

Salicylic acid application improves morpho-physiological responses, yield, and water productivity of lowland rice cultivars under normal and deficit irrigation

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Abstract

Background: The main constraint of rice cultivation in Mediterranean area is the limited irrigation water and consumes large. In addition, rice is very sensitive to drought conditions due to its effect on morpho-physiological traits with yield reduction. The application of salicylic acid (SA) has been noticed as very effective to alleviate the adverse effects of drought stress in rice. This investigation was conducted as split-split experiments based on randomized complete blocks design with two rice cultivars (Giza 177 and Giza 179), SA as a foliar application at four concentrations (0, 400, 700, and 1000 μM) under normal and drought conditions.

Results: plant growth, leaf photosynthetic pigments, yields, and most studied traits were significantly affected by irrigation conditions (I), cultivars (C), and SA concentrations ($p < 0.05$ or 0.01). The interaction effect of $I \times C \times SA$ was significant only on carotenoids content ($p < 0.05$). The reduction of grain yield and most studied traits was more pronounced under drought conditions. The Giza 179 proved to be a drought-tolerant cultivar under all SA concentrations in drought conditions, while cv Giza 177 is drought-sensitive. The application of SA at 700 μM recorded the best crop grain yield and all studied traits in both cultivars under drought conditions compared to other SA concentrations. Grain yield (t ha^{-1}) for normal irrigation (Y_p) and drought stress (Y_s) conditions were highly positively correlated with indices of mean productivity index (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 indices of SSI stress susceptibility index (SSI), stress tolerance index (TOL), yield reduction ratio (YR), stress susceptibility percentage index (SSPI) and abiotic tolerance index (ATI).

Conclusions: The results indicated that salicylic acid, as a growth regulator, could be used to alleviate the harmful effect of inadequate water availability in soil on rice cultivars as well as improve the growth, water productivity and yield of the crop

1 Introduction

Rice is ranked the second cereal crop after wheat and it is the most important food crop in the world and Egypt (El-Hashash and EL-Agoury, 2019).

Rice production in the world exceeded 700 x106 tonnes per year, with 167 million hectares under cultivation (FAOSTAT, 2018). Irrigated lowland rice provides more than 75% of rice production (Yuan et al. 2021). Rice has traditionally been produced in flooded conditions with a constant water depth of 5 to 10 cm (Bouman et al. 2007). Lowland rice is primarily direct-seeded or transplanted on puddled soils by ploughing under saturated water conditions, then harrowing and levelling management.

In many parts of the world, the supply of irrigation water for agriculture, particularly rice production, is challenged, not only by a global lack of water resources (Abd El Mageed et al., 2022), but also by rising urban and industrial demand (Boretti, and Rosa, 2019). Rice farming uses a lot of water than other crops around the world; it's estimated that irrigated rice uses roughly 40% of the global water utilised for irrigation (Hoekstra et al., 2011).

To ensure food security and reduce the water shortage in Egypt, the development of acceptable yield, drought-tolerant and water-saving rice cultivars have become increasingly important (El-Hashash and EL-Agoury, 2019).

Exploring the possibilities of drought-tolerant crops is the time required for all terrestrial crop species, especially in the climate change scenario (El-Hashash and Agwa, 2018). Drought stress is a major problem that limits the adoption of high-yielding rice genotypes in drought-prone rainfed rice environments (Lafitte et al., 2007), where moderate drought stress can be broadly characterized by a 31 to 64% loss in rice grain yield compared to normal irrigation conditions (Kumar et al. 2008). Hall (1993) define drought tolerance as the relative yield of genotype compared to other genotypes 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 (Solis et al. 2018). Blum, (1988) mentioned that drought resistance is obstructed by low heritability and deficiency of successful selection methods. Therefore, the selection of rice genotypes should be adapted to drought stress conditions (El-Hashash and EL-Agoury, 2019).

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 photosynthesis rate, antioxidant defense system, transpiration rates, proline metabolisms, stomatal closure reversal, signal transduction inhibition, seed germination promotion, induction of flowering and nutrient uptake (Hayat et al. 2010; Nazar et al. 2015; Chavoushi et al. 2019; Khan et al. 2019). Several researchers have shown the positive effect of SA application on rice, which leads to the improvement of morpho-physiological traits through protecting plants against drought stress, thus increasing rice grain yield (Hosain et al. 2020; Kimbembe et al. 2020; Ali et al. 2021; Rafiq et al. 2021).

The development of high-yielding genotypes requires detailed knowledge of the genetic variability present found in the germplasm of the crop, the correlation among yield components, input requirements, and agriculture practices (Dutta et al. 2013) as an application with SA. Also, the knowledge of the source of genetic variability for the rice grain yield and other traits is considered of great importance to improve the yield and the quality by evolving superior genotypes in rice (Hake and Bhoite, 2021). Heritability is essential for selection as it indicates the extent of transmissibility of a trait into future generations and the quality of phenotype data in multilocation trials (Sabesan et al. 2009). The genetic advance is yet another important selection parameter that aids breeders in a selection program (Shukla et al. 2004). Genotypic coefficient of variation (GCV %) and phenotypic coefficient of variation (PCV %) are useful in detecting the amount of variability present in the germplasm. Heritability coupled with high genetic advance would be more useful in predicting the resultant effect in the selection of the best genotypes for yield and yield contributing traits (El-Hashash and EL-Agoury, 2019). A combination of information relating to heritability with values of PCV% and GCV% makes it possible to identify the best genotype to obtain the desired characteristics in the descendants (Burton and Devane, 1953).

Some researchers believe in selection under favorable conditions (Betran et al. 2003) and some believe in selection under typical drought conditions (Ceccarelli and Grando 1991). Nevertheless, there exist numerous researchers that chose the midway and believe in selection under both favorable and stressed conditions (Clark et al. 1992; Fernandez 1992). To differentiate drought-tolerant genotypes, several drought indices have been suggested based on a mathematical relationship between yield under drought and non-stressed conditions. These indices are based on either drought resistance or drought susceptibility of genotypes (Clarke et al. 1984). The stress susceptibility index (SSI) suggested by Fischer and Maurer (1978), tolerance index (TOL) and mean productivity index (MP) by Rosielle and Hamblin (1981). The geometric mean productivity (GMP), by (Fernandez 1992), a stress tolerance index (STI) defined by Fernandez (1992). The yield index (YI) suggested by Gavuzzi et al. (1997), yield stability index (YSI) suggested by Bouslama and Schapaugh (1984), drought resistance Index (DI) by Lan (1998), Yield reduction ratio (YR) by Golestani–Araghi and Assad (1998), harmonic mean (HM) by Hossain et al. (1990) and Golden mean (GOL) by Moradi et al. (2012) in order to evaluate the stability of genotypes in both stress and non-stress conditions. The abiotic tolerance index (ATI) and stress susceptibility percentage index (SSPI) were introduced by Moosavi et al. (2008) for screening drought-tolerant genotypes in stress and non-stress conditions.

