INTRODUCTION

More than three percent of the entire land area in the world are salt affected and thus decreased crop production worldwide (FAO, 2019). The saturation extract (ECe) of the root zone of a saline soil has an electrical conductivity (EC) more than 4 dS/m at 25 °C and has an exchangeable sodium of 15 % (Shrivastava & Kumar, 2015). Most plants, including major agricultural crops are sensitive to salt stress, while some other plants have better tolerance level to the stress. Among cereals, foxtail millet (Setaria italica L. Beauv.) shows comparable tolerance to salinity (Kafi, Zamani, & Ghoraishi, 2009). This plant is also known for its tolerance to drought (Yu et al., 2018) and low nutrient condition (Nadeem et al., 2018). Not only having remarkable tolerance to abiotic stresses, foxtail millet is also valued for its nutritious grain (Bandyopadhyay, Muthamilarasan, & Prasad, 2017). The nutritional value of foxtail millet increases the importance of this crop not only as carbohydrate source, but also as functional food. Foxtail millet has a low glycemic index (Jali, Kamatar, Jali, Hiremath, & Naik, 2012), but high in dietary fiber and protein (Amadou, Gounga, & Le, 2013), contains antioxidants (Sharma, Saxena, & Riar, 2018; Suma & Urooj, 2012), it has been reported to be a potential anti-colon cancer (Shan et al., 2015) and a potential high-blood pressure medication (Chen et al., 2017). Despite the emerging potency of foxtail millet, the utilization of this cereal as food crop is still very limited in Indonesia. Developing foxtail millet variety with better tolerance to abiotic stresses, particularly salinity, should be the target of foxtail millet breeding program.

Salinity causes plant growth reduction due to osmotic stress and ionic toxicity. The osmotic

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Different Root Anatomical Changes in Salt-tolerant and Salt-sensitive Foxtail Millet Genotypes
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ABSTRACT

Foxtail millet is relatively tolerant to salinity stress and thus can be grown in salinity affected areas. This study was conducted to identify anatomical changes in the roots of foxtail millet genotypes with different tolerance level to salt stress. Four foxtail millet genotypes, namely ICERI-5 and ICERI-6 (salt-tolerant) and ICERI-4 and ICERI-10 (salt-sensitive), were grown hydroponically for 1 week prior to 60 and 120 mM salt stress treatments. Root anatomical changes were observed on the fifth day after treatments. The results showed that salt stress significantly induced some anatomical changes in the roots of foxtail millet, i.e. increased epidermis and cortex thickness, increased root diameter, and increased number of root hairs. The increase in epidermis thickness, root diameter and number of root hairs due to the salt application were more pronounced in the sensitive genotypes. Number of protoxylem in the tolerant genotypes significantly increased due to salt stress, however salinity significantly decreased the number of protoxylem among the sensitive genotypes. The different anatomical changes under salt stress between the tolerant- and sensitive genotypes indicated that some anatomical attributes of the roots might determine the salt tolerance level of foxtail millet.
pressure of the soil solution increases as the salt ion concentration increases and induces osmotic stress in the first phase of the stress, while ion toxicity takes place later after the toxic ion accumulated in photosynthetic tissues (Hanin, Ebel, Ngom, Laplaze, & Masmoudi, 2016). Plant evolves variety of strategies to cope with salinity (Munns & Gilliham, 2015). Plant root is the first organ being exposed to salinity, thus it will respond to the stress earliest. Salinity induced-reduction in root cell elongation due to nutrition and water uptakes inhibition in the root is general symptom caused by salt stress (Munns & Tester, 2008). Previous studies suggested that different roots from the same plant may respond differently to salt stress, as lateral root growth has been reported to be more suppressed by salinity compared to that of primary root in Arabidopsis thaliana (Ding & De Smet, 2013; Duan et al., 2013). A study in maize, as the representative of monocots plant, showed that the primary root was less affected by salinity stress than either the crown root or the seminal root (Zhang et al., 2015).

