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Physiological and Biochemical Responses of Moroccan Grapevine (*Vitis vinifera* L.) Varieties to Salinity Stress: Implications for Environmental Stress Adaptation

Karima Hbyaj [1,2], *Yassine Mouniane* [1], *Nouhaila El-Khayat* [1], *Naira Sbbar* [3], *Abdelilah Meddich* [3], *Fadma El Abdellaoui* [4], *Youssef Haouazine* [4], *Aicha El Oualkadi* [2], *Driss Hmouni* [1*]

[1] *Natural Resources and Sustainable Development Laboratory, Faculty of Sciences, Ibn Tofail University, Kenitra BP 133, Morocco*

[2] *Regional Center of Agricultural Research of Tangier, National Institute of Agricultural Research, Rabat 10090, Morocco*

[3] *Centre of Agrobiotechnology and Bioengineering, Research Unit Labeled CNRST (Centre AgroBiotech URL-CNRST 05), "Physiology of Abiotic Stresses" Team, Cadi Ayyad University, Marrakech 40000, Morocco*

[4] *Education, Environment & Health Research Laboratory, Centre Régional des Métiers de l'Éducation et de la Formation (CRMEF), Rabat 11000, Morocco*

ABSTRACT

Salinity is a major environmental constraint limiting grapevine growth, productivity, and long-term sustainability, particularly in arid and semi-arid regions. Understanding the mechanisms underlying varietal tolerance is therefore essential for improving vineyard resilience. This study evaluated four Moroccan grapevine varieties (Bezoul Awda, Sbiea Bnat, Zbarjel, and Krichi) subjected to increasing NaCl concentrations, using morphological, physiological, biochemical, and enzymatic indicators. Salinity significantly reduced shoot and root growth, biomass accumulation, photosynthetic pigments, and relative water content across all cultivars. Conversely, stress-related metabolites such as proline, malondialdehyde, and hydrogen peroxide increased markedly, reflecting enhanced oxidative pressure. Antioxidant enzymes (catalase, peroxidase, and polyphenol oxidase) exhibited strong activation, suggesting the

*CORRESPONDING AUTHOR:
Driss Hmouni, Natural Resources and Sustainable Development Laboratory, Faculty of Sciences, Ibn Tofail University, Kenitra BP 133, Morocco; Email: hmouni.driss@uit.ac.ma

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triggering of defense pathways to mitigate cellular damage. Correlation analysis revealed positive associations among growth and morpho-physiological traits, and negative correlations between these traits and stress indicators. Conversely, antioxidant activities were positively correlated with salinity intensity. Principal component analysis showed that Bezoul Awda and Krichi displayed better growth maintenance and water balance under stress, indicating stronger tolerance strategies compared with Sbiea Bnat and Zbarjel, which exhibited higher biochemical stress responses. Hierarchical clustering grouped SB and KC as tolerant cultivars, whereas ZL and BA formed a more sensitive group. Overall, the integration of morpho-physiological and biochemical markers proved effective in differentiating tolerance mechanisms and identifying grapevine genotypes adapted to saline environments.

Keywords: Salt Stress; Moroccan Grapevine; Oxidative Stress; Enzymatic Antioxidants; Salt Tolerance

1. Introduction

Because plants are sessile organisms, they are very prone to being affected by climate change and abiotic factors [1]; one of these factors would be salinity. Among all of these abiotic stresses, salinity is one of the most important types of salt-related stress that can severely limit agricultural production and food security [2,3]. Salinity has an impact currently on 20% of all arable land and 30% of all lands that are "irrigated," and as salinity continues to increase due to climate change and poor agricultural practices due to irrigation [4,5]. Prolonged exposure to salts has caused changes in osmotic balance and restricted water absorption and utilization due to high concentrations of Na^+ and Cl^- ions negatively interfering with essential biological processes such as photosynthesis, enzyme production and action, and pigment (chlorophyll/a/b) production [6,7]. Prolonged exposure to saline conditions causes osmotic and ionic stress to the plant, limiting the expansion of leaves and newly developing tissues and hastening the senescence (yellowing) and breakdown of chlorophyll; prolonged exposure to excessive saline conditions will have negative effects on plant growth, reproduction, and yield [8,9].