There is a need to use PCA to show the results of rice experiments and to select based on a combination of correlations and drought tolerance indices. So, many researchers such as El-Hashash et al. (2018), El-Hashash and EL-Agoury (2019), Bii et al. (2020), Ahmad et al. (2021), and Khan et al. (2022) have used the PCA to assess the relationship and diversity between several rice germplasm, in addition to knowing the relationships between yield and other quantitative traits of rice. This study hypothesized that exogenous application of SA may positively affect rice performance, drought tolerance indices, WP, and leaf photosynthetic pigments. Therefore, our experiment was designed to study the response of two 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 Rice Research and Training Center (RRTC), located (31° 30' 7.59" and 31° 9' 58.09" North and between 30° 20' 36.83" and 31° 17' 15.16 East) in Sakha experimental station, Kafr El-Sheikh Governorate at the northern part of the Nile Delta, between Rosetta and Damietta Nile branches. Data of weather for the studied field experiment as monthly average precipitation, minimum and maximum (mm) temperature (°C), Dew Point (°C), wind speed (km hr⁻¹), relative humidity (%) for the experimental duration (May–September) during both growing summer seasons (2019 and 2020), were presented in Figure (1).

2.2 Soil characteristics of the studied field region

Soil samples were collected for 0.0-0.50 cm depth before planting and air-dried, crushed thoroughly, sieved through a 2-mm sieve and physical and chemical characterization obtained through laboratory analysis. Particle size distribution (sand%, silt%, clay %) was also determined according to (Gee and Bauder 1996). The pH values of soil samples were measured in saturated soil-water paste using a Bekman pH meter (model Elico, LI120-UK) according to Page et al. (1982). The ECe values were determined in saturated soil-water paste extract and defined as dS m⁻¹ using CM25 conductivity meter (model 3200, YSI, Inc., Yellow Springs, Ohio) according to Page et al. (1982). Table 1 shows the characteristics (physical and chemical) analysis of the studied soil in both 2019 and 2020 seasons.

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

Physical and chemical properties		Sakha, Kafr El-Sheikh			
		Unite	2020	2019	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	
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 ⁺⁺		4.55	4.53	4.54	
Mn ⁺⁺		3.20	3.40	3.30	

2.3 Experimental design and treatment details

Two local rice cultivars (Giza 177 and 179) were obtained from Seeds Production Unit of the Rice Research and Training Center, Sakha Agricultural Research Station, Egyptian Ministry of Agriculture and Land Reclamation. The pedigree, origin, and varietal group of these utilized genotypes under study are mentioned in Table 2. Seeds of 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
A. Irrigations conditions (I)	
Normal	Rice plants were irrigated with full irrigation (10710 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)
B. Salicylic acid (SA)	
SA0 (SA0)	Without SA as control
SA400 (SA1)	400 µM of SA was foliar applied three times at 15, 30 and 45 days after transplanting.
SA700 (SA2)	700 µM of SA was foliar applied three times at 15, 30 and 45 days after transplanting.
SA1000 (SA3)	1000 µM of SA was foliar applied three times at 15, 30 and 45 days after transplanting.
C. Cultivars (C)	
Giza 177	Giza171/YomjoNo.1//PiNo.4 (Japonica type, sensitive to drought)
Giza 179	GZ6296/GZ1368 (Indica/Japonica type, moderate to drought)

The experimental design was a split-split-plot arrangement based on 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 m²) for two irrigation treatments with a spacing of 4 m apart each. Each main plot was divided into 4 subplots represented by 24 experimental units (288 m²) identified for four SA treatments (i.e., 0 (SA0), 400 (SA1), 700 (SA2), and 1000 (SA3) µM), which were foliar applied at 15, 30, and 45 days after transplanting.

Each subplot split into 2 sub-sub plots was represented by six experimental units (72 m²), which were randomly allocated for two rice cultivars i.e., Giza 177 and Giza 179. Thirty days old seedling of each cultivar was individually transplanted into the permanent field in 15 row/experimental units (12 m²; 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 land preparation of nursery as recommended agricultural practices, before sowing grains of Giza 177 and 179 rice cultivars were cleaned and soaked in tap water for 24 hours and incubated for 48 hours. After the seed treatment, pre-germinated seeds were broadcasted in the nursery on 20th of May. Seedling of 30 days old age (3–4 seedling hill⁻¹) was transplanted at the field experiment at 20 cm × 20 cm distance between hills and rows in both 2019 and 2020 seasons. The field experiment of each season was basally supplied with 36 kg of P₂O₅ ha⁻¹ (232 kg calcium super monophosphate contained 15.5% P₂O₅) during the preparation of the field. Also, nitrogen was applied with 220 kg N ha⁻¹ in form of urea (46% N). Nitrogen fertilizer was applied in two equal doses, the first one was incorporated into dry soil before flooding and the second one was applied after 30 days of transplanting. 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 Egyptian rain-fed conditions.

2.5 Agronomic traits and yield components

All plots were drained before 10 days of harvesting for ease of handling crop harvest and the plants were harvested manually at full maturity on 25th and 27th of September in 2019 and 2020 seasons, respectively.

Ten equidistance plants from each cultivar were randomly collected from each plot to measure 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 mm³ (determined by measuring the volume of water displaced by plant root system); Shoot and root were separated and dried in an oven at 70°C for five days. The heading date (HD) (recorded after flowering by daily count of panicle exertion), physiological maturity dates (recorded when 80% of grains turn into golden yellow color), NL, flag leaf area (FLA) in cm² (measured by taking maximum length × width × 0.75), NP, PL (cm), FGP, IGP, PW (g), 100-GW (g), and

GYP (g). While, all plants in each plot were harvested to determine grain yields and converted to yield t ha⁻¹. Water productivity (WP; kg m⁻³) was calculated by dividing grain yield by growing season irrigation water, (Sun et al., 2010).

