Abstract: MR219 transgenic rice line which overexpressed an auxin-binding protein (ABP57) and its wild-type cultivar, MR219, were screened under well-watered (WW) and drought stress (DS) conditions at the early reproductive stage. This study was conducted with the standard planting distance and under a normal environment to assess the yield advantages based on the field conditions. The aim of this study was to understand the response of these rice genotypes towards DS at morpho-physiological, biochemical, and agronomical levels. It was found that the DS had affected all these levels of the genotypes studied; however, the transgenic plant showed a higher number of tillers, flag leaf area, biomass, relative water content, total chlorophyll content, and antioxidative defense mechanism than the MR219 under DS. Compared to its wild-type, the transgenic plant showed an increased leaf photosynthetic rate by 7% under WW and 11% under DS. The transgenic plant also showed higher yields than MR219 under the WW (10%) and DS (59%). The results propose that drought tolerance is significantly improved in the MR219 transgenic rice line. It may develop a new opportunity for the drought-tolerant rice breeding programme via overexpression of ABP57.

Keywords: antioxidant enzyme; auxin-binding protein; drought tolerance; transgenic rice
gene, ABP57 [7]. The auxin-binding protein, ABP57, is known to be able to activate the plasma membrane H^+\text{-}ATPase that is important for cellular expansion, intracellular pH regulation, salinity tolerance, secondary transport, stomata aperture and osmoregulation [8]. The contribution of auxin and plasma membrane H^+\text{-}ATPase in the plant abiotic stress is thought to be involved in the drought tolerance mechanisms in plants [9,10]. A previous study has revealed that the activation of plasma membrane H^+\text{-}ATPase through the binding of auxin and ABP57 altered the auxin transport in the plant root architecture [9]. This response enhances the process of proton secretion which is prominent to sustain root elongation under drought conditions in drought-tolerant plants [10–13]. Ultimately, this process enables the roots to move towards moist soil region and absorb more water, consequently increasing drought tolerance in rice. Thus, expressing this auxin-binding protein gene into the rice variety may enhance its performance under drought stress.

Previously, we found that overexpressing ABP57 conferred improved physiological performance during drought stress in Arabidopsis [10]. The wild-type and non-transformant plants experienced severe wilting when exposed to 150 mM mannitol. In comparison, the transgenic line showed mild symptoms and retained higher chlorophyll content for two weeks. The osmoregulation of the transgenic plants was better than that of the control, indicating that the transgenic line was more capable to maintain osmotic homeostasis in the leaves. The transgenic line also showed higher biomass than the control plants, indicating that the ABP57 was involved in the plant growth process under drought stress.

Oryza sativa L. cv. MR219 is a commercial Malaysian Indica rice variety that is identified as susceptible to drought stress. Its growth is highly disrupted under high drought stress. Interestingly, the drought tolerance of MR219 improved when ABP57 was inserted into its genome to create the transgenic MR219, which was tested under drought conditions [14]. Our previous study proposed that ABP57 was involved in stimulating bioactive auxin which then triggered the development of lateral root, conferring drought stress tolerance [15]. Rice plants exposed to severe drought stress intensity may increase the level of auxin transport in the root tips, thus improving drought tolerance in rice.

Overall, transgenic plants overexpressing ABP57 gene show better drought tolerance than its wild-type. This tolerance may help in improving the yield of drought-susceptible varieties even when facing drought stress. In this study, we evaluated the growth performance, physiological responses and antioxidant enzyme activities between transgenic MR219 (overexpressing ABP57 gene) and wild-type MR219 from a previous study [15] under drought stress conditions to examine the effects of ABP57 on improving the drought tolerance in rice.

2. Materials and Methods

2.1. Plant Materials

MR219 transgenic rice line and MR219 were planted in a confined field trial at the Universiti Kebangsaan Malaysia in January–April 2020, with an average environmental temperature of 32 °C. The MR219 transgenic line was derived from the preliminary study on developing drought-tolerant rice plants using Agrobacterium-mediated transformation [15]. The seedlings were planted in a tray and the 21-day old seedlings were then transplanted into a confined field trial.

