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Incompatibility Selected Dwarf Rootstock and Scion of *Citrus* sp. regard to Abiotic Stress Tolerant

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ABSTRACT

This study aims to determine the response's incompatibility of selected dwarf citrus rootstock after treated by abiotic stresses such as drought, waterlogging, and acidity on a scion. The results of this study was selected seedlings for sub-optimal lands in Indonesia with dwarf growth characteristic and compatible with the grafted-scion. The preliminary study has shown that there were 3 (three) selected rootstock accessions with dwarf characteristics, namely Citromello (Cit), Volkameriana (Volk), and Cleopatra Mandarin (CM). The seeds of these accessions were treated with 8% Polyethylene Glycol (PEG), 150% FC; 9 mM Al_2SO_4 at eight months after planting. Each rootstock was grafted with Pontianak orange (*C. nobilis var. microcarpa*). The results showed that Citromello (Cit) and Volkameriana (Volk) are incompatible rootstock and dwarf potential seedling. However, Cleopatra Mandarin has a compatibility with the grafted scion and is tolerant of abiotic stress treatments. The effect of abiotic treatment on Citromello resulted in 15% higher root length higher than other accessions. Furthermore, this variety has 40% dry weight and 25% lateral root numbers, respectively, by Al_2SO_4 and PEG. Meanwhile, Volkameriana had the 40% higher number of leaves.

INTRODUCTION

Citrus sp. is a perenial plant that is vegetatively propagated using grafting method. The demand for citrus fruits gradually increased by 3.73–7.85% during 2016-2020 from fresh fruits to processed fruits in industrial scale (Pusat Data dan Sistem Informasi Pertanian, 2016). These conditions has made the production area are extended to marginal land. Currently, the centre production area were located at East Java, West Kalimantan, North Sumatra, Bali, and South Kalimantan.

The higher plant population was needed to increase the fruit production per area. These condition indicated that dwarf plant performance was preferred to reduce the planting distance. The dwarf plant has a shorter plant height for about 1.5 m and narrow canopy diameter for 1.5-2 m with more productive branches. The dwarf feature could be manipulated therough agronomic techniques like wrinkled-trees: pruning, root distortion and branch bending, as also commonly practiced in bonsai plants (Shukla, Sharma, Ramteke, Kashyap, & Kurrey, 2016).

Moreover, it is necessary to investigate that dwarf characteristics are also genetically inherited. In this case, these characteristics are also associated with other advanced features according to the existing environmental conditions, such as faster nutrient absorption, resistance to environmental stresses and resistance to pests and diseases (Bilova, Ryabova, & Anisimova, 2016). Thus, dwarf planting material is expected to be used to overcome problems in dry and saline land.

Water stress and salinity conditions can be done by modifying the PEG application (Widoretno, 2011). This is a step towards obtaining dwarf planting material. Drought modification using PEG on apple and orange rootstock plantlets can regulate tissue osmosis, is non-toxic and can reduce water

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potential in plants (Ashari, Nurcahyani, Qudus, & Zulkifli, 2018; Maslukah, Yulianti, Roviq, & Maghfoer, 2019). Another environmental condition which had inhibited plant growth and development is acidic soil with low pH <5.6, which potentially increases metals accumulation such as Al, Fe, and Mn and causes the decline of some macronutrients, particularly N, P, and S. The results research by Liao, Yang, Lu, Ye, & Chen (2015) show that citrus plants contain Aluminum in soil repressed the content of chlorophyll hence low absorption of light energy. In contrast, high aluminum accumulation in citrus grafting can reduce the dry weight of roots, stems, and leaves.

In the same conditions, aluminum content in soil can affect soil microorganisms activity, as depicted in an unhealthy rhizosphere (Bhuyan et al., 2019; Siecińska & Nosalewicz, 2016). Waterlogging can lower soil pH, thereby increasing micronutrients in plants, which affects lipid peroxidation metabolism and protein denaturation (Bhuyan et al., 2019). Ren, Zhang, Dong, Liu, & Zhao (2016) stated that waterlogging indicates a decline in physiological reaction to photosynthesis, including lower LAI, chlorophyll content, and the destruction of chloroplast, which repressed and changed the morphology of leaves such as shapes changed from long and oval to elliptical or circular. Based on the modified soil treatment, the seeds have dwarf characteristics and are tolerant to abiotic stress. The scion grafted on dwarf seed has the potential to obtain accession candidates for cultivating citrus on marginal land.

This research aims to determine the response's incompatibility of selected dwarf citrus rootstock after treated by abiotic stresses such as drought, waterlogging, and acidity on a scion. Therefore, this research is expected to obtain a positive contribution to increasing national citrus production with dwarf performance and tolerance to abiotic stress.