2.6 The photosynthetic pigments (Chlorophyll A, B and carotenoids)

A randomly selected fresh leaves of 85 days old plants from each cultivar to determine the chlorophyll a, b and carotenoid content according to Welburn (1994), the weighed fresh leaf 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 was centrifuged using the NüveFüj 647 model centrifuge at 1500 rpm for ten minutes. The supernatant was separated and the absorbance of acetone extracts was measured at 664 nm, 647 nm and 480 nm using an Analytik Jena Specord 200 model spectrophotometer. The Chlorophyll a, b and total content of carotenoids were calculated as the following equation:

$$\text{Chlorophyll A (mg/dm}^2\text{)} = (11.65 \times A_{664}) - (2.69 \times A_{647}) \times v/sp$$

$$\text{Chlorophyll B (mg/ dm}^2\text{)} = (20.8 \times A_{647}) - 3.14 \times A_{664} \times v/sp$$

$$\text{Carotenoids (mg/ dm}^2\text{)} = (1000 A_{480} - 1.28 \text{ Chlorophyll a} - 5.67 \text{ Chlorophyll b}) / 245 v/sp$$

2.7 Drought tolerance indices

Drought tolerance indices based on rice grain yield (t ha⁻¹) for normal irrigation (Y_p) and drought stress (Y_s) conditions for each cultivar under each SA concentration were calculated using the formulas cited in Table 3 to discriminate based on drought response in terms of rice grain yield (t ha⁻¹).

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

No.	Drought tolerance indices	Equation	Reference
1	Stress susceptibility index (SSI)	$[1 - (Y_s/Y_p)]/[1 - (\bar{Y}_s/\bar{Y}_p)]$	Fischer and Maurer (1978)
2	Stress tolerance index (TOL)	$Y_p - Y_s$	Rosielle and Hamblin (1981)
3	Mean productivity index (MP)	$(Y_p + Y_s)/2$	Rosielle and Hamblin (1981)
4	Geometric mean productivity (GMP)	$(Y_p \times Y_s)^{1/2}$	Fernandez (1992)
5	Stress tolerance index (STI)	$(Y_p \times Y_s) / (\bar{Y}_p)^2$	Fernandez (1992)
6	Yield index (YI)	Y_s / \bar{Y}_s	Gavuzzi et al. (1997)
7	Yield stability index (YSI)	Y_s / Y_p	Bousslama and Schapaugh (1984)
8	Drought resistance Index (DI)	$[Y_s \times (Y_s / Y_p)] / \bar{Y}_s$	Lan (1998)
9	Yield reduction ratio (YR)	$1 - (Y_s / Y_p)$	Golestani-Araghi and Assad (1998)
10	Abiotic tolerance index (ATI)	$\left[(Y_p - Y_s) / (\bar{Y}_p - \bar{Y}_s) \right] \times \left[\sqrt{Y_p \times Y_s} \right]$	Moosavi et al. (2008)
11	Stress susceptibility percentage index (SSPI)	$\left[(Y_p - Y_s) / 2(\bar{Y}_p) \right] \times 100$	Moosavi et al. (2008)
12	Harmonic mean (HM)	$\left[2(Y_p \times Y_s) \right] / (Y_p + Y_s)$	Hossain et al. (1990)
13	Golden mean (GOL)	$(Y_p + Y_s) / (Y_p - Y_s)$	Moradi et al. (2012)
Y_p and Y_s : grain yield of each cultivar under non-stress and stress conditions, respectively.			

\bar{Y}_p and \bar{Y}_s : mean grain yield of all cultivars in non-stress and stress conditions, respectively.

2.8 Statistical Approaches

The normality of data distribution was verified using the Komolgorov- Smirnov test. A combined analysis of variance was performed to determine the main and interactions 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 and Torrie (1997). The CV% estimates were categorized as very high (CV ≥ 21%), high (15% ≤ CV ≤ 21%),

moderate ($10\% < CV \leq 15\%$) and low ($CV < 10\%$) according to Gomes (2009). The obtained data were expressed as mean \pm standard error (SE) and multiple comparisons were determined using the least significant difference test (LSD) at 0.05 level of probability (Steel and Torrie, 1997). The variances components due to the main and interactions effects of three studied experimental factors were estimated with analysis of variance (ANOVA) by Searle et al. (2006). Genotypic (GCV %), phenotypic (PCV %) and error environmental (ECV %) coefficients of variation were calculated according to Burton (1952). Plot Pearson's correlation coefficient and principal component analysis (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 done 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 and interactions effects of cultivars and different SA concentrations under normal and drought stress conditions on quantitative traits were shown in Table 4. The combined results exhibited that the mean squares due to irrigation conditions (I), cultivars (C) and SA concentrations were significant at 0.01 probability level for all studied traits, except carotenoids content, plant height and heading date traits, respectively. The interaction $I \times C$ showed significant effects on all the investigated traits at 0.01 probability level, except No. of leaves and heading date. The effects of $I \times SA$ interaction had significant on variables of plant height, No. of leaves, flag leaf area, chlorophyll a, chlorophyll b and carotenoids, infertile grain panicle⁻¹ and 100-grain weight ($p \leq 0.05$ or 0.01). In addition, a significant difference was noticed by $C \times SA$ interaction on plant height, 100-grain weight ($p \leq 0.05$) and root dry weight ($p \leq 0.01$). The significant mean squares by the second-order interaction ($I \times C \times SA$) were found only on carotenoids content at 0.05 probability level. A large proportion of 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 infertile grain panicle⁻¹ 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).

Table 4

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

Traits	Mean Squares									
	Replications	Irrigation (I)		Cultivars (C)		I × C	Salicylic Acid (SA)	I × SA	C × SA	I × C × SA
df	2	1	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 ^{**}	12096.75 ^{**}	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}	18161.44 ^{**}	62040.71 ^{**}	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: insignificant differences; RL: Root Length, PH: Plant height (cm), RV: Root volume, NL: No. of Leaves, FLA: Flag leaf Area (cm²), SDW: Shoot dry weight, RDW: Root dry weight, Chl. A: Chlorophyll A, Chl. B: Chlorophyll B, NP: No. of Panicle; HD: Heading date, 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 (t ha⁻¹), WP: Water Productivity (kg m³).