2.2. Treatment

The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replications. The plot size was 5 × 8 m and was separated into two plots by a concrete barrier. Three seedlings were planted at a hill spacing of 0.30 × 0.30 m in each replication. The plot was filled with clay loam soil with pH 7.5, 12% sand, 46% silt and 50% clay. A basal application, N:P:K (150:60:50) kg ha\(^{-1}\) was applied. Nitrogen in a quantity of 1/3 was applied on three occasions at the basal, mid-tillering and panicle initiation stages. Insecticides and fungicides were applied prior to the nitrogen application to avoid damages from rice pests and blast disease. From 30 days after transplanting (DAT) until maturity, well-watered (WW) and drought stress (DS) treatments were applied. In the WW plot, ±5 cm
standing water was maintained. The DS treatment was imposed by draining out water at the early reproductive stage (30 DAT) and the soil water potential was sustained at $-30 \pm 5$ kilopascals (kPa). In the DS plot, three tensiometers (Takemura DM 8, Japan) were installed to regularly check the soil water potential at 30–40 cm depth and the detailed readings were recorded every day, every 3 h from 8:00 to 17:00. When the tensiometer readings reached the desired values, the plants were re-irrigated to retain the water potential values in the DS plot.

2.3. Morphological Analyses

Nine hills from each treatment were selected for sampling. At the maturity stage, the plant growth was measured based on the number of tillers (NOT), plant height (PH), and flag leaf area (FLA). The FLA was calculated by multiplying the leaf length and leaf width and the calibration factor (The calibration factor used for all cereal crops was 0.75). After harvesting, culms and leaves dry weight (CLDW), panicles dry weight (PDW), and total dry weight (TDW) of the whole plant were measured. Each plant part was dried at 80 °C up to a constant weight to record the dry weights measurement.

2.4. Physiological Analyses

Fresh leaf samples of 0.5 g for each genotype per treatment were kept in distilled water in a petri dish for 4 h to obtain the turgid weight. Then, each leaf sample was put in the oven for 48 h at 80 °C to attain the fixed weight. All measurements were made using an analytical scale. The values of the fresh weight (FW), turgid weight (TW) and dry weight (DW) were used to calculate the relative water content (RWC) following the protocols outlined by Turner [16].

Fresh leaf samples (0.2 g) were cut into 0.5 cm pieces to determine the contents of leaf chlorophyll. The leaf samples of each genotype were kept overnight in 25 mL tube with 80% acetone at $-10$ °C. The mixture was centrifuged at 12,000×g for 10 min and the absorbance reading of the supernatant obtained against an acetone blank was measured at 645 and 663 nm using an Eppendorf Biophotometer 6131 (Hamburg, Germany). The contents of the chlorophyll were calculated based on the method by Ashraf et al. [17].

The rate of photosynthesis ($P_n$) and stomatal conductance ($g_s$) was measured over a period of four months during the experiment at the peak flowering stage (85 DAT) using a portable infrared gas analyser (IRGA) systems (LI-COR-6400; LI-COR Inc., Lincoln, NE, USA). The setting of the CO$_2$ flow rate was 400 µmol m$^{-2}$s$^{-1}$ and the saturating photosynthetic photon flux density (PPFD) was 900 mmol m$^{-2}$s$^{-1}$. Three measurements were carried out using different plants in each treatment.

2.5. Biochemical Analyses

2.5.1. Proline Content

The free proline was extracted from 0.2 g fresh leaf sample by keeping it in 3% (w/v) aqueous sulfosalicylic acid. The mixture was boiled for 1 h at 100 °C and then, kept frozen in ice with added 4 mL toluene. The mixture was left to stand for 10 min. The absorbance developed by the red colour product was read at 520 nm against a toluene blank as reported by Bates et al. [18].

2.5.2. Antioxidant Enzymes Assay

The protein extraction was carried following the method by Aebi [19]. Third or fourth fully expanded leaves from the tip of the shoots in four plants per genotype in each treatment were sampled to extract enzymes at the peak flowering stage (85 DAT).

A total of 0.5 g of ground leaves samples were mixed with 5 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 0.4 M EDTA, 1 M ascorbate and 2% (w/v) polyvinylpyrrolidone. The crude mixture was homogenized at 15,000×g for one minute and centrifuged at 15,000×g for 20 min. The supernatant was used to determine the antioxidant enzyme activity. Catalase (CAT, EC 1.11.1.6),
ascorbate peroxidase (APX, EC 1.11.1.11) and guaiacol peroxidase (GPX, EC 1.11.1.7) activities were assayed according to Zarifith et al. [20].

