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Exogenously Applied Salicylic Acid Boosts Morpho-Physiological Traits, Yield, and Water Productivity of Lowland Rice under Normal and Deficit Irrigation

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Abstract: The main constraint on rice cultivation in the Mediterranean area is the limited irrigation and its large water consumption. In addition, rice is very sensitive to drought conditions because of drought stress on morpho-physiological traits and yield reduction. The application of salicylic acid (SA) has been noticed to be very effective in alleviating the adverse effects of drought stress on rice. The current investigation was conducted as a split-split arrangement under a randomized complete block design with two lowland rice cultivars (Giza177 and Giza179) and SA as a foliar application at four concentrations (0, 400, 700, and 1000 µM) under normal and drought conditions. The results showed that plant growth, leaf photosynthetic pigments, yields, and the most studied traits were significantly affected by irrigation (I), cultivar (C), and SA concentration ($p \leq 0.05$ or 0.01). The interaction effect of I × C × SA was only significant on the carotenoids content ($p \leq 0.05$). The reduction in grain yield and most studied traits was more pronounced under drought conditions. The Giza179 proved to be a drought-tolerant cultivar under all SA concentrations under drought conditions, while Giza177 was a drought-sensitive cultivar. The application of 700 µM SA gave the best grain yield in both rice cultivars under drought conditions compared to other SA concentrations. Grain yield for normal irrigation (Yp) and drought stress (Ys) conditions were highly positively correlated with indices of the mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI), yield index (YI), yield stability index (YSI), drought resistance index (DI), harmonic mean (HM), and golden mean (GOL). While they are highly negatively correlated with the indices of the stress susceptibility index (SSI), tolerance index (TOL), yield reduction ratio (YR), stress susceptibility percentage index (SSPI), and abiotic tolerance index (ATI). It could be concluded that SA, as a growth regulator, could be used to alleviate the harmful effect of inadequate water availability in soil on rice cultivars as well as to improve the growth, water productivity, and grain yield.

Keywords: cultivars; drought stress; drought tolerance indices; leaf photosynthetic pigments; rice; salicylic acid

1. Introduction

Rice is ranked the second important cereal crop after wheat and it is the most important food crop in the world and in Egypt [1]. Rice production in the world has exceeded

513 million metric tons, with 166.47 million hectares under cultivation [2]. It is grown in irrigated lowland in flooded conditions with a constant water depth of 5 to 10 cm [3]. Lowland rice is primarily directly seeded or transplanted on puddled soils by ploughing under saturated water conditions, then by harrowing and levelling management. In many parts of the world, the supply of irrigation water for agriculture particularly in rice production is challenged, not only by a global lack of water resources [4], but also by rising urban and industrial demand [5]. Rice farming consumes a lot more water than other crops around the world and it is estimated that irrigated rice uses roughly 40% of the global water utilized for irrigation [6]. To ensure food security and develop an acceptable yield in water shortage conditions in Egypt, drought-tolerant and water-saving rice varieties are becoming increasingly important. [1].

With the anticipation of future climate change, it is necessarily the time for exploring the possibilities of drought-tolerant crops for all crop species [7]. Drought stress is a major problem that limits the adoption of high-yielding rice genotypes in drought-prone rainfed rice environments [8], where moderate drought stress can be broadly characterized by a 31–64% loss in rice grain yield compared to normal irrigation conditions [9,10]. Hall [11] defines drought tolerance as the relative yield of a genotype compared to others subjected to the same drought stress. Drought resistance is a complex phenomenon, which is the manifestation of both drought tolerance (tissue tolerance, maintenance of photosystem, etc.) and drought avoidance (deep root, leaf rolling, etc.), traits that are governed by multiple genes [12]. Blum and Jordan [13] showed that drought resistance is obstructed by the low heritability and deficiency of successful selection methods. Therefore, the selection of rice genotypes should be adapted to drought stress conditions [1].

Salicylic acid (SA) is a promising phenolic compound and oxidative plant growth regulator. SA is associated with stress tolerance in plants through the regulation of multiple physiological processes under drought stress conditions, such as the photosynthesis rate, antioxidant defense system, transpiration rates, proline metabolisms, stomatal closure reversal, signal transduction inhibition, seed germination promotion, the induction of flowering, and nutrients uptake [14–16]. Several researchers have investigated the impact of exogenously foliar-applied substances, such as SA or nutrients, on the morpho-physiological traits and yield of field crops, like rice under abiotic stress, including drought stress [17–20].

Some researchers believe in selection under favorable conditions [21] and some believe in selection under typical drought conditions [22]. Nevertheless, there exist numerous researchers that chose the midway and believe in selection under both favorable and stressed conditions [23–25]. To determine drought-tolerant genotypes, several drought indices have been suggested on the basis on a mathematical relationship between yield under drought and non-stressed conditions. These indices are based on either the drought resistance or drought susceptibility of genotypes [26]. The stress susceptibility index (SSI) was suggested by Fischer and Maurer [27], whilst the tolerance index (TOL) and mean productivity index (MP) were suggested by Rosielle and Hamblin [28]. The geometric mean productivity (GMP) and stress tolerance index (STI) were defined by Fernandez [25]. The yield index (YI) was suggested by Gavuzzi et al. [29], the yield stability index (YSI) was suggested by Bouslama and Schapaugh [30], drought resistance index (DI) was proposed by Lan [31], the yield reduction ratio (YR) was proposed by Golestani–Araghi and Assad [32], the harmonic mean (HM) was proposed by Hossain et al. [33], and the golden mean (GOL) was proposed by Moradi et al. [34] in order to evaluate the stability of genotypes under both stress and non-stress conditions. The abiotic tolerance index (ATI) and stress susceptibility percentage index (SSPI) were introduced by Moosavi et al. [35] for screening drought-tolerant genotypes under stress and non-stress conditions.

There is a need to use principle competent analysis (PCA) to show the results of rice experiments and to select based on a combination of correlations and drought tolerance indices. Thus, many researchers such as [1,36–39] have used PCA to assess the relationship and diversity between several rice germplasms, in addition to knowing the relationships between yield and other quantitative traits of rice. The current study hypothesized that the exogenous application of SA may positively affect rice performance, drought tolerance indices, water productivity, and leaf photosynthetic pigments. Therefore, our main objective was to study the response of two lowland rice cultivars grown under normal and drought stress conditions.

2. Materials and Methods

2.1. Location and Climatic Data

Two experiments were conducted across 2019 and 2020 summer seasons at the Rice Research and Training Center (RRTC), located (31°30′7.59″ and 31°9′58.09″ N and between 30°20′36.83″ and 31°17′15.16″ E) in Sakha experimental station, Kafr El-Sheikh Governorate, Egypt at the northern part of the Nile Delta, between Rosetta and Damietta Nile branches. Weather data for the studied field experiment including the monthly average precipitation, minimum and maximum temperatures, dew point, wind speed, and relative humidity for the experimental duration (May–September) during both growing summer seasons (2019 and 2020) are presented in Figure 1.

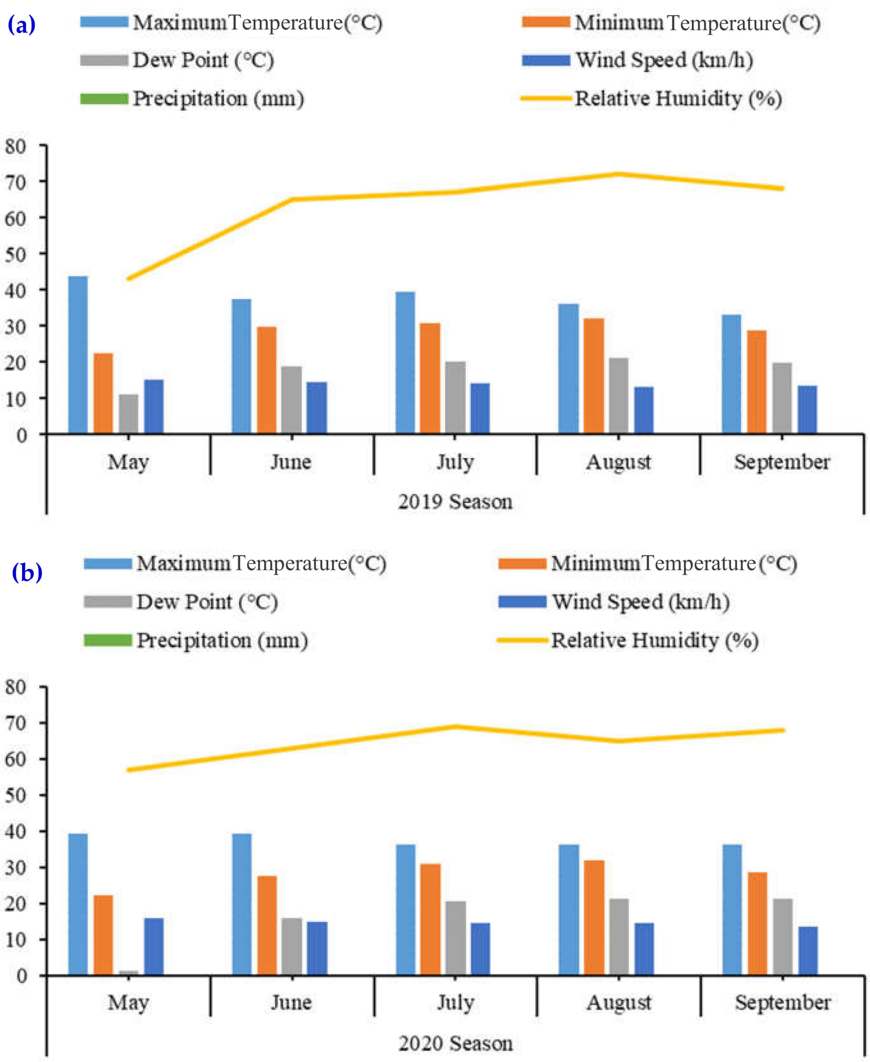

Figure 1. Monthly weather data for the experiment field during both growing summer seasons. (2019 (**a**) and 2020 (**b**)).