3.2 Main effects of three factors and their second-order interaction on rice traits

Data regarding morpho-physiological and grain yield traits are given in Tables 5 and 6, respectively. In this study, all investigated traits were significantly affected ($p < 0.01$) by irrigation conditions, cultivars and SA concentrations, except carotenoids content, plant height and heading date traits, respectively. The normal irrigation conditions caused a significant increase in all studied traits, except traits of carotenoids, infertile grain panicle⁻¹ and water productivity as compared to the drought irrigation conditions. Giza 179 cultivar increased all measured studied, except infertile grain panicle⁻¹ and 100-grain weight traits as compared to Giza 177 cultivar. Regarding the SA effects, the application of 700 μM produced the minimum values for heading date and infertile grain panicle⁻¹ and the maximum values for grain yield and other studied traits, followed by the application of 400 μM , 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 on fertile grain panicle⁻¹ significant at 5% probability level. The results indicated negative effects of SA application with 1000 μM rate on grain yield and other studied traits of the two cultivars under normal and drought irrigation conditions.

The interaction effect of I × C × SA had significant on carotenoids content at 5% probability level and insignificant on grain yield and other studied traits. The highest decreases (desirable) for heading date were obtained by the interaction effect of Giza 177 fertilized with 700 μM and 1000 μM of SA during normal and drought irrigation conditions, respectively (Table 5). While plant height and 100-grain weight were increased by Giza 177 cultivar fertilized with 700 μM of

SA under normal and drought irrigation conditions. On the other hand, the interaction of Giza 179 cultivar fertilized with 700 μM of SA under normal and drought irrigation conditions produced more grain yield, water productivity and other measured traits than the other interactions $I \times C \times SA$ in this study.

Generally, Giza 179 cultivar under irrigation conditions and SA concentrations recorded best mean performances for all investigated traits, except 100-grain weight as compared to Giza 177 cultivar. Under drought conditions, the best mean performance of grain yield, water productivity and most measured traits were registered by the Giza 179 cultivar fertilized with 700 μM of SA, while the undesirable mean performance by application of SA at 0 μM and 1000 μM on Giza 177 cultivar. These results confirmed that SA has a positive role in plant tolerance to drought stress conditions (Tables 5 and 6).

Table 5
Morph physiological traits of two rice cultivars as affected by irrigations conditions, salicylic acid and their interaction.

Factors	RL (cm)	PH	RV (mm ³)	NL	FLA (cm ²)	SDW (g)	RDW	Chl. A (mg/dm ²)	Chl. B
Irrigation conditions (I)									
Normal (NI)	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 ^c
Drought (DI)	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 ^t
Cultivars (C)									
Giza177 (C1)	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	23.1
Giza179 (C2)	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	27.6
Salicylic acid concentrations (SA)									
SA0	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
SA1	23.22 ± 1.35 ^{cb}	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 ^t
SA2	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 ^ε
SA3	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 ^ε
P-value									
I	**	**	**	**	**	**	**	**	**
C	**	NS	**	**	**	**	**	**	**
SA	**	**	**	**	**	**	**	**	**
I x C x SA SA	NS	NS	NS	NS	NS	NS	NS	NS	NS
Statistically significant differences at * $p \leq 0.05$ and ** $p \leq 0.01$, ns: indicate the non-significant difference. RL: Root Length, PH: Plant height (cm), RV: Root Volume, FLA: Flag leaf area (cm ²), SDW: Shoot dry weight, RDW: Root dry weight, Chl. A: Chlorophyll A, Chl. B: Chlorophyll B.									

Table 6
Grain yield and its components traits of two rice cultivars as affected by irrigations conditions, salicylic acid and their interaction.

Factors	NP	HD	PL	FGP	IGP	PW	100-GW	GY plant ⁻¹	GY	WP
		(day)	(cm)	(Panicle ⁻¹)		(g)			(t ha ⁻¹)	Kg m ³
Irrigation conditions (I)										
Normal (NI)	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 (DI)	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 (C1)	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 (C2)	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 concentrations (SA)										
SA0	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
SA1	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
SA2	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
SA3	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 ^{b,c}	9.75 ± 0.59 ^c	1.39 ± 0.11 ^b
P-value										
I	**	**	**	**	**	**	**	**	**	**
C	**	**	**	**	**	**	**	**	**	**
SA	**	NS	**	**	**	**	**	**	**	**
I x C x SA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Statistically significant differences at *p ≤ 0.05 and **p ≤ 0.01; ns: indicate the non-significant difference. NP: No. of Panicle; HD: Heading date, 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 (t ha ⁻¹), WP: Water Productivity (kg m ³).										

3.3 Drought tolerance indices

To assess the drought tolerance of two rice cultivars fertilized with different SA concentrations, tolerance indices under normal (Yp) and stress (Ys) irrigation conditions based on grain yield (t ha⁻¹) 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 ATI index was low in Giza 177 and Giza 179 cultivar fertilized with 0 μM and 1000 μM of SA, respectively. Compared with Giza 177 cultivar, the Giza 179 cultivar had the highest grain yield and the best values of drought stress indices under normal and drought irrigation conditions. Based on drought tolerance Indices, the Giza 179 fertilized with 700 μM of SA had been identified as a drought-tolerant under drought irrigation conditions in Egypt.

Principal component analysis (PCA) was used to assess the relationship between drought tolerance indices based on grain yield for two rice 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), thus which can be used as the basis for assessing the relationship between drought tolerance indices (Fig. 3). Only, PC1 and PC2 extracted had eigenvalues larger than one (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 indices of Yp, Ys, MP, GMP, STI, YI, YSI, DI, HM and GOL under Giza 179 cultivar with SA concentrations. PC2 accounted for 10.71% of the total variance and positively correlated with indices of Yp, SSI, TOL, YR, SSPI and ATI under Giza 177 cultivar with SA concentrations. In biplot analysis (Fig. 3), the sharp angle (below 90 degrees) and the obtuse angle (above 90 degrees) between the variables indicate the positive and negative correlation between variables, respectively. Under Yp and Ys, positive correlations were observed among indices of MP, GMP, STI, YI, YSI, DI, HM and GOL, as well as among indices of SSI, TOL, YR, SSPI and ATI. The indices of MP, GMP, STI, YI, YSI, DI, HM and GOL highly positively correlated with Giza 179 fertilized by 700 μM of SA under normal and drought irrigation conditions.

Table 7

Comparison of drought indices for two rice cultivars fertilized with different salicylic acid rates based on grain yield ($t\ ha^{-1}$) under normal (Y_p) and drought (Y_s) conditions (averaged over 2 years).

Salicylic acid		Drought Tolerance Indices														
		Y_p	Y_s	SSI	TOL	MP	GMP	STI	YI	YSI	DI	YR	ATI	SSPI	HM	GOL
Giza 177	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
Giza 179	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

Yp: grain yield under normal, Ys: grain yield under drought, SSI: susceptibility stress 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, GOL: golden mean.