2.6. Agronomical Traits and Drought Tolerance Indices Determination

Grain yield, 1000-grain weight and spikelets/panicle of each rice genotype in each treatment were determined at the final harvest. Seven drought tolerance indices including the relative yield index (REI), mean productivity index (MPI), mean relative performance (MRP), stress tolerance level (TOL), stress tolerance index (STI), stress susceptibility index (SSI), stress intensity (SI) and drought tolerance efficiency (DTE), were calculated using the following equations [21–25]. The SSI value was categorized based on the report by Hossain et al. [26] (Table 1).

\[
\text{REI} = \left( \frac{Y_i}{Y_s} \right) \times \left( \frac{Y_{is}}{Y_{ns}} \right)
\]

(1)

\[
\text{MPI} = \left( \frac{Y_i + Y_s}{2} \right)
\]

(2)

\[
\text{MRP} = \left( \frac{Y_i}{Y_s} \right) + \left( \frac{Y_{is}}{Y_{ns}} \right)
\]

(3)

\[
\text{TOL} = Y_{ns} - Y_i
\]

(4)

\[
\text{STI} = \left( \frac{Y_{ns} + Y_i}{(Y_{ns})^2} \right)
\]

(5)

\[
\text{SSI} = 1 - \left( \frac{Y_i}{Y_{ns}} / \text{SI} \right)
\]

(6)

\[
\text{SI} = 1 - \left( \frac{Y_s}{Y_{ns}} \right)
\]

(7)

\[
\text{DTE} = \left( \frac{Y_i}{Y_{ns}} \right) \times 100
\]

(8)

where \( Y_{ns} \) is the mean yield of all evaluated genotypes under WW condition; \( Y_s \) is the mean yield of all evaluated genotypes under DS condition; and \((Y_i)_{ns}\) and \((Y_i)s\) denote with respect to the yield of the \(i\)th genotype under the WW and DS conditions, respectively.

| SSI Value       | Rate              |
|-----------------|-------------------|
| Less than 0.50  | Highly drought-tolerant |
| 0.51–0.75       | Drought-tolerant |
| 0.76–1.00       | Moderately drought-tolerant |
| More than 1.00  | Drought-susceptible |

2.7. Statistical Analysis

The mean data for all observations were compiled in each treatment by taking the average value over randomly selected plants from all the replications. The data obtained were analysed using the Statistical Analysis System (SAS) software. The analysis of variance (ANOVA) for each trait under study was used to determine the level of variation due to the genotypes, water treatments, and genotypes by water treatments. The mean and coefficient of variation (CV) were calculated for each trait. Duncan’s New Multiple Range Test (DNMRT) was used to define significant differences among treatment means. The CORR procedure of SAS was used to evaluate the correlations among the traits.
The drought tolerance indices comprising of REI, MPI, MRP, TOL, STI, SSI, and DTE were determined on the basis of the mean yield under the WW and DS treatments.

3. Results

3.1. Drought Stress Effects on Morphological Performances

Table 2 shows the results obtained using ANOVA for all the morphological traits. There was a significant difference (p ≤ 0.05) for water treatments (W), genotypes (G), and interaction between water treatments and genotypes (G × W) in FLA. The traits of PDW and TDW were significantly different (p ≤ 0.05) for W and G. The G also showed significant difference (p ≤ 0.05) in NOT and CLDW. However, the W, G and G × W were not significant for PH trait.

| Source of Variation | df | FLA (cm²) | NOT (cm) | PH (cm) | CLDW (g plant⁻¹) | PDW (g plant⁻¹) | TDW (g plant⁻¹) |
|---------------------|----|-----------|----------|---------|------------------|----------------|----------------|
| Replications (R)    | 2  | 0.02      | 0.25     | 2.27    | 0.57             | 2.46           | 0.31           |
| Water treatments (W)| 1  | 458.80 *  | 126.75   | 61.20   | 102.55           | 1017.71 *      | 1864.76 *      |
| R × W               | 2  | 2.77      | 0.25     | 0.96    | 0.03             | 1.97           | 6.37           |
| Genotypes (G)       | 1  | 705.33 *  | 630.75 * | 8.17    | 225.51 *         | 4186.94 *      | 6541.27 *      |
| G × W               | 1  | 83.21 *   | 0.75     | 3.31    | 12.94            | 185.57         | 337.61         |
| Error               | 4  | 0.27      | 1.75     | 0.74    | 0.91             | 1.68           | 3.04           |

* The mean difference is significant at the 0.05 level.

