The production and tolerance level of 22 accessions of Sukabumi robusta coffee to water stress

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Abstract. Extensification of the Robusta coffee to enhance national productivity can be expanded to marginal areas with limited soil water. Robusta coffee clones that have a good tolerance for these conditions are needed. This study aimed to evaluate production and tolerance level of 22 Sukabumi Robusta coffee accessions to water stress. The study was conducted in a greenhouse at Pakuwon Experimental Station, altitude of 450 m asl with Latosol type of soil and B type of climate according to Schmidt and Fergusson classification, start from January 2015 until July 2016. Randomized complete block design with 22 treatments of Robusta coffee accessions and two replications was performed. Variables observed were the height of seedlings, number of leaves, and diameter of stems at 15 and 45 days after water stress treatment (DAS), as well as the percentage of viable seedlings at 60 and 75 DAS. The results showed that seven Robusta coffee accessions were classified as high production accessions (ROS 212, 335, 337, 508, 514, 519, and ROS 520), while two accessions were classified as tolerant to water stress (ROS 010 and ROS 507). However, there is no accession that classified as high production and tolerant to water stress.

1. Introduction
Breeding programs to produce superior varieties of coffee require genetic resources with characteristics that support the purpose. Genetic resources with desirable traits need to be identified and discovered through a series of activities including exploration, collection, characterization and evaluation of germplasms. Germplasms evaluation activities are more directed at examining genetic materials against biotic and abiotic stresses. Of which, biotic stresses are caused by plant pests and diseases, while abiotic stresses are caused by limited environmental conditions (land, water, climate, etc.).

In Indonesia, the breeding activities of Robusta coffee had begun since the 1920s, about 20 years after being introduced to Indonesia. Robusta coffee introduced to Indonesia were classified as Coffea canephora, or C. canephora Pierre var. Robusta Cheval, originated from Congo and Guinea, with different characteristics. Another type of Robusta coffee, namely C. canephora Quilou was also introduced to Indonesia. Before the Second World War, Besoekish Proefstation, a research institute located in Jember, East Java had been performed research on developing superior variety of coffee and produced several superior Robusta coffee clones, e.g. BP 39 and BP 42 which were often used as standard clones. In the 1940s, BP 42 clone was considered as one of the Robusta coffee clones recommended to be planted on a large scale in Indonesia. One of the advantages of BP 42 clone is relatively tolerant to drought [1].
Indonesian Industrial and Beverage Crops Research Institute (IIBCRI) of IAARD, Ministry of Agriculture that located in Sukabumi, collected several Robusta coffee accessions which were generated from seedlings obtained from farmer’s garden in Sukabumi area. Observation on accession numbers of collected Robusta coffee in 2015 at Pakuwon experimental station, showed that 22 accession numbers revealed in high production (>3 kg/plant). However, others genetic character e.g. the potential source for water stress tolerant has not been evaluated, yet.

The availability of Robusta coffee genetic materials with high productivity and tolerant to water stress conditions would be able to support the enhancement of national Robusta coffee productivity. In addition, the selected genetic materials are a source of germplasms that can be utilized to assembly the desired variety of Robusta coffee. The chances to obtain Robusta coffee genetic materials tolerant to drought are relatively open, considering that this type of coffee has an obligate open pollination which led to a high genetic variation on morphological, physiological, and biochemical characters[2]. This study aimed to analyze the production and tolerance level of 22 accessions of Sukabumi Robusta coffee under water stress condition.

2. Materials and methods

The research was conducted from January 2015 to July 2016, at Pakuwon Experimental Garden, IIBCRI, Sukabumi, West Java, at altitude of 450 m asl with Latosol soil types and B climate types (Schmidt and Fergusson).

2.1. Plant materials

The genetic material of Robusta coffee was obtained from farmers’ garden in South Sukabumi. This coffee plant is a descendant of the 3rd generation, of which the propagation for each generation was conducted through seedlings (generative). For each generation, farmers selected from several coffee plants that are considered to have high productivity. According to the farmers, the basic genetic material of Robusta coffee they developed was obtained from the Sukabumi Plantation Institute, and the clone was a BP 42 previously obtained from Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember.