MATERIALS AND METHODS

This study was carried out in Punten Experimental greenhouse garden in Batu, Indonesia from September to November 2021. This garden covers 2.7 ha of land at 950 m asl. It was started with the previous pre-research by sowing eight rootstock accession, i.e., Japansche Citron (JC), Rough Lemon (RL), Kanci, Citromello (Cit), Carizzo (CC), Volkameriana (Volk), Troyer, and Cleopatra Mandarin (CM) for five months. The selection was carried out by showing dwarf phenotypes with the parameters of slower seedling growth, short internode distances, smaller leaves, and narrower canopy diameter.

The rootstock accession with dwarfism is Citromello (Cit), Volkameriana (Volk), and Cleopatra Mandarin (CM). Hardening was carried out for three months on the three accession. They were then given abiotic stress treatment, i.e., control, 8% of PEG; 3.5% of NaCl, 150% FC Waterlogging (WL); 9 mM of Al_2SO_4. This research was conducted for two months.

The tools used are Olympus microscopes, microtomes, preparation tools, stationery writing, and field equipment. The used materials are rootstock accession of Citromello (Cit), Volkameriana (Volk), and Cleopatra Mandarin (CM), grafting plastic, and a bud of Pontianak citrus (*C. nobilis var. microcarpa*).

The stage of rootstock-scion grafting: (1) The measurement bud distance is 15 cm from the base of the stem (2) make a horizontal incision of 1.5 cm, (2) The bud is inserted into the incision and tied with a transparent plastic rope, (4) After four weeks, the plastic rope is opened, and shoot observation.

This research used a randomized group design with a single factor being rootstock accession that had been stressed and grafted with Pontianak citrus (*C. nobilis var. microcarpa*) and repeated five times. With five units, the material required was 5 x 3 x 3 = 45 rootstock seedlings. Observations were carried out once a week for two months. Tukey post hoc test was used with a confidence level of 95%.

RESULTS AND DISCUSSION

This advanced study of abiotic stress was modified by PEG 8%, FC Waterlogging 150%, and 9 mM Al_2SO_4. The results present morphological and physiological character changes due to abiotic stress treatment. Moreover, regarding those treatments, some information on seedling incompatibility as rootstocks selected dwarf with the Pontianak citrus as a scion (*C. nobilis var. microcarpa*) and their effect. The results of this study are expected to obtain information and technology of rootstock propagation that has dwarf and tolerant under abiotic stress, such as drought, waterlogging, and acidic soils.

Success Proportion

The percentage of vigor seedlings is a preference for the success of grafting on rootstocks with scions as cultivated crop production. The initial growth phase is indicated by the successful union of buds attached to the selected rootstock incision. Scion induces vegetative shoot emergence and seedling development. The desired rootstock requirements are diameter, age, root strength, and resistance to disease. Indeed, it must be tolerant to biotic and abiotic stress. The results show the percentage of vigor seedlings in Cleopatra Mandarin under treatment Al_2SO_4 and waterlogging (WL), which were 75-77.3% and 75-82.5%, respectively, lower than the control presented in Fig. 1.

Citromello and Volkameriana had the lowest percentage of seedling vigor under drought stress (PEG) compared to other treatments. It is known that PEG is a chemical agent as osmotic potential regulates similar conditions in drought. Furthermore, PEG with different molecular weights, e.g., 6000, can inhibit root growth in plants. Hence root has less responsive to water penetration (Jung et al., 2015). In that case, the root system indicates plant tolerance to drought. Rough lemon rootstock has deep roots that can adapt to dry land conditions (Cimen & Yesiloglu, 2016). This study shows that deep root allows plants to tolerate drought.

Numbers of Leaf and Shoot Length

The number of leaves in Volk and CM significantly differs from Cit at about five leaves under PEG and WL. While those accessions have the same number of leaves, around eight under Al_2SO_4, all the treatments were lower than the control instead of Cit at around 9 (Fig. 2). Length of shoot Cit, Volk, and CM have significantly different lower than control at about 2 mm under WL and Al_2SO_4, while the lowest length of shoot reached by PEG treatment. All treatments affected by abiotic stress present lower on a shoot than control, only shown by CM (Fig. 3).

Schmitt, Watanabe, & Jansen (2016) stated that under Al, different supply levels affect organs and tissue in *S. paniculata,* which affects the development of bark tissue and leaves. Moreover, Al induces some morphological changes such as repressed on root and shoots system, chlorosis of leaves, and stunted growth. Neogy, Datta, Roy, & Mukherji (2002) reported different concentrations of $Al_2(SO_4)^3$ inhibit both shoot and root length, leaf area, and fresh and dry weight. The influence of Al can reduce the number of leaves and shoot dry weight per plant by around 22% and 30%. In this case, that photosynthetic capacity declined along with the reduction of the number of leaves and chlorophyll content in the unit leaf area (Robin et al., 2021).