As can be seen, the vegetative growth data for the MR219 transgenic line and wild-type MR219 were significantly reduced under the drought stress (DS) treatment except for PH (Table 3). Under the DS treatment, the MR219 transgenic line showed higher values of FLA (42.8%) and NOT (50.0%) when compared to the wild-type MR219. Although drought stress significantly reduced dry weight production for all the genotypes, the MR219 transgenic line consistently showed higher CLDW, PDW, and TDW than the wild-type in all water treatments (Table 3). For instance, the transgenic line had 18.6% and 39.7% more total dry weight on average than the wild-type under the WW and DS treatments, respectively.

| Genotype/Treatment | FLA (cm²) | NOT (cm) | PH (cm) | CLDW (g plant⁻¹) | PDW (g plant⁻¹) | TDW (g plant⁻¹) |
|--------------------|-----------|----------|---------|------------------|----------------|----------------|
| MR219 transgenic    | WW        | DS       | WW      | DS               | WW            | DS            |
| line               | 41.5      | 34.4     | 28      | 21               | 95.1          | 91.6          |
|                    | a         | a        | a       | a                | b             | a             |
| Wild-type MR219     | WW        | DS       | WW      | DS               | WW            | DS            |
|                    | 31.4      | 13.8     | 13      | 7                | 97.8          | 92.2          |
|                    | b         | b        | b       | a                | a             | a             |
| Mean               | 36.4      | 24.1     | 21      | 14               | 96.4          | 91.9          |
| CV (%)             | 15.3      | 47.0     | 40.3    | 55.1             | 1.66          | 1.38          |

Notes: Means followed by the different letters (a and b) within a column are significantly different from each other according to the DNMRT at p ≤ 0.05.

3.2. Drought Stress Effects on Physiological Responses

The RWC, chlorophyll content and gs varied significantly (p ≤ 0.05) among the W, G and G × W. There was a significant (p ≤ 0.05) difference for W and G in Pn. However, this trait did not show a significant (p ≤ 0.05) difference for G × W (Table 4).
The results showed that the chlorophyll content in leaf decreased under the DS treatment, with a greater reduction in the wild-type MR219 compared to WW treatment. Under the DS treatment, MR219 transgenic line showed greater capability in maintaining higher relative water content than the wild-type MR219. Similar to leaf RWC, the chlorophyll content in leaf decreased under the DS treatment, with a greater reduction in the wild-type MR219 (30.8%) than in the transgenic line (11.2%) (Figure 1). The results showed that the MR219 transgenic line had the highest chlorophyll content values under the WW and DS treatments as compared to wild-type MR219.

Figure 1 shows the progression of leaf RWC, chlorophyll content, Pn, and gs under WW and DS treatments. The average RWC of the leaf recorded a decrease of 22.7% under the DS treatment compared to WW treatment. Under the DS treatment, MR219 transgenic line showed greater capability in maintaining higher relative water content than the wild-type MR219. Similar to leaf RWC, the chlorophyll content in leaf decreased under the DS treatment, with a greater reduction in the wild-type MR219 (30.8%) than in the transgenic line (11.2%) (Figure 1). The results showed that the MR219 transgenic line had the highest chlorophyll content values under the WW and DS treatments as compared to wild-type MR219.

The Pn of the MR219 transgenic line and wild-type MR219 showed a significant reduction under the DS treatment. There was a significant difference in Pn between the transgenic and wild-type as the transgenic line recorded the highest value under both WW (10.66 µ mol CO₂ m⁻²s⁻¹) and DS (6.09 µ mol CO₂ m⁻²s⁻¹) treatments compared to its wild-type, with 9.22 µ mol CO₂ m⁻²s⁻¹ and 4.83 µ mol CO₂ m⁻²s⁻¹ under the WW and DS treatments, respectively (Figure 1). The MR219 transgenic line also showed higher gs when compared to the wild-type under the DS treatment (Figure 1). The gs of the wild-type MR219 between WW treatment (0.58 mol H₂O m⁻²s⁻¹) and DS treatment (0.37 mol H₂O m⁻²s⁻¹) showed the highest reduction.

* The mean difference is significant at the 0.05 level.