At the level of plant cells and molecular structure, salt stress produces an excessive production of ROS (reactive oxygen species), superoxide, and hydrogen peroxide, which causes oxidative damage to plants and other more complex organisms [10]. Due to the oxidative damage caused by salt stress, plants utilize their antioxidant defence mechanisms, whether through the induction of enzymatic mechanisms (SOD, CAT, POD, APX), or non-enzymatic defence mechanisms such as phenolic compounds, Ascorbic Acid, Carotenoids, and signalling pathways (like SOS (Salt Overly Sensitive)) [11–13]. The SOS pathway signifies AP2/ERF transcriptional regulators that can regulate (A) ion homeostasis, (B) osmotic balance, and (C) hormonal balance via the regulation of stress tolerance. Even though plants continuously adapt to the stresses imposed on them by salinity, they still show tremendous diversity in their ability to tolerate stress both among species and cultivars within the same species [14]. The take-home message from this is that identifying and characterizing stress-tolerant genotypes is critical [15].

The grapevine (*Vitis vinifera* L.) is one of the most important fruit crops in the world, grown over more than 7.5 million hectares, and plays an important role in both agriculture and industry [16,17]. Unfortunately, salinity is also a growing threat to viticulture, especially in arid and semi-arid regions where irrigation is required [18]. Salinity stress in grapevines can manifest both morphologically, by decreasing shoot growth, leaf expansion, and vigor, and physiologically, by reducing chlorophyll content, stomatal conductance, and photosynthetic capacity [19–21]. Salt stress also creates large biochemical changes, including osmolyte accumulation (e.g., glycine betaine, proline, trehalose) and changes in antioxidant enzyme activity [22]. Roots are subject to salt influence and are the first body part that interacts with saline soils; roots are the first line and last line of tolerance, by being responsible for the regulation of ion transport into the plant and subsequently compartmentalizing Na^+ into vacuoles and the regulation of both water and nutrient uptake [23]. However, research on grapevine salinity tolerance is limited by an incomplete understanding of underlying physiological and molecular mechanisms and by the scarcity of studies on autochthonous cultivars.

Salinity is a big problem in Morocco as it affects about 30% of the land area [24]. Viticulture, considering it, is an important cultural heritage, and sector of the economy, a fragile culture and cultivated plant [25]. Indigenous Moroccan grape varieties form an important genetic resource [16], their response to salinity is poorly researched in comparison to the widely studied international cultivars [26]. Therefore, it is important to investigate their physiological and biochemical responses, so we can identify tolerant varieties that may provide rootstocks or agro-breeding parents that could enhance resilience to environmental stresses [27].

The focus of the present study is to assess the morphological, physiological, and biochemical responses in four Moroccan vine varieties to different levels of salinity stress caused by NaCl. The study aims to examine their tolerance mechanisms by considering growth traits, photosynthetic pigments, osmolyte content, and antioxidant enzyme activity. The results will hopefully help develop viable viticultural adaptation strategies for saline environments and protect the sustainability of Moroccan viticulture in an environment of challenges.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Four indigenous Moroccan grapevine (*Vitis vinifera* L.) cultivars originate from the Zoumi region in north-western Morocco, with the following GPS localization: 34°80′61040″N 5°26′29510″W were used in this study. Woody cuttings were obtained from mother plants of each cultivar and propagated through vegetative multiplication. Several cuttings are treated with salinity, while another control group is subject to morphological description concerning the adult leaf.

Cuttings were planted in plastic pots (25 cm diameter, 30 cm depth) containing a substrate mixture of soil, peat, and compost (1:1:1, v/v/v), which has been reported to promote optimal rooting and plant establishment [28]. Plants were maintained in a greenhouse located at the Faculty of Sciences in Kenitra, Ibn Tofail University, Morocco.

The propagation phase lasted three months, during which time the cuttings developed a sufficient root system and vegetative growth. At the end of this period, the plants were pruned and standardized to obtain a uniform size and morphology for all treatments. A completely randomized design was then applied, including the four grape varieties and four salt treatments, with each treatment replicated three times with five plants per repetition.

2.2. Morphological Description

Cluster samples were collected during the fruiting period, and ten adult leaves from each variety were collected for description. These plant organs are described using established qualitative descriptors [29] (**Table 1**).

Table 1. Qualitative descriptors and codes for the morphological description of bunches, berries and mature leaf [29].

Plant Part	Descriptors	OIV Code
Bunch	Density	O-204
Berry	Shape	O-223
	Color of skin	O-225
	Presence of seeds	O-241
Mature leaf	Shape of the blade	O-067
	Number of lobs	O-068
	Density of erect hairs between the main veins on the lower surface of the blade	O-085
	Profile of the cross-section of the blade	O-074
	Anthocyanin coloration area on the main veins on the upper surface of the blade	O-070
	Shape of teeth	O-076
	Opening/overlapping of petiole sinus	O-079
	Shape of base of petiole sinus	O-080
	Degree of openness/overlap of upper side sinus	O-082

2.3. Salinity Treatments

After the establishment phase, the plants were subjected to salt stress for 21 days. Sodium chloride (NaCl) was added to the irrigation water at four concentrations: 0 mM (control), 17 mM, 34 mM, and 85 mM NaCl. Subsequently, the plants were irrigated 2–3 times per week with the corresponding saline solutions throughout the experimental period.

