Changes in Physiological and Relative Genes Expression Response of Mandarin Citrus (*Citrus reticulata* Blanco) cv Rimau Gerga Lebong (RGL) Grafted onto Different Citrus Rootstocks

Farida Yulianti¹, Afifuddin Latif Adiredjo², Lita Soetopo², Sumeru Ashari²

**ABSTRACT**

**Background:** RGL mandarin is one of the important mandarin citrus varieties in Indonesia. The tolerance of RGL mandarin citrus to water deficit can be induced by the rootstock. This study aimed to characterize the physiological responses and transcriptional gene expression of RGL mandarin citrus grafted onto three rootstock genotypes during the dry and the rainy seasons.

**Methods:** Three-years-old mandarin citrus trees cv. Rimau Gerga Lebong (RGL) grafted onto three citrus (JC, Cit and K) were planted at the experimental field of the ICISFRI. The experiment was conducted with a randomized block design and each scion-rootstock combination contained five replications. All of the physiological and relative gene expression parameters observed were conducted at the last of the dry season (September 2019) and the mid of the rainy season (January 2020). Water deficit was induced by no watering plants during the dry season.

**Result:** The results of physiological responses and relative gene expression analyses showed that RGL-Cit combination had better than RGL-JC combination and RGL-JC combination had better than RGL-K combination. RGL-Cit combination showed less wilt than RGL-JC and RGL-K combinations. The RGL-Cit combination had the highest stomatal density, stomatal aperture, stomatal conductance and photosynthetic rate in the dry season. Different plant combinations showed different gene expressions. RGL-JC and RGL-K combinations were upregulated in almost all the primers related to drought responses tested in the dry season. RGL-Cit combination only upregulated the PIP1, PIP2 and ACS2 and unregulated the others in the dry season. Therefore, citrumelo seems to be a valuable type of rootstock.

**Key words:** Drought tolerance, Gene expression, Physiology, RGL mandarin, Rootstock.

**INTRODUCTION**

Citrus is one of the important fruit commodities in the world market. Currently, Indonesia is the second-largest citrus importer in Association of Southeast Asian Nations (ASEAN) after Malaysia with import value was 137.585 tons in 2019 (BPS-Statistics Indonesia, 2020). Therefore, accelerating national citrus production will have an important urgency because it is in addition to increasing people’s income, employment opportunities and fruit consumption. Rimau Gerga Lebong (RGL) mandarin is one of the important mandarin citrus varieties in Indonesia that has good characteristics, such as big size, bright skin colors, good taste and high productivity.

Considering the citrus plants are perennial crops, the tolerance of citrus plants to drought stress is very important. Drought stress causes decreasing in plant growth and productivity (Santana-Vieira *et al*., 2016). The physiological responses of scion grafted onto the rootstocks have been reported by several researchers. Sweet orange grafted onto different rootstock presented variability on turgor, osmotic potential, osmotic adjustment and bulk modulus of elasticity in leaves (Gonçalves *et al*., 2016). Grapefruit cv ‘Marsh Seedless’ and ‘Redblush’ grafted on nine rootstocks presented variation gas exchange parameters, relative water content and enzyme content (Sharma *et al*., 2015). Moreover, Santos *et al.* (2019) also reported that ‘Rangpur Santa Cruz’ lime and ‘Sunki Maraviha’ mandarin grafted on different rootstock presented variation in tree physiology, enzyme activity and chlorophyll contents. Hence, selection and improvement of rootstock is a valuable strategy to stabilize plant production in changing climate (Berdeja *et al*., 2015).
Plant response to drought stress is a complex mechanism. In stress conditions, plants adapt with express several gene which is influence biochemical and physiological pathways (Krasensky and Jonak, 2012). Several studies verifying the genes expression in citrus were carried out using the RT-qPCR technique (Gonçalves et al., 2016; Hu et al., 2018; Pajon et al., 2017).