2.2. Soil Characteristics of the Experiment Field

Soil samples were collected at 0.0–0.50 cm depth before planting, it was air-dried, then crushed thoroughly, after that sieved through a 2 mm sieve, and the physical and chemical characterization were obtained through laboratory analysis. Particle size distribution (sand%, silt%, and clay %) was also determined according to Gee and Bauder [40]. The pH values of soil samples were measured in saturated soil–water paste using Beckman a pH meter (model Elico, LI120-UK) according to Page et al. [41]. The electrical conductivity (ECe) values were determined in saturated soil–water paste extract and defined as dS m^{-1} using CM25 conductivity meter (model3200, YSI, Inc., Yellow Springs, OH, USA) according to Page et al. [41]. The characteristics (physical and chemical) analysis of the studied soil in both the 2019 and 2020 seasons are presented in Table 1.

Table 1. Physical and chemical properties of the experiment soil at Sakha Research Station in the 2019 and 2020 years.

Property		Unit	2019	2020	Average
Particle size distribution	Clay	(%)	55.20	55.10	55.15
	Silt		32.30	32.30	32.3
	Sand		12.50	12.60	12.55
Texture			Clayey		
Organic matter		(%)	1.37	1.37	1.37
pH			8.20	8.20	8.20
Electrical conductivity ECe		(dS m^{-1})	3.33	3.31	3.32
Total N		(ppm)	513.00	516.00	514.50
Available P			15.39	15.83	15.60
K^+			16.00	15.00	15.50
Fe^{2+}			4.55	4.53	4.54
Mn^{2+}			3.20	3.40	3.30

2.3. Experimental Design and Treatment Details

Two local rice cultivars (Giza177 and Giza179) were obtained from Seeds Production Unit of the Rice Research and Training Center, Sakha Agricultural Research Station, Egyptian Ministry of Agriculture, and Land Reclamation, Egypt. The pedigree, origin, and varietal group of these utilized genotypes under study are mentioned in Table 2. The seeds of the rice genotypes were sown in a nursery on 20 May and transplanted into the permanent field after 30 days in the 2019 and 2020 seasons.

Table 2. Description of irrigation conditions (I), salicylic acid (SA) treatments, and rice cultivars (C) in the studied cultivated site.

Treatments	Description
	Irrigations (I)
Normal	Rice plants were irrigated with full irrigation (10,710 m^3 ha^{-1}), every 4 days (595 m^3 ha^{-1} per one irrigation) through a surface irrigation system (n = 18 irrigation)
Drought	Rice plants were irrigated with flush irrigation (4510 m^3 ha^{-1}), every 10 days (643 m^3 ha^{-1} per one irrigation) through a surface irrigation system (n = 7 irrigation)
	Cultivars (C)
Giza177	Giza171/YomjoNo.1//PiNo.4 (Japonica type, sensitive to drought)
Giza179	GZ6296/GZ1368 (Indica/Japonica type, moderate to drought)
	Salicylic acid (SA)
SA_0	Distilled water (control) was foliar sprayed three times at 15, 30, and 45 days after transplantation.
SA_1	400 µM of SA was foliar sprayed three times at 15, 30, and 45 days after transplantation.
SA_2	700 µM of SA was foliar sprayed three times at 15, 30, and 45 days after transplantation.
SA_3	1000 µM of SA was foliar sprayed three times at 15, 30, and 45 days after transplantation.

The experimental design was conducted using a split-split-plot arrangement under a randomized complete block design with triplicates. The main plots were randomly allocated to irrigation treatments, i.e., full irrigation (every 4 days) and deficit irrigation (flush irrigation every 10 days). Each main plot was represented by 48 units (576 m^2) for two irrigation treatments, each spaced of 4 m apart. Each main plot was divided into four subplots represented by 24 experimental units (288 m^2) identified for four SA treatments (i.e., 0 (SA_0), 400 (SA_1), 700 (SA_2), and 1000 (SA_3) μM), which were foliar applied at 15, 30, and 45 days after transplantation.

To secure the effectual and even spraying of each SA level on the rice plants, a very little amount of Tween-20 (C58 H114 O26; 0.3% v/v) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was added to the applied-SA solution as a nonionic polyoxymethylene agent. The spray solution was applied at a dosage of 480 L ha^{-1} using a 20 L motorized knapsack motor (Kubota, Osaka, Japan) mist blower sprayer. Each subplot was divided into two sub-sub plots and was represented by six experimental units (72 m^2), which were randomly allocated for two rice cultivars, i.e., Giza177 and Giza179. Thirty-day-old seedlings of each cultivar were individually transplanted into the permanent field in a 15 row experimental $unit^{-1}$ (12 m^2; 3 m in width and 4 m in length) with the spacing of 20 cm between rows and 20 cm between plants within rows.

2.4. Agronomical Management Practices

After the land preparation of the nursery following the recommended agricultural practices, the rice grains of Giza177 and Giza179 were cleaned and soaked in tap water for 24 h and incubated for 48 h before sowing time. After that, the pre-germinated seeds were broadcasted in the nursery on the 20th of May. The 30-day-old seedlings (3–4 seedling $hill^{-1}$) were transplanted in the permanent experimental field and spaced at a distance of 20 cm apart within rows and 20 cm apart between rows during both the 2019 and 2020 seasons.

The experimental field in each season was basally supplied with 36 kg of P_2O_5 ha^{-1} (232 kg calcium super monophosphate contained 15.5% P_2O_5) during the preparation of the field. Additionally, nitrogen was applied with 220 kg N ha^{-1} in the form of urea (46% N). Nitrogen fertilizer was applied in two equal doses: the first dose was incorporated into dry soil before flooding and the second dose was applied after 30 days of transplantation. Other pre- or post-stand establishment management such as land preparation, fertilizer application, weeding, pest control, and other agricultural practices were applied as usual in rice fields under the Egyptian conditions.

2.5. Agronomic Traits and Yield Components

All plots were drained 10 days before harvesting for the ease of handling the crop harvest. The plants were manually harvested at full maturity on the 25th and 27th of September during the 2019 and 2020 seasons, respectively. Ten plants from each cultivar were randomly collected from each plot to measure the plant height (PH) in cm (measured from the soil surface to the tip of the tallest panicle of each plant); root length (RL) in cm; (measured by root length from the base of the plant to the tip of the longest root); root volume (RV) in mm^3 (determined by measuring the volume of water displaced by the plant root system).

Shoot and root were separated and dried in an oven at 80 °C for 24 h. The heading date (HD) (recorded after flowering by the daily count of panicle exertion), physiological maturity dates (recorded when 80% of grains turn into golden yellow color), the number of leaves (NL), flag leaf area (FLA) in cm^2 (measured by taking maximum length × width × 0.75), number of panicle (NP, cm), panicle length (PL, cm), fertile grain $panicle^{-1}$ (FGP), infertile grain $panicle^{-1}$ (IGP), panicle weight (PW, g), 100-grain weight (100-GW, g), and grain yield $plant^{-1}$ (GYP g) served to determine the grain yields, while all plants in each plot were harvested and converted to yield t ha^{-1}. Water productivity (WP; kg m^{-3}) was calculated by dividing the grain yield by growing season irrigation water [42].

2.6. The Photosynthetic Pigments (Chlorophyll A and B as Well as Carotenoids)

To determine the chlorophyll A (*Chl.* A), chlorophyll B (*Chl.* B), and carotenoid contents ($mg\ g^{-1}$ fresh weight; FW) from the 85-day-old plants of each cultivar [43], 200 mg leaf blade samples were extracted with 100% acetone and were homogenized with the B-Brawn type homogenizer at 1000 rpm for one minute. The homogenate was filtered by two-layer cheese cloths and centrifuged using a Beckman Coulter refrigerated centrifuge (Brea, CA, USA) at 15,000× g for 10 min. The supernatant was separated, and the absorbance of acetone extracts was measured at 663, 645, and 470 nm using an Analytik Jena Specord 200 model spectrophotometer. The *Chl.* A, *Chl.* B, and total content of carotenoids were calculated using the following equation [43]:

$$\textit{Chl. } \mathrm{A}\ (\mathrm{mg\ g^{-1}\ FW}) = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times V/(1000 \times W) \quad (1)$$

$$\textit{Chl. } \mathrm{B}\ (\mathrm{mg\ g^{-1}\ FW}) = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V/(1000 \times W) \quad (2)$$

$$\mathrm{Carotenoids}\ (\mathrm{mg\ g^{-1}\ FW}) = [(1000\ A_{470}) - (2.27\ \textit{Chl. } \mathrm{A}) - (81.4\ \textit{Chl. } \mathrm{B})/226] \times V/(1000 \times W) \quad (3)$$

where A_{663}, A_{645}, and A_{670} are the corresponding wavelengths of the light density value, respectively, whilst V is the volume of extracting liquid and W is the weight of fresh leaf sample in grams.

2.7. Drought Tolerance Indices

Drought tolerance indices based on rice grain yield per hectare (GYH) for normal irrigation (Yp) under drought stress (Ys) conditions were calculated for each cultivar and for each SA concentration using the formulas cited in Table 3 to differentiate between rice cultivars based on the drought response in terms of rice GYH.

Table 3. Drought tolerance indices used for the evaluation of rice cultivars to drought conditions.