Root performance in acquiring water and nutrients is determined by the anatomy of the root, such as the size and number of xylem vessels, root cortex width, root hairs number, and suberin formation in the root (Acosta-Motos et al., 2017). Roots anatomy of many species have been reported to change by salinity, including orange (Mohammad, Shiraishi, & Ono, 1999), wheat (Akram, Akhtar, Javed, Wahid, & Rasul, 2002), radish (Çavuşğolu, Kiliç, & Kabar, 2008), lentil (Panuccio, Logoteta, De Lorenzo, & Muscolo, 2011), and finger millet (Krishnamurthy et al., 2014). The changes in root anatomy under salinity might determine the salinity tolerance level of the plant. Despite of the better relative salinity-tolerance level of foxtail millet compared to other major grain-crops (Goron & Raizada, 2015), foxtail millet genotypes varied in their tolerance level to salinity (Ardie, Khumaida, Nur, & Fauziah, 2015; Kafi, Zamani, & Ghoraishi, 2009). Two foxtail millet genotypes, ICERI-5 and ICERI-6, were reported to have better salinity tolerance compared to the other two genotypes (ICERI-4 and ICERI-10) (Ardie, Khumaida, Nur, & Fauziah, 2015). Widyawaran, Khumaida, Kitashiba, Nishio, & Ardie (2018) also reported that the salinity-tolerant genotypes, ICERI-5 and ICERI-6, were also possessed tolerance to drought. The two tolerant genotypes were clustered in one cluster by a diversity assessment using RAPD markers (Ardie, Khumaida, Fauziah, & Yudiansyah, 2017). These tolerant and sensitive genotypes previously identified would serve as good models to understand the different changes in salt-induced root anatomy between salt-tolerant and -sensitive genotypes, which should deepen the understanding of salt-tolerance mechanism of foxtail millet. This study was conducted to observe salinity-induced root anatomy change in foxtail millet genotypes with different salinity tolerance level. The results should give an insight of root anatomical traits that are important in salinity stress-responses in foxtail millet.

MATERIALS AND METHODS

This study was conducted from July to August 2015 in the greenhouse of Cikabayan Experimental Field (240 m asl, 6°33'05.7"S 106°42'50.3"E), Bogor, West Java, Indonesia. A completely randomized design with two factors and five replications was used as statistical arrangement. Foxtail millet genotype consisted of four (4) genotypes from Indonesian Cereals Research Institute (ICERI) was the first factor. The four foxtail millet genotypes were choosen according the study of Ardie, Khumaida, Nur, & Fauziah (2015), namely ICERI-5 and ICERI-6 (tolerant genotypes) and ICERI-4 and ICERI-10 (sensitive genotypes). The second factor was NaCl concentration in the culture solution consisted of 0, 60 (6-7 dS/m), and 120 (9-10 dS/m) mM NaCl. Each replication was a 2 L pot containing 5 seedlings. Seeds of plants were germinated in a planting tray containing rice charcoal husk and compost (1:1 v/v) mixture as planting medium for 14 days. Seedlings (14 days-old) were then transferred into 2 L pots containing nutrient solution in the greenhouse. The roots of each seedling were washed by tap water and each seedlings’ base were wrapped carefully using foam. Seedlings were floated in the nutrient culture solution using styrofoam. Pots were covered with black plastic sheet to prevent light penetration to the rooting area, and each pot was aerated using an aerator. The nutrient solution contained macro- and micro-nutrients as described by Ohki (1987) with slight modification. The nutrient solutions were renewed at 7 days after transplant with nutrient solution containing various concentrations of NaCl as described before. The pH and EC of the nutrient culture were monitored every 3 days using portable pH-meter AD-110 and portable TDS meter AD-310. The addition of 1 M HCl or 1 N NaOH was done to adjust the pH of nutrient solution to 6.8 ± 0.05.
The average temperature in the greenhouse was 31 ºC, while the average relative humidity was 59.7%. Roots anatomical observation was conducted at 5 days after NaCl treatment on minimum three seedlings per replication. Fresh material was sectioned by hand with a razor blade at ± 3 mm behind the root tip. The observed variables included epidermis thickness, cortex thickness, stele diameter, root diameter, metaxylem number and diameter, protoxylem number, and root hair number. Roots’ fresh sections were directly observed and micrographed under a Olympus microscope BX51 with a camera DP25 (DP2-BSW).

