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# Phenotypes of *Citrus Sp.* As a Selected in Dwarf Rootstock Material Regard to Abiotic Stress Tolerance

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**Abstract:** Research on abiotic stress tolerant horticultural plants has been widely carried out. However, there has not been much research on the citrus phenotype with rootstock as planting material, which has advantages in overcoming abiotic stress and dwarf performance. This research provides citrus rootstock that is tolerant to some of the marginal land in Indonesia especially on drought, salinity, waterlogging, and acidity. This morphological research was conducted at Punten Experimental Garden in Batu city, Indonesia. Anatomical observations were carried out in the laboratories of Indonesian Balitjestro and Universitas Brawijaya. This has done pre-treatment selection lasted for 8 months and then abiotic stress treatment lasted for 2 months, from July to September 2021. This study consisted of 15 treatment combinations: three rootstock varieties, i.e., *Citromello* (Cit), *Volkameriana* (Volk), and *Cleopatra mandarin* (CM), and five abiotic stress treatments, i.e., control, PEG 10%, NaCl 3.5%, waterlogging 150% FC, and 9 mM $Al_2SO_4$. The results showed that abiotic stress, especially NaCl and waterlogging, caused phenotypic changes such as in leaf shape, i.e., leaf lamina shape, reduced leaf area, chlorophyll content, stomata density, and canopy diameter compared to other abiotic stresses. The best stomatal density and open stomata percentage were for *Cleopatra mandarin* (CM). This was also shown by the increase in proline content when plants are subjected to abiotic stresses, especially in *Cleopatra mandarin* under NaCl stress, *Volkameriana* under $Al_2SO_4$ stress, and *Citromello* under waterlogging (WL). The palisade size decreased, its vascular bundles in the leaves increased, and the pore distribution changed. The results showed all rootstock candidates were resistant to several abiotic stresses and had dwarf performance. It can be concluded that the best tolerant of abiotic stress rootstock variety of abiotic stress is *Cleopatra mandarin*, while *Volkameriana* and *Citromello* are better tolerant on acidic soil.

**Keywords:** rootstock, abiotic stress, proline, phenotype, marginal land.

# 柑橘属的表型。作为一种在非生物胁迫耐受性方面选择的矮化。木材料

**摘要：**耐非生物胁迫园艺植物的研究已广泛开展。然而，以砧木为种植材料的柑橘表型研究较少，在克服非生物胁迫和矮化性能方面具有优势。这项研究提供的柑橘砧木能够耐受印度尼西亚的一些边缘土地，尤其是干旱、盐分、内涝和酸度。这项形态学研究是在印度尼西亚巴图市的蓬腾实验花园进行的。在印度尼西亚巴利杰斯特罗和布拉维加亚大学的实验室进行了解剖观察。从2021年7月到2021年9月，预处理选择持续了8个月，然后非生物胁迫处理持续了2个月。本研究包括15种处理组合：三个砧木品种，即西特罗梅洛(花旗)、沃尔卡梅里亚纳(大众)和埃及艳后柑橘(厘米)，以及五种非生物胁迫处理，即对照、聚乙二醇10%、氯化钠3.5%、涝渍150%足球俱乐部和9毫米Al2SO4。结果表明，与其他非生物胁迫相比，非生物胁迫，尤其是氯化钠和涝渍，导致叶片形状（即叶片形状）、叶面积、叶绿素含量、气孔密度和冠层直径等表型变化。最佳气孔密度和开放气孔百分比是埃及艳后柑（厘米）。当植物受到非生物胁迫时，脯氨酸含量的增加也表明了这一点，特别是在氯化钠胁迫下的克娄

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巴特拉普通话、Al2SO4胁迫下的沃尔卡梅里亚纳和涝渍(WL)下的西特罗梅洛。栅栏尺寸减小，叶片中的维管束增多，孔隙分布发生变化。结果表明，所有候选砧木都对几种非生物胁迫具有抗性并且具有矮化性能。可以得出结论，非生物胁迫的砧木品种对非生物胁迫的耐受性最好的是埃及艳后，而沃尔卡梅里亚纳和西特罗梅洛对酸性土壤的耐受性较好。

**关键词：**砧木, 非生物胁迫, 脯氨酸, 表型, 边缘土地。

# 1. Introduction

Rootstock is the support for the plants growing on it. It functions in the transport of nutrients, hormones, and photosynthates. In citrus cultivation, seedlings are generally propagated vegetatively, namely, rootstock-scion grafting. Rootstock is disease resistant and able to survive in unfavorable environments is needed to increase fruit production. The increasing consumption of fresh oranges and processed oranges in Indonesia, i.e. 11.58% per year according to [1], has urged stakeholders to strive for the fruit's availability. Therefore, policies must be directed toward expanding citrus plantations in the marginal lands.

BBSDLP released in 2015 [2] that marginal land in Indonesia reached 285.86 million ha; consisting of dry soil, saline soil, waterlogged soil, and acidic soil. Plants grown on such land will experience stress in their phenotypes and physiology.

This study simulates abiotic stress, which is close drought, salinity, waterlogging, and acidity. PEG (polyethylene glycol) and NaCl are osmotic stress agents used to stimulate drought stress conditions [3] and salinity stress conditions [4]. PEG is a polymer that causes plant tissues not to be easily penetrated, so it interferes with water absorption in the root zone, lowers osmosis potentials and makes plants under water shortage [5]. It is used in rootstock breeding technology as a fusion to fuse plant cell protoplasm to test drought resistance [6]. High salinity can increase proline content and reduce Fe content in pistachio rootstock [7].

Waterlogging simulates waterlogged soil, causing stress on plants because of excessive water. This condition causes the oxygen concentration around the roots to decrease, i.e. the hypoxia [8, 9]. Acid soils are associated with low soil pH; it causes poisoning by metal ions such as Al, Fe, Zn, Cu, and Mn. This reduces the absorption of important soil nutrients such as P, Ca, Na, Mg, and K [10]. Al toxicity in citrus is characterized by a decreased rate of photosynthesis in scion-rootstock plants [11].

Rootstocks tolerant to abiotic stress will experience phenotypic and physiological changes in their growth stage to maintain their life. This study aimed to observe phenotypic changes in several rootstock varieties that experience abiotic stress such as drought, salinity, waterlogging, and acidity. The results of this study are expected to be useful in citrus farming to meet the national citrus needs in sub-optimal land and limited areas.