2.4. Soil EC Measurement

The electrical conductivity of the soil (EC) was determined using a soil-water suspension in a ratio of 1:5. In brief, 50 g of oven-dried and sieved (2 mm) soil was mixed with 250 mL of distilled water, stirred manually for 1 min four times at 30-minute intervals, and then left to equilibrate for 4 h. The suspension was then filtered through Whatman No. 42 filter paper, and the EC was measured in the filtrate using a calibrated digital conductivity meter [30].

2.5. Morphological Measurements

In assessing morphological parameters, the plants that were collected after harvest were analyzed to quantify growth and development. Total biomass was determined using a precision scale. Shoot length was determined by measuring from the collar to the apical tip of the shoot. Root length was measured from the collar to the tip of the main root.

2.6. Physiological Parameters

Relative water content was determined using the classical formula based on fresh, turgid, and dry weights of leaves [31]. Photosynthetic pigments, including chlorophylls and carotenoids, were extracted from fresh leaf tissue using 80% acetone and quantified spectrophotometrically [32,33].

2.7. Biochemical Assays

Proline content was estimated using the ninhydrin method [34]. A number of proline standard solutions were made. 2 mL of the proline sample was then mixed with acetic acid and ninhydrin solution. The sample was heated to boil in a water bath for 30 min, cooled, and then mixed with 4 mL of toluene. Absorbance at 520 nm was used to generate the standard curve. Indeed, the soluble sugar content in the dried leaves was measured using the method described by Dubois et al. [35]. Soluble protein concentration in the crude enzyme extract was determined using the Bradford method with modifications [36].

2.8. Oxidative Stress Indicators

The malondialdehyde (MDA) content was determined following the method of de Vos et al. [37]. A 0.15 g sample was ground and extracted with a phosphate buffer (ph 7.8, 0.05 mM). It was subsequently centrifuged at 4 °C, with the supernatant mixed with 0.5 mL of 0.5 % thiobarbituric acid solution at a 100 °C water bath for 20 min and again centrifuged and subsequently measured species spectrophotometrically at wavelengths of 450, 532, and 600 nm. Indeed, Hydrogen peroxide (H_2O_2) content was determined following the procedure described by Velikova et al. [38].

2.9. Antioxidant Enzyme Activities

The activities of key antioxidant enzymes were determined in fresh leaf extracts. Polyphenol oxidase (PPO) activity was measured following Simões et al. [39]. Indeed, Peroxidase (POX) activity was determined according to the method of Kar and Mishra [40]. Moreover, the catalase (CAT) activity was determined according to the specification laid out by de Azevedo Neto et al. [41]. The assay solution contained 1 mL of 0.3 % H_2O_2, 1.9 mL of distilled water, and 0.1 mL of enzyme extract. The change in absorbance at 240 nm was measured at 30–60 s intervals for 210 s.

2.10. Statistical Analyses

All statistical analyses were performed to evaluate the effects of salinity stress and varietal differences on the morpho-physiological and biochemical traits of grapevine. First, descriptive statistics (mean, standard deviation, and percentage variation compared to the control) were calculated for each parameter. A two-way analysis of variance (ANOVA) was then conducted to test the main effects of the two factors, i.e., salinity level (0, 17, 34, and 85 mM

NaCl) and variety (Bezoul Awda, Sbiea Bnat, Zbarjel, and Krichi), as well as their interaction. In addition, pearson's correlation matrix was computed to explore the relationships among morphological, physiological, and biochemical variables under salinity stress. Principal component analysis (PCA) was subsequently applied to reduce dimensionality and to identify the main variables contributing to the discrimination of varieties under salt stress conditions. Finally, hierarchical cluster analysis (HCA) based on Ward's method and Euclidean distances was performed at the highest salinity level (85 mM NaCl) to classify the four grapevine varieties according to their overall response to salinity stress.

3. Results

3.1. Morphological Description

Figure 1 and **Table 2** present the results of the qualitative description for clusters, berries and mature leaf. We observe that the only variety exhibiting the loose clusters is Krichi. Whereas other cultivars have compact and very compact clusters. The shape of the berries is Narrow ellipsoid for Krichi and Sbiea Bnat, obovoid for Zbarjel, while Bezoul Awda cultivars have Broad ellipsoid berries. Berry's color ranges from yellow green for Krichi and Sbiea Bnat to blue-black for Zbarjel, however Bezoul Awda have a dark red violet berry.