Table 4. Analysis of variance for physiological traits.

| Source of Variation | df | Mean Square | RWC (%) | Chlorophyll Content (mg g⁻¹ FW) | Pn (µ mol CO₂ m⁻²s⁻¹) | gs (mol H₂O m⁻²s⁻¹) |
|---------------------|----|-------------|---------|-------------------------------|----------------------|---------------------|
| Replications (R)    | 2  | 9.33        | 0.19    | 0.01                          | 0.01                 |
| Water treatments (W)| 1  | 3300.08 *   | 53.78 * | 60.21 *                       | 0.02 *               |
| R × W               | 2  | 4.33        | 0.14    | 0.01                          | 0.01                 |
| Genotypes (G)       | 1  | 1140.75 *   | 76.93 * | 5.50 *                        | 0.04 *               |
| G × W               | 1  | 784.08 *    | 36.04 * | 0.02                          | 0.06 *               |
| Error               | 4  | 2.17        | 0.06    | 0.01                          | 0.01                 |

Figure 1. Effects of drought stress treatment on RWC, chlorophyll content, Pn, and gs in rice genotypes. Vertical bars represent the standard error of the mean. Values within the drought stress treatment with the different letters (a and b) are significantly different based on comparison using DNMRT at \( p \leq 0.05 \) \((n = 12)\).
3.3. Drought Stress Effects on Biochemical Changes

There was a significant difference \( (p \leq 0.05) \) in the proline content and all antioxidant enzyme activities for W, G, and G \( \times \) W interaction (Table 5). The transgenic MR219 showed a significant increase in the proline content between WW and DS treatments (Figure 2). The transgenic plant also recorded higher proline content than the wild-type plant. In comparison with WW treatments, the proline content increased under the DS treatment by 51.2% for the MR219 transgenic line, but only by 29.9% for MR219. The effects of drought on the antioxidative enzyme activities in the form of CAT, APX and GPX were investigated (Figure 2). The CAT activity increased under DS, suggesting the adaptation of photorespiratory \( H_2O_2 \) production caused by drought. Compared with the wild-type plants, transgenic plants showed significantly higher activity of CAT under the DS treatment \( (p \leq 0.05) \). Similar to CAT activity, APX and GPX activities were higher for the MR219 transgenic line than the wild-type plant under the DS treatment \( (p \leq 0.05) \), suggesting an enhanced adaptation to drought stress.

| Source of Variation          | df | Proline Content (mg g\(^{-1}\)) | CAT (Units mg Protein\(^{-1}\)) | APX (Units mg Protein) | GPX (Units mg Protein\(^{-1}\)) |
|-----------------------------|----|---------------------------------|-------------------------------|------------------------|---------------------------------|
| Replications (R)            | 2  | 0.07                            | 0.01                          | 0.06                   | 0.03                            |
| Water treatments (W)        | 1  | 0.97 *                          | 7.24 *                        | 4.94 *                 | 26.58 *                         |
| R \( \times \) W           | 2  | 0.02                            | 0.01                          | 0.02                   | 0.02                            |
| Genotypes (G)               | 1  | 0.37 *                          | 2.22 *                        | 2.41 *                 | 16.10 *                         |
| G \( \times \) W           | 1  | 0.24 *                          | 2.22 *                        | 1.79 *                 | 12.20 *                         |
| Error                       | 4  | 0.04                            | 0.03                          | 0.06                   | 0.03                            |

\* The mean difference is significant at the 0.05 level.

**Figure 2.** Effects of drought stress treatment on proline, CAT, APX, and GPX activities in rice genotypes. Vertical bars represent standard error of the mean. Values within drought stress treatment with the different letters (a and b) are significantly different based on comparison using DNMRT at \( p \leq 0.05 \) (\( n = 12 \)).
3.4. Drought Stress Effects on Agronomical Performances

3.4.1. Yield and Yield Attributes

There were significant differences \((p \leq 0.05)\) in grain yield, 1000-grain weight and spikelets/panicle for W, G and G \(\times\) W interaction (Table 6). The mean grain yield under WW and DS treatments was significantly different for both genotypes, indicating a considerably different performance under WW and DS (Table 7). The MR219 transgenic line showed higher grain yield than the wild-type MR219 in both WW and DS treatments. On average, the M219 transgenic line had 10.4% and 59.8% higher grain yield than the wild-type MR219 under the WW and DS treatments, respectively, indicating that the grain yield of the transgenic rice plant had improved in drought conditions. The 1000-grain weight and spikelets/panicles were also affected by the DS treatment (Table 7). In comparison with the WW treatment, both yield attributes decreased under the DS treatment for both genotypes. However, the MR219 transgenic line exhibited higher 1000-grain weight (16.4%) and spikelets/panicles (30.0%) than the wild-type variety under the DS treatment.