# 2. Methodology

This research was conducted in a greenhouse in the Punten Experimental Garden of Batu city. This garden covers 2.7 ha of land on 950 AMSL. This research was started by sowing eight rootstock varieties, i.e. *Japansche Citron, Rough Lemon*, *Kanci*, *Citromello, Carizzo*, *Volkameriana, Troyer*, *and Cleopatra Mandarin*, for 5 months. Then, the dwarf phenotype selection was carried out with the parameters of slower seedling growth, short internode distances, smaller leaves, and narrower canopy diameter.

The rootstock varieties with dwarfism are *Citromello* (Cit), *Volkameriana* (Volk), and *Cleopatra Mandarin* (CM). Hardening was carried out for 3 months on the three varieties. They were then given abiotic stress treatment, i.e. control, 8% PEG; 3.5% NaCl, 150% FC Waterlogging (WL); 9 mM $Al_2SO_4$. This research was conducted for 2 months.

This research used Olympus microscopes, microtome, prepared slides and kits, writing stationery, field equipment, LAM (Leaf Area Meter) Type Li-Cor 3100C, LC-MS/MS Spectrophotometer (Thermo Scientific), and a pH soil meter. The used materials are rootstock varieties of *Citromello* (Cit), *Volkameriana* (Volk), and *Cleopatra mandarin* (CM), PEG, NaCl, water, $Al_2SO_4$, staining blue, and nail polish.

This study used a factorial randomized block design. The first factor is variety, and the second factor is abiotic stress. The treatment combinations are shown in Table 1.

Table 1 Treatment combinations

| V/C | C1 (PEG) | C2 (NaCl) | C3 (WL) | C4 ($Al_2SO_4$) | C0 (Control) |
|---|---|---|---|---|---|
| V1 (Cit) | V1/C1 | V1/C2 | V1/C3 | V1/C4 | V1/C0 |
| V2 (Volk) | V2/C1 | V2/C2 | V2/C3 | V2/C4 | V2/C0 |
| V3 (CM) | V3/C1 | V3/C2 | V3/C3 | V3/C4 | V3/C0 |

The experiment was repeated three times with five units, so the material required was 5 x 5 x 3 x 3 = 225 rootstocks. Observations were made once a week for two months. Tukey post hoc test was used with a confidence level of 95%.

## 3. Results and Discussion

Phenotypic changes often occur when plants are under extreme environmental changes. The changes were made to survive and tolerate the environment. Mandarins show significant physiological and biochemical symptoms when they lack in water [12]. Citrus plants are classified as plants very sensitive to salinity [13]. Therefore, information on citrus rootstocks tolerant of several types of abiotic stress is needed. Changes in phenotype in citrus can provide information about the ability of certain rootstock varieties to adapt to less normal environments.

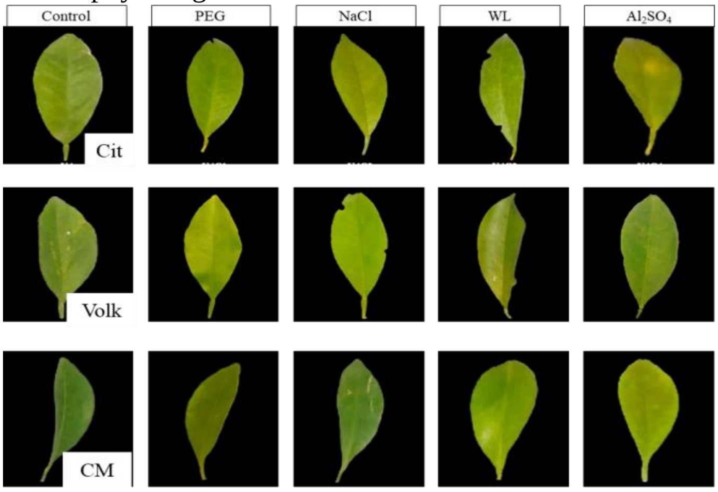


Fig. 1 The shape of leaves in stress treatments (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$)

Leaf shape changes under abiotic stress can be seen in Fig. 1. The leaf lamina shape of *Citromello* changes from lanceolate obovate and elliptic under abiotic stress. In *Volkameriana* and *Cleopatra mandarin*, elliptic shape turned to ovate, especially under NaCl and $Al_2SO_4$ treatment (Table 2).

Table 2 Description of the anatomy of citrus leaves under abiotic stress treatment (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$)

| **Cit** | **C0** | **PEG** | **NaCl** | **WL** | **$Al_2SO_4$** |
|---|---|---|---|---|---|
| Leaf lamina attachment | Brevipetolate | Brevipetolate | Brevipetolate | Brevipetolate | Brevipetolate |
| Leaf lamina shape | Lanceolate | Obovate | Elliptic | Elliptic | Elliptic |
| Leaf lamina margin | Dentate | Dentate | Dentate | Dentate | Dentate |
| Petiole wings | Presence | Presence | Presence | Presence | Presence |
| | Medium | Medium | Medium | Medium | Medium |
| Petiole wing shape | Obdeltate | Obdeltate | Obdeltate | Obdeltate | Obdeltate |
| **Volk** | **C0** | **PEG** | **NaCl** | **WL** | **$Al_2SO_4$** |
| Leaf lamina attachment | Brevipetolate | Brevipetolate | Brevipetolate | Brevipetolate | Brevipetolate |
| Leaf lamina shape | Elliptic | Elliptic | Ovate | Elliptic | Elliptic |
| Leaf lamina margin | Dentate | Dentate | Dentate | Dentate | Dentate |
| Petiole wings | Presence medium | Presence medium | Presence medium | Presence medium | Presence medium |
| Petiole wing shape | Obdeltate | Obdeltate | Obdeltate | Obdeltate | Obdeltate |
| **CM** | **C0** | **PEG** | **NaCl** | **WL** | **$Al_2SO_4$** |
| Leaf lamina attachment | Brevipetolate | Brevipetolate | Brevipetolate | Brevipetolate | Brevipetolate |
| Leaf lamina shape | Elliptic | Elliptic | Elliptic | Ovate | Ovate |
| Leaf lamina margin | Acute | Acute | Acute | Acute | Acute |
| Petiole wings | Presence Narrow | Presence Narrow | Presence Narrow | Presence Narrow | Presence Narrow |
| Petiole wing shape | Obovate | Obovate | Obovate | Obovate | Obovate |