The plant materials used was 22 accessions of Robusta coffee obtained from individual selection of 1115 plants derived from seedlings as basic genetic materials. The selection was conducted in 2015 with the criteria of fresh cherries production (>3 kg /plant). Based on the production data, a frequency distribution was made to classify the level of production/plant to be low, medium and high. Furthermore, the 22 selected accessions were propagated vegetatively through rooted cuttings, and the propagated seedlings were then evaluated for their resistance to water stress. The normal growth seedlings (after 2 months in the nursery) were then transferred to a black polybag with a size of 25 x 15 cm which contains planting medium of a soil mixture with cow manure (1: 1). The mixing of planting media was carried out evenly and then sifted with a rather coarse sieve in order to obtain good and relatively uniform planting media. Each polybag was filled with planting media up to 2/3 of the polybag, and then the seedlings were planted carefully to prevent the roots from being cut off. The seedlings were well maintained until they grow optimally at the age of 5 months after planting in a polybag. Furthermore, the seedlings were treated to water stressed.

2.2. Research Design and Data Analysis

The experiment was arranged in Randomized Complete Block Design (RCBD) with 22 treatments of Robusta coffee accessions which were repeated twice, and each experimental plot consisted of five plants. These 22 accessions of Robusta coffee generated from a selection conducted in 2015, which was then propagated through rooted cuttings. The 22 accessions of Robusta coffee used in this study were: (1) ROS 002, (2) ROS 010, (3) ROS 132, (4) ROS 212, (5) ROS 295, (6) ROS 307, (7) ROS 335, (8) ROS 337, (9) ROS 403, (10) ROS 436, (11) ROS 446, (12) ROS 504, (13) ROS 507, (14)
ROS 508, (15) ROS 509, (16) ROS 510, (17) ROS 511, (18) ROS 514, (19) ROS 516, (20) ROS 519, (21) ROS 520, and (22) ROS 523.

Water stress treatment was carried out by not watering the seedlings for about 75 days. Observations were carried out on two parameters: (1) Vegetative characters (plant height, number of leaves and stem diameter at 15 and 45 days after water stress treatment/DAS), and (2) Seeds viability (percentage of alive seedlings at 60 and 75 DAS). The first variable was analyzed by ANOVA, while the 2nd variable was analyzed using a frequency distribution to classify the tolerance level of coffee accessions to water stress. The tolerance levels to water stress are divided into three classifications, namely intolerance, moderately tolerant, and tolerant.

3. Results and discussion

3.1. Production Classification of Parental Genotypes for Accession Selection

Based on the production data of fresh cherry/plant, originating from 1115 parental genotypes, which is ranging from 3.05 to 5.30 kg/plant, a frequency distribution can be classified into three, i.e. low (3.05-3.79 kg), medium (3.80-4.55 kg), and high (4.56-5.30 kg) (Table 1).

Table 1. Frequency distribution to determine the level of Sukabumi Robusta coffee production

| No. | The range of fresh cherry production/plant (kg) | Classification of production level |
|-----|-----------------------------------------------|-----------------------------------|
| 1.  | 3.05 – 3.79                                   | Low                               |
| 2.  | 3.80 – 4.55                                   | Medium                            |
| 3.  | 4.56 – 5.30                                   | High                              |

Through frequency distributions as presented in Table 1, we could determine the classification of production from selected Robusta coffee accessions. We found seven accessions classified as the high production (ROS 212, 335, 337, 508, 514, 519, and ROS 520), 4 accessions as the medium production (ROS 307, 403, 504, and 523), and the remaining 11 accessions were low production (Figure 1). The high diversity in the production of 22 coffee accessions may due to generative (seedling) propagation of the genetic material of Robusta coffee through 3 generations, Robusta coffee is a type of cross-pollinated coffee, thus the progenies derived from generative propagated remained vary. This variation will be shown in various characters including production and their resistance to water stress [2–5].
3.2. Effect of Water Stress on Seedlings Growth

The results of the analysis showed that vegetative growth (seed height, number of leaves, and stem diameter) up to 45 DAS were not significantly different for the 22 accessions tested (Table 2). It could be due to the soil conditions up to 45 DAS are still able to hold water even though the amount is relatively limited, therefore the vegetative growth of coffee seedlings has not been affected, yet. Furthermore, this research was conducted in a greenhouse of which the evapotranspiration process was not too high as compared to the field, thus the water loss runs slowly. Therefore, tolerance level of 22 coffee accessions to water stress could not detect at vegetative growth significantly. This research was in accordance to several researchers of which the coffee plants that are considered tolerant to water stress conditions was generally shows specific and different morphological, physiological, and biochemical characters from intolerant ones [6–9]. The response of coffee accessions tested to water stress is likely to be shown after the water stress continued to higher level, which indicated by the seedling leaves begun to turn yellow, and then dried and eventually died.