RESULTS AND DISCUSSION

Changes in anatomical traits of plant roots have been widely considered as important adaptation determinant under salinity (Rewald, Shelef, Ephrath, & Rachmilevitch, 2012). In this study, the first most observable change after NaCl stress application was the swollen root tip (Fig. 1). The swollen root tip was more significantly observed under 120 mM NaCl treatment, compared to those under 0 and 60 mM NaCl. In accordance with the swollen root tip, an increase in NaCl concentration also increased root tip diameter (Table 1). Root tip diameter increase in foxtail millet was caused by the increase in epidermis and cortex thickness as the NaCl concentration increase. The correlation between root tip diameter and epidermis thickness was significant \((r = 0.98**)\), as well as between root tip and cortex thickness \((r = 0.98**)\). Hajibagheri, Yeo, & Flowers (1985) reported that an increase in root tip diameter under salinity was also observed in the halophytic plant, *Suaeda maritima* (L.) Dum. The increase in root tip diameter in *Suaeda maritima* (L.) Dum. was caused by the increase in cortex thickness due to the increase in the volume of cortical cells. Further observation revealed that the development of vacuole was responsible to the cell size increase in the root tip under salt stress. Koyro (1997) also reported cell size increase due to vacuole development under salt stress in the root of sorghum. Different results were reported in Rhodes grass (Céccoli, Ramos, Ortega, Acosta, & Perreta, 2011), wheat (Akram, Akhtar, Javed, Wahid, & Rasul, 2002), and maize (Farhana, Rashid, & Karmoker, 2014). The cortex thickness or cortical area in the root tip of these three species decreased as the NaCl concentration increases.

<table>
<thead>
<tr>
<th>Foxtail Millet Genotype</th>
<th>ICERI-5</th>
<th>ICERI-6</th>
<th>ICERI-4</th>
<th>ICERI-10</th>
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<tbody>
<tr>
<td>0 mM</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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<tr>
<td>60 mM</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>120 mM</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
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</table>

Fig. 1. Swollen root tip of four foxtail millet genotypes subjected to 5-day-long of NaCl treatments
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Root hairs play an important role in nutrient uptake and it is likely that the toxic effect of Na⁺ is partly due to the impairment of nutrient acquisition. The detrimental effect of salt stress on root hair was reported by Wang et al. (2008) in Arabidopsis thaliana as salt stress decreased its root hair length and density. The inhibition of root hair growth and development was caused specifically by Na⁺ ion toxicity and not by osmotic stress, thus it was considered as an adaptive mechanism to avoid excessive Na⁺ uptake. However, Tanaka et al. (2014) further reported that the root-hairless mutant of A. thaliana, NR23, showed impaired salt tolerance due to lower capacity in water and potassium (K⁺) absorption. This study found that salinity enhanced the number of root hairs in all foxtail millet genotypes tested (Table 1, Fig. 2). It indicates that foxtail millet does not avoid excessive Na⁺ uptake by limiting root hairs growth and development, but might be by tight regulation of Na⁺ and K⁺ absorption. Islam M. et al. (2011) reported that Na⁺ concentration increased significantly in the root, stem and leaf tissues of foxtail millet under salt stress compared to the control condition, while K⁺ concentration remained unchanged. Once Na⁺ ions enter the roots, it needs to be compartmentalized in the vacuole. Foxtail millet seems to let the Na⁺ ions entering the root and compartmentalized them in the vacuole, as shown by the increase of cortical cell thickness (Table 1) and larger cortical cell size (Fig. 2) under salt stress.