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 grain yield and other studied traits. The number of positive correlations among studied traits during the drought irrigation conditions was higher than during the normal irrigation conditions (Fig. 4a, b).

Under normal stress, the traits of root volume, No. of leaves, shoot dry weight, root dry weight, chlorophyll a, chlorophyll b, carotenoids, No. of panicle, panicle length, fertile grain panicle⁻¹ and panicle weight showed a significant correlation among them ($p < 0.05$ or 0.01). The RL positively and significantly correlated with all studied traits except plant height, No. of leaves, heading date, fertile grain panicle⁻¹ and infertile grain panicle⁻¹ traits ($p < 0.05$ or 0.01). the plant height with flag leaf area, 100-grain weight and grain yield plant⁻¹ ($p < 0.05$), flag leaf area with 100, grain yield ($t\ ha^{-1}$) ($p < 0.05$) and grain yield plant⁻¹ ($p < 0.01$), the traits of shoot dry weight, root dry weight and panicle length with grain yield ($t\ ha^{-1}$) and water productivity ($p < 0.05$), grain yield plant⁻¹ with panicle length ($p < 0.05$) and grain yield ($t\ ha^{-1}$) ($p < 0.01$), and grain yield ($t\ ha^{-1}$) with water productivity ($p < 0.05$) showed a positive and significant correlation (Fig. 4a). Regarding the drought irrigation conditions, positive and significant correlations were observed among chlorophyll a, chlorophyll b, carotenoids, 100-grain weight and plant height as well as among root length, root volume, No. of leaves, flag leaf area, shoot dry weight, root dry weight, No. of panicle, panicle length, fertile grain panicle⁻¹, panicle weight, grain yield plant⁻¹, grain yield ($t\ ha^{-1}$), water productivity ($p < 0.01$) and heading date ($p < 0.05$). RL and PH significantly positive correlation with carotenoids and root dry weight ($p < 0.05$), respectively. Plant height, carotenoids and chlorophyll b with grain yield plant⁻¹, grain yield ($t\ ha^{-1}$) and water productivity had positive and significant correlations ($p < 0.05$), (Fig. 4b). Concerning both irrigation treatments, infertile grain panicle⁻¹ was negatively associated with all measured traits, except with heading date had a positive correlation under the normal irrigation conditions. Contrary to normal irrigation conditions, strong and positive significant correlations of grain yield in drought irrigation conditions were found with all studied traits except chlorophyll a and 100-grain weight.

3.5 Principal component analysis (PC)

Principal component analysis (PCA) was used to assess the relationship between 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 10. The first three main PCs extracted had eigenvalues higher than one (15.58, 2.90 and 1.39, respectively), and they explain 99.38% of the total variance of variables. In contrast, the fourth and fifth PCs had eigenvalues less than one (Eigenvalue < 1). The 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 grain yield and all studied traits, except infertile grain panicle⁻¹ and water productivity traits. The PC2 is strongly correlated with flag leaf area, chlorophyll b, carotenoids and water productivity traits. The PC3 is highly correlated with plant height, chlorophyll b, carotenoids, and 100-grain weight traits.

Table 8

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

Variables	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, PH: Plant height (cm), RV: Root Volume, NL: No. of Leaves, FLA: Flag Leaf Area (cm ²), SDW: Shoot Dry Weight, RDW: Root Dry Weight, Chl. A: Chlorophyll A, Chl. B: Chlorophyll B, NP: No. of Panicle; HD: Heading date, 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 (t ha ⁻¹), WP: Water Productivity (kg/m ³).					

The five PCs for the main effects of two rice cultivars, SA concentrations and irrigation conditions are shown in Table 11. In PC1, the higher positive correlations with Giza 179 cultivar and the application of SA at 700 μM under normal irrigation conditions. Regarding PC2, Giza 179 cultivar and the application of SA at 400 μM and 700 μM under drought irrigation conditions recorded the highest positive correlation. While Giza 177 cultivar and the application of SA at 400 μM and 700 μM under drought irrigation conditions positively correlated with the PC3.

Table 9

Results of PCs for the studied factors based on the studied traits during the normal and drought stress conditions.

Factors	PC1	PC2	PC3	PC4	PC5
Normal	5.26	-2.21	-0.09	-0.01	-0.04
Drought	-5.25	2.20	0.10	0.01	0.04
Giza 177	-4.14	-1.96	1.22	-0.03	0.03
Giza 179	4.14	1.97	-1.20	0.04	-0.01
SA at 0 μM	-1.61	-0.99	-1.53	-0.17	0.34
SA at 400 μM	0.04	0.48	0.31	-0.50	-0.30
SA at 700 μM	3.59	1.13	1.95	0.17	0.19
SA at 1000 μM	-2.03	-0.61	-0.76	0.49	-0.25

The PC1 and PC2 were employed to draw a biplot and the correlation between studied traits were calculated under the main effects of two rice cultivars, SA concentrations and irrigation conditions (Fig. 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, but they differed in their degree and consistency in quantity. In biplot analysis (Fig. 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 includes grain yield and all studied traits except infertile grain panicle⁻¹ and water productivity, which are strongly positively associated with the Giza 179 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 infertile grain panicle⁻¹ and water productivity under normal and drought irrigation conditions.

While the second group is related to PC2 and includes infertile grain panicle⁻¹ and water productivity, which had a strong positive correlation with the Giza 179 cultivar and the application of SA at 700 µM (first quarter) under drought irrigation conditions (second quarter). Infertile grain panicle⁻¹ was strongly positively correlated with water productivity. On the other hand, the Giza 177 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 Giza 179 fertilized with 700 µM of SA were located near the grain yield and most studied traits under normal and drought irrigation conditions. The PCA scree plot for the main effects of two rice fertilized with different SA concentrations under normal and drought irrigation conditions on grain yield and other traits evaluated showed that the PC1 and PC2 eigenvalues correspond to the whole percentage of the variance in the dataset (Fig. 6).

4 Discussion

Drought stress is a principal constraint to rice production worldwide and in Egypt. Rice production is being ravaged by drought in arid and semi-arid ecosystems of the world, which drought affects grain yield and other important traits in rice (Ali et al. 2021). In the present work, the two rice cultivars under normal and drought irrigation have been subjected to different concentrations of SA in order to investigate their effects on grain yield and studied traits, to estimate genetic parameters and to find the relationship between these studied traits.