### Table 6. Analysis of variance for agronomical traits.

| Source of Variation     | df | Grain Yield (g plant\(^{-1}\)) Mean Square | 1000-Grain Weight (g) Mean Square | Spikelets/Panicle Mean Square |
|-------------------------|----|------------------------------------------|-----------------------------------|-------------------------------|
| Replications (R)        | 2  | 3.82                                     | 0.01                              | 3.00                          |
| Water treatments (W)    | 1  | 2679.94 *                               | 45.86 *                           | 602.08 *                      |
| R \(\times\) W          | 2  | 0.82                                     | 0.01                              | 2.33                          |
| Genotypes (G)           | 1  | 4918.73 *                               | 83.32 *                           | 1850.08 *                     |
| G \(\times\) W          | 1  | 1625.18 *                               | 19.92 *                           | 352.08 *                      |
| Error                   | 4  | 2.54                                     | 0.02                              | 1.33                          |

* The mean difference is significant at the 0.05 level.

### Table 7. The grain yield, 1000-grain weight, and spikelets/panicle of the rice genotypes under well-watered (WW) and drought stress (DS) treatments.

| Genotype/Treatment       | Grain Yield (g plant\(^{-1}\)) | 1000-Grain Weight (g) | Spikelets/Panicle |
|--------------------------|-------------------------------|-----------------------|-------------------|
| WW          | DS         | WW         | DS         | WW         | DS         |
| MR219 transgenic line    | 91.77a                      | 85.15a                | 29.19a          | 27.86a     | 81a        | 78a        |
| Wild-type MR219          | 74.55b                      | 21.39b                | 26.50b          | 20.01b     | 67b        | 42b        |
| Mean                    | 83.16                       | 53.27                 | 27.85           | 23.94      | 74         | 60         |
| CV (%)                  | 11.55                       | 65.58                 | 5.31            | 17.96      | 10.41      | 32.56      |

Notes: Means followed by the different letters (a and b) within a column are significantly different from each other according to the DNMRT at \(p \leq 0.05\).

3.4.2. Drought Tolerance Indices

The mean of the relative yield (RY) in the DS treatment was significantly less than the WW treatment for all genotypes. The MR219 transgenic line showed high RY under the DS treatment (mean RY < RY) while the wild-type MR219 had low RY (mean RY > RY) (Table 8). The MR219 transgenic line ranked first for all the drought tolerance indices which recorded the lowest TOL (6.61) and SSI (0.55) values under the DS treatment (Table 9). The transgenic line also showed the highest values of STI (0.94) and DTE (72.79%) for grain yield.
Table 8. The genotypes mean yield, relative yield (RY) performance and mean relative yield of the rice genotypes in response to drought stress treatment.

| Treatment                | Transgenic MR219 | Wild-Type MR219 |
|--------------------------|------------------|-----------------|
| Well-watered plant       |                  |                 |
| Mean yield (g)           | 91.77a           | 74.55b          |
| RY (g)                   | 1.00             | 0.81            |
| Mean RY (g)              | 0.91             |                 |
| Drought stress plant     |                  |                 |
| Mean yield (g)           | 88.15a           | 21.39b          |
| RY (g)                   | 1.00             | 0.25            |
| Mean RY (g)              | 0.63             |                 |

Notes: Means followed by the different letters within a row are significantly different from each other according to the DNMRT at $p \leq 0.05$.

Table 9. The drought tolerance indices of genotypes under drought stress condition.

| Genotype          | REI    | MPI    | MRP    | TOL    | STI    | SSI    | DTE (%) |
|-------------------|--------|--------|--------|--------|--------|--------|---------|
| Transgenic MR219  | 1.76 (1)| 88.43 (1)| 2.70 (1)| 6.61 (1)| 0.03 (1)| 0.20 (1)| 92.81 (1)|
| Wild-type MR219   | 0.36 (2)| 47.97 (2)| 1.30 (2)| 53.16 (2)| 0.01 (2)| 1.98 (2)| 28.69 (2)|

Notes: REI = relative yield index, MPI = mean productivity index, MRP = mean relative performance, TOL = stress tolerance level, STI = stress tolerance index, SSI = stress susceptibility index, DTE = drought tolerance efficiency, ( ) = ranking between rice genotypes for each index.

3.5. Phenotypic Correlations among Morpho-Physiological, Biochemical and Agronomical Traits

Table S1 shows the simple phenotypic correlation coefficients between the traits. The FLA, CLDW, PDW, TDW, RWC, 1000-grain weight, and spikelets/panicle traits showed positive and high phenotypic correlations ($r = 0.894–0.997$) with the grain yield while NOT and $P_n$ showed a significant correlation ($r = 0.867$ and 0.719, respectively) with the grain yield.