By contrast, some plants did not show any symptoms of Al toxicity though they grow in acid soils and have amounts of Al (Huang, Yuan, Wang, Tan, & Niu, 2017). For instance, buckwheat leaves can contain up to 10 g/kg Al after 12 weeks (Shen, Chen, & Ma, 2006).

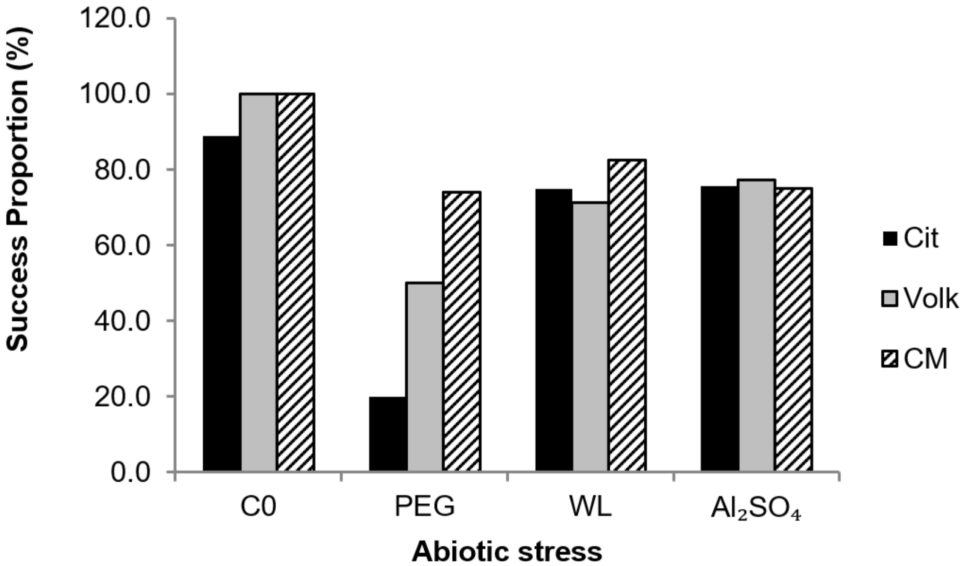

Fig. 1. The percentage of bud life grafting in Pontianak citrus (*C. nobilis var. microcarpa*) grafted on rootstocks (Citromello (Cit), Volkameriana (Volk), and Cleopatra Mandarin (CM))

Root Dry Weight

Plant roots uptake water and nutrients from the soil, which is important for growth and development. Environmental factors such as insufficient water, waterlogging, and soil acidity can change plant morphological and anatomical characteristics, particularly the length of root, the number of lateral roots, and the dry weight of roots. It is shown that the dry weight of rootstock is lower than the control (Fig. 4).

Under Al_2SO_4, the dry weight of roots on Cit and CM had higher than 41% compared to Volk. However, their dry weight was lower under waterlogging stress. By contrast, dry weight on Volk had a higher value at about 16% under waterlogging, followed by PEG and Al_2SO_4 treatments (Fig. 4).

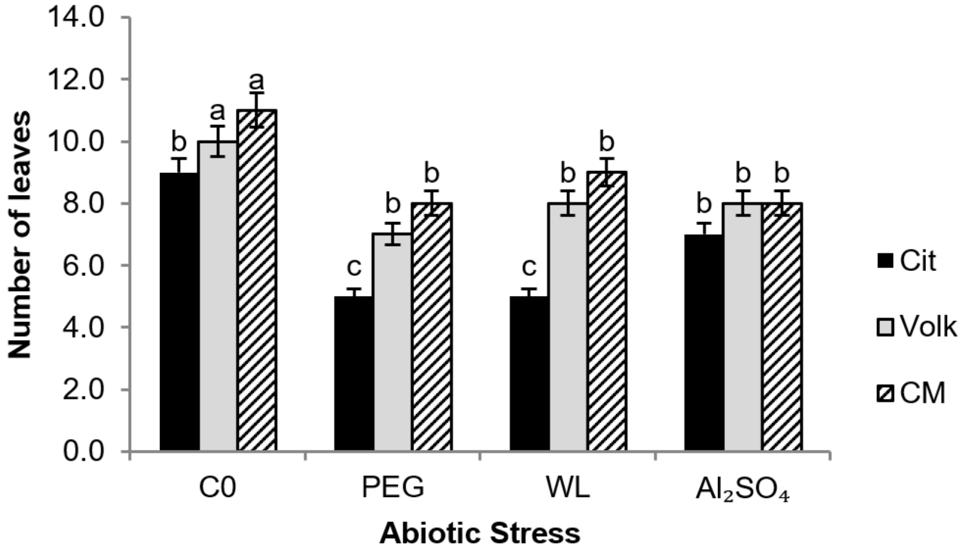

Fig. 2. The number of leaves of Pontianak citrus (*C. nobilis var. microcarpa*) grafted on rootstocks (Citromello (Cit), Volkameriana (Volk) and Cleopatra Mandarin (CM))