In this study, 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, were observed. Significant effects of cultivars, irrigation conditions, SA and their interactions on rice quantitative traits have been previously reported by Yang et al. (2019); Hosain et al. (2020); Ahmad et al. (2021); Ali et al. (2021); Rafiq et al. (2021); El-Mouhamady et al. (2022). The irrigation conditions, followed by cultivars and SA concentrations were showed a large proportion of total variation for grain yield and most studied traits. Garg et al. (2017) reported that under drought stress conditions are expected to increase variations, where 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 (Rasheed et al. 2020). Under higher osmotic stress levels, the variation of SA concentrations showed more pronounced effects (Rafiq et al. 2021). These indicate that there was sufficient variability and desirable in the two rice cultivars responses with 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 content, infertile grain panicle⁻¹ and water productivity, and significantly decreased grain yield and other studied traits as compared to the normal conditions. These results are accordance with the findings of El-Hashash et al. (2018), Torres and Henry (2018), Sohag et al. (2020); El-Mowafi et al. (2021); Hussain et al. (2021). Significant differences in the averages between the drought-stressed and well-watered conditions lead to variations in rice grain yield (Bii et al. 2020). 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 (Abdalla et al. 2007; Abdelaal et al. 2020), due to the decrease in leaves numbers and plant growth (Boyer 1988).

Giza 179 cultivar showed remarkably superiority in the grain yield and all studied traits over the Giza 177 cultivar under both irrigation conditions, except 100-grain weight trait. Similar results were also obtained by El-Hashash et al. (2018); Gaballah et al. (2021). Under drought conditions, reduced rice grain yield by 24% and 13%, while increased water productivity by 19% and 29% in Giza 177 and Giza 179, respectively as compared to normal irrigation conditions. The cv Giza 179 showed relatively higher morpho-physiological traits along with high water productivity, whereas Hatfield and Dold (2019) cleared that high photosynthetic rate and water use efficiency are important traits for an effective drought-tolerant genotype. This indicates that tolerance to drought in Giza 179 cultivar has a common genetic background, and it may be a good source of drought tolerance genes, thus it can be used for the development of cultivars to drought tolerance. Drought-tolerant genotypes can evolve a set of mechanisms that are more effective in protecting their structure and membrane functions compared to drought-sensitive genotypes (Grzesiak et al. 2019). The cultivars that exhibited the highest drought tolerance are often used to investigate drought tolerance (Rasheed et al. 2020).

Compared with control, the grain yield and all studied traits were significantly increased with applying 400 µM of SA, then reached a maximum with applying 700 µM of SA, then decreased with increasing rate of 0 µM into 1000 µM. Applying 700 µM of SA led to a desirable significant decrease in heading date and infertile grain panicle⁻¹ traits. Applying 700 µM of SA increased rice grain yield and water productivity by 8% in Giza 179 than in Giza 177 during drought conditions. Rice yield contributing and morpho-physiological traits are positively and significantly affected by the application of different concentrations of SA (Issak et al. 2017; Hosain et al. 2020; Ali et al. 2021), thus SA significantly increased 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 to drought stress conditions (Khalvandi et al. 2021). Hayat and Ahmad (2007), Mutlu et al. (2016), Pirasteh-Anosheh (2015), Wang et al. (2019) and Khalvandi et al. (2021) reported that SA maybe plays the main role in promoting drought tolerance in plants through increased elements uptake, increased photosynthetic rate, improved enzymatic and nonenzymatic antioxidant activity, decreased oxidative stress or conceal the reactive oxygen species (ROS), reserve water in plant cells, improved cell membrane stability and provide protection for cell structure. SA could be used as a potential protectant to regulating the drought response of plants, thus, improving plant growth and increasing yield traits under drought stress conditions (Hosain et al. 2020). In many other studies, the application of SA led to increased osmotic potential under drought conditions, thus increased morpho-physiological traits, improved yield traits and changes in protein expression in rice under drought conditions (for example Wang et al. 2016; Issak et al. 2017; Kimbembe et al. 2020). 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 sustainable productivity of rice and other crops under drought stress.

I × C × SA interaction had significant effects on carotenoids content, but not on grain yield and all studied traits. Rice grain yield and its components are greatly affected by the combined influence of drought stresses and SA application (Hosain et al. 2020). The cultivar Giza 179 fertilized with 700 µM of SA was the most tolerant to drought stress, leading to a severely increased grain yield and all studied traits, as a result of which this cultivar became the most tolerant under drought irrigation conditions compared to cv Giza 177. The drought tolerance of 100-grain weight in cultivar Giza 177 fertilized with 700 µM of SA was observed. So, the performance of Giza 177 and Giza 179 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 lowest values of SSI, TOL, YR, ATI and SSPI indices, were observed in cv Giza 179 fertilized with 700 µM of SA. Hence, these indices were useful in identifying cv Giza 179 as more drought tolerant as compared to cv Giza 177, also indicating the higher importance of applying 700 µM of SA in drought tolerance of wheat than other applications of SA concentrations. PCA of drought tolerance indices exhibited that the highest indices of PC1 and the lowest indices of PC2 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 (1992), indices of MP, GMP, STI, YI, YSI, DI, HM and GOL had the best indices of drought tolerance, due to that have a high correlation with rice grain yield under both normal and drought irrigation conditions. Also, 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 are in agreement with those obtained by Chaeikar et al. (2018); El-Hashash et al. (2018); El-Hashash and EL-Agoury (2019); Basavaraj et al. (2021); Hussain et al. (2021). Generally, PCA of drought tolerance indices exhibited that 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 related to cv Giza 179 with fertilized 700 µM of SA can be referred to as the drought-tolerant high-yield components.

Positive correlations between the two traits indicate that selection for the increased value of one trait will result in an increase in the value of the other (Yehia and El-Hashash 2021). Strong positive correlations among most studied traits were observed under normal and drought irrigation conditions. These previous results have been reported in several studies such as El-Mowafi et al. (2021); Hussain et al. (2021). The highest number of positive correlations among studied traits during the drought conditions were recorded compared to during the normal irrigation conditions, this may be a response to drought stress. A statistically significant correlation was found between rice grain yield and all studied traits under drought stress conditions, except chlorophyll a, infertile grain panicle⁻¹ and 100-grain weight, indicating that rice grain yield can be improved and increased by increasing these traits. Falconer and Mackay (1996) reported that correlations of these traits indicated that their drought tolerance abilities are controlled by genes in linkage disequilibrium and/or with pleiotropic effects.