4. Discussion

Previous works have discovered that an increase in drought stress can influence the development of plant growth, thus leading to a reduction in FLA, NOT and PH of rice plants [27–29]. In the present study, the MR219 transgenic line showed higher FLA, NOT and PH compared to the wild-type MR219 variety, which was in agreement with the results obtained by Zariith et al. [20] for the advanced mutant rice MR219-4 and MR219-9. These results might be ascribed to the higher efficiency of biomass allocation among plant parts in the MR219 transgenic line [30]. The allocation of photosynthates among plant parts may influence plant productivity [31]. The MR219 transgenic line also showed higher CLDW, PDW, and TDW in comparison with other studied rice genotypes. Drought stress can affect the mechanism of biomass accumulation within the plants [32]. Significant differences in FLA, NOT, CLDW, PDW, and TDW between MR219 transgenic line and wild-type under the DS treatment have proven that the MR219 transgenic line has resisted drought stress better than the wild-type (Table 3). This result may indicate that the product of $ABP57$ gene in the MR219 transgenic line may be involved in the drought tolerance mechanism through the initiation of the auxin signaling pathways that regulate the growth of cells, thus increasing the growth rate of the upper plant parts [15] and leading to a higher FLA, NOT, CLDW, PDW and TDW.

The reduction in the leaf RWC of all studied rice genotypes under drought conditions might be caused by an indirect effect on the osmotic adjustment [33]. The DS treatment reduces the osmotic potential of the external microenvironment and hence, reverses the direction of water influx in the cell, resulting in dehydration as observed from the values of RWC in leaves in all rice genotypes in this experiment (Figure 1). However, the MR219 transgenic line had the highest value of RWC under the drought condition, and the values were slightly lower as compared to the WW condition in contrast with wild-type plants. The least reduction in cellular water potential below the external
water potential of the transgenic MR219 indicates that this transgenic rice has better cellular osmotic adjustability, allowing the appropriate osmotic homeostasis into the cell. The DS treatment also reduced the relative chlorophyll content in the leaves of transgenic MR219 and wild-type. This observation might be caused by the oxidative damage of the chloroplast which may occur during the drought stress [34,35]. The transgenic MR219’s recording higher chlorophyll content compared to the wild-type was similar to that of transgenic Arabidopsis thaliana as reported by Tan [15]. The transgenic MR219 may experience less chlorophyll degradation than the wild-type MR219, possibly contributing to the higher $P_n$ observed in the MR219 transgenic line compared to the wild-type MR219. The decline in $P_n$ was consistent with the reduction in the $g_s$. The significant reduction in $P_n$ and $g_s$ under the drought conditions for all rice genotypes studied was in concordance to the results reported by Zheng et al. [36] for neem plants (Azadirachta indica A. Juss). The decrease in $P_n$ under drought stress conditions may be due to the direct effect of water dehydration on leaf water potential tolerance via stomatal closure. Consequently, good gas exchange in the MR219 transgenic line reduces the rate of transpiration that eventually prevents more water loss from the plant. Nonetheless, the MR219 transgenic line showed higher $P_n$ than the wild-type, indicating that drought caused a minor effect on the photosynthetic apparatus of the MR219 transgenic line than the wild-type. Overexpressing ABP57 gene may indirectly stimulate the plant receptor function by increasing the stomatal closure and lowering the stomatal conductance and transpiration rate, leading to improve water-use efficiency of the transgenic plants.

It was reported that drought stress resulted in increased proline accumulation in other plant species [37]. The DS treatment in this study had increased proline content in all tested genotypes (Figure 2). The proline accumulation may contribute to maintaining the osmotic status of cells and to avoid cellular damages during the drought stress. The MR219 transgenic line had the highest accumulation of PC under the DS compared to the wild-type, thus indicating better osmoregulation in the MR219 transgenic line and conferring better tolerance to drought stress. This was consistent with the results obtained by Zarifth et al. [20], who found that improved mutant rice genotypes exhibited higher proline content when exposed to drought stress. The antioxidant enzyme activities such as CAT, APX, and GPX increased in the leaves under the DS treatment, suggesting that these antioxidant enzymes played important roles to protect plant cells from the oxidative damages. In the present study, the MR219 transgenic line showed higher CAT, APX, and GPX activities than the wild-type under DS (Figure 2). According to Anjum et al. [38] and Lum et al. [39], increased CAT activity under drought stress conditions suggested the mechanism of adaptation intended for scavenging the photorespiratory $H_2O_2$ production. As reported by Lum et al. [39], the APX had reduced $H_2O_2$, indicating that this antioxidant enzyme contributed to the mechanism of antioxidative defense of rice against drought. In this study, the higher APX activity in the MR219 transgenic line under the DS treatment suggested that the activity of APX in the transgenic plant leaves might be attributed to the protection of the chloroplast-localized enzymes in the leaf tissues from oxidative damages. The increased activity of GPX under the drought condition may be associated with the removal of the malondialdehyde compound and control of $H_2O_2$ accumulation [38–40]. In accordance with previous findings [20,38], under drought stress, the transgenic plant had higher GPX (Figure 2), which might be vital for the decomposition of $H_2O_2$. The higher CAT, APX, and GPX activities in the MR219 transgenic line suggest that a better antioxidation mechanism may contribute to the better performance under drought stress.