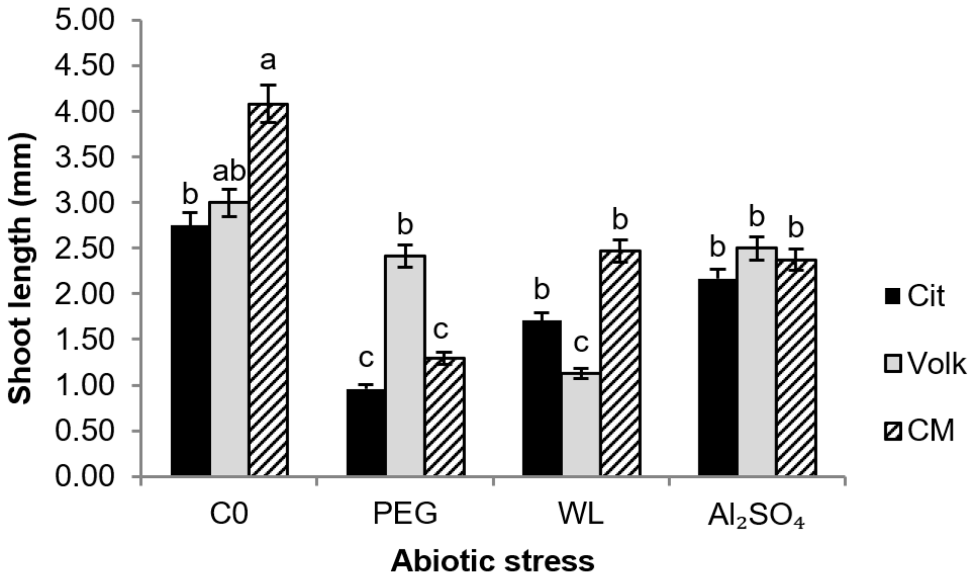

Fig. 3. The shoot length of the Pontianak citrus (*C. nobilis var. microcarpa*) grafted on rootstock (Citromello (Cit), Volkameriana (Volk) and Cleopatra Mandarin (CM))

The experiment results by Huang, Yuan, Wang, Tan, & Niu (2017) show that a supply Al concentration of 4.0 mM cause repressed dry weight of roots and lateral roots of the oil tea plant. Meanwhile, in drought conditions, a modification of 5% PEG on rootstock/scion combination pepper cutting caused a decrease in the dry weight of roots and leaves (Lopez-Serrano et al. 2019). The dry root weight and length decrease under waterlogging are assumed to inhibit roots from penetrating in oxic soil (Cardoso, de la Cruz Jiménez, & Rao, 2014).

Root Lenght

Root development of rootstock affected by abiotic stress conditions, such as root length, number of lateral roots, and dry weight of roots, had morphology changes. This research showed that the CM root length had shorter than 40% under waterlogging. However, has higher under PEG and Al_2SO_4, respectively, at 21 cm and 19 cm. Meanwhile, Cit and Volk are higher at 18-21 cm under abiotic treatment (Fig. 5). The abiotic stress impacts root architecture and the properties of rootstock, thus affect to the growth of the scion. Root length shows the ability of roots to absorb water and nutrients go to the oxygen availability

In drought conditions, the roots grow deeper into the soil layer with sufficient water (Fig. 5). Gandullo, Ahmad, Darwish, Karlova, & Testerink (2021) and Karlova, Boer, Hayes, & Testerink (2021), state that insufficient water in soil induces elongated root growth following the gravity direction. By contrast, the waterlogging treatment causes the anaerobic condition. However, the roots are trying to reach the top of the soil to get oxygen.

According to Schmitt, Watanabe, & Jansen (2016), some plants have various strategies to grow in acidic soil. Thus, they can adaptable and grow well. Waterlogging conditions induced changes in root architecture by restricting maximum penetration of the root and reducing the lateral root, root dry weight, and root length (Cardoso, de la Cruz Jiménez, & Rao, 2014).

Number of Lateral Roots

The function of lateral roots as secondary roots are water absorption and nutrient organs in soil. The result shows inhibition of lateral root growth under abiotic treatment. Meanwhile, the number of lateral roots is repressed by a water saturation condition of about 5.5. By contrast, the Lateral root was higher from 7 to 10 under the PEG condition. Moreover, the lateral root had lower at about 13.5%, respectively, by waterlogging and Al_2SO_4 condition (Fig. 6).