In this study, statistical analysis PCA has been used to identify 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 PCs extracted 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 Bii et al. (2020); Laraswati et al. (2021); Khan et al. (2022). The PC1 accounted for 77.92% of the total variance of all analyzed variables, followed by PC2 and PC3. So, PC1 can be the basis in the weighting of selection variables such as genotypes and SA concentrations under both conditions. In other studies of rice, PC1 contributed the highest variance proportion with a value of 51.10%, 57.65, 58.83% and 96.46% of the total variability (Ahmad et al. 2021; Khan et al. 2022; Laraswati et al. 2021; Bii et al. 2020, respectively) According to the PCA plot, Giza 179 cultivar and the application of SA at 700 µM had the maximum and positive weight on PC1, which are strongly positively with grain yield and all analyzed variables, except infertile grain panicle⁻¹ and water productivity measures. Therefore, the PC1 can be referred to as the drought-tolerant high-yield component and is important to increase rice grain yield under drought stress conditions. As for PC2, infertile grain panicle⁻¹ and water productivity measures have the same eigenvector direction and variance with the Giza 179 cultivar and the application of SA at 700 µM. PCA confirmed a positive correlation was observed among all studied traits except infertile grain panicle-1 and water productivity under the normal and drought irrigation conditions. Generally, all analyzed variables by PCA indicate the cv Giza 179 positively correlated with grain yield traits and with morpho-physiological traits of rice under applying 700 µM of SA and drought irrigation conditions. Khan et al. (2017 and 2022) suggested that analyzed variables by PCA which contribute the highest for of the total variance could be manipulated during yield improvement programs in rice. Based on our results, the cv Giza 179 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

The analysis of variance indicated the existence of wide variability due to the main effects of two cultivars and SA concentrations on grain yield and most yield contributing morpho-physiological traits during normal stress and drought conditions. This indicates that the size of differences in these materials was enough to select from them against drought stress. A decrease in grain yield and most investigated traits were observed in drought conditions compared with the normal irrigation conditions. Based on all studied traits, the cv Giza 179 is a drought-resistant cultivar under all SA concentrations in drought conditions, while the cv Giza 177 is susceptible to drought. In comparison with 0 µM, 400 µM and 1000 µM SA, the best performer for grain yield and all studied traits were obtained by 700 µM SA in both cultivars under drought conditions. Based on Pearson's correlation analysis, the measures of root length, root volume, No. of leaves, shoot dry weight, root dry weight, No. of panicle, heading date, fertile grain panicle⁻¹, panicle weight, water productivity, chlorophyll b, carotenoids, panicle length and grain yield can be used as direct selection criteria to improvement genotypes under drought stress conditions. In general, MP, GMP, STI, YI, YSI, DI, HM and GOL indices and PCA analysis could be used as suitable methods for studying the drought tolerance mechanisms in rice and were useful in

identifying the cv Giza 179 as drought-tolerant with high yield potential under 700 μM of SA and both irrigation conditions, this is recommended under drought stress conditions in Egypt.

Declarations

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: Heba Abd El hameed El Sherbiny, Randa Samir Nofal, and Eman Mohamed Bleih; investigation, methodology, and data curation: Heba Abd El hameed El Sherbiny, Randa Samir Nofal, and Eman Mohamed Bleih; preparing original draft: Essam Fathy El-Hashash, Moamen Mohamed Abou El-Enin and Ahmed Shaaban; review and final editing: Moamen Mohamed Abou El-Enin, Ahmed Shaaban, and Taia A. Abd El-Mageed. All authors read and approved the final manuscript.

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Tables

Tables 10-11 are not available with this version

Figures

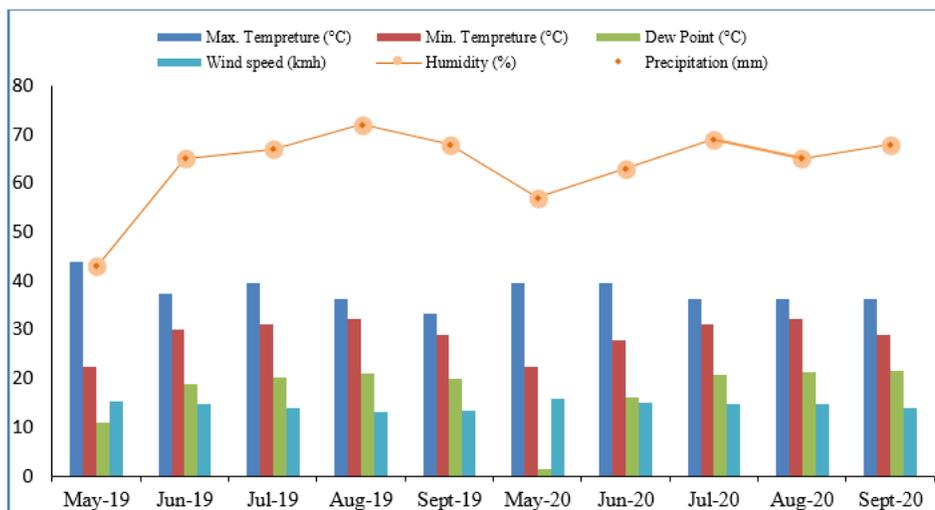


Figure 1

Monthly weather data for the studied field experiment during both growing summer seasons (2019 and 2020).