Citrus fruit production areas in Indonesia generally use Japansche citroen (JC) (*Citrus limonia* Osbeck) as a rootstock. Citrumelo (*Citrus paradisi* Macf. cv. Duncan x Poncirus trifoliata (L.) Raf) and Kanci (*Fortunella* sp.) are the potential citrus rootstock to be used in citrus cultivation system in Indonesia. Citrumelo is tolerant to Citrus Tristeza Virus (CTV) (Castle et al., 2000), nematodes, Phytophthora spp., CSD (Bové and Ayres, 2007) and cause the superior fruit quality of scion. The use of *Fortunella* as rootstock caused tree size and canopy volume of citrus scion smaller than other rootstock (Donadio et al., 2019). Unfortunately, there is still no study of the effect of rootstocks on the physiology of citrus plants in Indonesia. Therefore, this study aimed to characterize the physiological and gene expression of mandarin citrus cv RGL grafted onto three different rootstocks genotypes.

**MATERIALS AND METHODS**

Fifteen plants of three-year-old mandarin citrus trees cv. Rimau Gerga Lebong (RGL) were grafted onto three citrus rootstocks (Japansche citroen (JC), Citrumelo (Cit) and Kanci (K)). All plants were planted in the experimental field of the Indonesian Citrus and Subtropical Fruits Research Institute (ICISFRI).

The experiment was conducted with a randomized block design and each scion-rootstock combination contained five replications. All of the parameters observed were conducted at the last of the dry season (September 2019) and the mid of the rainy season (January 2020). In this experiment, the plants were not watered during the dry season to impose water deficit stress.

**Relative gene expression analysis**

**Total RNA isolation**

Total RNA was isolated from the harvested young leaf of each plant observed. The RNA isolation was using Ribospin Plant (Gene All), following the manufacturer’s instructions. The quality and quantity of total RNA were determined by Nano Quant M200 Pro (Tecan).

**The cDNA synthesis**

The isolated RNAs were synthesized into cDNA following the protocol described in the technical manual of iScript™ cDNA Synthesis Kit (Bio Rad).

**Relative gene expression analysis**

The analysis was conducted using RT-qPCR. The 10 µL of each RT-qPCR reaction contained of 5 µL SYBR Green fluorophore (Bio Rad), 0.25 µL (10mM) of each primer (Table 1), 2 µL cDNA and 2.75 µL sterile ultrapure water. The amplification was performed in a CFX-96 Real Time Thermal Cycler (Bio-Rad) equipment with the amplification conditions: 95°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 1 min with the insertion of the melting curve from 65°C to 95°C. The relative genes expression in samples were calculated using a reference gene as the normalizer (Pfaffl, 2001). In this study, the reference gene used was GAPDH. Quantification of the gene expression estimation and clustergram construction were performed by Bio-Rad CFX Maestro software.

**Stomatal Measurement**

Leaf sampling for stomatal analysis was conducted in the morning (08.00 AM) when leaf stomata were expected to open. The twelve mature healthy leaves per plant were sampling from the fourth node of the four cardinal directions of each plant observed. The leaves were cleaned with 70%