No.	Index	Equation	Reference
1	Stress susceptibility index (SSI)	$[1-(Y_s/Y_p)]/[1-(\overline{Y}_s/\overline{Y}_p)]$	Fischer and Maurer [27]
2	Tolerance index (TOL)	$Y_p - Y_s$	Rosielle and Hamblin [28]
3	Mean productivity (MP)	$(Y_p + Y_s)/2$	
4	Geometric mean productivity (GMP)	$(Y_p \times Y_s)^{1/2}$	Fernandez [25]
5	Stress tolerance index (STI)	$(Y_p \times Y_s)/(\overline{Y}_p)^2$	
6	Yield index (YI)	$Y_s/\overline{Y}_s$	Gavuzzi et al. [29]
7	Yield stability index (YSI)	Y_s/Y_p	Bouslama and Schapaugh [30]
8	Drought resistance index (DI)	$[Y_s \times (Y_s/Y_p)]/\overline{Y}_s$	Lan [31]
9	Yield reduction ratio (YR)	$1-(Y_S/Y_p)$	Golestani–Araghi and Assad [32]
10	Harmonic mean (HM)	$[2(Y_p \times Y_s)]/(Y_p + Y_s)$	Hossain et al. [33]
11	Golden mean (GOL)	$(Y_p + Y_s)/(Y_p - Y_s)$	Moradi et al. [34]
12	Abiotic tolerance index (ATI)	$[(Y_p - Y_s)/(\overline{Y}_p - \overline{Y}_s)] \times [\sqrt{Y_p \times Y_s}]$	Moosavi et al. [35]
13	Stress susceptibility percentage index (SSPI)	$[(Y_p - Y_s)/2(\overline{Y}_p)] \times 100$	

Y_p and Y_s indicates the grain yield of each cultivar under non-stress and stress conditions, respectively. $\overline{Y}_p$ and $\overline{Y}_s$ indicates the mean grain yield of all cultivars under non-stress and stress conditions, respectively.

2.8. Statistical Analysis

The normality of data distribution was verified using the Kolmogorov–Smirnov test. A combined analysis of variance was performed to determine the main interaction effects of cultivars and different SA concentrations under normal and drought stress conditions on quantitative traits over two years and computed according to the method of Steel et al. [44]. The CV% estimates were categorized as very high ($CV \geq 21\%$), high ($15\% \leq CV \leq 21\%$), moderate ($10\% \leq CV \leq 15\%$) and low ($CV \leq 10\%$) according to Gomes [45]. The obtained data were expressed as the mean ± standard error (SE) and multiple comparisons were determined using the least significant difference test (LSD) at a 0.05 level of probability [44]. Plot Pearson's correlation coefficient and PCA were applied for a better understanding of the relationship among studied traits across experimental factors. The ANOVA, plot Pearson's correlation coefficient, and PCA were performed using a computer software program SPSS version 25 and Origin Pro 2021 version b 9.5.0.193.

3. Results

3.1. Combined ANOVA

The results of the combined ANOVA of the main interaction effects of cultivars and different SA concentrations under normal and drought stress conditions on quantitative traits were shown in Table 4. The combined results showed that the mean squares due to irrigation conditions (I), cultivars (C), and SA concentrations were significant at the 0.01 probability level for all studied traits, except the carotenoids content, PH and HD traits, respectively. The interaction I × C showed significant effects on all the investigated traits at the 0.01 probability level, except NL and HD. The effects of the I × SA interaction was significant on the variables of PH, NL, FLA, *Chl.* A, *Chl.* B, and carotenoids, IGP and 100-GW ($p \leq 0.05$ or 0.01).

Table 4. Combined analysis of variance for the various quantitative traits of two rice cultivars and different salicylic acid under normal and drought stress conditions.

Trait	Mean Square									CV (%)
	Replicates	Irrigation (I)	Cultivars (C)	I × C	Salicylic Acid (SA)	I × SA	C × SA	I × C × SA	Error	
df	2	1	1	1	3	3	3	3	30	
RL	0.26 ^{ns}	598.90 **	195.42 **	44.18 **	48.54 **	1.96 ^{ns}	3.78 ^{ns}	1.23 ^{ns}	2.71	7.03
PH	2.73 ^{ns}	3998.93 **	8.77 ^{ns}	35.23 **	114.96 **	82.22 **	15.57 *	8.74 ^{ns}	3.69	2.23
RV	2.94 ^{ns}	3206.32 **	2203.30 **	216.77 **	52.83 **	3.18 ^{ns}	0.26 ^{ns}	0.25 ^{ns}	1.85	3.89
NL	11.85 ^{ns}	3468.00 **	12,096.75 **	60.75 ^{ns}	305.44 **	33.92 *	11.89 ^{ns}	20.17 ^{ns}	11.38	5.90
FLA	1.51 ^{ns}	62.99 **	606.54 **	758.09 **	60.65 **	13.59 **	7.87 ^{ns}	2.89 ^{ns}	2.86	4.42
SDW	0.12 ^{ns}	826.56 **	537.98 **	66.99 **	38.61 **	2.55 ^{ns}	0.43 ^{ns}	1.91 ^{ns}	1.57	6.15
RDW	3.27 *	300.73 **	336.74 **	10.65 **	31.22 **	0.16 ^{ns}	4.36 **	0.15 ^{ns}	0.80	6.28
Chl. A	0.51 ^{ns}	964.68 **	744.70 **	735.82 **	116.85 **	6.55 **	5.60 ^{ns}	0.28 ^{ns}	1.98	0.51
Chl. B	6.61 *	64.52 **	219.01 **	142.04 **	57.97 **	6.94 *	3.90 ^{ns}	4.28 ^{ns}	1.81	5.27
Carotenoids	0.54 ^{ns}	1.09 ^{ns}	165.61 **	76.10 **	44.23 **	6.39 **	2.05 ^{ns}	4.82 *	1.31	7.03
NP	0.61 ^{ns}	621.36 **	378.00 **	14.85 **	8.63 **	0.46 ^{ns}	0.15 ^{ns}	0.17 ^{ns}	0.84	5.52
HD	1.57 ^{ns}	44.08 **	31.69 **	2.08 ^{ns}	3.17 ^{ns}	0.99 ^{ns}	2.90 ^{ns}	0.54 ^{ns}	1.14	1.15
PL	1.48 ^{ns}	177.58 **	86.20 **	41.12 **	9.54 **	0.64 ^{ns}	0.09 ^{ns}	0.65 ^{ns}	0.47	3.33
FGP	13.09 ^{ns}	18,161.44 **	62,040.71 **	544.56 **	840.58 **	22.80 ^{ns}	14.16 ^{ns}	17.76 ^{ns}	26.63	4.14
IGP	7.50 ^{ns}	6210.75 **	1326.15 **	1419.19 **	415.12 **	62.67 *	51.27 ^{ns}	29.55 ^{ns}	17.98	22.85
PW	0.02 ^{ns}	9.20 **	31.60 **	1.35 **	0.52 **	0.05 ^{ns}	0.04 ^{ns}	0.01 ^{ns}	0.05	7.33
100-GW	0.01 ^{ns}	0.63 **	0.06 **	0.07 **	0.09 **	0.08 **	0.04 *	0.02 ^{ns}	0.01	1.30
GYP	4.37 ^{ns}	2544.29 **	171.63 **	148.46 **	78.01 **	0.63 ^{ns}	3.81 ^{ns}	0.04 ^{ns}	4.07	5.00
GYH	0.04 ^{ns}	150.93 **	12.96 **	7.41 **	4.36 **	0.05 ^{ns}	0.14 ^{ns}	0.03 ^{ns}	0.27	5.17
WP	0.01 ^{ns}	6.82 **	0.65 **	0.26 **	0.20 **	0.03 ^{ns}	0.02 ^{ns}	0.03 ^{ns}	0.03	12.66

df: degree of freedom; CV: coefficient of variation; * and **: statistically significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively; ns: non-significant differences; RL: root length (cm); PH: plant height (cm); RV: root volume (mm^3); NL: number of leaves; FLA: flag leaf area (cm^2); SDW: shoot dry weight (g); RDW: root dry weight (g); *Chl.* A: chlorophyll A (mg g^{-1} FW); *Chl.* B: chlorophyll B (mg g^{-1} FW); NP: number of panicle; HD: heading date (day); PL: panicle length (cm); FGP: fertile grain $panicle^{-1}$; IGP: infertile grain $panicle^{-1}$; PW: panicle weight (g); 100-GW: 100-grain weight (g); GYP: grain yield $plant^{-1}$ (g); GYH: grain yield per hectare (t); and WP: water productivity (kg m^3).

In addition, a significant difference was noticed at the C × SA interaction on PH, 100-GW ($p \leq 0.05$), and RDW ($p \leq 0.01$). The significant mean squares by the second-order interaction (I × C × SA) were only found on the carotenoid content at a 0.05 probability level. A large proportion of the total variation for grain yield and most studied traits was due to the irrigation conditions, followed by cultivars and SA concentrations, while the lowest proportion was due to the second-order interaction. A very high coefficient of variation was noticed for IGP with a value of 22.85%. In contrast to the other measured traits, the values of CV% were low (CV ≤ 10%) under the experimental factors studied (Table 4).

3.2. Main Effects of Three Factors and Their Second-Order Interaction on Rice Traits

Data regarding the morpho-physiological and grain yield traits are given in Tables 5 and 6, respectively. In this study, all investigated traits were significantly affected ($p \leq 0.01$) by irrigation conditions, cultivars, and SA concentrations, except the carotenoid content, PH, and HD traits, respectively. The normal irrigation conditions caused a significant increase in all studied traits, except the traits of carotenoids as compared to the drought irrigation conditions. The Giza179 cultivar increased all measured traits, except IGP and 100-GW traits as compared to the Giza177 cultivar.

Table 5. Morpho-physiological traits of two rice cultivars (C) as affected by irrigation (I), salicylic acid (SA), and their interaction.