The leaf shape is pentagonal for all varieties, while Sbiea Bnat exhibits a Kidney-shaped leaf. All four varieties display 5 lobes except for Krichi cultivars, which possess 7 lobes. We also notice that all cultivars share similar characteristics, namely a flat cross-sectional profile of the mature leaf, the absence of anthocyanin pigmentation in the veins of the upper leaf surface, and an open/closed upper lateral sinus. However, Sbiea Bnat and Zbarjel exhibit none or very few erect hairs between the main veins on the lower leaf surface, whereas this density is moderate for Bezoul awda and high for the Zbarjel cultivar.

The tooth shape is biconvex for Sbiea Bnat and Zbarjel cultivars, straight on both sides for Bezoul Awda, and intermediate, combining straight and convex sides for the Krichi cultivar.

The two cultivars Bezoul awda and Zbarjel have an open petiole sinus, while it 'is open closed for Krichi whereas Sbiea bnat have a very wide-open petiole sinus.

The outline of the petiolar sinus is U-shaped for Bezoul Awda and Krichi cultivars, V-shaped for Zbarjel, and bracket-shaped for Sbiea Bnat. The depth of the lateral sinus ranges from medium for Bezoul awda and Zbarjel, to deep for Sbiea Bnat, reaching a very deep sinus for the Krichi cultivar.

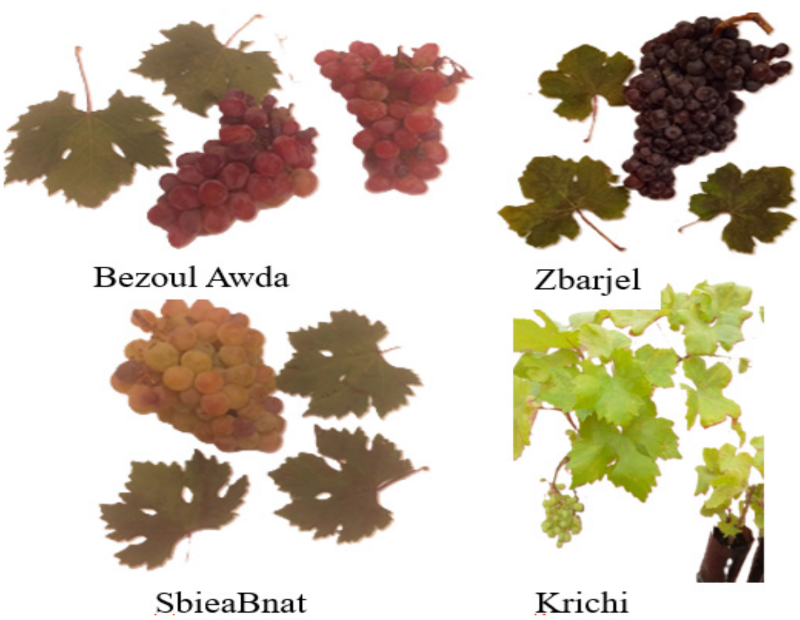

Figure 1. Photos of grapes and leaf of cultivar study.

Table 2. Morphological description of bunches, berries and mature leaf [29]. Mode value.

Varieties	OIV 204	OIV 223	OIV 225	OIV 241	O-067	O-068	O-085	O-074	O-070	O-076	O-079	OIV 080	O-082	OIV 094
Bezoul Awda	7 = Dense	3 = Broad ellipsoid	5 = Dark red violet	1 = Present	3 = Pentagonal	3 = Five	5 = Medium	1 = Flat	1 = Absent	2 = Both sides straight	3 = Open	1 = U-Shaped	4 = Open / Closed	5 = Medium
Krichi	3 = Loose	4 = Narrow ellipsoid	1 = Green yellow	1 = Present	3 = Pentagonal	4 = Seven	7 = High	1 = Flat	1 = Absent	5 = Mixture between both sides straight and both sides convex	4 = Open / closed	1 = U-Shaped	4 = Open / Closed	9 = Very deep
Sbiea Bnat	9 = Very dense	4 = Narrow ellipsoid	1 = Green yellow	1 = Present	5 = Kidney-shaped	3 = Five	1 = None or very low	1 = Flat	1 = Absent	3 = Both sides convex	1 = Very wide open	2 = Brace-shaped({)	4 = Open / Closed	7 = Deep
Zbarjel	9 = Very dense	8 = Obovoid	6 = Blue black	1 = Present	3 = Pentagonal	3 = Five	1 = None or very low	1=Flat	1 = Absent	3 = Both sides convex	3 = Open	3 = V-Shaped	4 = Open / Closed	5 = Medium