Waterlogging conditions inhibit growth caused by hypoxic plants or lack of oxygen for the roots inclined to rise and absorb available oxygen or upper layer soil. According to Karlova, Boer, Hayes, & Testerink (2021), flooded conditions induce auxin to bring roots to the soil surface and inhibit the elongation of lateral roots.

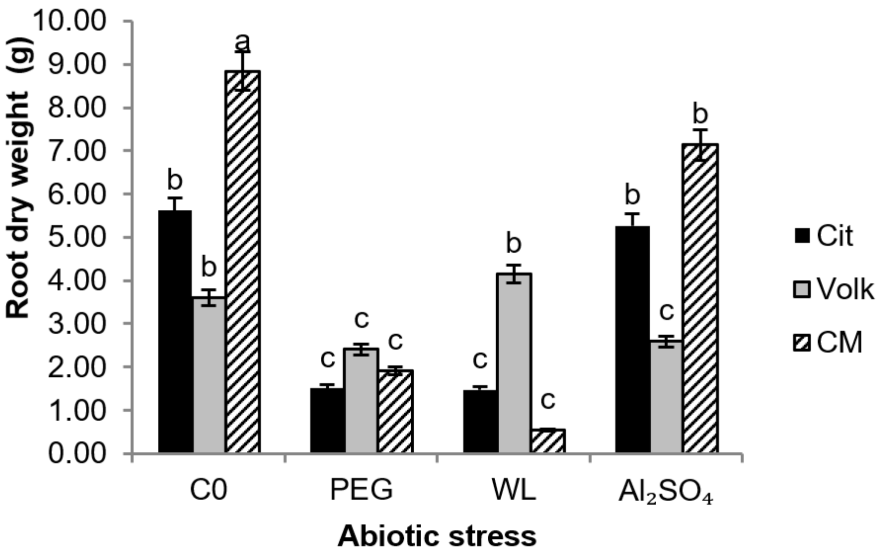

Fig. 4. The root dry weight of citrus rootstocks (Citromello (Cit), Volkameriana (Volk), and Cleopatra Mandarin (CM)) grafting with Pontianak citrus (*C. nobilis var. microcarpa*)

Lateral root development near the root base was facilitated by the oxygen provided by aerenchyma from the nodal root (Cardoso, de la Cruz Jiménez, & Rao, 2014). Meanwhile, in saline conditions, at a concentration of 100 mM, NaCl has the same symptoms under drought conditions. This condition can reduce the auxin content of roots and inhibit primary roots and lateral roots length (Smolko et al., 2021; Zou, Zhang, & Testerink, 2022).

According to Robin et al. (2021), PEG-induced osmotic stress increased root dry weight, root hair density, and increased main axis and lateral root lengths in wheat genotypes. In order to reduce osmotic stress, the density of root hairs on lateral roots was increased thereby increasing the ability of the roots to absorb water. Under insufficient environments, plants initially expand the surface area of their root hairs to uptake water and nutrients (Brown, George, Dupuy, & White, 2013).

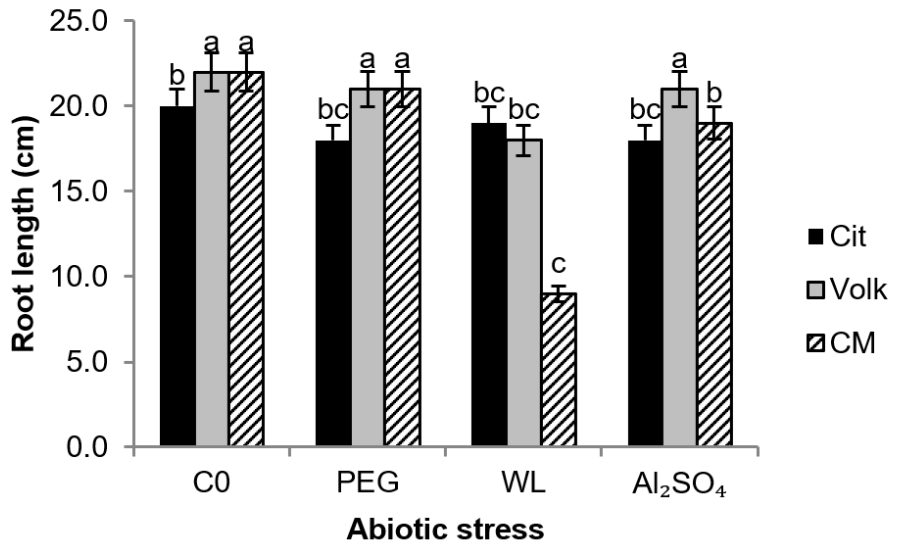

Fig. 5. The root length of the citrus rootstock (Citromello (Cit), Volkameriana (Volk), and Cleopatra Mandarin (CM)), which was grafted with Pontianak citrus (*C. nobilis var. microcarpa*)