Factor	RL	PH	RV	NL	FLA	SDW	RDW	*Chl.* A	*Chl.* B	Carotenoids
	(cm)		(mm^3)		(cm^2)	(g)			($mg\ g^{-1}$ FW)	
Irrigation (I)										
Normal	26.94 ± 0.58 ^{a}	95.35 ± 0.34 ^{a}	43.09 ± 1.03 ^{a}	65.71 ± 3.21 ^{a}	39.44 ± 0.33 ^{a}	24.48 ± 0.64 ^{a}	16.72 ± 0.58 ^{a}	51.40 ± 1.75 ^{a}	26.69 ± 0.97 ^{a}	16.13 ± 0.81 ^{a}
Drought	19.88 ± 0.74 ^{b}	77.10 ± 1.17 ^{b}	26.74 ± 1.94 ^{b}	48.71 ± 3.79 ^{b}	37.15 ± 1.73 ^{b}	16.18 ± 1.00 ^{b}	11.71 ± 0.74 ^{b}	42.45 ± 0.67 ^{b}	24.37 ± 0.48 ^{b}	16.43 ± 0.42 ^{a}
Cultivars (C)										
Giza177	21.39 ± 1.06 ^{b}	85.80±2.33 ^{b}	28.14 ± 2.19 ^{b}	41.33 ± 2.24 ^{b}	34.74 ± 1.26 ^{b}	16.98 ± 1.19 ^{b}	11.56 ± 0.75 ^{b}	43.00 ± 0.54 ^{b}	23.39 ± 0.50 ^{b}	14.43 ± 0.52 ^{b}
Giza179	25.43 ± 0.70 ^{a}	86.65 ± 1.82 ^{a}	41.69 ± 1.35 ^{a}	73.08 ± 2.01 ^{a}	41.85 ± 0.73 ^{a}	23.68 ± 0.73 ^{a}	16.86 ± 0.51 ^{a}	50.85 ± 1.90 ^{a}	27.66 ± 0.80 ^{a}	18.14 ± 0.50 ^{a}
Salicylic acid (SA)										
SA_0	21.84 ± 1.23 ^{b}	84.03 ± 3.59 ^{b}	33.34 ± 3.28 ^{c}	53.29 ± 5.49 ^{c}	36.59 ± 1.89 ^{c}	19.06 ± 1.66 ^{c}	13.20 ± 1.21 ^{b}	44.25 ± 2.01 ^{c}	23.58 ± 0.72 ^{c}	14.67 ± 0.63 ^{c}
SA_1	23.22 ± 1.35 ^{b,c}	85.21 ± 3.13 ^{b}	34.78 ± 3.29 ^{b}	57.79 ± 5.74 ^{b}	38.41 ± 1.50 ^{b}	20.19 ± 1.68 ^{b}	13.71 ± 1.15 ^{b}	47.25 ± 1.98 ^{b}	26.40 ± 1.07 ^{b}	17.10 ± 0.81 ^{b}
SA_2	26.30 ± 1.37 ^{a}	90.81 ± 1.73 ^{a}	37.92 ± 3.18 ^{a}	64.13 ± 5.22 ^{a}	41.44 ± 1.41 ^{a}	22.91 ± 1.74 ^{a}	16.61 ± 0.92 ^{a}	51.18 ± 2.35 ^{a}	28.23 ± 0.84 ^{a}	18.62 ± 0.67 ^{a}
SA_3	22.28 ± 1.38 ^{b}	84.85 ± 2.88 ^{b}	33.62 ± 3.39 ^{c}	53.63 ± 5.69 ^{c}	36.76 ± 2.06 ^{c}	19.15 ± 1.65 ^{c}	13.33 ± 1.27 ^{b}	45.02 ± 2.40 ^{c}	23.90 ± 1.31 ^{c}	14.75 ± 0.98 ^{c}
p-Value										
I	**	**	**	**	**	**	**	**	**	NS
C	**	NS	**	**	**	**	**	**	**	**
SA	**	**	**	**	**	**	**	**	**	**
I × C × SA	NS	NS	NS	NS	NS	NS	NS	NS	NS	*

* $p \leq 0.05$ and ** $p \leq 0.01$. NS indicates a non-significant differences. SA_0, SA_1, SA_2, and SA_3 represent 0, 400, 700, and 1000 μM of SA, respectively. RL: root length; PH: plant height; RV: root volume; NL: number of leaves; FLA: flag leaf area; SDW: shoot dry weight; RDW: root dry weight; *Chl.* A: Chlorophyll A; and *Chl.* B: Chlorophyll B. Means followed by the same letter for each studied factor in each column are not significantly differ according to LSD test ($p \leq 0.05$).

Table 6. Grain yield and its components traits of two rice cultivars (C) as affected by irrigation (I), salicylic acid (SA), and their interaction.

Factor	NP	HD (day)	PL (cm)	FGP (Panicle^{-1})	IGP	PW	100-GW (g)	GY Plant^{-1}	GYH (t)	WP kg m^3
Irrigation (I)										
Normal	20.25 ± 0.51 ^{a}	93.73 ± 0.29 ^{a}	22.63 ± 0.20 ^{a}	144.25 ± 7.00 ^{a}	7.18 ± 0.85 ^{b}	3.49 ± 0.14 ^{a}	2.72 ± 0.02 ^{a}	47.81 ± 0.57 ^{a}	11.83 ± 0.15 ^{a}	1.07 ± 0.05 ^{b}
Drought	13.05 ± 0.74 ^{b}	91.81 ± 0.30 ^{b}	18.78 ± 0.53 ^{b}	105.35 ± 8.41 ^{b}	29.93 ± 2.88 ^{a}	2.61 ± 0.21 ^{b}	2.49 ± 0.02 ^{b}	33.12 ± 0.94 ^{b}	8.28 ± 0.24 ^{b}	1.83 ± 0.06 ^{a}
Cultivars (C)										
Giza177	13.85 ± 0.89 ^{b}	91.96 ± 0.34 ^{b}	19.37 ± 0.63 ^{b}	88.85 ±4.99 ^{b}	23.81 ± 3.92 ^{a}	2.24 ± 0.13 ^{b}	2.64 ± 0.04 ^{a}	38.64 ± 2.01 ^{b}	9.53 ± 0.48 ^{b}	1.34 ± 0.08 ^{b}
Giza179	19.46 ± 0.68 ^{a}	93.58 ± 0.28 ^{a}	22.05 ± 0.30 ^{a}	160.75 ± 3.92 ^{a}	13.30 ± 1.58 ^{b}	3.86 ± 0.09 ^{a}	2.57 ± 0.02 ^{b}	42.30 ± 1.25 ^{a}	10.57 ± 0.31 ^{a}	1.57 ± 0.10 ^{a}
Salicylic acid (SA)										
SA_0	15.90 ± 1.46 ^{b}	92.67 ± 0.60 ^{a}	20.02 ± 0.82 ^{b}	116.54 ± 11.85 d	23.87 ± 5.05 ^{c}	2.89 ± 0.28 ^{b}	2.55 ± 0.05 ^{c}	38.17 ± 2.45 ^{c}	9.54 ± 0.61 ^{c}	1.32 ± 0.15 ^{b}
SA_1	16.67 ± 1.38 ^{b,c}	92.63 ± 0.46 ^{a}	20.55 ± 0.82 ^{b}	125.78 ± 12.72 b	17.19 ± 3.69 ^{b}	3.02 ± 0.28 ^{b}	2.60 ± 0.04 ^{b}	40.37 ± 2.44 ^{b}	10.01 ± 0.59 ^{b}	1.47 ± 0.12 ^{b}
SA_2	17.83 ± 1.34 ^{a}	92.29 ± 0.37 ^{a}	22.00 ± 0.71 ^{a}	136.00 ± 12.50 ^{a}	10.84 ± 3.06 ^{a}	3.35 ± 0.30 ^{a}	2.70 ± 0.03 ^{a}	43.98 ± 2.28 ^{a}	10.91 ± 0.54 ^{a}	1.62 ± 0.14 ^{a}
SA_3	16.21 ± 1.45 ^{b}	93.50 ± 0.51 ^{a}	20.26 ± 0.78 ^{b}	120.87 ± 12.68 ^{c}	22.33 ± 5.18 ^{c}	2.94 ± 0.29 ^{b}	2.56 ± 0.04 ^{c}	39.35 ± 2.41 ^{bc}	9.75 ± 0.59 ^{c}	1.39 ± 0.11 ^{b}
p-Value										
I	**	**	**	**	**	**	**	**	**	**
C	**	**	**	**	**	**	**	**	**	**
SA	**	NS	**	**	**	**	**	**	**	**
I × C × SA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

** $p \leq 0.01$. NS indicates a non-significant difference. SA_0, SA_1, SA_2, and SA_3 represent 0, 400, 700, and 1000 μM of SA, respectively. NP: number of panicle; HD: heading date; PL: panicle length; FGP: fertile grain panicle^{-1}; IGP: infertile grain panicle^{-1}; PW: panicle weight; 100-GW: 100-grain weight; GYP: grain yield plant^{-1}; GYH: grain yield per hectare; and WP: water productivity. Means followed by the same letter for each studied factor in each column are not significantly differ according to LSD test ($p \leq 0.05$).

Regarding the SA effects, the application of 700 μM produced the minimum values for HD and IGP and the maximum values for the grain yield and other studied traits, followed by the application of 400 μM, and then the applications of 0 μM and 1000 μM. The effects of 0 μM and 1000 μM applications were not significant on all studied traits except FGP, which was significant at the 5% probability level. The results indicated the negative effects of SA application with a 1000 μM rate on the grain yield and other studied traits of the two cultivars under normal and drought irrigation conditions.

The Supplementary Tables S1 and S2 have detailed information on the interaction effect of I × C × SA. The interaction effect of I × C × SA had significant effect on the carotenoids content at the 5% probability level and non-significant effect on grain yield and other studied traits. The highest (desirable) decreases for HD were obtained by the interaction effect of Giza177 fertilized with 700 μM and 1000 μM of SA during normal and drought irrigation conditions, respectively (Supplementary Table S2). Whilst, PH and 100-GW were increased by the Giza177 cultivar fertilized with 700 μM of SA under normal and drought irrigation conditions. On the other hand, the interaction of Giza179 cultivar fertilized with 700 μM of SA under normal and drought irrigation conditions produced more grain yield, WP and other measured traits than the other interactions I × C × SA in the present study (Supplementary Tables S1 and S2).