**Table 1**: Names of the PCR primer, sequences and gene database accession numbers.

| Name of Primer | Sequences | Gene target and accession number |
|---------------|-----------|---------------------------------|
| CsNF-YA5      | CATTTCAGAATGGGGAAATCAT | Citrus sinensis nuclear transcription factor Y subunit A-4-like (XM_006482808.3) |
|               | TTCTTCCTCACACCAAAAG    |                                  |
| Osmotin       | CAACGACCTGTCCTGCAACACCG| Citrus jambhiri RlemTLP mRNA for thaumatin-like protein (AB467316.1) |
|               | ACGCTGTCGTGATACAAAC    |                                  |
| PIP1          | AGGATTACACGAGAGCCACCCT | Citrus sinensis putative aquaporin (PIP1) (MK08479.1) |
|               | TGTTTTGAGATTTGACCGG    |                                  |
| PIP2          | TGTTGTTGATGTTGCACTTGG  | Citrus sinensis putative aquaporin (PIP2) (MK084797.1) |
|               | TGATGTTGTCACACCCGAGAAG |                                  |
| CHLASE        | ACCGCTTGGGACACTGGAAG   | Citrus sinensis chloroplast chlorophyllase (NM_001288890.1) |
|               | TGTCCATATCTCCTGATG     |                                  |
| ACS2          | CACAGTGTTTCAAGAAGAGCT | Citrus sinensis partial mRNA for ACC synthase (AJ276295.1) |
|               | CGAGTGAATGACCCGACCGTAA |                                  |
| ETR1          | CAGGAGAGAAGCCGAAAACAG | Citrus sinensis partial mRNA for ethylene receptor (AJ276294.1) |
|               | GCTCGGCTGATCATCAGT     |                                  |
| GAPDH         | CGTCTGCTGAGATGACTCT    | Citrus sinensis clone G05 glyceraldehyde 3-phosphate dehydrogenase (GU911363.1) |
|               | GGAGGTCAAGATGCGAATCAA  |                                  |
ethanol. Five small parts of the abaxial leaf surface were coated with transparent nail polish, left for about 10 minutes to dry, swiftly removed using a transparent adhesive tape and then placed on an object slide. The samples observed under a trinocular microscope (Olympus-BX51) at 400x magnification. The density of stomata was counted manually and the stomatal size was measured using Olympus cellSens Standard software attached to the microscopy system.

**Leaf chlorophyll index**

Leaf chlorophyll index was analyzed using a portable “Chlorophyll Content Meter” (CCM-200 plus, Opti-Sciences). Measurements were taken on twelve leaves that have fully opened from the four cardinal directions of each plant observed.

**Gas exchange parameters**

Gas exchange parameters were recorded between 9.00 to 11.00 AM, with the help of a portable photosynthesis meter (ADC type Lcpro-sd, ADC Bioscientific Ltd). Measurements were taken on twelve points of observation from the four cardinal directions of each plant observed.

**Statistical analysis**

Statistical analysis of the average of physiological data was done following the methods of analysis of variance (two-way ANOVA. The means were separated using LSD at $\alpha \leq 0.05$ level of significance.

**RESULTS AND DISCUSSION**

**Relative gene expression analysis**

Water deficit occurred in the soil caused stress in the roots. This stress activated the signaling pathways, altered the gene expression levels and the plant physiology (Yamaguchi-Shinozaki and Shinozaki, 2006). The response of plants to drought stress was influenced by rootstock used. In our study, RGL mandarin grafted onto different rootstocks (JC, Citrumelo and Kanci) resulted different relative gene expression during the dry and the rainy seasons (Fig 1).

For all plant combinations tested, almost all relative gene expressions in the dry season were higher than that in the rainy season. The relative gene expression level among the combinations were different. For almost all primer tested, RGL-K combination resulted the highest value of relative gene expression and RGL-Cit combination resulted the lowest one in the both seasons except in ACS2 and PIP2 primers. The difference of genes expression of the scion showed that rootstocks have different abilities to respond to environmental changes. The difference of relative gene expressions level may be influenced by transcription of RNA, hormones and nutritional components from the rootstock.

The difference expression of each gene presented the difference respond mechanism of the plant to drought stress. The ACS2 and ETR1 induced ethylene productions as respond to drought stress (Agusti et al., 2008). The CHLASE activated the chlorophyllase enzyme to catalyzes the chlorophyll hydrolysis (Distefano et al., 2009). The CsNFYA5 regulated the size of the stomatal apertured and activated stress responsive genes, such as genes involved in oxidative stress response (Li et al., 2008; Petroni et al., 2013). The osmotin was a protein related to osmotic stress tolerance that has a function in osmoregulation and food preservation (Hakim et al., 2018). Water exchange in the plant cells going through the aquaporins or PIP (Yaneff et al., 2015). Under drought stress, the overexpression of PIP genes leading to involve in the control of plant’s transpiration through regulating of water flow (Opazo et al., 2020).