3.2. Variation in Electrical Conductivity Under Salt Stress

Soil electrical conductivity (EC) increased significantly with rising NaCl concentrations for all four Moroccan grapevine cultivars (**Figure 2**). At 0 mM NaCl, EC showed the lowest values, ranging from 0.26 mS/cm in Krichi (KC) to 1.08 mS/cm in Zbarjel (ZL). In contrast, exposure to 85 mM NaCl resulted in high salinity levels, with EC values of 2.13 mS/cm in Bezoul Awda (BA), 2.53 mS/cm in Sbiea Bnat (SB), and 2.93 mS/cm in Krichi, reaching a maximum of 3.90 mS/cm in Zbarjel. These results indicate that soils associated with ZL experienced the highest salinity, whereas BA soils were the least affected, suggesting differential soil–plant interactions among cultivars. Analysis of variance (ANOVA) confirmed significant effects of cultivar, salinity level, and their interaction (**Table 3**).

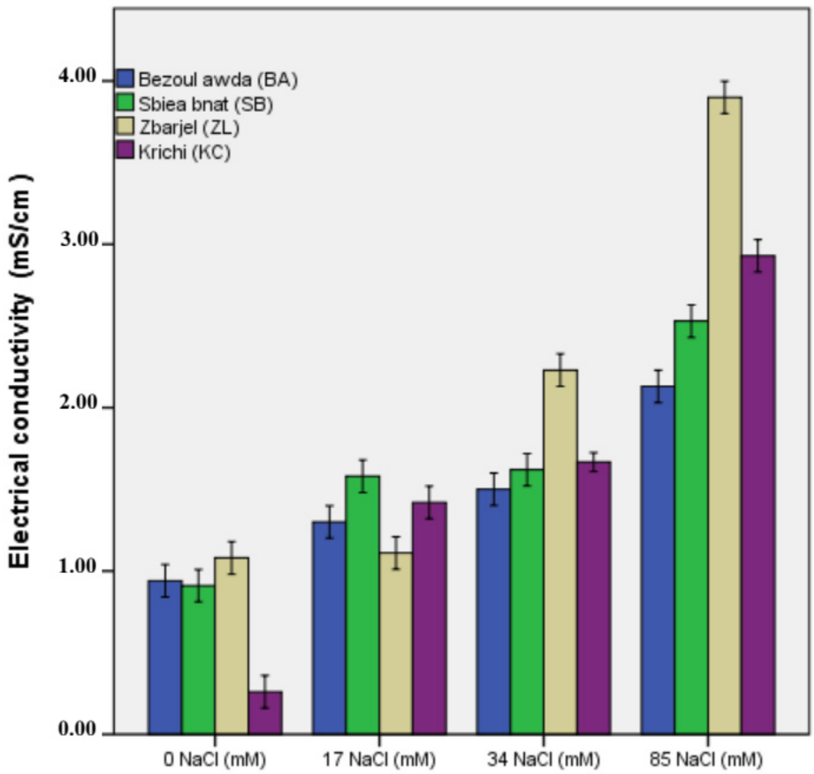

Figure 2. Variation in electrical conductivity in four Moroccan grapevine cultivars under salt stress.

Table 3. ANOVA of the effects of variety and NaCl concentration on electrical conductivity (EC, mS/cm) in four indigenous Moroccan grapevine (*Vitis vinifera* L.) cultivars under salt stress.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	2.604	3	0.868	566.185	0.000*
NaCl	27.754	3	9.251	6033.435	0.000*
Variety × NaCl	5.087	9	0.565	368.641	0.000*

Note: * Significant effect of variety, NaCl, and their interaction ($p < 0.05$).

3.3. Effect of Salt Stress on Morphological Parameters

Morphological traits of all cultivars were strongly affected by salinity, with clear differences between varieties and NaCl treatments (**Table 4**). Biomass decreased under salt stress in Bezoul Awda (BA), dropping from 78.12 g at 0 mM NaCl to 40.15 g at 85 mM. In contrast, Krichi (KC) maintained comparatively higher biomass under stress (55.48 g at 85 mM NaCl). Sbiea Bnat (SB) and Zbarjel (ZL) exhibited lower mean biomass under salinity, averaging 41.96 g and 32.87 g, respectively.