Generally, the Giza179 cultivar under irrigation conditions and SA concentrations recorded the best mean performances for all investigated traits, except 100-GW as compared to Giza177 cultivar. Under drought conditions, the best mean performance of grain yield, WP, and most measured traits were recorded for the Giza179 cultivar fertilized with 700 μM of SA, while an undesirable mean performance was recorded for the application of SA at 0 μM and 1000 μM on Giza177 cultivar. These results confirmed that SA plays a positive role in plant tolerance to drought stress conditions (Tables 5 and 6).

3.3. Drought Tolerance Indices

To assess the drought tolerance of two rice cultivars fertilized with different SA concentrations, the tolerance indices under normal (Yp) and stress (Ys) irrigation conditions based on GYH were calculated and illustrated in Table 7. The highest Yp, Ys, MP, GMP, STI, YI, YSI, DI, HM, and GOL drought tolerance indices and the lowest SSI, TOL, YR, and SSPI drought tolerance indices were obtained from the two fertilized with 700 μM of SA, while the abiotic tolerance index (ATI) index was low in the Giza177 and Giza179 cultivar fertilized with 0 μM and 1000 μM of SA, respectively. Compared with the Giza177 cultivar, the Giza179 cultivar had the highest grain yield and the best values of drought stress indices under normal and drought irrigation conditions. Based on the drought tolerance indices, the Giza179 fertilized with 700 μM of SA was identified as a drought-tolerant combination under drought irrigation conditions in Egypt.

PCA was used to assess the relationship between drought tolerance indices based on grain yield for two rice cultivars fertilized with different concentrations of SA over two years. PCA has condensed the grain yields (Yp and Ys) and drought indices to only two components (PC1 and PC2), which can thus be used as the basis for assessing the relationship between drought tolerance indices (Figure 2). Only the extracted PC1 and PC2 had eigenvalues larger than 1 (13.37 and 1.61, respectively) and explain 99.85% of the total variance of variables. PC1 explains 89.14% of the total variance of variables and is positively correlated with the indices Yp, Ys, MP, GMP, STI, YI, YSI, DI, HM, and GOL under the Giza179 cultivar with SA concentrations. PC2 accounted for 10.71% of the total variance and was positively correlated with indices of Yp, SSI, TOL, YR, SSPI, and ATI under Giza177 cultivar with SA concentrations.

Table 7. Comparison of drought indices for two rice cultivars fertilized with different salicylic acid rates based on grain yield under normal and drought conditions (averaged over two years).

Cultivar	Salicylic Acid (SA)	Index														
		Yp	Ys	SSI	TOL	MP	GMP	STI	YI	YSI	DI	YR	ATI	SSPI	HM	GOL
Giza177	SA_0	11.21	6.65	1.36	4.56	8.93	8.63	0.53	0.80	0.59	0.48	0.41	11.09	19.28	8.35	3.92
	SA_1	11.65	7.28	1.25	4.37	9.47	9.21	0.61	0.88	0.62	0.55	0.38	11.34	18.47	8.96	4.33
	SA_2	12.57	8.52	1.07	4.05	10.55	10.35	0.77	1.03	0.68	0.70	0.32	11.81	17.12	10.16	5.21
	SA_3	11.38	7.02	1.28	4.36	9.20	8.94	0.57	0.85	0.62	0.52	0.38	10.98	18.43	8.68	4.22
Giza179	SA_0	11.52	8.79	0.79	2.73	10.16	10.06	0.72	1.06	0.76	0.81	0.24	7.74	11.54	9.97	7.44
	SA_1	12.00	9.10	0.81	2.90	10.55	10.45	0.78	1.10	0.76	0.83	0.24	8.54	12.26	10.35	7.28
	SA_2	12.62	9.94	0.71	2.68	11.28	11.20	0.90	1.20	0.79	0.95	0.21	8.46	11.33	11.12	8.42
	SA_3	11.68	8.94	0.78	2.74	10.31	10.22	0.75	1.08	0.77	0.83	0.23	7.89	11.58	10.13	7.53
Maximum		12.62	12.62	9.94	1.36	4.56	11.28	11.20	0.90	1.20	0.79	0.95	0.41	11.81	19.28	11.12
Minimum		11.21	11.21	6.65	0.71	2.68	8.93	8.63	0.53	0.80	0.59	0.48	0.21	7.74	11.33	8.35
Mean		11.83	11.83	8.28	1.01	3.55	10.06	9.88	0.70	1.00	0.70	0.71	0.30	9.73	15.00	9.72

SA_0, SA_1, SA_2, and SA_3 represent 0, 400, 700, and 1000 µM of SA, respectively. Yp: grain yield under normal conditions; Ys: grain yield under drought conditions; SSI: stress susceptibility index; TOL: tolerance index; MP: mean productivity; GMP: geometric mean productivity; STI: stress tolerance index; YI: yield index; YSI: yield stability index; DI: drought resistance index; YR: yield reduction ratio; ATI: abiotic tolerance index; SSPI: stress susceptibility percentage index; HM: harmonic mean; and GOL: golden mean.

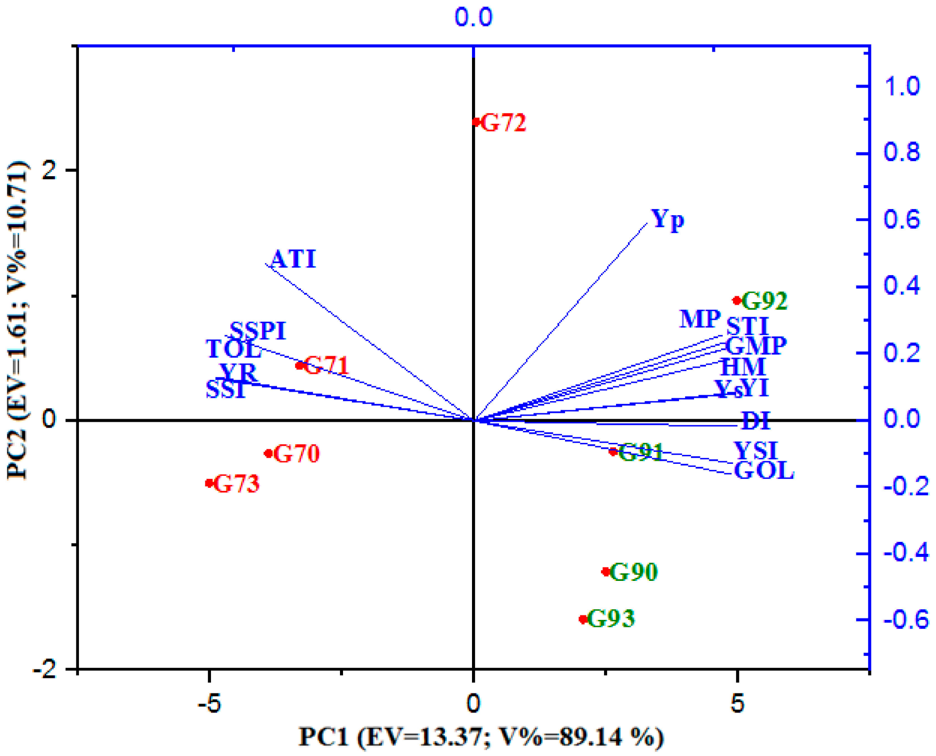

Figure 2. Biplot diagram based on principal component analysis (PC1) and PC2 shows similarities and dissimilarities in relationships among the drought indices for two rice cultivars fertilized with different salicylic acid (SA) rates based on the grain yield under normal (Yp) and drought (Ys) conditions; G90, G91, G92, and G93: Giza179 fertilized with 0, 400, 700, and 1000 µM of SA, respectively; G70, G71, G72, and G73: Giza177 fertilized with 0, 400, 700, and 1000 µM of SA, respectively. SSI: stress susceptibility index; TOL: tolerance index; MP: mean productivity; GMP: geometric mean productivity; STI: stress tolerance index; YI: yield index; YSI: yield stability index; DI: drought resistance index; YR: yield reduction ratio; ATI: abiotic tolerance index; SSPI: stress susceptibility percentage index; HM: harmonic mean; and GOL: golden mean.

In biplot analysis (Figure 2), the sharp angle (below 90 degrees) and the obtuse angle (above 90 degrees) between the variables indicated the positive and negative correlation between variables, respectively. Under Yp and Ys, positive correlations were observed among the indices of MP, GMP, STI, YI, YSI, DI, HM, and GOL, as well as among the indices of SSI, TOL, YR, SSPI, and ATI. The indices of MP, GMP, STI, YI, YSI, DI, HM, and GOL were highly positively correlated with Giza179 fertilized by 700 µM of SA under normal and drought irrigation conditions.

3.4. Pearson's Correlation Coefficient

Based on the main effects of two rice cultivars and SA concentrations under normal and drought irrigation conditions, Pearson's correlations analysis was performed to study the relationship between the grain yield and other studied traits. The number of positive correlations among studied traits during the drought irrigation conditions was higher than during normal irrigation conditions (Figures 3 and 4).