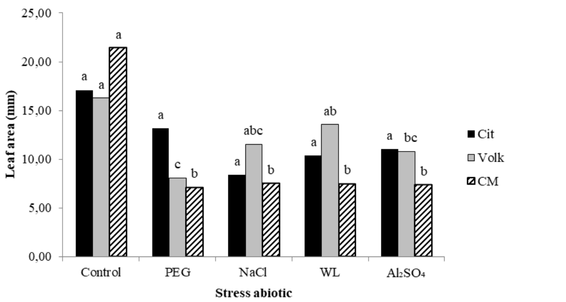


Fig. 2 Leaf areas in three rootstock varieties (*Citromello*, *Volkameriana*, dan *Cleopatra Mandarin*) given the abiotic stress treatment (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$). Tukey test with the confidence level of 95%

The results show that leaf area decreased in all rootstock varieties (*Citromello, Volkameriana, and Cleopatra Mandarin*) after the stress treatment compared with controls (Fig 2). *Volkameriana* has the highest leaf area under waterlogging treatment (WL), i.e. 13.54 mm, and the lowest under PEG treatment, i.e. 8.06 mm. *Citromello* had the highest leaf area under PEG treatment, i.e. 13.15 mm, and the lowest under NaCl treatment, i.e. 8.37 mm. *Cleopatra Mandarin* tended to be stable under all abiotic stress treatment, but lower than the control, i.e. 7.10–7.35 mm (Fig 2).

The first response that we can see from a plant when it is under stress is the decrease in leaf area. This is caused by a decrease in turgor pressure in the cell, so the cell volume decreases due to an increase in the concentration of solutes in the cell. Decreasing leaf area is one of plants' ways to reduce water transpiration and their first way to fight drought. Generally, plants give early signals by reducing leaf area when plants experience salinity, waterlogging, and drought stress [14]. Drought stress on paprika plants causes plants to reduce their leaf area and number of stomata [15].

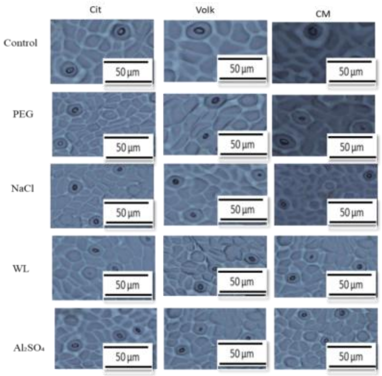


Fig. 3 Cross-section of stomata in *Citromello, Volkameriana,* and *Cleopatra Mandarin* on the eighth week under four abiotic stress treatments (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$). 400x magnification

Plants' initial defense when they are subjected to an abnormal environment, apart from decreasing leaf area, is closing their stomata. Partially closed stomata and smaller stomata under all abiotic stress treatments compared to control plants can be seen in Fig 3 and Table 3.

*Cleopatra mandarin* has higher stomatal density and more open stomata than other varieties, but the ratio of open/closed stomata and its percentage of opening stomata are lower (Table 4). This shows that plants given the abiotic stress treatments of PEG, NaCl, WL, and $Al_2SO_4$ are trying to survive by reducing excessive evaporation, i.e., avoiding dehydration due to evaporation and transpiration [16].

Saline soils, which contain NaCl, reduce groundwater potential, which causes the dehydration of plants. The symptoms that appear in plants are the same as the symptoms when they experience drought stress. Anaerobic conditions, i.e., waterlogged soil, cause oxidative damage that reduces the chlorophyl content and stomatal conductance [8].

Table 3 Stomata size after abiotic stress treatment (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$)

| **Variety** | **Abiotic stress** | **Stomata size (µm)** |
|---|---|---|
| Cit | Control | 22.43 |
| | PEG | 17.47 |
| | NaCl | 15.95 |
| | WL | 16.71 |
| | Al2SO4 | 18.98 |
| Volk | Control | 24.10 |
| | PEG | 17.73 |
| | NaCl | 18.29 |
| | WL | 16.31 |
| | Al2SO4 | 17.17 |
| CM | Control | 26.89 |
| | PEG | 17.94 |
| | NaCl | 15.56 |
| | WL | 16.59 |
| | Al2SO4 | 16.56 |

Table 4 Stomata density, open-close ratio, percentage of opened stomata under abiotic stress treatment (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$)

| **Varieties** | **Stress** | **Stomata Density** | **The ratio of open and closed stomata** | **Percentage of open stomata (%)** |
|---|---|---|---|---|
| Cit | Control | 505,97 | 1,5 | 60 |
| | PEG | 303,58 | 0,53 | 34,62 |
| | NaCl | 252,99 | 0,38 | 27,27 |
| | WL | 269,85 | 0,52 | 34,04 |
| | $Al_2$ $SO_4$ | 320,45 | 0,58 | 36,54 |
| Volk | Control | 522,84 | 2,58 | 72,09 |
| | PEG | 303,58 | 0,75 | 42,86 |
| | NaCl | 202,39 | 0,57 | 36,36 |
| | WL | 269,85 | 0,64 | 39,02 |
| | $Al_2$ $SO_4$ | 320,45 | 0,66 | 39,58 |
| CM | Control | 556,57 | 1,27 | 55,93 |
| | PEG | 320,45 | 0,68 | 40,43 |
| | NaCl | 320,45 | 0,58 | 36,54 |
| | WL | 337,31 | 0,65 | 39,22 |
| | $Al_2$ $SO_4$ | 404,78 | 0,67 | 40 |

Regarding the plant growth inhibition due to abiotic stress, in addition to decreasing leaf area, stomata size, the percentage of open stomata, the contents of a and b chlorophyll were also reduced in all abiotic stress treatments. *Cleopatra mandarin* contains chl a and b better in $Al_2SO_4$ and *Volkameriana* in PEG treatment (Fig 4). Plants began to turn yellow in the second week, especially in the waterlogging treatment (WL), because the plant roots were hypoxic, so the gas exchange in the leaves and the photosynthesis were disrupted.