Xylem network plays a crucial role in maintaining water and nutrient flow from the roots to the other parts of the plant. Water and nutrient absorbed by the roots moves radially from small protoxylem vessels to larger metaxylem vessels (Kim, Park, & Hwang, 2014). In this study, stele diameter, number of metaxylem and width of metaxylem of foxtail millet genotypes were not significantly affected by NaCl at the concentration applied (Table 1). Salinity effects on xylem attributes in the roots were reported to be varied, depending on the plant species. Metaxylem vessels number and diameter in the roots of Kikuyu (Pennisetum clandestinum) seedlings increased when they were grown in salinized Hoagland solution (Muscolo, Sidari, Santonoceto, & De Santis, 2004). In contrast the area of metaxylem in the root of wheat genotypes decreased significantly as the NaCl concentration increased (Akram, Akhtar, Javed, Wahid, & Rasul, 2002).

Table 1. Root anatomical traits of four foxtail millet genotypes subjected to 5-day-long of NaCl treatments

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tolerant Genotype</th>
<th>Sensitive Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET (µm)</td>
<td>17.9±1.4† 19.3±2.1 22.2±3.1 17.6±0.6 19.3±0.4 20.9±1.1</td>
<td>16.6±1.1 21.7±2.2 26.2±0.2 16.8±0.5 21.7±0.5 29.7±1.7</td>
</tr>
<tr>
<td>CT (µm)</td>
<td>70.9±5.2 114.6±12.9 122.8±29.9 71.2±4.5 107.7±11.8 119.6±5.7</td>
<td>102±20.1 125.7±20.6 68.9±7.4 90.0±6.9 139.4±25.1</td>
</tr>
<tr>
<td>SD (µm)</td>
<td>158.3±3.3 145.9±35.5 158.1±33.9 159.6±11.7 129.9±11.9 147.8±4.9</td>
<td>133.2±7.7 143.8±13.6 158.6±7.7 132.1±14.3 118.2±12.2 135.0±26.7</td>
</tr>
<tr>
<td>RD (µm)</td>
<td>332.9±33.9 411.5±60.8 426.1±96.3 320.2±16.0 373.3±26.6 431.1±17.6</td>
<td>275.6±14.0 388.1±76.1 448.7±38.8 278.1±24.5 331.7±25.9 478.3±64.7</td>
</tr>
<tr>
<td>MD (µm)</td>
<td>36.2±3.7 39.9±11.1 35.4±4.6 37.6±7.5 33.5±3.1 28.8±1.7</td>
<td>7.7±1.5 11.0±1.0 10.3±0.6 4.3±0.6 9.3±0.6 8.0±3.5</td>
</tr>
<tr>
<td>MX</td>
<td>2.3±1.1 2.3±1.1 3.3±1.1 2.7±0.6 2.3±1.1 2.7±1.1</td>
<td>1.3±0.6 15.3±5.0 21.3±2.3 1.0±0.0 13.0±2.6 19.3±1.1</td>
</tr>
<tr>
<td>PX</td>
<td>3.6±3.7 35.9±11.1 35.4±4.6 37.6±7.5 33.5±3.1 28.8±1.7</td>
<td>7.7±1.5 11.0±1.0 10.3±0.6 4.3±0.6 9.3±0.6 8.0±3.5</td>
</tr>
<tr>
<td>RH</td>
<td>332.9±33.9 411.5±60.8 426.1±96.3 320.2±16.0 373.3±26.6 431.1±17.6</td>
<td>4.7±2.9 25.4±3.6 38.3±3.2 1.7±0.6 25.3±6.1 42.3±2.1</td>
</tr>
</tbody>
</table>