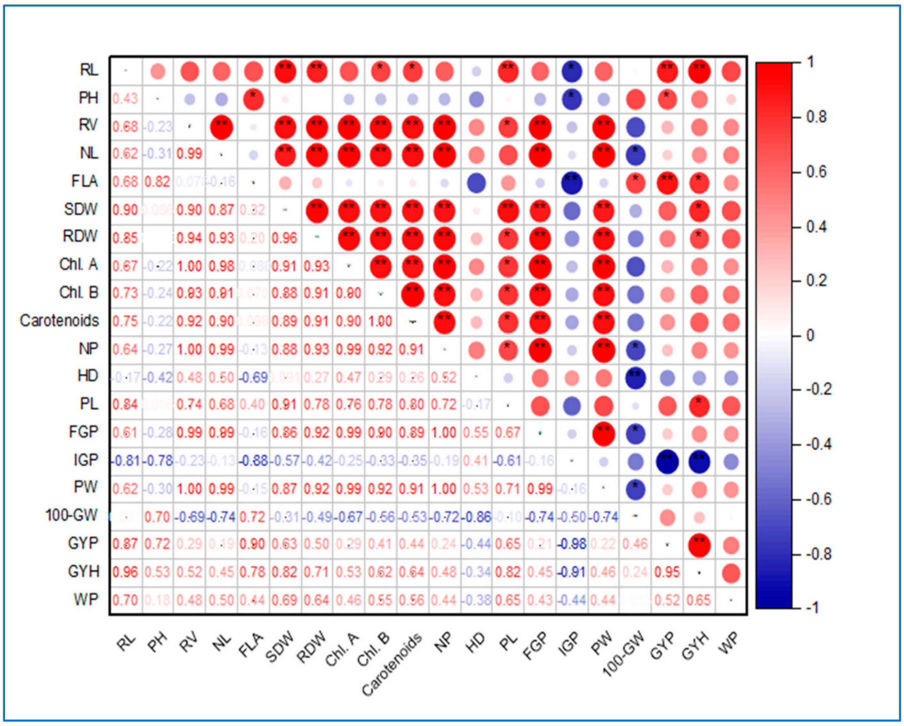

Figure 3. Heat map correlation plot describing Pearson's correlation between the studied traits of rice cultivars under normal irrigation conditions. RL: root length (cm); PH: plant height (cm); RV: root volume (mm^3); NL: number of leaves; FLA: flag leaf area (cm^2); SDW: shoot dry weight (g); RDW: root dry weight (g); *Chl.* A: chlorophyll A (mg g^{-1} FW); *Chl.* B: chlorophyll B (mg g^{-1} FW); NP: number of panicle; HD: heading date (day); PL: panicle length (cm); FGP: fertile grain $panicle^{-1}$; IGP: infertile grain $panicle^{-1}$; PW: panicle weight (g); 100-GW: 100-grain weight (g); GYP: grain yield $plant^{-1}$ (g); GYH: grain yield per hectare (t); and WP: water productivity (kg m^3). The large and medium red (positive) and blue (negative) circles indicates a significant (* $p \leq 0.05$) or highly significant correlation (** $p \leq 0.01$), while the small red (positive) and blue (negative) circles indicates non-significant correlations.

Under normal irrigation conditions, the traits of RV, NL, SDW, RDW, *Chl.* A, *Chl.* B, carotenoids, NP, PL, FGP, and PW showed a significant correlation among them ($p \leq 0.05$ or 0.01). The RL showed significant positive correlation with all studied traits except PH, NL, HD, FGP, and IGP traits ($p \leq 0.05$ or 0.01). The following traits showed positive correlation with both, PH with FLA, 100-GW, and GYP ($p \leq 0.05$); and FLA with 100, GYH ($p \leq 0.05$), and GYP ($p \leq 0.01$); the traits of SDW, RDW, and PL with GYH and WP ($p \leq 0.05$); GYP with PL ($p \leq 0.05$) and GYH ($p \leq 0.01$); and GYH with WP ($p \leq 0.05$) (Figure 3).

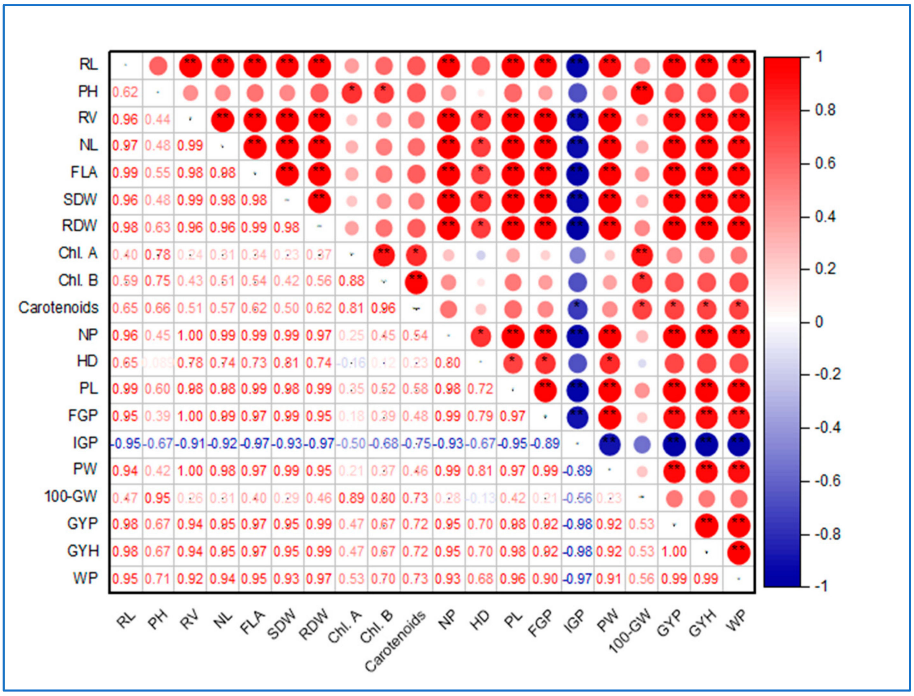

Figure 4. Heat map correlation plot describing Pearson's correlation between the studied traits of rice cultivars under drought irrigation conditions. RL: root length (cm); PH: plant height (cm); RV: root volume (mm^3); NL: number of leaves; FLA: flag leaf area (cm^2); SDW: shoot dry weight (g); RDW: root dry weight (g); *Chl.* A: chlorophyll A (mg g^{-1} FW); *Chl.* B: chlorophyll B (mg g^{-1} FW); NP: number of panicle; HD: heading date (day); PL: panicle length (cm); FGP: fertile grain $panicle^{-1}$; IGP: infertile grain $panicle^{-1}$; PW: panicle weight (g); 100-GW: 100-grain weight (g); GYP: grain yield $plant^{-1}$ (g); GYH: grain yield per hectare (t); and WP: water productivity (kg m^3). The large and medium red (positive) and blue (negative) circles indicates a significant (* $p \leq 0.05$) or highly significant correlation (** $p \leq 0.01$), while the small red (positive) and blue (negative) circles indicates non-significant correlations.

Regarding the drought irrigation conditions, significant positive correlations were observed among *Chl.* A, *Chl.* B, carotenoids, 100-GW, and PH as well as among RL, RV, NL, FLA, SDW, RDW, NP, PL, FGP, PW, GYP, GYH, WP ($p \leq 0.01$), and HD ($p \leq 0.05$). RL and PH had significant positive correlations with carotenoids and RDW ($p \leq 0.05$), respectively. PH, carotenoids, and *Chl.* B had significant positive correlations with GYP, and GYH and WP were significantly positively correlated ($p \leq 0.05$) (Figure 4). Concerning both irrigation treatments, IGP was negatively associated with all measured traits, except for HD with which it had a positive correlation under normal irrigation conditions. In contrast to normal irrigation conditions, strong and significantly positive correlations of grain yield in drought irrigation conditions were found with all studied traits, except *Chl.* A and 100-GW.

3.5. Principal Component Analysis (PCA)

PCA was used to assess the relationship between the studied traits under the main effects of two rice cultivars fertilized with different SA concentrations across normal and drought irrigation conditions over two years. The five PCs for studied traits affected by the two rice cultivars and SA concentrations under normal and drought irrigation conditions are given in Table 8. The first three main extracted PCs had eigenvalues higher than one (15.58, 2.90, and 1.39, respectively), and they explained 99.38% of the total variance of variables.

Table 8. Results of the principal component analyses (PCAs) in the first five PCs for the studied traits during the main effects of experimental factors.

Trait	PC1	PC2	PC3	PC4	PC5
RL	0.25	−0.05	0.12	0.04	0.28
PH	0.21	−0.29	0.20	0.27	−0.03
RV	0.25	−0.03	−0.16	0.08	−0.24
NL	0.23	0.19	−0.18	0.06	−0.15
FLA	0.22	0.30	0.04	0.07	0.29
SDW	0.25	−0.01	−0.04	0.15	−0.11
RDW	0.25	0.07	−0.01	0.34	0.33
Chl. A	0.25	0.05	0.08	−0.14	0.15
Chl. B	0.22	0.25	0.23	−0.31	−0.32
Carotenoids	0.16	0.42	0.25	−0.41	−0.06
NP	0.25	−0.05	−0.16	−0.01	−0.22
HD	0.17	−0.15	−0.57	−0.39	0.47
PL	0.25	−0.03	0.02	0.31	−0.12
FGP	0.23	0.17	−0.26	−0.02	−0.08
IGP	−0.25	0.08	−0.14	0.30	0.05
PW	0.23	0.17	−0.23	0.28	−0.20
100-GW	0.17	−0.29	0.48	0.03	0.14
GYP	0.23	−0.21	0.10	−0.15	0.13
GYH	0.24	−0.20	0.09	−0.07	0.07
WP	−0.08	0.54	0.17	0.23	0.36
Eigenvalues	15.58	2.90	1.39	0.08	0.04
Variance %	77.92	14.50	6.96	0.40	0.22
Cumulative %	77.92	92.42	99.38	99.78	100.00

RL: root length (cm); PH: plant height (cm); RV: root volume (mm^3); NL: number of leaves; FLA: flag leaf area (cm^2); SDW: shoot dry weight (g); RDW: root dry weight (g); *Chl.* A: chlorophyll A (mg g^{-1} FW); *Chl.* B: chlorophyll B (mg g^{-1} FW); NP: number of panicle; HD: heading date (day); PL: panicle length (cm); FGP: fertile grain $panicle^{-1}$; IGP: infertile grain $panicle^{-1}$; PW: panicle weight (g); 100-GW: 100-grain weight (g); GYP: grain yield $plant^{-1}$ (g); GYH: grain yield per hectare (t); and WP: water productivity (kg m^3).