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Figure 2

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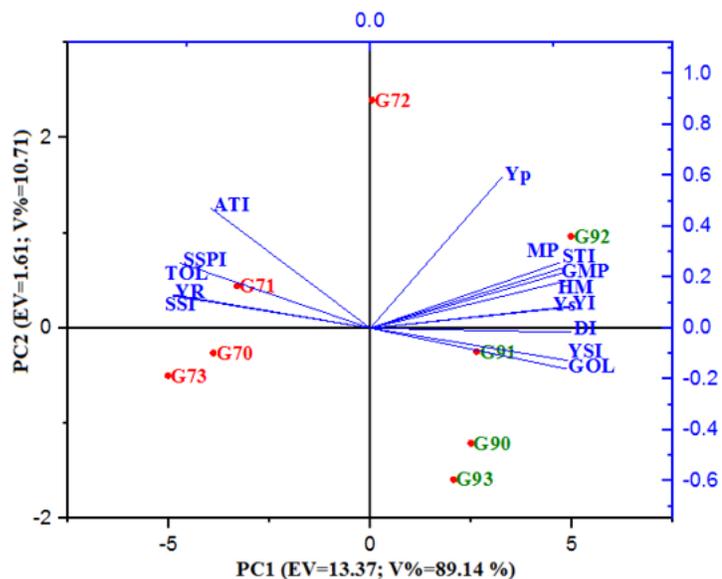
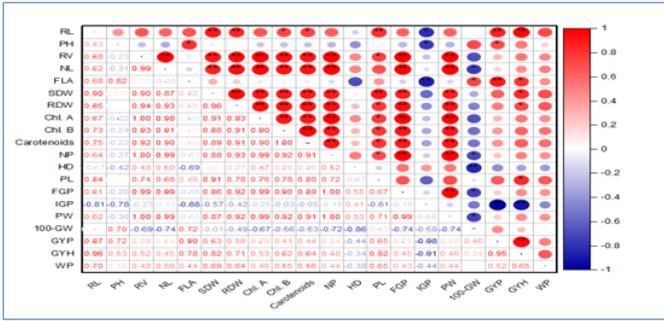
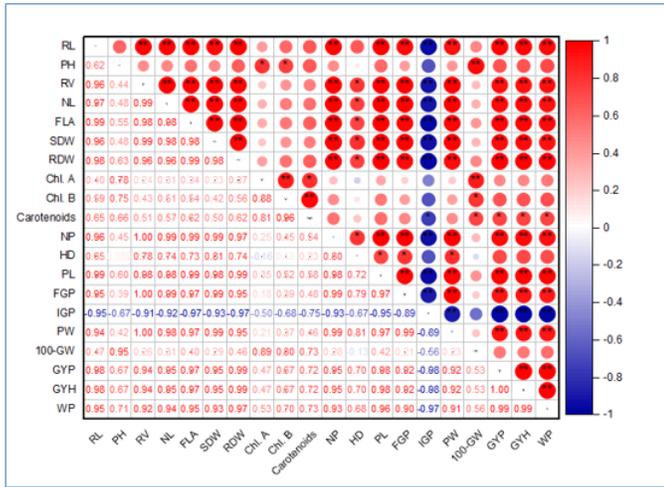


Figure 3

Biplot diagram based on PC1 and PC2 shows similarities and dissimilarities in relationships among the drought indices for two rice cultivars fertilized with different SA rates based on grain yield under normal (Yp) and drought (Ys) conditions; G90, G91, G92 and G93: Giza 179 fertilized with 0, 400, 700 and 1000 μM of SA, respectively; G70, G71, G72 and G73: Giza 177 fertilized with 0, 400, 700 and 1000 μM of SA (SA0, 1, 2, 3), respectively. SSI: susceptibility stress 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, GOL: golden mean.



(a)



(b)

Figure 4

a. Heat map correlation plot describing Pearson's correlation between studied traits of rice cultivars under normal irrigation conditions. RL: Root Length; PH: Plant height (cm); RV: Root Volume; NL: No. of Leaves; FLA: Flag Leaf Area (cm²); SDW: Shoot Dry Weight.; RDW: Root Dry Weight; Chl. A: Chlorophyll A; Chl. B: Chlorophyll B; NP: No. of Panicle; HD; Heading date; 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); GYP: Grain Yield (t ha⁻¹); WP: Water Productivity (kg/m³). The large and medium red (positive) and blue (negative) circles indicate a significant (* p<0.05) or highly significant (** p<0.01), while the small red (positive) and blue (negative) circles indicate non-significant correlation.

b. Heat map correlation plot describing Pearson's correlation between studied traits of rice cultivars under drought irrigation conditions. RL: Root Length; PH: Plant height (cm); RV: Root Volume; NL: No. of Leaves; FLA: Flag Leaf Area (cm²); SDW: Shoot Dry Weight.; RDW: Root Dry Weight; Chl. A: Chlorophyll A; Chl. B: Chlorophyll B; NP: No. of Panicle; HD; Heading date; 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); GYP: Grain Yield (t ha⁻¹); WP: Water Productivity (kg/m³). The large and medium red (positive) and blue (negative) circles indicate a significant (* p<0.05) or highly significant (** p<0.01), while the small red (positive) and blue (negative) circles indicate non-significant correlation.

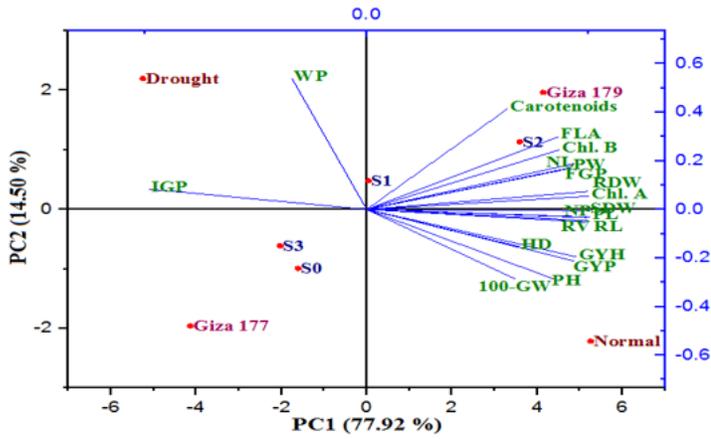


Figure 5
 A biplot diagram based on PC1 and PC2 shows similarities and dissimilarities relationships among the measured traits across two rice Egyptian cultivars and different salicylic acid concentrations in normal and drought stress conditions. S0, S1, S2, S3: SA0, SA400, SA700 and SA700 μM , respectively. RL: Root Length; PH: Plant height (cm); RV: Root Volume; NL: No. of Leaves; FLA: Flag Leaf Area (cm^2); SDW: Shoot Dry Weight.; RDW: Root Dry Weight; Chl. A: Chlorophyll A; Chl. B: Chlorophyll B; NP: No. of Panicle; HD: Heading date; 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); GYP: Grain Yield (t ha^{-1}); WP: Water Productivity (kg/m^3).

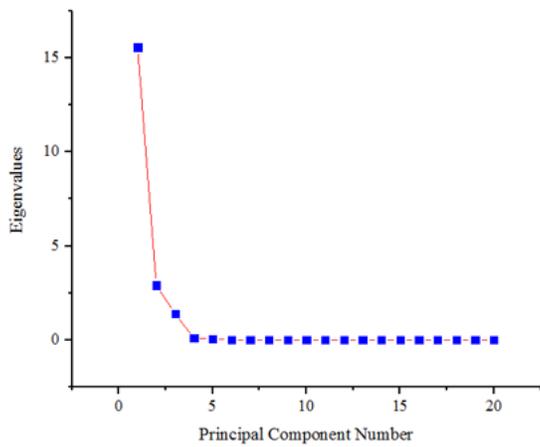


Figure 6
 Scree plot of PCA between respective eigenvalues % and components number.