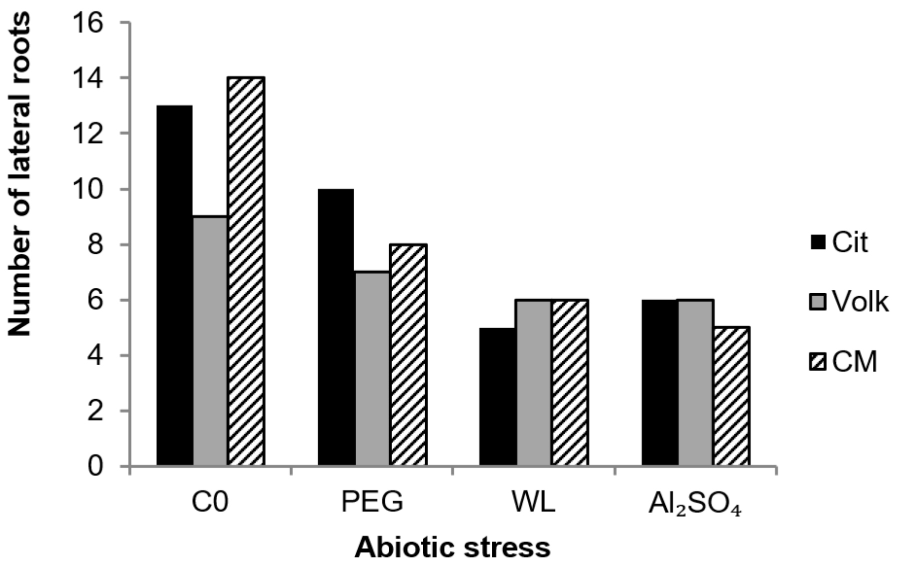

Fig. 6. The number of lateral roots of Pontianak citrus (*C. nobilis var. microcarpa*) grafted on rootstocks (Citromello (Cit), Volkameriana (Volk) and Cleopatra Mandarin (CM))

Cross Section of Rootstock-Scion

The result shows that CM significantly differs from other rootstocks under abiotic stress. Meanwhile, in Cit and Volk, it is suspected to show rootstock and scion incompatibility (Fig. 7). incomplete cambium attachment or delayed cambium formation is seen in Cit and Volk accession. Moreover, disrupted cambium formation is caused by a lack of lignification at the union of the cell wall between rootstock and scion (Adhikari, Xu, & Notaguchi, 2022). Thus, root growth under abiotic stress affects water flow and causes nutrients to be disrupted. Hence the shape diameter of the stem rootstock becomes swollen (elephant's foot), or the diameter of the rootstock is smaller than the scion (stork's leg). This condition causes the plants to become stunted when planted in the field.

On the other states, Incompatibilities on rootstock and scion physiologically have higher phenolic compounds than stems with good compatibility. Hence, phenolic compounds bring on from the vacuole to the cytoplasm to inhibit the lignification process at the beginning of union formation (Rasool et al., 2020).

Addressed to Huang, Deslauriers, & Rossi (2014), a combined cambium is formed due to incision wounds, then the callus grows and forms a cambium. The hormone auxin induces cambium formation as a secondary growth stage in plants. Meanwhile, according to Chen et al. (2016; 2019), the presence of wounds in plants accumulates that auxin induction. In plants that are grafting, the presence of wounds can be a trigger in the accumulation of auxin on the stem of the plant. Rootstock and scion grafting compatibility in almonds, linked by a cambium formed from callus cells and begins to develop for 2-3 weeks after grafting, then rootstock and scion grafting growth becomes perfect, particularly 6-8 weeks after grafting (Özdemir, Yilmaz, Büyükkartal, & Okay, 2019).