The decrease in chlorophyll content and stomatal conductance occurred in *Citrange Carrizo* grafted with navel orange under waterlogging [17]. The high NaCl in plant tissue interferes with the overall growth. In addition to inhibiting nutrient uptake in roots, $Na^+$ and $Cl^-$ ions are toxic when they accumulate in leaves, so photosynthesis is disrupted. Drought stress also causes the same thing, in which the photosynthesis is disrupted because the stomata close, which reduces the chlorophyl content [14].

Acidity stress also causes the degradation of chlorophyll content, so the photosynthesis rate is inhibited due to $Al^+$ ion toxicity. Here, plants cannot absorb Ca and Mg. Additionally, Al induction in the rootstock of *C. sinensis* can reduce the contents of a and b chlorophyll, which slows down photosynthesis [18].

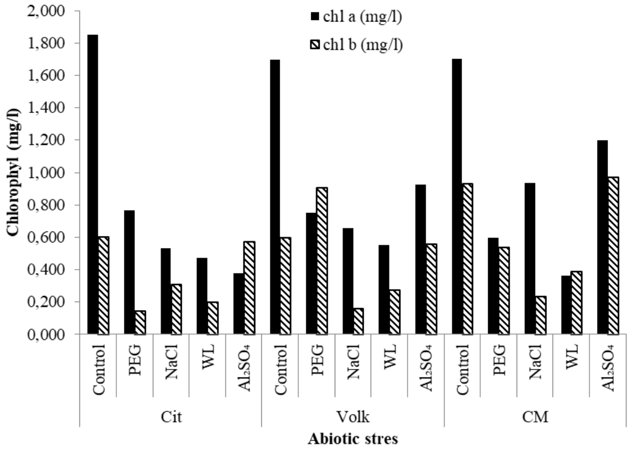


Fig. 4 Chlorophyll content of *Citromello, Volkameriana,* and *Cleopatra Mandarin*) in the eighth week under four abiotic stress treatments (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$). Tukey test with a confidence level of 95%

The canopy diameter of all varieties is smaller under all abiotic stress treatments than the control, i.e. 4–15 mm on the average. The narrowest canopy diameter is found in plants under salinity stress (NaCl). *Cleopatra Mandarin* (CM) has the widest diameter of all other varieties in all abiotic stress treatments, i.e. 7–10 mm. *Volkameriana* has a wide canopy diameter under all abiotic stress treatments, i.e. 9–15 mm, except in salinity stress, i.e. 4 mm (Fig. 5).

The disruption of metabolic processes due to salinity, drought, waterlogging, and acidity stress reduces growth rate, plant height, leaf area due to self-defense from the transpiration process, and canopy diameter (Fig 5). Further, the stem diameters of all varieties are smaller than the control, but higher in PEG treatment. NaCl stress has a smaller effect than other abiotic stresses (Table 5). Drought stress causes roots to grow lengthwise toward water sources and away from drought in the upper layers [19].

In woody plants, osmotic stress decreases stem diameter and an increase in lignin –a constituent of cell walls in plants [20,21]. In the grafted citrus, stem cross-sectional area and plant height correlate with canopy diameter [22].

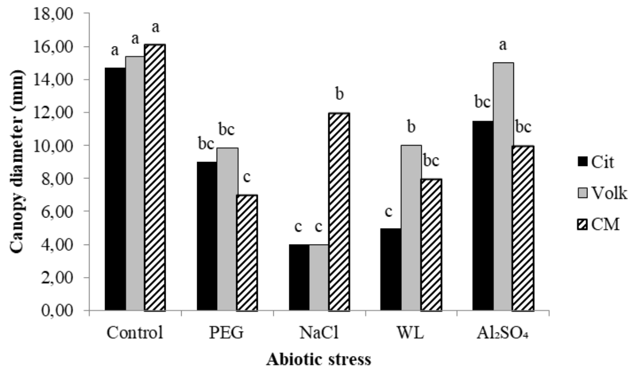


Fig. 5 Canopy diameter of *Citromello, Volkameriana,* and *Cleopatra Mandarin*) in the eighth week under four abiotic stress treatments (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$). Tukey test with a confidence level of 95%

Table 5 Stem diameter, root length, root dry weight under abiotic stress (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$)

| **Varieties** | **Abiotic stress** | **Stem diameter (mm)** | **Root length (cm)** |
|---|---|---|---|
| Cit | C0 | 4,67 | 20,0 |
| | PEG | 3,16 | 21,0 |
| | NaCl | 3,24 | 19,0 |
| | WL | 3,44 | 19,0 |
| | $Al_2SO_4$ | 3,54 | 19,0 |
| Volk | C0 | 5,05 | 22,0 |
| | PEG | 3,34 | 23,0 |
| | NaCl | 2,81 | 19,0 |
| | WL | 3,35 | 19,0 |
| | $Al_2SO_4$ | 3,81 | 21,0 |
| CM | C0 | 5,25 | 22,0 |
| | PEG | 4,07 | 23,0 |
| | NaCl | 3,55 | 19,0 |
| | WL | 3,95 | 10,0 |
| | $Al_2SO_4$ | 4,01 | 20,0 |

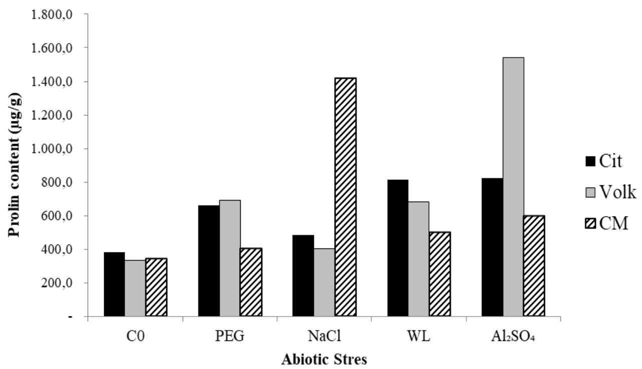


Fig. 6 Proline in the leaves of plants under abiotic stress treatment (control; 8% PEG; 3.5% NaCl, 150% FC Waterlogging (WL); 9 mM $Al_2SO_4$)

The analysis using LC-MS/MS indicated that the highest proline concentration was in *Cleopatra mandarin* under NaCl stress, i.e. 1,420.8 (µg/g), and *Volkameriana* under $Al_2SO_4$ stress, i.e. 1,539.6 (µg/g). *Citromello* has the highest proline content under waterlogging treatment (WL), i.e. 816.3 (µg/g).