Shoot length also declined markedly, with BA reduced from 62.0 cm in the control to 19.8 cm at 85 mM NaCl, and ZL from 25.1 cm to 8.5 cm. KC, however, retained a relatively longer shoot length under stress (40.0 cm at 85 mM NaCl). Root length followed a similar trend in most cultivars, decreasing from 68.0 to 39.5 cm in BA, 40.2 to 26.0 cm in SB, and 78.0 to 27.7 cm in ZL. Conversely, KC showed greater tolerance, increasing its root length from 38.0 cm in the control to 51.5 cm at 85 mM NaCl. Overall, KC displayed the greatest morphological resilience to salinity, whereas ZL was the most negatively affected. ANOVA confirmed significant effects of cultivar, salinity level, and their interaction ($p \leq 0.001$) on biomass, shoot length, and root length (**Table 5**).

Table 4. Descriptive statistics of growth parameters of four Moroccan grapevine cultivars under NaCl stress.

Variety	NaCl (mM)	Biomass (g) (Mean ± SD)	Shoot Length (cm) (Mean ± SD)	Root Length (cm) (Mean ± SD)
Bezoul awda (BA)	0	78.12 ± 0.04	62.00 ± 0.04	68.00 ± 0.04
	17	59.44 ± 0.04	28.60 ± 0.04	69.00 ± 0.04
	34	56.55 ± 0.04	20.80 ± 0.04	49.33 ± 0.36
	85	40.15 ± 0.04	19.80 ± 0.04	39.50 ± 0.04
Sbiea bnat (SB)	0	42.25 ± 0.04	19.50 ± 0.04	40.20 ± 0.04
	17	51.71 ± 0.04	32.50 ± 0.04	37.10 ± 0.04
	34	36.73 ± 0.04	5.20 ± 0.04	32.80 ± 0.04
	85	37.16 ± 0.04	18.60 ± 0.04	26.00 ± 0.04
Zbarjel (ZL)	0	38.68 ± 0.04	25.10 ± 0.04	78.00 ± 0.04
	17	22.33 ± 0.04	15.50 ± 0.40	34.00 ± 0.04
	34	49.29 ± 0.04	14.01 ± 0.05	34.72 ± 0.24
	85	21.16 ± 0.04	8.50 ± 0.04	27.70 ± 0.40
Krichi (KC)	0	43.31 ± 0.04	81.00 ± 0.04	38.00 ± 0.04
	17	47.80 ± 0.04	13.70 ± 0.04	38.50 ± 0.04
	34	60.77 ± 0.04	36.50 ± 0.04	47.00 ± 0.04
	85	55.48 ± 0.04	40.00 ± 0.04	51.50 ± 0.04

Table 5. ANOVA of growth parameters (Biomass, Shoot length, Root length) in four Moroccan grapevine cultivars under NaCl stress.

Source of Variation	Biomass (g) (*p*-Value)	Shoot Length (cm) (*p*-Value)	Root Length (cm) (*p*-Value)
Variety	<0.001	<0.001	<0.001
NaCl	<0.001	<0.001	<0.001
Variety × NaCl	<0.001	<0.001	<0.001

3.4. Effect of Salt Stress on Physiological Parameters

Physiological parameters revealed a marked effect of salinity on relative water content (RWC), chlorophyll levels, and carotenoid concentrations, with responses varying among cultivars. Overall, RWC decreased substantially under salt stress (**Table 6**). Bezoul Awda (BA) and Sbiea Bnat (SB) declined from 72.58% and 68.04% under control conditions to 14.56% and 57.96% at 85 mM NaCl, respectively, indicating that SB is relatively more tolerant than BA. Zbarjel (ZL) maintained moderate RWC values (62.71% at 17 mM and 53.29% at 85 mM), whereas Krichi (KC) showed the greatest reduction (from 59.45% to 38.30%).

Photosynthetic pigments generally declined with increasing salinity. Chlorophyll content decreased sharply, particularly in BA and ZL, dropping from 22.49 mg/g to 2.53 mg/g in BA and from 23.47 mg/g to 2.63 mg/g in ZL. SB showed a more moderate reduction (19.95 mg/g to 8.04 mg/g), suggesting greater pigment stability. Carotenoid content followed a similar trend, decreasing substantially in KC (10.66 mg/g to 0.13 mg/g) and BA (11.62 mg/g to 1.17 mg/g), while remaining relatively higher in SB (17.93 mg/g to 2.75 mg/g) across treatments.

These results suggest that cultivars differ in physiological tolerance to salinity, with SB and ZL showing higher resistance, whereas BA and KC were more sensitive. ANOVA confirmed highly significant effects of salinity on RWC and chlorophyll content, as well as on the interaction between variety and NaCl treatment ($p < 0.001$), while the effect on carotenoids was not significant ($p > 0.05$) (**Table 7**).