In contrast, the fourth and fifth PCs had eigenvalues less than one (eigenvalue < 1). PC1, PC2, and PC3 explained 77.92%, 14.50%, and 6.96% of the total variance of variables, respectively. Thus, PC1 and PC2 can be used as the basis for assessing the relationship between investigated traits under the main effect of the experimental factors. The PC1 had a high positive correlation with the grain yield and all studied traits, except IGP and WP traits. The PC2 was strongly correlated with FLA, *Chl.* B, carotenoids, and WP traits. The PC3 was highly correlated with PH, *Chl.* B, carotenoids, and 100-GW traits.

The five PCs for the main effects of two rice cultivars, SA concentrations, and irrigation conditions are shown in Table 9. In PC1, there are higher positive correlations with the Giza179 cultivar and the application of SA at 700 µM under normal irrigation conditions. Regarding PC2, the Giza179 cultivar and the application of SA at 400 µM and 700 µM under drought irrigation conditions recorded the highest positive correlation, while the Giza177 cultivar and the application of SA at 400 µM and 700 µM under drought irrigation conditions was positively correlated with PC3.

PC1 and PC2 were employed to draw a biplot and the correlation between the studied traits was calculated under the main effects of two rice cultivars, namely SA concentrations and irrigation conditions (Figure 5). Under the contribution of irrigation conditions, cultivars, and SA concentrations, a sharp angle between most variables in this study was found, indicating a positive correlation between these variables, which differed in their degree and consistency in quantity.

Table 9. Results of principal component analyses (PCAs) for the studied factors based on the studied traits during the normal and drought stress conditions.

Factors	PC1	PC2	PC3	PC4	PC5
Irrigation					
Normal	5.26	−2.21	−0.09	−0.01	−0.04
Drought	−5.25	2.20	0.10	0.01	0.04
Cultivar					
Giza177	−4.14	−1.96	1.22	−0.03	0.03
Giza179	4.14	1.97	−1.20	0.04	−0.01
Salicylic acid (SA)					
SA_0	−1.61	−0.99	−1.53	−0.17	0.34
SA_1	0.04	0.48	0.31	−0.50	−0.30
SA_2	3.59	1.13	1.95	0.17	0.19
SA_3	−2.03	−0.61	−0.76	0.49	−0.25

SA_0, SA_1, SA_2, SA_3 represent 0, 400, 700, and 1000 µM of SA, respectively.

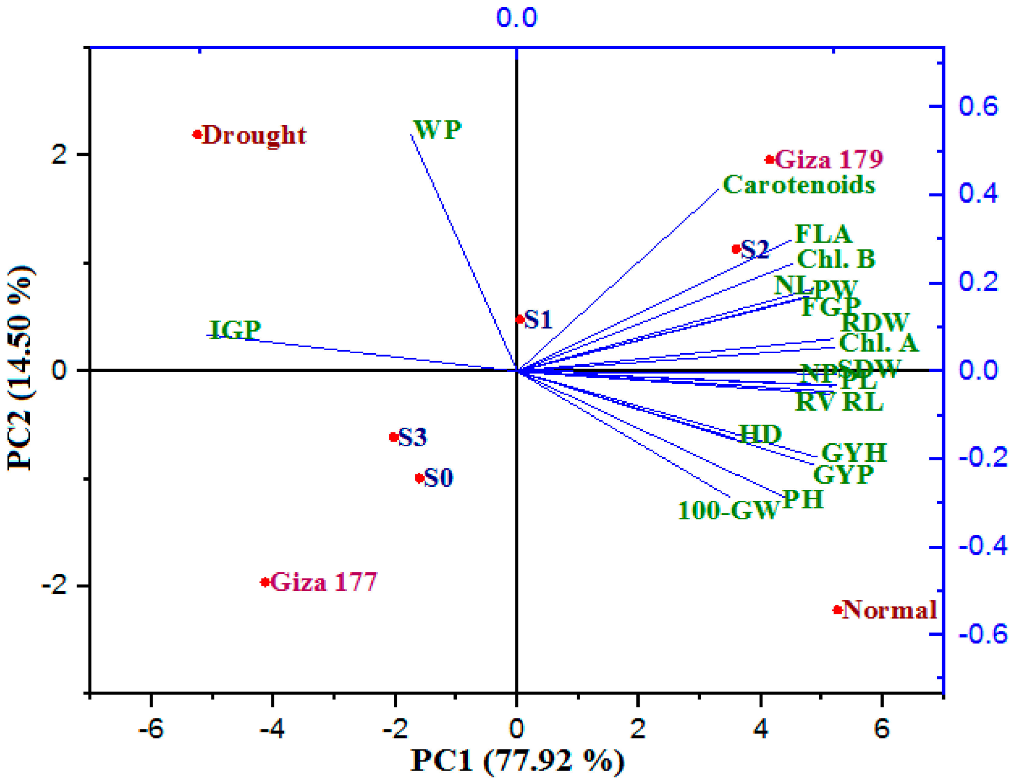

Figure 5. A biplot diagram based on principal component analyses (PC1) and PC2 shows similarity and dissimilarity relationships among the measured traits across two Egyptian rice cultivars and different salicylic acid (SA) concentrations under normal and drought stress conditions. S0, S1, S2, and S3 represent 0, 400, 700, and 1000 µM of salicylic acid, respectively. RL: root length (cm); PH: plant height (cm); RV: root volume (mm^3); NL: number of leaves; FLA: flag leaf area (cm^2); SDW: shoot dry weight (g); RDW: root dry weight (g); *Chl.* A: chlorophyll A (mg g^{-1} FW); *Chl.* B: chlorophyll B (mg g^{-1} FW); NP: number of panicle; HD: heading date (day); PL: panicle length (cm); FGP: fertile grain $panicle^{-1}$; IGP: infertile grain $panicle^{-1}$; PW: panicle weight (g); 100-GW: 100-grain weight (g); GYP: grain yield $plant^{-1}$ (g); GYH: grain yield per hectare (t); and WP: water productivity (kg m^3).

In biplot analysis (Figure 5), the PC1 and PC2 had mainly distributed and distinguished the studied traits into two groups according to their degree of correlations. The first group was related to PC1 and included the grain yield and all studied traits except IGP and WP, which are strongly positively associated with the Giza179 cultivar and the application of SA at 700 µM (first quarter) under normal irrigation conditions (fourth quarter). A positive correlation was observed among all studied traits except for IGP and WP under normal and drought irrigation conditions.

The second group was related to PC2 and included IGP and WP, which had a strong positive correlation with the Giza179 cultivar and the application of SA at 700 µM (first quarter) under drought irrigation conditions (second quarter). IGP was strongly and positively correlated with WP. On the other hand, the Giza177 cultivar was associated with the application of SA at 0 µM and 1000 µM under normal and drought irrigation conditions and occupied the third quarter.

Generally, the Giza179 fertilized with 700 µM of SA was located near the grain yield and most studied traits under normal and drought irrigation conditions. The PCA scree plot for the main effects of fertilizing two rice cultivars with different SA concentrations under normal and drought irrigation conditions on the grain yield and other evaluated traits showed that the PC1 and PC2 eigenvalues corresponded to the whole percentage of the variance in the dataset (Figure 6).

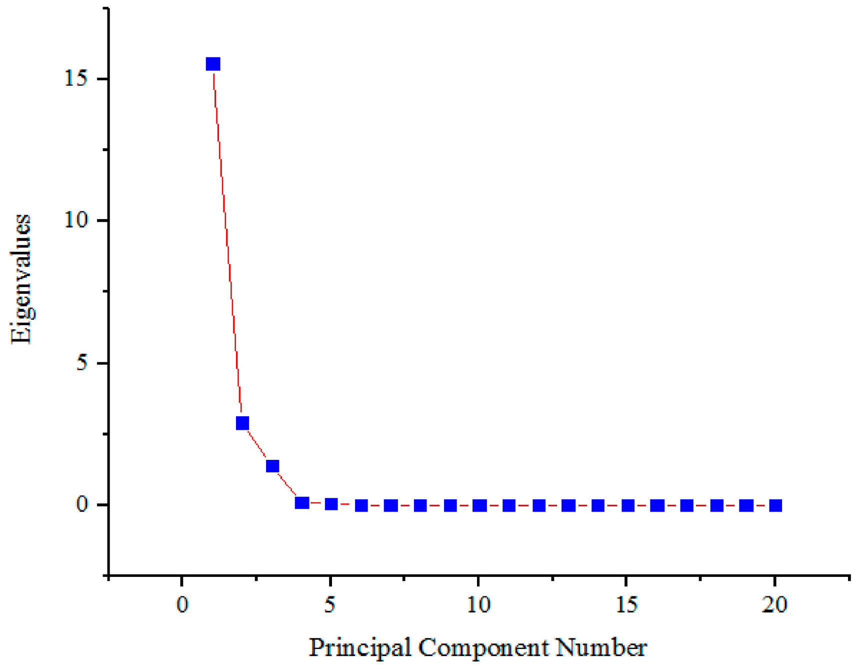

Figure 6. Scree plot of principal component analyses (PCA) between respective eigenvalues % and components number.

4. Discussion

Drought stress is a principal constraint on rice production worldwide and in Egypt. Rice production is being ravaged by drought in the arid and semi-arid ecosystems of the world, as drought affects grain yield and other important traits of rice [20]. In the present work, the two rice cultivars under normal and drought irrigation were subjected to different concentrations of SA to investigate their effects on the grain yield and studied traits, and to find the relationship between these studied traits.

In this study, a significant mean square due to the main effects of irrigation conditions, cultivars, SA, as well as their interactions on grain yield and most studied traits was observed. The significant effects of cultivars, irrigation conditions, SA, and their interactions on rice quantitative traits were previously reported by [18,20,21,38,46,47]. The irrigation conditions, followed by cultivars and SA concentrations, determined a large proportion of the total variation in the grain yield and most studied traits. Garg et al. [48] reported that variations are expected to increase under drought stress conditions and various genotypes respond differentially. The genetic variation between rice cultivars is fundamental to the development of drought tolerance cultivars because they react reversibly to drought stress [49]. Under higher osmotic stress levels, the variation of SA concentrations shows more pronounced effects [21]. These indicate that there was sufficient desirable variability in the two rice cultivars' responses to SA concentrations under normal and drought irrigation conditions, which may be utilized in improving the rice grain yield under drought regions in Egypt.