The grain yield decreased proportionally to the DS treatment (Table 6). The grain yield was reduced as a result of the reduced amount of water supplied and photosynthesis capacity. This result suggests that the yield determinant is influenced by the amount of water provided. In the present study, it was found that the reduction in grain yield might be attributed to the reduction in the 1000-grain weight and spikelets/panicle as a result of low soil moisture content during the reproductive stage. Water shortage prior and during early flowering has been reported to reduce the number of spikelets, leading to decreasing final grain yield production [41]. Furthermore, the increment of soil water tension during the reproductive stage would increase spikelet abortion, thus reducing the spikelets/panicle [20]. In addition to that, grain yield reduction might also be related to the reduction in leaf area, lower
photosynthetic rate and high evaporation demand [42]. According to Zarifth et al. [20], drought stress had reduced yield in rice. In this study, the grain yield per plant for the MR219 transgenic line was the least affected by DS compared to the wild-type due to the better drought stress tolerance mechanism adapted by the MR219 transgenic line.

The genotype with high yield production under WW and DS conditions can be identified by assessing its STI value (Table 9). The genotype with high STI value indicates a high tolerance ability against drought stress. The results of the highest STI value for MR219 transgenic line showed that this transgenic rice can be considered as drought-tolerant genotypes. Wild-type MR219 is known to be highly susceptible to drought stress as shown by the lowest STI value under drought stress conditions. The best performer for TOL and SSI under DS condition was the MR219 transgenic line (Table 9). A lower TOL value implies the ability of a given genotype to show higher stress tolerance. Similar findings were stated by Hossain et al. [26] and Raman et al. [43]. The SSI is used to identify the yield stability under unfavorable and favorable environments. A lower value of SSI implies that the yield differences between WW and DS conditions are low, meaning that a genotype is more tolerant to drought. The results were in good agreement with previously reported SSIs under drought conditions [43,44]. It was evident that the MR219 transgenic line possessed a high level of drought tolerance under DS conditions. According to Zarifth et al. [20], the genotypes demonstrated smaller yield reduction under the DS compared to WW conditions, which recorded low values of SSI; in other words, a value of less than one can be considered as drought-tolerant genotypes. Using the ranking described above, the value of TOL and SSI obtained can be used to identify genotypes that perform well under stress conditions. The rankings were made for different genotypes based on the value of SSI and the direction of desirability as indicated in Tables 1 and 9. Transgenic rice plants overexpressing ABP57 were identified as drought-tolerant genotypes (SSI: 0.51–0.75) for grain yield. Drought resistance mechanisms can be measured through DTE value. In most cases, the selection of stable genotypes under DS can be more appropriately determined using the DTE value. The MR219 transgenic line recorded high yield performances under the DS condition, and this transgenic rice was stable compared to the wild-type based on the STI values (Table 9). Therefore, STI is also a useful drought tolerance index to identify genotypes with high yield under stress conditions. TOL, SSI, and DTE are the drought tolerance indices to select genotypes with good yield potential under DS conditions. Therefore, in this study, the MR219 transgenic line showed better performance under DS conditions.

5. Conclusions

The MR219 transgenic line overexpressing the ABP57 gene exhibited higher grain yield than the wild-type MR219, especially under DS conditions. Enhanced performance in grain yield and higher DTE of the MR219 transgenic line were correlated with greater $P_n$ and $g_s$ in leaves, higher RWC, chlorophyll content and proline content, and higher activities of antioxidant enzymes, CAT, APX, and GPX. These results provide the experimental confirmation that the MR219 transgenic line may show improved grain yield, especially under drought conditions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/10/1530/s1, Table S1: Phenotypic correlations of morpho-physiological and agronomical traits during stress condition.

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