Table 6. Descriptive statistics of physiological parameters of four Moroccan grapevine cultivars under NaCl stress.

Variety	NaCl (mM)	Relative Water Content (%) (Mean ± SD)	Chlorophyll (mg/g FW) (Mean ± SD)	Carotenoids (mg/g FW) (Mean ± SD)
Bezoul awda (BA)	0	72.58 ± 0.04	22.49 ± 0.12	11.63 ± 0.29
	17	67.90 ± 0.03	9.43 ± 1.29	5.76 ± 1.52
	34	46.17 ± 0.04	10.08 ± 4.39	4.08 ± 1.92
	85	14.56 ± 0.04	2.53 ± 0.07	1.17 ± 0.28
Sbiea bnat (SB)	0	68.04 ± 0.04	19.95 ± 0.73	17.94 ± 3.47
	17	64.17 ± 0.04	8.74 ± 0.43	8.55 ± 1.41
	34	53.10 ± 0.04	6.87 ± 0.83	6.64 ± 1.56
	85	57.96 ± 0.04	8.04 ± 2.66	2.75 ± 1.13
Zbarjel (ZL)	0	54.38 ± 0.04	23.47 ± 0.17	13.07 ± 3.41
	17	62.71 ± 0.04	10.35 ± 0.79	8.41 ± 0.20
	34	66.84 ± 0.04	8.89 ± 0.03	6.03 ± 2.80
	85	53.29 ± 0.04	2.63 ± 0.05	1.28 ± 0.47
Krichi (KC)	0	59.45 ± 0.04	13.03 ± 1.84	10.67 ± 3.78
	17	44.28 ± 0.04	9.37 ± 0.70	6.68 ± 0.30
	34	33.05 ± 0.04	7.01 ± 1.23	3.04 ± 0.34
	85	38.30 ± 0.04	1.76 ± 0.09	0.14 ± 0.09

Table 7. ANOVA of physiological parameters (RWC, Chlorophyll, Carotenoids) in four Moroccan grapevine cultivars under NaCl stress.

Source of Variation	Relative Water Content (%) (*p*-Value)	Chlorophyll (mg/g FW) (*p*-Value)	Carotenoids (mg/g FW) (*p*-Value)
Variety	<0.001	<0.001	<0.001
NaCl	<0.001	<0.001	<0.001
Variety × NaCl	<0.001	<0.001	0.349

3.5. Effect of Salt Stress on Biochemical Parameters

Biochemical analysis showed a clear increase in proline accumulation with rising NaCl concentrations, from an average of 7.41 µmol/g at 0 mM to 15.98 µmol/g at 85 mM, representing more than a twofold increase (**Table 8**). All cultivars followed this trend, with Sbiea Bnat displaying the highest proline level (17.65 µmol/g) and Krichi the lowest (8.20 µmol/g).

Total sugars exhibited strong inter-varietal variability: Zbarjel and Bezoul Awda recorded much higher sugar contents (315.7 and 261.4 µmol/g at 0 mM, respectively), while Sbiea Bnat maintained very low levels, not exceeding 24.4 µmol/g even at 85 mM NaCl. Soluble protein content decreased progressively with increasing salinity, from 2.87 mg/g at 0 mM to 0.68 mg/g at 85 mM, with Bezoul Awda showing the greatest reduction (−80%).

ANOVA revealed highly significant effects of NaCl and cultivar on all three biochemical parameters ($p < 0.001$), as well as a significant variety × NaCl interaction for proline and total sugars, but not for soluble proteins (**Table 9**).

Table 8. Descriptive statistics of biochemical parameters in four Moroccan grapevine cultivars under NaCl stress.

Variety	NaCl (mM)	Proline (µmol/g FW) (Mean ± SD)	Total Sugars (µmol/g FW) (Mean ± SD)	Proteins (mg/g FW) (Mean ± SD)
Bezoul awda (BA)	0	7.31 ± 0.39	261.40 ± 1.23	3.32 ± 0.21
	17	8.53 ± 0.49	235.60 ± 1.08	2.10 ± 0.18
	34	9.94 ± 0.76	234.50 ± 1.45	1.35 ± 0.27
	85	15.03 ± 0.36	285.63 ± 1.42	0.66 ± 0.17
Sbiea bnat (SB)	0	7.76 ± 0.21	164.03 ± 1.05	3.17 ± 0.56
	17	8.97 ± 0.36	9.46 ± 1.13	1.54 ± 0.44
	34	12.32 ± 0.31	21.10 ± 1.10	1.06 ± 0.18
	85	17.66 ± 0.50	24.40 ± 1.10	0.71 ± 0.17
Zbarjel (ZL)	0	7.68 ± 0.43	315.70 ± 0.10	2.60 ± 0.65
	17	8.85 ± 0.56	15.10 ± 0.10	1.44 ± 0.42
	34	11.68 ± 0.25	177.90 ± 0.10	0.68 ± 0.05
	85	15.27 ± 0.21	164.20 ± 0.10	0.68 ± 0.10
Krichi (KC)	0	6.91 ± 0.32	131.93 ± 1.06	2.42 ± 0.43
	17	8.32 ± 0.07	184.13 ± 1.42	1.44 ± 0.35
	34	9.37 ± 0.67	200.00 ± 1.10	0.42 ± 0.09
	85	13.33 ± 0.24	265.63 ± 3.23	0.62 ± 0.10