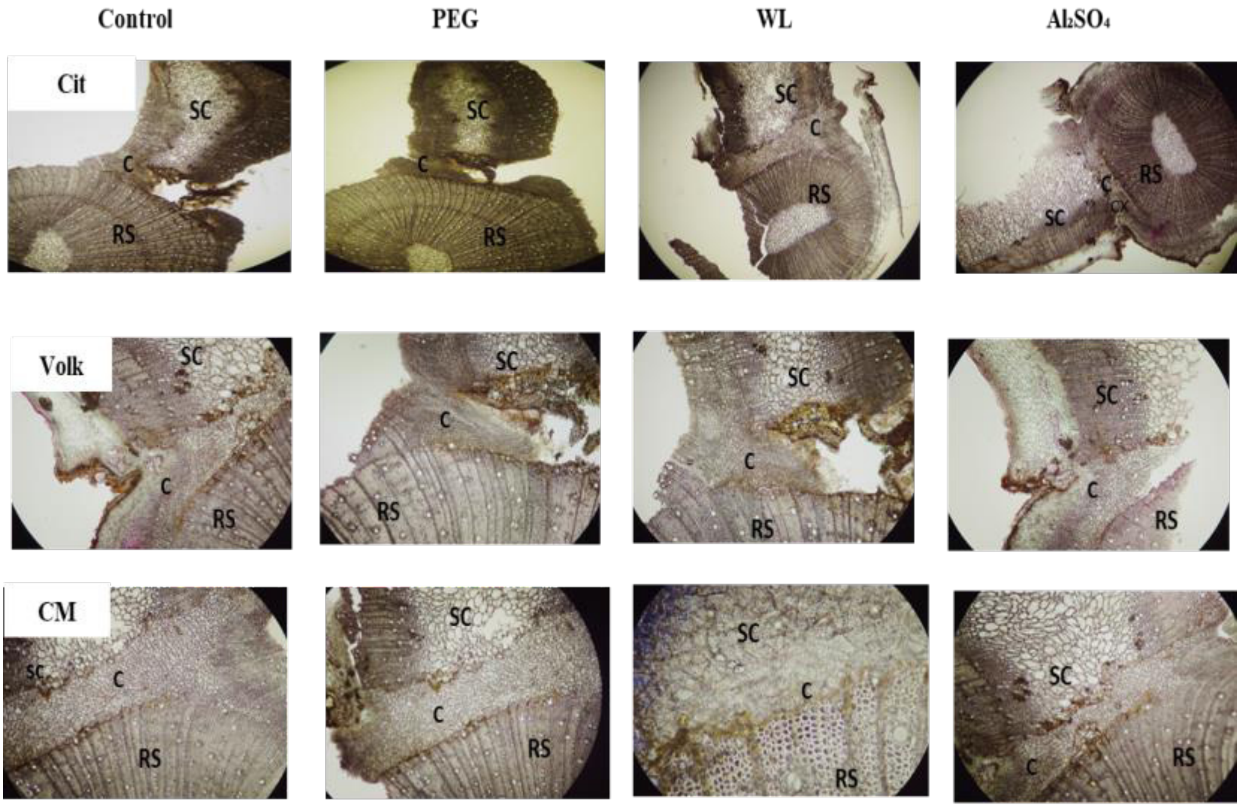

Fig. 7. Cross-sectional union of Pontianak citrus (*C. nobilis var. microcarpa*), citrus rootstock (Citromello (Cit), Volkameriana (Volk) and Cleopatra Mandarin (CM)) RS: Rootstock; SC: Scion; C: Cambium. 400x magnification

In water-deficient rootstock cells, cortex cells as a store of food reserves turn out to be thinner and flattened, parenchymal cells are irregularly shaped or damaged, and parenchymal turgor of parenchymal cells is also reduced due to dehydration (Terletskaya et al., 2021).

Rootstock-Scion Compatibility and Incompatibility Performance

Dwarf rootstock selection from the beginning of seedling to abiotic stress treatment provides the performance of rootstocks that have a small canopy and short plant height. The results showed that the combination of rootstock grafting had vigor growth in the abiotic treatment (Fig. 8). Moreover, plant roots also improved under abiotic stress and their performance remained dwarfed as expected. Therefore, the rootstock character affected the growth of the scion. Thus, the number of leaves is less, ranging from 5 to 8, and the shoot length was shorter than the control at 0.96-2.50 mm.