Proline is synthesized in the cytoplasm or chloroplast from L-glutamate and L-Ornithine, and it is degraded in the mitochondria. When a plant is stressed, proline synthesis begins. Proline as a compound for an osmoprotectant defense mechanism under abiotic stress is accumulated in leaves. It is important as a metal chelator, antioxidant defense, and stress tolerance agent [23]. In citrus plantlets, under dry conditions, PEG 4%, proline increases to 0.525 ml/l [24]. *Cleopatra mandarin* showed the least damage under a salinity stress of 100-mm NaCl and had the highest proline level during seedling [25].

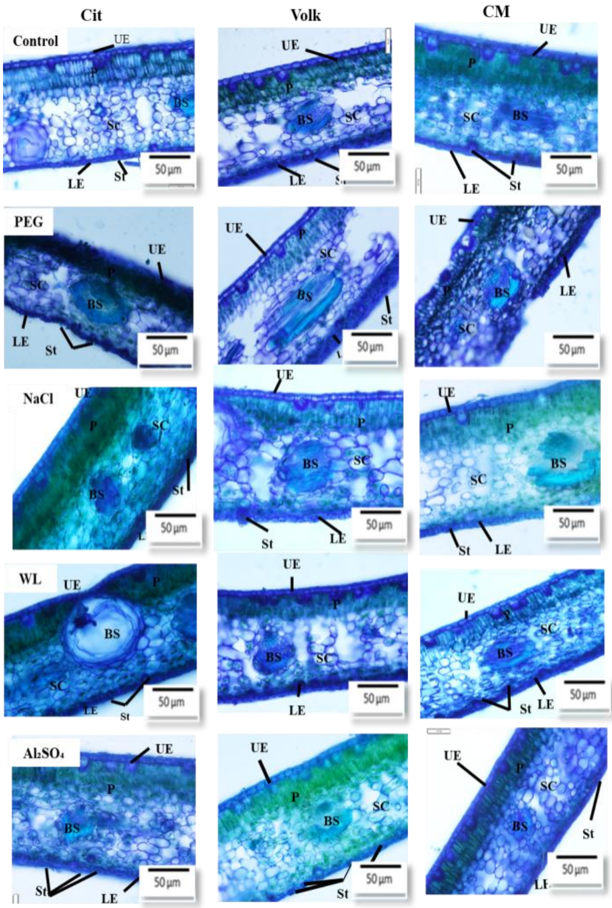


Fig. 7 Cross-section of leaves in *Citromello, Volkameriana,* and *Cleopatra Mandarin* on the eighth week under four abiotic stress treatments (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$). 400x magnification

The cross-section of the leaves of the plants under some abiotic stress also shows changes in their vascular bundle consisting of xylem and phloem. Theirs are bigger than those of the control, but it was not the case for plants under waterlogging treatment (WL). Their palisade tissue was smaller than the tissue of control plants (Fig. 7). The palisade is the location of the cytoplasm and photosynthesis chloroplast organelles. The low chlorophyll content under abiotic stress indicates that the palisade function is less optimal during the period. [26] revealed that the size of palisade and spongy cells decreased significantly when plants were under high light stress; this also causes the chloroplasts to shrink.

Cell changes in leaves also occur when plants experience drought stress. This stress degrades the size of the palisade and bundle sheath tissues. However, if tolerance toward drought stress increases, the palisade tissue, bundle sheath, and leaves will thicken, and stomata will be constricted [27].

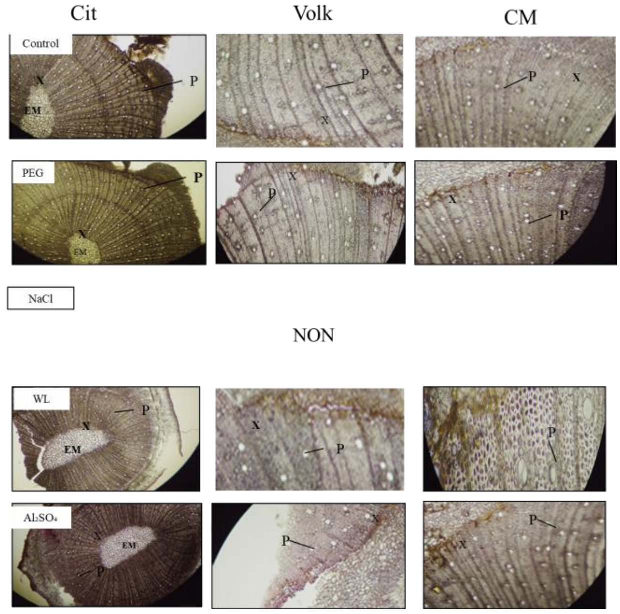


Fig. 8 Cross-section of rootstock under abiotic stress treatments (control; 8% PEG; 3.5% NaCl, 150% FC waterlogging (WL); 9 mM $Al_2SO_4$). X: xylem, Em: Pith, P: vessel .400x magnification

Based on the cross-sectional image of rootstocks under abiotic stress, the plants' porous vessels are categorized as single pore surrounded by wood fibers; each has pith as a food reserve network in dicotyledonous plants. Xylem transports water and nutrients in plants. In *Cleopatra Mandarin*, single pores turned into grouped pores under waterlogging (WL) treatments. All rootstock varieties died of NaCl treatment. In *Volkameriana*, a single pore changed to radial pore multiple under $Al_2SO_4$ treatment; the two pores appeared to intersect with each (Fig 8).

Environmental changes, especially drought stress combined with high temperature and CO2, affect the vascular system and water regulation of plants. Here the size and density of xylem, the size of cambium, and the cell wall are degrading [28]. Under drought and salinity stress, the sizes of vessel cells and xylem in Populus trichocarpa become smaller [21].

## 4. Conclusion

Phenotypic changes occur in plants that live in unfavorable areas and cause stress symptoms. The phenotypic changes that occur regarding abiotic stress to citrus rootstocks include the following.