Based on clustergram, the genotypes grouped into two clusters related to the season. The primers grouped into three clusters, the CHLASE, ETR1, CsNFYA5, PIP1 and ACS2 primers grouped into one cluster, whereas osmotin and PIP2 were other clusters. The CHLASE, ETR1, CsNFYA5, PIP1 and ACS2 primers were upregulated in RGL-JC and RGL-K combinations in the dry season and downregulated in the rainy season. RGL-Cit combination only upregulated the PIP1, PIP2 and ACS2 and unregulated the others in the dry season (Fig 2). The overexpression of CHLASE, ETR1, CsNFYA5 and PIP1 in the RGL-JC and RGL-K combinations in the dry season caused physiological changes, such as decreased of chlorophyll index, stomatal apertured and transpiration and caused the plant more wilt than RGL-Cit combination (Fig 3). Drought stress caused harmful changes to physiological, morphological and chlorophyll fluorescence parameters (Ghoulam and Khadraji, 2016; Zadražnik and Šuštar-Vozlič, 2020).
Changes in Physiological and Relative Genes Expression Response of Mandarin Citrus (*Citrus reticulata* Blanco) cv Rimau...

**Stomatal density, stomatal size and percentage of stomata apertured**

The results of the physiological character observations on RGL mandarin are presented in Table 2. Different types of rootstocks caused differences in stomatal density, stomatal size and percentage of stomatal apertured. These differences indicated that rootstock have different responses to drought stress. Drought stimulates decreasing stomatal size and aperture and increasing stomatal density (Arve *et al.*, 2011; Zhao *et al.*, 2015). In this study, drought only affected the stomata density and the percentage of stomatal apertured, while the size of the stomata was not affected.

The stomata density of RGL-JC and RGL-Cit combinations were higher than RGL-K in the dry season but no difference in the rainy season. The percentage of stomatal apertured of RGL-Cit combination in the dry season was higher than that of other combination. In the rainy season it was same with RGL-JC and higher than RGL-K combination. The size of the stomata of RGL-K combination was the lowest in both seasons. The differences of stomatal characters may be influenced by CsNFYA5 gene expression. The overexpression of the CsNFYA5 gene in RGL-JC and RGL-K combinations (Fig 1) induced their stomata to close to reduce the transpiration. The reduced evapotranspiration surface by the increased number of closed stomata was the avoidance mechanism to drought stress (Lestari *et al.*, 2019).

**Leaf chlorophyll index**

In terms of the chlorophyll index character, the leaves chlorophyll index in the rainy season was higher than the dry season. RGL-Cit combination had the highest chlorophyll index in the dry season. RGL-JC combination had the highest chlorophyll index in the rainy season. RGL-K had the lowest chlorophyll index in both of seasons (Table 2). Drought induced senescence of the plants by producing ethylene and hydrolysis of chlorophyll. As explained above that overexpression of CHLASE gene regulated the chlorophyll hydrolysis become phytol and lutein (Distefano *et al.*, 2009), overexpression of the CHLASE gene in RGL-K combination caused this plant had the lowest chlorophyll index. The lowest value of chlorophyll index of RGL-K combination in water deficit stress indicated that this plant was sensitive to drought stress.

**Gas exchange parameters**

The water pressure in the cell (DH$_2$O) of RGL leaves in all combinations did not differ in the same season. The differences occur due to differences of seasons, the value in the rainy season was higher than in the dry season. Using of different rootstocks also has no effect on plant respiration rate. Rootstock affected CO$_2$ concentration in the cell, stomatal conductance and photosynthetic rate. In the dry season, the RGL-JC combination accumulates the least amount of CO$_2$ while in the rainy season it occurs in the RGL-K combination. The accumulation of CO$_2$ in cells affects the stomatal conductance and the rate of photosynthesis. The small accumulation of CO$_2$ causes a small stomatal
Changes in Physiological and Relative Genes Expression Response of Mandarin Citrus (*Citrus reticulata* Blanco) cv Rimau...