Table 2. Height, number of leaves, and stem diameter of seedlings of 22 Sukabumi Robusta coffee accessions at 15 and 45 days after water stress treatment (DAS)

| Coffee accessions | 15 DAS | 45 DAS |
|------------------|--------|--------|
|                  | Seedlings height | No. of leaves | Stem diameter | Seedlings height | No. of leaves | Stem diameter |
| ROS 002          | 66.72   | 31.78  | 7.47      | 63.84         | 23.24         | 7.72         |
| ROS 010          | 62.85   | 27.83  | 7.34      | 62.14         | 21.36         | 7.61         |
| ROS 132          | 63.20   | 25.29  | 7.42      | 61.25         | 21.42         | 7.34         |
| ROS 212          | 64.30   | 21.87  | 7.06      | 61.68         | 20.14         | 7.40         |
| ROS 295          | 62.96   | 22.92  | 7.35      | 58.23         | 22.69         | 7.46         |
| ROS 307          | 65.12   | 24.91  | 7.46      | 62.82         | 22.38         | 8.03         |
| ROS 335          | 64.35   | 23.11  | 7.12      | 61.32         | 22.19         | 6.93         |
| ROS 337          | 64.57   | 25.00  | 7.46      | 62.84         | 22.71         | 7.80         |
| ROS 403          | 61.91   | 24.40  | 7.53      | 60.54         | 20.52         | 7.76         |
| ROS 436          | 63.12   | 23.87  | 7.47      | 61.57         | 19.24         | 7.31         |
Scientists divided plant tolerance to drought into several basic groups, namely drought avoidance, drought tolerance, drought escape [9–11], and drought recovery [9,10]. Plant species can have a different response to drought through: (1) the mechanism of rapid yield, (2) reduce the process of dehydration by limiting growth, and (3) delay dehydration by growing deep roots and/or through the effectiveness of controlling water loss such as the process of stomatal closure and reduction in leaf area, thus it could be improved the status of plant water and maintain turgor [3,6,8,12].

### 3.3. Classification of Production and Tolerance Level to Water Stress

Up to 60 days after water stress treatment (DAS), the seedlings of the 22 accessions tested did not show any drought symptoms based on the viability of the seedlings. The seedlings tested were still 100% alive (data not shown), even though the soil conditions of growing medium were already dry.

Data analysis based on frequency distribution of alive seedling at 75 DAS resulted in three classifications of tolerance levels to water stress, e.g. intolerant, moderately tolerant, and tolerant (Table 3). The results of the subsequent analysis based on the average value of the two replications showed that the 22 Robusta coffee accessions tested were classified into two classes, namely intolerant and moderately tolerant. Two accessions (ROS 010 and ROS 507) were moderately tolerant, while the remaining 20 accessions were intolerance (Table 4; Figure 2).

**Table 3. Frequency distribution to determine the tolerance level of Sukabumi Robusta coffee seedlings to water stress**

| No. | The range of alive seedlings (%) | Classification of tolerance level |
|-----|----------------------------------|----------------------------------|
| 1.  | 0 – 20                           | Intolerant                       |
| 2.  | 21 – 40                          | Moderately tolerant              |
| 3.  | 41 – 60                          | Tolerant                         |

Note: Minimum value = 0 % (no seedlings lives), and maximum value = 60% (3 live seedlings)
Table 4. Production and tolerance level of 22 Sukabumi Robusta coffee accessions to water stress