Changes in leaf shape when plants experience abiotic stress, i.e. on leaf lamina shape, do occur. The leaf area and the canopy diameter of all varieties tended to decrease, except for *Volkameriana* (Volk). It has an insignificant canopy diameter under $Al_2SO_4$ treatment compared to that of control plants. It has smaller stomata that are closed and partially closed. The best stomata density and the best open stomata percentage are for *Cleopatra mandarin* (CM). Its stem diameter is smaller, and its root is shorter than, as compared to their condition of PEG treatment.

The highest proline content was on *Cleopatra mandarin* under NaCl stress, on *Volkameriana* under $Al_2SO_4$ stress, and on *Citromello* under waterlogging (WL). Their xylem and phloem were larger than those of control plants, but that is not the case under waterlogging (WL). All rootstock varieties died on NaCl treatment .The palisade tissue became smaller than the tissue of control plants. In *Cleopatra Mandarin*, single pores turned into grouped pores under waterlogging (WL). In *Volkameriana*, single pores become radial pores multiple under $Al_2SO_4$ treatment, in which two pores intersect each other

The results of the phenotypic research showed that all rootstock candidates were resistant to several abiotic stresses, and could be recommended on four marginal lands, especially on dry land, salinity, waterlogging, and acid soils with dwarf performance. However, as information based on the data above, the best tolerant variety is *Cleopatra mandarin*, while *Volkameriana* and *Citromello* are better tolerant of acidic soil.

This research is expected to continue in grafting with scions as a production plant, so that its compatibility with scions is known, the stability of both growth and productivity.

## References


[1] CENTER FOR AGRICULTURAL DATA AND INFORMATION SYSTEMS. *Citrus Outlook; Horticulture Sub-Sector Agricultural Commodities*. Ministry of Agriculture, Jakarta, 2016.

[2] AGENCY FOR AGRICULTURAL RESEARCH AND DEVELOPMENT. *Indonesia's Agricultural Land Resources Extensive, Distribution, and Potential Availability*. IAARD Press, Jakarta, 2015.

[3] CASTAÑEDA V., & GONZÁLEZ E. M. Strategies to Apply Water-Deficit Stress: Similarities and Disparities at the Whole Plant Metabolism Level In Medicago Truncatula. *International Journal of Molecular Sciences*, 2021, 22: 2813. https://doi.org/10.3390/ijms22062813

[4] AKBARI M., KATAM R., HUSAIN R., FARAJPOUR M., MAZZUCA S., and MAHNA N. Sodium Chloride Induced Stress Responses of Antioxidative Activities in Leaves and Roots of Pistachio Rootstock. *Biomolecules*, 2020, 10: 189. https://doi.org/10.3390/biom10020189

[5] JUNG J. S., MUHAMMAD Z., LEE K.-W., MUN J. Y., PARK H. S., KIM Y. J., KIM W. H., LEE S.-H., and LEE S.-H. 2015. Effects of polyethylene glycol-induced water stress on the physiological and biochemical responses of different Sorghum genotypes. Proceedings of the the XXIII International Grassland Congress (Sustainable use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection), New Delhi, 2015. https://uknowledge.uky.edu/igc/23/4-1-2/4/

[6] CIMEN B., & YESILOGLU T. *Rootstock Breeding for Abiotic Stress Tolerance in Citrus*. Intech, London, 2016. http://dx.doi.org/10.5772/62047

[7] RAHNESHAN Z., NASIBI F., and MOGHADAM A.A. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (Pistacia vera L.) rootstocks. *Journal of Plant Interactions*, 2018, 13(1): 73–82. https://doi.org/10.1080/17429145.2018.1424355

[8] AMADOR M. L., SANCHO S., BIELSA B., GOMEZ-APARISI J., and RUBIO-CABETAS M. J. Physiological and biochemical parameters controlling waterlogging stress tolerance in Prunusbefore and after drainage. *Physiologia Plantarum*, 2012, 144: 357–368. https://doi.org/10.1111/j.1399-3054.2012.01568.x

[9] MORALES-OLMEDO M., ORTIZ M., and SELLES G. Review: Effects of transient soil waterlogging and its importance for rootstock selection. *Chilean Journal of Agricultural Research*, 2015, 75(1): 45–56. http://dx.doi.org/10.4067/S0718-58392015000300006

[10] BHUYAN M. H. M. B, HASANUZZAMAN M., NAHAR K., AL MAHMUD J., PARVIN K., BHUIYAN T.F., and FUJITA M. Plant Behavior under Soil Acidity Stress: Insight into Morphophysiological, Biochemical, and Molecular Responses. In: *Plant Abiotic Stress Tolerance*. Springer Nature, Cham, 2019: 35–82. https://doi.org/10.1007/978-3-030-06118-0_2

[11] XIN-YAN L., LIN-TONG Y., YI-BIN L., YE X., and LI-SONG C. Roles of rootstocks and scions in aluminum-tolerance of Citrus. *Acta Physiologiae Plantarum,* 2015, 37: 1743. https://doi.org/10.1007/s11738-014-1743-1

[12] DOS SANTOS I. C., DE ALMEIDA A. A. F., PIROVANI C. P., COSTA M. G. C., DA CONCEIÇÃO A. S., FILHO W. S. S., FILHO M. A. C., and GESTEIRA A. S. Physiological, biochemical and molecular responses to drought conditions in field-grown grafted and ungrafted citrus plants. *Environmental and Experimental Botany*, 2019, 162: 406-420. https://doi.org/10.1016/j.envexpbot.2019.03.018

[13] ABOUTALEBI A., & HASANZADEH H. Salinity and Citrus Rootstocks and Interstocks. *International Journal of Plant, Animal and Environmental Sciences*, 2014, 4: 654–672. https://www.cabdirect.org/cabdirect/abstract/20143220344

[14] KORDROSTAMI M., & MAFAKHERI M. Consequences of Water Stress and Salinity on Plants/Crops; Physiobiochemical and Molecular Mitigation Approaches. In: PESSARAKLI M. (ed.) *Handbook of Plant and Crop Physiology*. CRC Press, Boca Raton, 2022: 789–814. https://doi.org/10.1201/9780367554545-37

[15] PADILLA Y. G., GISBERT-MULLOR R., LÓPEZ-SERRANO L., LÓPEZ-GALARZA S., and A. CALATAYUD. Grafting Enhances Pepper Water Stress Tolerance by Improving Photosynthesis and Antioxidant Defense Systems. *Antioxidants*, 2021, 10(576): 1–15. https://doi.org/10.3390/antiox10040576