### Table 2: Stomatal density, stomatal size, percentage of stomata apertured and chlorophyll index of RGL mandarin.

| Scion-rootstock combination | Stomatal density/mm² | Percentage of stomata apertured (%) | Stomatal size (µm²) | Chlorophyll index |
|-----------------------------|----------------------|-------------------------------------|---------------------|------------------|
| **The dry season**          |                      |                                     |                     |                  |
| RGL-JC                      | 509.5 b              | 36.03 b                             | 75.19 c             | 57.93 b          |
| RGL-Cit                     | 510.3 b              | 46.10 c                             | 74.67 c             | 64.44 c          |
| RGL-K                       | 464.7 a              | 37.37 b                             | 71.03 b             | 48.86 a          |
| **The rainy season**         |                      |                                     |                     |                  |
| RGL-JC                      | 479.9 ab             | 50.90 c                             | 72.73 bc            | 82.25 e          |
| RGL-Cit                     | 486.4 ab             | 48.82 c                             | 67.82 b             | 78.51 d          |
| RGL-K                       | 506.5 b              | 24.47 a                             | 63.99 a             | 51.34 a          |

Means in each column followed by the different letter are significantly different at α<0.05.

### Table 3: Gas exchange parameter of RGL mandarin as responses to water deficit.

| Scion-Rootstock Combination | Leaf H₂O pressure (mbar) | CO₂ substomatal (vpm) | Stomatal conductance mol.m⁻².s⁻¹ | Respiration rate mmol.m⁻².s⁻¹ | Photosynthetic rate mmol.m⁻².s⁻¹ |
|-----------------------------|---------------------------|-----------------------|----------------------------------|-------------------------------|-------------------------------|
| **The dry season**          |                           |                       |                                  |                               |                               |
| RGL-JC                      | 4.033 a                   | 10.67 ab              | 0.062 a                          | 2.00 a                        |                               |
| 3.392 ab                    |                           |                       |                                  |                               |                               |
| RGL-Cit                     | 4.417 a                   | 19.00 c               | 0.105 ab                         | 1.54 a                        | 6.078 c                       |
| RGL-K                       | 5.767 a                   | 15.50 abc             | 0.097 ab                         | 1.41 a                        | 4.940 abc                     |
| **The rainy season**         |                           |                       |                                  |                               |                               |
| RGL-JC                      | 13.38 b                  | 18.17 bc              | 0.171 b                          | 2.12 a                        | 5.838 bc                      |
| RGL-Cit                     | 14.91 b                  | 14.78 abc             | 0.183 b                          | 2.25 a                        | 4.75 abc                      |
| RGL-K                       | 12.97 b                  | 7.92a                 | 0.142 ab                         | 1.97 a                        | 2.551 a                       |

Means in each column followed by the different letter are significantly different at α<0.05.

conductance and respiration rate. In the dry season, the RGL-Cit combination has the highest photosynthesis rate, while in the rainy season it occurs in the RGL-JC combination (Table 3).

The stomatal character and behavior affected the gas exchange directly in the leaf. Stomata act in gas exchange which is necessary for photosynthesis and transpiration (Daszkowska-Golec and Szarejko, 2013). High stomatal density and percentage of stomata apertured increased respiration rate and decreased leaf H₂O pressure. Stomatal closure as a response to water deficit reduced the stomatal conductance. This condition indirectly affected the photosynthetic process. Scion-rootstock combination with high stomatal conductance had high CO₂ content in leaf. High concentrate of CO₂ resulted in high photosynthetic rate. Physiological control of stomatal conductance allowed plants to balance CO₂-uptake for photosynthesis (Haworth et al., 2016). The highest gas exchange parameter value RGL-Cit combination in the dry season indicated that this plant was tolerant to drought stress.