Remarks: ET = epidermis thickness; CT = cortex thickness; SD = stele diameter; RD = root diameter; MX = number of metaxylem; MD = metaxylem diameter; PX = number of protoxylem; RH = number of root hair. † = Data are means ± standard errors from five replications.
Comparison in the changes of anatomical traits between foxtail millet genotypes contrasting in salinity tolerance levels might partly explain the salt tolerance mechanism of foxtail millet. The increase in epidermis thickness and the number of root hairs due to the salt application were more pronounced in the sensitive genotypes compared to the tolerant genotypes (Fig. 3). The epidermis thickness of the sensitive genotypes, ICERI-4 and ICERI-10, increased up to 57 % and 76 % under 120 mM NaCl, respectively. Meanwhile, the epidermis thickness of the tolerant genotypes, ICERI-5 and ICERI-6, only slightly increased up to 26 % and 16 % under 120 mM NaCl, respectively (Fig. 3A). Younis et al. (2013) conducted hydroponics experiment to study the root anatomical changes of Alternanthera bettzickiana under salt stress and found that the changes of epidermis thickness depend on cultivars and NaCl concentration applied. The epidermis thickness of the less-salt tolerant A. bettzickiana ‘Aurea’ increased as the NaCl concentration increased from 0 to 150 mM NaCl, while it further decreased when the NaCl concentration increased to 200 mM. In contrast, the epidermis thickness of the salt tolerant A. bettzickiana ‘Green’ decreased gradually as the NaCl concentration increased from 0 to 200 mM. The study of Younis et al. (2013) and the results indicated that the increase of root epidermis thickness of salt sensitive genotypes under salt stress were more pronounced than the tolerant genotypes.

Tip-growing extensions of epidermal cells formed root hairs (Dolan & Costa, 2001). Similar to the epidermis thickness, the number of root hair also significantly increased in the sensitive foxtail millet genotypes. The number of root hair of the sensitive genotypes, ICERI-4 and ICERI-10, increased up to 7.2 % and 24 % under 120 mM NaCl, respectively. Meanwhile, the number of root hair of the tolerant genotypes, ICERI-5 and ICERI-6, only slightly increased up to 1.5 % and 2.8 % under 120 mM NaCl, respectively (Fig. 3B).
Furthermore, while salt stress induced a significant increase in the number of protoxylem in the tolerant genotypes, it significantly decreased the number of protoxylem in the sensitive genotypes (Fig. 3C). The number of protoxylem of the tolerant genotypes, ICERI-5 and ICERI-6, increased up to 34% and 84% under 120 mM NaCl, respectively. Meanwhile, the number of protoxylem of the sensitive genotypes, ICERI-4 and ICERI-10, decreased up to 47% and 48% under 120 mM NaCl, respectively. The observation in this study was conducted in the seedling stage (28 days after planting), thus the vascular tissues observed were still in their early development. Protoxylem are the first cells to develop during plant development and they are mature before the surrounding organs elongates (Esau, 1977), thus the decrease in the number of protoxylem in the sensitive genotypes might lead to disturbance in the root-to-shoot water and beneficial nutrient transports of foxtail millet seedlings. Furthermore, protoxylem development seems to be tightly regulated by salinity. Hameed et al. (2013) reported that protoxylem area in the roots of *Cynodon dactylon* increased by salinity, but the Salt Range (high saline area) population showed greater response to salinity than its counterpart from the Faisalabad region (non-saline area).

Plants can use several strategies to cope with salinity. However, since plant root is the first organ exposed to salinity, special attention need to be addressed to this plant organ. Epidermis thickness, root diameter, number of root hairs and the number of protoxylem have shown to be anatomical traits that respond differently between salt-tolerant and salt-sensitive foxtail millet genotypes under salinity. Deeper study on how these anatomical traits contributed to the salinity tolerance of the plant will be an interesting field to do research on.

**CONCLUSION AND SUGGESTION**

The research data clearly showed that epidermis thickness, root diameter, number of root hairs and the number of protoxylem were differently affected by salinity in the tolerant- and sensitive-genotypes of foxtail millet. These root anatomical traits are suggested to be the focuses on plant physiology studies revealing the salt tolerance mechanisms in foxtail millet.

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**REFERENCES**


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