[16] PIRASTEH-ANOSHEH H., SAED-MOUCHESHI A., PAKNIYAT H., and PESSARAKL M. Stomatal responses to drought stress. In: AHMAD, P. (ed.) *Water Stress and Crop Plants: A Sustainable Approach Volume 1*. John Wiley & Sons, Hoboken, 2016: 24-40. https://doi.org/10.1002/9781119054450.ch3

[17] OTERO A., & GOÑI C. Short hypoxia period affects photosynthesis of citrus scion leaves under different rootstocks. *Citrus Research & Technology*, 2016, 37(1): 19-25. http://dx.doi.org/10.4322/crt.ICC110

[18] LIAO X. Y., YANG L. T., LU Y. B., YE X., and CHEN L. S. Roles of rootstocks and scions in aluminum-tolerance of Citrus. *Acta Physiologiae Plantarum*, 2015, 37: 1743. https://doi.org/10.1007/s11738-014-1743-1

[19] GANDULLO J., AHMAD S., DARWISH E., KARLOVA R., and TESTERINK C. Phenotyping tomato root developmental plasticity in response to salinity in soil rhizotrons. *Plant Phenomics*, 2021, 2021: 1-14. https://doi.org/10.34133/2021/2760532

[20] POLLE A., CHEN S. L., ECKERT C., and HARFOUCHE A. Engineering Drought Resistance in Forest Trees. *Frontiers in Plant Science*, 2019, 9: Article 1875. https://doi.org/10.3389/fpls.2018.01875

[21] HORI C., YU X., MORTIMER J. C., SANO R., MATSUMOTO T., KIKUCHI J., DEMURA T., and OHTANI M. Impact of abiotic stress on the regulation of cell wall biosynthesis in Populus trichocarpa. *Plant Biotechnology*, 2020, 37: 273–283. https://doi.org/10.5511/plantbiotechnology.20.0326a

[22] MARTÍNEZ-CUENCA M. R., PRIMO-CAPELLA A., and GINER M. A. F. Influence of Rootstock on Citrus Tree Growth: Effects on Photosynthesis and Carbohydrate Distribution, Plant Size, Yield, Fruit Quality, and Dwarfing Genotypes. In: RIGOBELO E. C. (ed.) *Plant Growth*. InTech Open, London, 2016: 107-129. http://dx.doi.org/10.5772/64825

[23] SINGH A., SHARMA M.K., and SENGAR R.S. Osmolytes: Proline metabolism in plants as sensors of abiotic stress. *Journal of Applied and Natural Science*, 2017, 9(4): 2079-2092. http://dx.doi.org/10.31018/jans.v9i4.1492

[24] ASHARI A., NURCAHYANI E., HARDOK O., QUDUS I., and ZULKIFLI. Analisis Kandungan Prolin Planlet Jeruk Keprok Batu 55 (Citrus Reticulata Blanco Var. Crenatifolia) Setelah Diinduksi Larutan Atonik Dalam Kondisi Cekaman Kekeringan Secara in Vitro. *Analit: Analytical and Environmental Chemistry*, 2018, 3(01): 69–78. http://dx.doi.org/10.23960%2Faec.v3i1.2018.p

[25] MAHMOUD L. M., DUTT M., VINCENT C. I., and GROSSER J. W. Salinity-Induced Physiological Responses of Three Putative Salt Tolerant Citrus Rootstocks. *Horticulturae*, 2020, 6, 90. https://doi.org/10.3390/horticulturae6040090

[26] ZECHMANN B. Ultrastructure of plastids serves as reliable abiotic and biotic stress marker. *PLoS ONE*, 2019, 14(4). https://doi.org/10.1371/journal.pone.0214811

[27] PRASAD V., & RAO R. Various plant's responses and strategies to cope with the water deficit: A review. *Journal of Pharmacognosy and Phytochemistry*, 2019, 8(1): 159–168. https://www.phytojournal.com/archives/2019/vol8issue1/PartC/7-6-441-914.pdf

[28] QADERY M. M., MARTEL A. B., and DIXON S. L. Environmental Factors Influence Plant Vascular System and Water Regulation. *Plants*, 2019, 8: 65. https://doi.org/10.3390/plants8030065

**参考文:**

[1] 农业数据和信息系统中心. 柑橘展望；园艺子部门农产品。 雅加达农业部, 2016.

[2] 农业研究与发展署. 印度尼西亚的农业土地资源广泛、分布和潜在可用性。印度尼西亚农业研究与发展署出版社，雅加达, 2015.

[3] CASTAÑEDA V., 和 GONZÁLEZ E. M. 应用缺水胁迫的策略：蒺藜苜蓿全株代谢水平的异同。国际分子科学杂志, 2021, 22: 2813. https://doi.org/10.3390/ijms22062813

[4] AKBARI M., KATAM R., HUSAIN R., FARAJPOUR M., MAZZUCA S., 和 MAHNA N. 氯化钠诱导出开心果木叶和根抗氧化活性的应激反应。 生物分子, 2020, 10: 189. https://doi.org/10.3390/biom10020189

[5] JUNG J. S., MUHAMMAD Z., LEE K.-W., MUN J. Y., PARK H. S., KIM Y. J., KIM W. H., LEE S.-H., 和 LEE S.-H. 2015. 聚乙二醇诱导水分胁迫对不同高粱基因型生理生化反应的影响。第二十三届国际草原大会（草料生产、生物多样性和环境保护的草原资源的可持续利用）会议记录，新德里, 2015. https://uknowledge.uky.edu/igc/23/4-1-2/4/

[6] CIMEN B., & YESILOGLU T. 柑橘抗非生物胁迫。英泰克，伦敦, 2016. http://dx.doi.org/10.5772/62047

[7] RAHNESHAN Z., NASIBI F., 和 MOGHADAM A.A. 盐分胁迫对两种开心果（真正的开心果)木部分生长、生理、生化参数和养分的影响。 植物相互作用杂志, 2018, 13(1): 73–82. https://doi.org/10.1080/17429145.2018.1424355

[8] AMADOR M. L., SANCHO S., BIELSA B., GOMEZ-APARISI J., 和 RUBIO-CABETAS M. J. 控制李子排水前后耐胁迫的生理生化参数. 植物生理学, 2012, 144: 357–368. https://doi.org/10.1111/j.1399-3054.2012.01568.x