Drought stress significantly increased the carotenoids, IGP, and WP, and significantly decreased the grain yield and other studied traits as compared to the normal conditions. These results are in accordance with the findings of [36,50–53]. Significant differences in the averages between drought-stressed and well-watered conditions lead to variations in rice grain yield [37]. The detrimental effect of drought stress on the growth and yield traits might be related to the role of water in physiological processes resulting in a reduction in the photosynthetic rate, cell division, and nucleic acid synthesis [54,55], due to the decrease in the number of leaves and plant growth [56].

The Giza179 cultivar showed remarkable superiority in the grain yield and all studied traits over the Giza177 cultivar under both irrigation conditions, except the 100-GW trait. Similar results were also obtained by [36,57]. Under drought conditions, the rice grain yield reduced by 24% and 13%, while WP increased by 19% and 29% in Giza177 and Giza179, respectively, compared to normal irrigation conditions. Giza179 showed relatively higher morpho-physiological traits along with high WP, whereas Hatfield and Dold [58] found that the high photosynthetic rate and water use efficiency are important traits for an effective drought-tolerant genotype. This indicates that the Giza179 cultivar may have drought tolerance in its genetic background and be a good source of drought-tolerance genes; thus, it may be used in the development of drought tolerant cultivars. Drought-tolerant genotypes can develop a set of mechanisms that are more effective in protecting their structure and membrane functions compared to drought-sensitive genotypes [59]. The cultivars that exhibit the highest drought tolerance are often used to investigate drought tolerance [49].

Compared with the control, the grain yield and all studied traits were significantly increased by applying 400 μM of SA, reached a maximum with 700 μM of SA, and then decreased with the increasing rate of 700–1000 μM. Applying 700 μM of SA led to a desirable significant decrease in HD and IGP traits. Applying 700 μM of SA increased the rice grain yield and WP by 8% more in Giza179 than in Giza177 under drought conditions. The rice yield contributed morpho-physiological traits and were positively and significantly affected by the application of different concentrations of SA [18,20,60]; therefore, SA significantly increases the rice grain yield.

Many aspects of physiological and biochemical processes are affected by SA; thus, SA is a promoted growth regulator to increase plant tolerance under drought stress conditions [61]. Khalvandi et al. [61], Hayat et al. [62], Mutlu et al. [63], Pirasteh-Anosheh et al. [64], and Wang et al. [65] reported that SA may play a main role in promoting drought tolerance in plants through increased elements uptake, increasing the photosynthetic rate, improving the enzymatic and nonenzymatic antioxidant activity, decreasing oxidative stress, concealing the reactive oxygen species (ROS), reserving water in plant cells, improving cell membrane stability, and providing protection for cell structure. SA could be used as a potential protectant to regulate the drought response of plants, thus improving plant growth and increasing yield traits under drought stress conditions [18].

In many other studies, the application of SA led to increased osmotic potential under drought conditions, by increasing morpho-physiological traits, improving yield traits, and inducing changes in the protein expression in rice under drought conditions, for example [19,60,66]. According to our results, the application of SA seems to be beneficial in coping with drought stress conditions, through ameliorating the negative effects of drought stress and improving plant growth and the sustainable productivity of rice and other crops under drought stress.

The I × C × SA interaction had significant effects on the carotenoids content, but not on grain yield and all studied traits. The rice grain yield and its components are greatly affected by the combined influence of drought stresses and SA application [18]. The cultivar Giza179 fertilized with 700 µM of SA was the most tolerant cultivar to drought stress, which severely increased its grain yield and all the other studied traits, as a result of which this cultivar became the most tolerant under drought irrigation conditions compared to cultivar Giza177. A drought tolerance of 100-GW was observed in cultivar Giza177 fertilized with 700 µM of SA. Thus, the performance of Giza177 and Giza179 might depend upon the application of SA, apart from their genetic architecture under drought stress conditions.

The combination of drought tolerance indices under the different concentrations of SA may provide a more useful criterion to evaluate the drought tolerance of the two cultivars studied. The highest values of Yp, Ys, MP, GMP, STI, YI, YSI, DI, HM, and GOL indices, as well as the lowest values of SSI, TOL, YR, ATI, and SSPI indices were observed in cultivar Giza179 fertilized with 700 µM of SA. Hence, these indices were useful in identifying cultivar Giza179 as more drought-tolerant compared to cultivar Giza177, also indicating the higher importance of applying 700 µM of SA in the drought tolerance of wheat compared to other applications of SA concentrations. The PCA of drought tolerance indices exhibited that the highest indices of PC1 and the lowest indices of PC2 which can be referred to as the drought-tolerant high-yield component. The relationship between grain yield (Yp and Ys) and drought tolerance indices is a useful criterion for screening the best indices and identifying superior genotypes under normal and drought conditions. Based on the biplot diagram and according to Fernandez [25], indices of MP, GMP, STI, YI, YSI, DI, HM, and GOL had the best indices of drought tolerance, due to their high correlations with rice grain yield under both normal and drought irrigation conditions.

Additionally, the Yp, Ys, MP, GMP, STI, YI, YSI, DI, HM, and GOL indices were in the opposite direction to SSI, TOL, YR, ATI, and SSPI indices, indicating their adverse correlation with each other. These findings agree with those obtained by [1,36,53,67,68]. Generally, the PCA of drought tolerance indices exhibited the highest indices of PC1 (Yp, Ys, MP, GMP, STI, YI, YSI, DI, HM, and GOL) and the lowest indices of PC2 (SSI, TOL, YR, SSPI, and ATI), and can be referred to as the drought-tolerant high-yield components in relation to Giza179 fertilized with 700 µM of SA.

Positive correlations between the two traits indicated that the selection for the increased value of one trait will result in an increase in the value of the other [69]. Strong positive correlations among most studied traits were observed under normal and drought irrigation conditions. These previous results were reported in several studies [52,53]. The highest positive correlations were found among the studied traits under drought conditions and under normal irrigation conditions and were compared to determine the response to drought stress. A statistically significant correlation was found between the rice grain yield and all studied traits under drought stress conditions, except *Chl.* A, IGP, and 100-GW, indicating that the rice grain yield can be improved and increased by increasing these traits. Falconer and Mackay [70] reported that the correlations of these traits indicated that their drought tolerance abilities are controlled by genes in linkage disequilibrium and/or with pleiotropic effects.

In the current study, the statistical PCA was used to identify the drought tolerance in two rice cultivars under SA concentrations and both normal and drought irrigation conditions, and to estimate the relationships between the studied traits across these variables. The first two extracted PCs had eigenvalues higher than one and contributed 92.42% of the total diversity for combined data during normal and drought irrigation conditions. These findings were consistent with [37,39,71]. The PC1 accounted for 77.92% of the total variance of all analyzed variables, followed by PC2 and PC3. Thus, PC1 can be the basis in the weighting of the selection of variables such as genotypes and SA concentrations under both conditions. In other studies of rice, PC1 contributed the highest variance proportion with 51.10%, 57.65%, 58.83%, and 96.46% of the total variability [37–39,71], respectively.

According to the PCA plot, the Giza179 cultivar and the application of 700 µM SA had the maximum and positive weight on PC1, which are strongly and positively correlated with the grain yield and all analyzed variables, except IGP and WP measures. Therefore, the PC1 can be referred to as the drought-tolerant high-yield component and is important to increase the rice grain yield under drought stress conditions. As for PC2, the IGP and WP measures have the same eigenvector direction and variance as the Giza179 cultivar and the application of SA at 700 µM. PCA confirmed that a positive correlation was observed among all studied traits except IGP and WP under the normal and drought irrigation conditions.

Generally, all analyzed variables by PCA indicated that the cultivar Giza179 was positively correlated with grain yield traits and with the morpho-physiological traits of rice under the application of 700 µM of SA and drought irrigation conditions. The variables analyzed by PCA which contributed the highest for of the total variance could be manipulated during yield improvement programs in rice as suggested by [39,72,73]. Based on our results, the cultivar Giza179 fertilized with 700 µM under drought conditions has the potential to improve plant growth and increase the sustainable productivity of rice in Egypt.

5. Conclusions

Drought stress markedly reduced the photosynthetic pigments (chlorophylls and carotenoids) and plant growth, which ultimately leads to rice grain yield reduction and poor yield contributing traits. The tested rice cultivars have a distinct genetic variation which was mirrored in their differential responsiveness to drought stress. The cultivar Giza179 seems to be a drought-resistant genotype with overall better yield performance under all applied SA levels used under drought conditions, while Giza177 was susceptible to drought stress. In comparison with the other SA levels, the best performer for grain yield and all studied traits was obtained by 700 µM in both cultivars under drought conditions. Based on Pearson's correlation analysis, the measures of root length, root volume, number of leaves, shoot dry weight, root dry weight, number of panicle, heading date, fertile grain panicle^{-1}, panicle weight, water productivity, photosynthetic pigments, panicle length, and grain yield can be used as direct selection criteria to improve genotypes under drought stress conditions. In general, the drought tolerance indices calculated and PCA analysis could be used as suitable methods for studying the drought tolerance mechanisms in rice and were useful in identifying the Giza179 cultivar as drought tolerant with a high yield potential under 700 µM of salicylic acid under drought stress conditions in Egypt.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12081860/s1, Table S1: Morphophysiological traits of two Egyptian rice cultivars (C) as affected by irrigation (I), salicylic acid rates (SA), and their interaction; Table S2: Grain yield and its components traits of two Egyptian rice cultivars (C) as affected by irrigations (I), salicylic acid rates (SA), and their interaction.

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