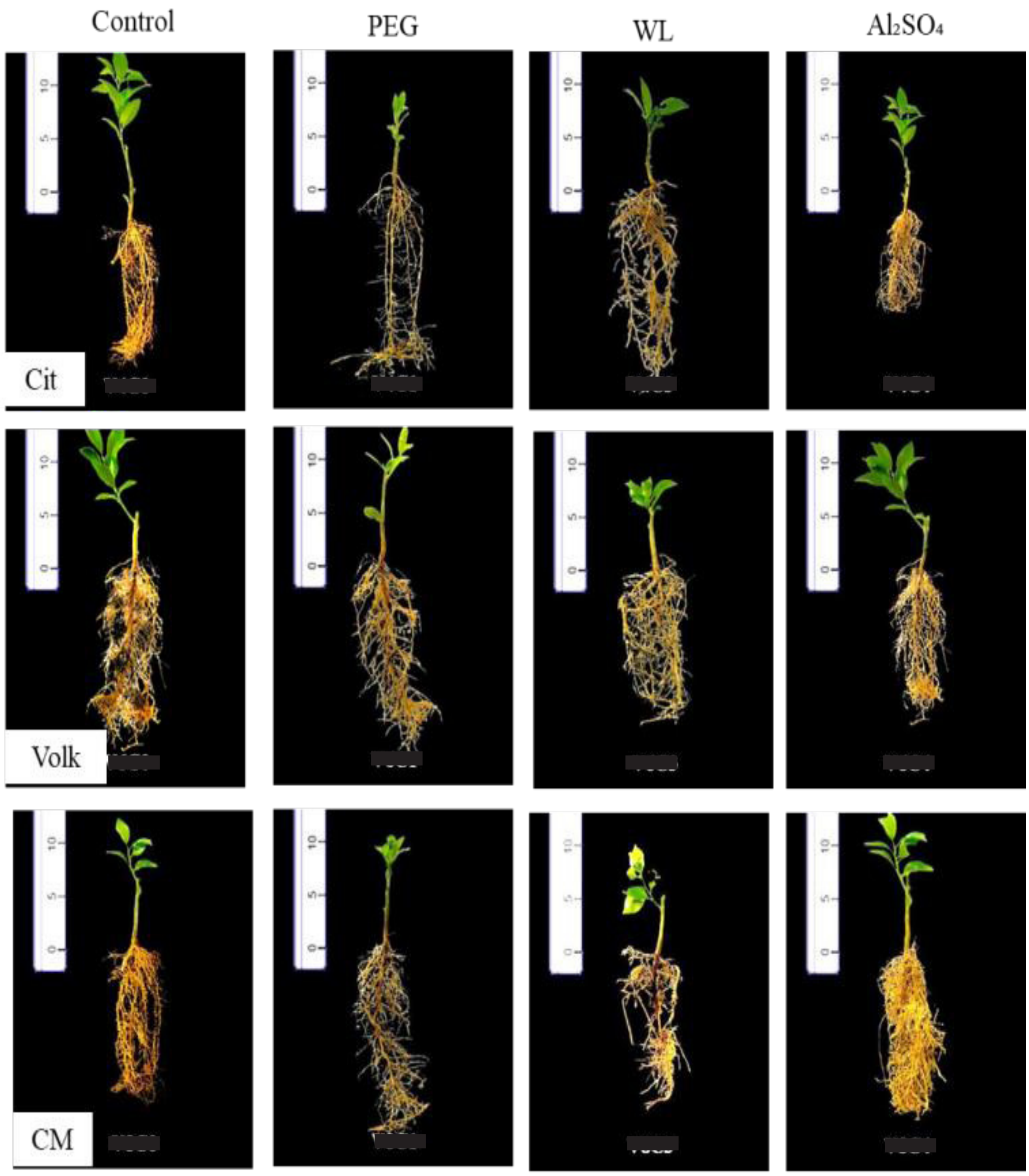

Fig. 8. The grafting performance of Pontianak citrus (*C. nobilis var. microcarpa*) and rootstocks (Citromello (Cit), Volkameriana (Volk) and Cleopatra Mandarin (CM)

Some different rootstock properties, such as vigor, fruit size, and precocity, while others may be selected by their characteristics, such as drought resistance, pest roots, and diseases. The ability of the rootstock is selected based on its adaptation to the environment, such as tolerance to dry and flooded soil, acidity and alkalinity soil (Donadio, Lederman, Roberto, & Stucchi, 2019).

The combination of rootstock and scion becomes compatible and incompatible under abiotic stress. Thus, rootstock affects scion growth, such as tree strength, tolerance under inadequate environment, fruit formation, and tree performance (Jayswal & Lal, 2020). However, Incompatibilities can induce performance dwarfism caused by reduced nutrient and water transport activity in graft unions (Darikova, Savva, Vaganov, Grachev, & Kuznetsova, 2011). Thus, the incompatible rootstock had a dwarf performance as expected.

CONCLUSION AND SUGGESTION

All rootstock accession under abiotic treatment had compatibility and incompatibility with Pontianak citrus. The incompatibility rootstock has a dwarf with potential performance. Citromello (Cit) and Volkameriana (Volk) are incompatibility selected rootstock and had tree performance as dwarf potential seedlings. Cleopatra Mandarin has developed compatibility and was tolerant to abiotic stress treatment. The abiotic treatment affected to the root length of Citromello, had 15% higher than the other accessions. Furthermore, this variety reaches dry weight at around 40% and lateral root number at about 25%, respectively, by Al_2SO_4 and PEG. Meanwhile, the number of leaves of Volk had a higher 40% under all abiotic treatments than other accessions.

For future research, it is recommended to identify molecular studies on the incompatibility and potential of dwarf rootstock with some citrus scions and investigate their effect on production. Thus, some information can be found about the potential of dwarf rootstock to be cultivated under abiotic stress on limited agricultural land with dense cropping systems

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