[9] MORALES-OLMEDO M., ORTIZ M., 和 SELLES G. 评论：瞬时土壤渍的影响及其对木选择的重要性。智利农业研究杂志, 2015, 75(1): 45–56. http://dx.doi.org/10.4067/S0718-58392015000300006

[10] BHUYAN M. H. M. B, HASANUZZAMAN M., NAHAR K., AL MAHMUD J., PARVIN K., BHUIYAN T.F., 和 FUJITA M. 土壤酸胁度和的植物行为：洞察形态生理学、生化和分子反应。在：植物非生物胁迫耐受性。施普林格自然，湛, 2019: 35–82. https://doi.org/10.1007/978-3-030-06118-0_2

[11] XIN-YAN L., LIN-TONG Y., YI-BIN L., YE X., 和 LI-SONG C. 在柑橘耐铝性中，摘木和接穗的作用。植物生理学杂志, 2015, 37: 1743. https://doi.org/10.1007/s11738-014-1743-1

[12] DOS SANTOS I. C., DE ALMEIDA A. A. F., PIROVANI C. P., COSTA M. G. C., DA CONCEIÇÃO A. S., FILHO W. S. S., FILHO M. A. C., 和 GESTEIRA A. S. 大田嫁接和非嫁接柑橘植物对干旱条件的生理、生化和分子反应。植物学的环境和实验植物学, 2019, 162: 406-420. https://doi.org/10.1016/j.envexpbot.2019.03.018

[13] ABOUTALEBI A., 和 HASANZADEH H. 盐度和柑橘(橘木)和中间木。国际植物、动物和环境科学杂志, 2014, 4: 654–672. https://www.cabdirect.org/cabdirect/abstract/20143220344

[14] KORDROSTAMI M., & MAFAKHERI M. 水分胁迫和盐分对植物/作物的影响；物理生化和分子缓解方法。在：PESSARAKLI M.（编辑）植物和作物生理学手册。化学橡胶公司出版社，博卡拉顿, 2022: 789–814. https://doi.org/10.1201/9780367554545-37

[15] PADILLA Y. G., GISBERT-MULLOR R., LÓPEZ-SERRANO L., LÓPEZ-GALARZA S., 和 A. CALATAYUD. 嫁接通过改善光合作用和抗氧化防御系统来增强辣椒对

水分胁迫的耐受性。抗氧化剂, 2021, 10(576): 1–15. https://doi.org/10.3390/antiox10040576
[16] PIRASTEH-ANOSHEH H., SAED-MOUCHESHI A., PAKNIYAT H., 和 PESSARAKL M. 气孔对干旱胁迫的反应。在：AHMAD, P.（编辑）水资源压力和作物植物：一种可持续的方法第1卷。约翰威利父子，霍博肯, 2016: 24-40. https://doi.org/10.1002/9781119054450.ch3
[17] OTERO A., 和 GOÑI C. 短缺氧期影响着不同的(名为)柑橘接穗叶片的光合作用。柑橘研究与技术, 2016, 37(1): 19-25. http://dx.doi.org/10.4322/crt.ICC110
[18] LIAO X. Y., YANG L. T., LU Y. B., YE X., 和 CHEN L. S. 在柑橘耐铝性中, 摘木和接穗的作用。植物生理学报, 2015, 37: 1743. https://doi.org/10.1007/s11738-014-1743-1
[19] GANDULLO J., AHMAD S., DARWISH E., KARLOVA R., 和 TESTERINK C. 表型番茄根发育可塑性响应土壤根际盐分。植物表型, 2021, 2021: 1-14. https://doi.org/10.34133/2021/2760532
[20] POLLE A., CHEN S. L., ECKERT C., 和 HARFOUCHE A. 林木抗旱工程。植物科学前沿, 2019, 9: 文章1875. https://doi.org/10.3389/fpls.2018.01875
[21] HORI C., YU X., MORTIMER J. C., SANO R., MATSUMOTO T., KIKUCHI J., DEMURA T., 和 OHTANI M. 非生物胁迫对毛果杨细胞壁生物合成调控的影响。植物生物技术, 2020, 37: 273–283. https://doi.org/10.5511/plantbiotechnology.20.0326a
[22] MARTÍNEZ-CUENCA M. R., PRIMO-CAPELLA A., 和 GINER M. A. F. (柑橘树)生长的影响：对光合作用和碳水化合物分布、植物大小、产量、果实品质和矮化基因型的影响。在：RIGOBELO E. C.（编辑）植物生长。英泰公开赛，伦敦，2016: 107-129. http://dx.doi.org/10.5772/64825
[23] SINGH A., SHARMA M. K., 和 SENGAR R. S. 渗透剂：植物中的脯氨酸代谢作为非生物胁迫的传感器。应用与自然科学杂志, 2017, 9(4): 2079-2092. http://dx.doi.org/10.31018/jans.v9i4.1492
[24] ASHARI A., NURCAHYANI E., HARDOK O., QUDUS I., 和 ZULKIFLI. 体外干旱胁迫条件下诱导无张力溶液后小株橘子石头55(网纹柑橘变种石榴)的脯氨酸含量分析。分析物：分析和环境化学, 2018, 3(01): 69–78. http://dx.doi.org/10.23960%2Faec.v3i1.2018.p
[25] MAHMOUD L. M., DUTT M., VINCENT C. I., 和 GROSSER J. W. 三种推定的耐盐柑橘木的盐度诱导生理反应。园艺科, 2020, 6, 90. https://doi.org/10.3390/horticulturae6040090
[26] ZECHMANN B. 质体的超微结构可作为可靠的非生物和生物胁迫标记。公共科学图书馆一, 2019, 14(4). https://doi.org/10.1371/journal.pone.0214811
[27] PRASAD V., 和 RAO R. 各种植物应对缺水的反应和策略：回顾。生药学与植物化学杂志, 2019, 8(1): 159–168. https://www.phytojournal.com/archives/2019/vol8issue1/PartC/7-6-441-914.pdf
[28] QADERY M. M., MARTEL A. B., 和DIXON S. L. 环境因素影响植物血管系统和水分调节。植物，2019，8: 65. https://doi.org/10.3390/plants8030065