity. Several studies have also shown that very high tolerance is found in

chickpeas (compared to cowpeas and soybeans) (Blamey et al. 2017) and *Arabis paniculata* (compared to *Arabidopsis thaliana*) (Tang 2021) caused by the restricted absorption of Mn. Paco et al. (2020) reported that higher sensitivity of the tropical legume species 'Medicago' compared to sub clover (*Trifolium subterraneum* L.) was associated with higher rates of Mn uptake and less retention of Mn on the roots. Meanwhile, the exceptionally high tolerance of maize to Mn (compared to peanuts) was related to reducing Mn translocation from roots and stems to leaves (Benac 1976). Yamaji et al. (2013) also stated that tolerance to Mn was associated among other things with the limited Mn translocation to the shoots.

Furthermore, Singh et al. (2015) added that heavy metal retention in the roots is attributed to the formation of metal complexes in the roots. On the roots, the formation of metal complexes (soluble or insoluble) can reduce the potential for Mn toxicity because it can change the form of Mn to be less toxic (Yan et al. 2020). An internal mechanism of genotype Mirah likely plays an essential role in dealing with excess Mn.

The results of this study can be concluded that Mn tolerance was derived from the genotype traits of tomatoes. Tolerance ranks of four tomato genotypes under excess Mn toxicity were Mirah > Opal > Ratna > Mutiara, respectively. These show that genetic composition, Mn uptake, and Mn translocations from roots to shoots of genotype play an essential role in Mn toxicity tolerance of tomato genotypes. High tolerance in Mirah might be due to its reduced Mn uptake and Mn retention into its roots.

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