### CONCLUSION

Different rootstock effected different physiological and transcriptional gene expression of RGL mandarin grafted on it. The transcriptional gene expression value affected the physiological changes. Based on physiological, transcriptional gene expression variation and morphological changes, RGL-Cit combination was more drought tolerant than RGL-JC combination and RGL-JC combination was more tolerant than RGL-K combination. The RGL-Cit combination had the highest chlorophyll index, stomatal density, stomatal aperture, stomatal conductance and photosynthetic rate in the dry season. This combination also had the lowest transcriptional gene expression in response to water deficit in the dry season. Therefore, citrumelo seems to be valuable type of rootstock. However different genotypes should be tested in the same layout for its confirmation.

ACKNOWLEDGEMENT

The authors would like to thank to ICISFRI for providing the plant materials and to the IAARD for the financial support of the doctoral study program.

REFERENCES

Agustí, J., Merelo, P., Cercós, M., Tadeo, F.R., and Talón, M. (2008). Ethylene-induced differential gene expression during abscission of citrus leaves. Journal of Experimental Botany. 59(10): 2717-2733. https://doi.org/10.1093/jxb/ern138.

Arve, L., Torre, S., Olsen, J., and Tanino, K. (2011). Stomatal Responses to Drought Stress and Air Humidity. In Abiotic Stress in Plants - Mechanisms and Adaptations (Vol. 395, pp. 116–124). InTech. https://doi.org/10.5772/24661.
Berdeja, M., Nicolas, P., Kappel, C., Dai, Z.W., Hilbert, G., Peccoux, A., Lafontaine, M., Ollat, N., Gomès, E. and Delrot, S. (2015). Water limitation and rootstock genotype interact to alter grape berry metabolism through transcripome reprogramming. Horticulture Research. 2(1): 15012. https://doi.org/10.1038/hortres.2015.12.

Bové, J.M. and Ayres, A.J. (2007). Etiology of Three Recent Diseases of Citrus in São Paulo State: Sudden death, variegated chlorosis and huanglongbing. IUBMB Life. 59(4): 346-354. https://doi.org/10.1080/15216540701293926.

BPS-Statistics Indonesia. (2020). Statistics of Annual Fruit and Vegetable Plants (Indonesia).

Castle, W.S., Stover, E. and Castle, B.B. (2000). Rootstock Reflections / Swingle Citrumelo Update Rootstock Reflections / Swingle citrumelo update. Citrus Industry. 81: 18-20.

Daszkowska-Golec, A. and Szarejko, I. (2013). Open or Close the Gate - Stomata Action under the Control of Phytohormones in Drought Stress Conditions. Frontiers in Plant Science. 4(MAY): 1-16. https://doi.org/10.3389/fpls.2013.00138.

Distefano, G., Casas, G., Las, Caruso, M., Todaro, A., Rapisarda, P., La Malfa, S., Gentile, A., Tribulato, E., Giuseppina, L., Caruso, D., Todaro, A., Rapisarda, P., Malfa, S.L.A., Gentile, A. and Tribulato, E. (2009). Physiological and molecular analysis of the maturation process in fruits of clementine mandarin and one of its late-ripening mutants. Journal of Agricultural and Food Chemistry. 57(17): 7974-7982. https://doi.org/10.1021/jf900710v.

Donadio, L.C., Lederman, I.E., Roberto, S.R. and Stucchi, E.S. (2019). Dwarfing-canopy and rootstock cultivars for fruit trees. Revista Brasileira de Fruticultura. 41(3): 1-12. https://doi.org/10.1590/0100-29452019997.

Ghoulam, C. and Khadraji, A. (2016). Effect of drought on growth, physiological and biochemical processes of chickpea- rhizobia symbiosis. Legume Research - An International Journal. 40(OF): 94-99. https://doi.org/10.18805/lr.v0iof.3771.