| No. | Coffee accessions | Fresh cherry production (kg/plant) | Alive seedlings at 75 DAS (%) | Tolerance level to water stress |
|-----|-------------------|------------------------------------|------------------------------|-------------------------------|
| 1.  | ROS 002           | 3.05 *                             | 20.00                        | Intolerant                    |
| 2.  | ROS 010           | 3.60 *                             | 30.00                        | Moderately tolerant           |
| 3.  | ROS 132           | 3.60 *                             | 10.00                        | Intolerant                    |
| 4.  | ROS 212           | 5.00 ***                           | 20.00                        | Intolerant                    |
| 5.  | ROS 295           | 3.60 *                             | 0.00                         | Intolerant                    |
| 6.  | ROS 307           | 4.30 **                            | 20.00                        | Intolerant                    |
| 7.  | ROS 335           | 4.70 ***                           | 0.00                         | Intolerant                    |
| 8.  | ROS 337           | 4.80 ***                           | 0.00                         | Intolerant                    |
| 9.  | ROS 403           | 3.90 **                            | 0.00                         | Intolerant                    |
| 10. | ROS 436           | 3.60 *                             | 10.00                        | Intolerant                    |
| 11. | ROS 446           | 3.60 *                             | 0.00                         | Intolerant                    |
| 12. | ROS 504           | 3.80 **                            | 10.00                        | Intolerant                    |
| 13. | ROS 507           | 3.10 *                             | 30.00                        | Moderately tolerant           |
| 14. | ROS 508           | 4.70 ***                           | 10.00                        | Intolerant                    |
| 15. | ROS 509           | 3.25 *                             | 10.00                        | Intolerant                    |
| 16. | ROS 510           | 3.10 *                             | 10.00                        | Intolerant                    |
| 17. | ROS 511           | 3.25 *                             | 0.00                         | Intolerant                    |
| 18. | ROS 514           | 4.80 ***                           | 0.00                         | Intolerant                    |
| 19. | ROS 516           | 3.60 *                             | 0.00                         | Intolerant                    |
| 20. | ROS 519           | 5.30 ***                           | 10.00                        | Intolerant                    |
| 21. | ROS 520           | 4.95 ***                           | 0.00                         | Intolerant                    |
| 22. | ROS 523           | 4.35 **                            | 10.00                        | Intolerant                    |
|     | Average           | 4.00                               | 8.59                         |                               |

Note: *** = high production; ** = medium production; * = low production; DAS = days after water stress treatment

Figure 2. Performance of Robusta coffee accessions with varying tolerance levels to water stress

The study on tolerance to water stress is important for plant, because water is the largest part of protoplasm, (85-90%), and plays an important role in photosynthesis and other physiological
processes. Water is also a solvent for salts, gases, and other materials that move in plant tissues through cell walls and other tissues. The availability of water is important to ensure the presence of turgidity, cell growth, stability of leaf shape, and the process of opening and closing stomata, as well as the continuity of movement of plant structures. Water stress will affect the rate of plant growth, reduce yield, and in extreme conditions cause plants dead. Anim-Kwapong, Anim-Kwapong and Adomako [13] suggested that the presence of "leaf-scorching" (leaves damage due to water stress) in coffee plants could be used as an important attribute in assessing the tolerance level of coffee plants to drought.

Water requirements for plants vary depending on several factors, including differences in plant species, plant growth and development, soil moisture content, and other climate elements. Plants that experience drought stress can be caused of lack of water supply in plant root areas and excessive water demand by leaves due to the rate of evapotranspiration exceeding the rate of water absorption by plant roots. In the field, even though enough water in the ground, plants can experience water stress if the absorption rate of water by the plant roots is lower than the loss of water through the transpiration process [14]. The ability of plants to minimize water loss could be managed through the stomata mechanism. The process of transpiration is a factor that has a major contribution in the concept of plant tolerance to drought [15,16].

Based on Table 4 which combines the production ability and tolerance to water stress demonstrated that no single accession of Robusta coffee tested was categorized as high production and tolerance to water stress. However, knowing coffee accessions that have high productivity or accessions that have moderate levels of tolerance are valuable information in an effort to enrich the collection of Robusta coffee germplasms. Selected coffee accessions, either high production or tolerance to water stress, are basic genetic materials that can be used as genetic stock in the crossing program.

This study was a preliminary research which was conducted at the greenhouse under controlled environmental conditions. Therefore, in order to obtain more convincing results, needs to be developed further study in the field. This research is in accordance to [6], of which in general, the research on coffee drought tolerance carried out in greenhouses, but the results are often extrapolated to the field level. Therefore, with various levels of field conditions and many other influential factors, further research should be carried out in the field with various levels of field conditions and any other influenced factor. In addition, the observed parameters need to be added because according to [15] [6] [8] [17] [18] [2] [19] [20] and [9], in drought tolerance of coffee plants, it is important to observe leaf and root morphology, root and shoot systems, water potential of soil and plants, proline and abscisic acid (ABA) contents, and dry matters of plant, as well as photosynthesis, respiration, photorespiration, and antioxidant systems.

4. Conclusion

Sukabumi Robusta coffee seedlings showed their response to water stress at 75 DAS, which demonstrated by dried seedlings and then died. The results showed that there were seven Sukabumi Robusta coffee accessions (ROS) classified into high production (ROS 212, 335, 337, 508, 514, 519, and ROS 520), and two accessions classified as moderately tolerant to water stress (ROS 010 and ROS 507). No single accession that can be classified as high production and tolerant to water stress.

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