Gonçalves, L.P., Alves, T.F.O., Martins, C.P.S., de Sousa, A.O., dos Santos, I.C., Pirovani, C.P., Almeida, A.A.F., Filho, M.A.C., Gesteira, A.S., Soares Filho, W. dos S., Girardi, E.A. and Costa, M.G.C. (2016). Rootstock-induced physiological and biochemical mechanisms of drought tolerance in sweet orange. Acta Physiologiae Plantarum. 38(7). https://doi.org/10.1007/s11738-016-2198-3.

Gong, X., Zhang, J. and Liu, J.H. (2014). A stress responsive gene of Fortunella crassifolia FcSISP functions in salt stress resistance. Plant Physiology and Biochemistry. 83: 10-19. https://doi.org/10.1016/j.plaphy.2014.07.003.

Hakim, Ullah, A., Hussain, A., Shaban, M., Khan, A.H., Alarjqi, M., Gul, S., Jun, Z., Lin, S., Li, J., Jin, S. and Munis, M.F.H. (2018). Osmotin: A plant defense tool against biotic and abiotic stresses. Plant Physiology and Biochemistry. 123 (December). 149-159. https://doi.org/10.1016/j.plaphy.2017.12.012.

Haworth, M., Killi, D., Materassi, A., Raschi, A. and Centritto, M. (2016). Impaired stomatal control is associated with reduced photosynthetic physiology in crop species grown at elevated [CO₂]. Frontiers in Plant Science. 7: 1-13. https://doi.org/10.3389/fpls.2016.01568.

Hu, J., Jiang, J. and Wang, N. (2018). Control of citrus huanglongbing via trunk injection of plant defense activators and antibiotics. Phytopathology. 108(2): 186-195. https://doi.org/10.1094/PHYTO-05-17-0175-R.

Krasensky, J. and Jonak, C. (2012). Drought, salt and temperature stress-induced metabolic rearrangements and regulatory networks. Journal of Experimental Botany. 63(4): 1593-1608. https://doi.org/10.1038/jxb.err460.

Lestari, M.W., Arfarita, N., Sharma, A. and Purkait, B. (2019). Tolerance mechanisms of Indonesian plant varieties of yardlong beans (Vigna unguiculata sub sp. sesquipedalis) against drought stress. Indian Journal of Agricultural Research. 53(2): 223-227. https://doi.org/10.18805/IJARe.A-369.

Li, W.X., Oono, Y., Zhu, J., He, X.J., Wu, J.M., Iida, K., Lu, X.Y., Cui, X., Jin, H. and Zhu, J.K. (2008). The Arabidopsis NFYA5 transcription factor is regulated transcriptionally and posttranscriptionally to promote drought resistance. Plant Cell. 20(8): 2238-2251. https://doi.org/10.1105/tpc.108.059444.

Mahantesh, S., Ramesh Babu, H.N., Ghanthi, K. and Raddy, P.C. (2018). Identification of drought tolerant genotypes based on physiological, biomass and yield response in groundnut (Arachis hypogaea L.). Indian Journal of Agricultural Research. 52(3): 221-227. https://doi.org/10.18805/IJARe.A-4984.

Opazo, I., Toro, G., Salvatierra, A., Pastenes, C. and Pimentel, P. (2020). Rootstocks modulate the physiology and growth responses to water deficit and long-term recovery in grafted stone fruit trees. Agricultural Water Management. 228: 105897. https://doi.org/10.1016/j.agwat.2019.105897.

Pajon, M., Febres, V.J. and Moore, G.A. (2017). Expression patterns of flowering genes in leaves of ‘Pineapple’ sweet orange [Citrus sinensis (L.) Osbeck] and pummelo (Citrus grandis Osbeck). BMC Plant Biology. 17(1): 146. https://doi.org/10.1186/s12870-017-1094-3.

Petroni, K., Kummoto, R.W., Gnesutta, N., Calvenzani, V., Fornari, M., Tonelli, C., Holt, B.F. and Mantovani, R. (2013). The promiscuous life of plant NUCLEAR FACTOR Y transcription factors. Plant Cell. 24(12): 4777-4792. https://doi.org/10.1105/tpc.112.105734.

Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research. 29(9): 45-45. https://doi.org/10.1093/nar/29.9.e45.

Santana-Vieira, D.D.S., Freschi, L., Da Hora Almeida, L.A., Moraes, D.H.S. De, Neves, D. M., Dos Santos, L.M., Bertolde, F.Z., Soares Filho, W.D.S., Coelho Filho, M.A. and Gesteira, A.D.S. (2016). Survival strategies of citrus rootstock cultivars for fruit trees. Revista Brasileira de Fruticultura. 41(3): 1-12. https://doi.org/10.18805/lr.v0iof.3771.

Santos, I.C. dos, Almeida, A.A.F. de, Pirovani, C.P., Costa, M.G.C., Petroni, K., Kumamoto, R.W., Gnesutta, N., Calvenzani, V., Fornari, M., Tonelli, C., Holt, B.F. and Mantovani, R. (2013). The promiscuous life of plant NUCLEAR FACTOR Y transcription factors. Plant Cell. 24(12): 4777-4792. https://doi.org/10.1105/tpc.112.105734.

Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research. 29(9): 45-45. https://doi.org/10.1093/nar/29.9.e45.

Santana-Vieira, D.D.S., Freschi, L., Da Hora Almeida, L.A., Moraes, D.H.S. De, Neves, D. M., Dos Santos, L.M., Bertolde, F.Z., Soares Filho, W.D.S., Coelho Filho, M.A. and Gesteira, A.D.S. (2016). Survival strategies of citrus rootstock cultivars for fruit trees. Revista Brasileira de Fruticultura. 41(3): 1-12. https://doi.org/10.18805/lr.v0iof.3771.
Sharma, R.M., Dubey, A.K. and Awasthi, O.P. (2015). Physiology of grapefruit (Citrus paradisi Macf.) cultivars as affected by rootstock. The Journal of Horticultural Science and Biotechnology. 90(3): 325-331. https://doi.org/10.1080/14620316.2015.11513190.

Tan, F., Tu, H., Liang, W., Long, J., Wu, X., Zhang, H. and Guo, W. (2015). Comparative metabolic and transcriptional analysis of a doubled diploid and its diploid citrus rootstock (C. junos cv. Ziyang xiangcheng) suggests its potential value for stress resistance improvement. BMC Plant Biology. 15(1): 89. https://doi.org/10.1186/s12870-015-0450-4.

Wei, X., Chen, C., Yu, Q., Gady, A., Yu, Y., Liang, G. and Gmitter, F.G. (2014). Novel expression patterns of carotenoid pathway-related genes in citrus leaves and maturing fruits. Tree Genetics and Genomes. 10(3): 439-448. https://doi.org/10.1007/s11295-013-0688-7.

Yamaguchi-Shinozaki, K., and Shinozaki, K. (2006). Transcriptional Regulatory Networks in Cellular Responses and Tolerance To Dehydration and Cold Stresses. Annual Review of Plant Biology. 57(1): 781-803. https://doi.org/10.1146/annurev.arplant.57.032905.105444.

Yanoff, A., Vitali, V., and Amodeo, G. (2015). PIP1 aquaporins: Intrinsic water channels or PIP2 aquaporin modulators? FEBS Letters, 589(23): 3508-3515. https://doi.org/10.1016/j.febslet.2015.10.018.

Zadražnik, T., and Šuštar-Vozliè, J. (2020). Impact of drought stress on physiological characteristics and isolation of chloroplasts in common bean (Phaseolus vulgaris L.). Legume Research. 43(1): 50-55. https://doi.org/10.18805/LR-455.

Zhao, W., Sun, Y., Kjelgren, R. and Liu, X. (2015). Response of stomatal density and bound gas exchange in leaves of maize to soil water deficit. Acta Physiologiae Plantarum. 37(1). https://doi.org/10.1007/s11738-014-1704-8.