Table 9. Analysis of variance for biochemical parameters.

Source of Variation	Proline (µmol/g FW) (*p*-Value)	Total Sugars (µmol/g FW) (*p*-Value)	Proteins (mg/g FW) (*p*-Value)
Variety	< 0.001	< 0.001	< 0.001
NaCl	< 0.001	< 0.001	< 0.001
Variety × NaCl	< 0.001	< 0.001	0.263

3.6. Assessment of Oxidative Stress Indicators

The oxidative stress assessment showed that malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) concentrations increased with rising NaCl levels, with notable differences among cultivars (**Table 10**). Under control conditions (0 mM NaCl), MDA values were low and relatively similar, ranging from 5.16 nmol/g FW in Krichi (KC) to 6.88 nmol/g FW in Sbiea Bnat (SB). Under severe salt stress (85 mM NaCl), MDA increased markedly, with Bezoul Awda (BA) showing the highest level (27.53 nmol/g FW), followed by KC (23.66 nmol/g FW), while SB (14.52 nmol/g FW) and Zbarjel (ZL) (16.14 nmol/g FW) exhibited lower increases.

A similar trend was observed for H_2O_2. Control values ranged from 19.99 nmol/g FW in KC to 40.59 nmol/g FW in SB, but increased substantially under salinity, reaching 184.05 nmol/g FW in SB, 124.58 nmol/g FW in ZL, and 114.78 nmol/g FW in BA. KC showed the lowest increase (90.31 nmol/g FW).

ANOVA indicated highly significant effects of NaCl, cultivar, and their interaction for both oxidative markers (MDA: F(Variety) = 3.27, $p = 0.034$; F(NaCl) = 147.00, $p < 0.001$; F (Interaction) = 13.32, $p < 0.001$; H_2O_2: F(Variety) = 31.54, $p < 0.001$; F(NaCl) = 311.71, $p < 0.001$; F (Interaction) = 12.86, $p < 0.001$), confirming a strong model fit.

Table 10. Descriptive statistics of biochemical parameters in four Moroccan grapevine cultivars under NaCl stress.

Variety	NaCl (mM)	MDA (nmol/g FW) Mean ± SD	H_2O_2 (nmol/g FW) Mean ± SD
Bezoul Awda (BA)	0	6.19 ± 2.06	22.31 ± 0.82
	17	7.91 ± 1.58	49.72 ± 4.21
	34	14.45 ± 1.79	61.35 ± 20.62
	85	27.53 ± 2.59	114.78 ± 8.02
Sbiea Bnat (SB)	0	6.88 ± 1.19	40.59 ± 0.85
	17	6.30 ± 2.10	53.71 ± 3.98
	34	19.96 ± 0.60	66.11 ± 3.67
	85	14.52 ± 1.97	184.05 ± 13.67
Zbarjel (ZL)	0	6.19 ± 2.06	27.52 ± 3.02
	17	9.29 ± 1.03	50.44 ± 12.39
	34	17.89 ± 2.60	65.01 ± 14.44
	85	16.14 ± 2.90	124.58 ± 1.52
Krichi (KC)	0	5.16 ± 1.03	19.99 ± 7.18
	17	7.23 ± 1.03	45.46 ± 0.91
	34	11.35 ± 2.73	58.47 ± 2.34
	85	23.66 ± 1.93	90.31 ± 1.35
Source of variation		(F, *p*-value)	
Variety		3.27, $p = 0.034$	31.54, $p < 0.001$
NaCl		147.00, $p < 0.001$	311.71, $p < 0.001$
Variety × NaCl		13.32, $p < 0.001$	12.86, $p < 0.001$

3.7. Assessment of Antioxidant Enzyme Activities

Antioxidant enzyme activities showed a strong induction in response to increasing salinity, with marked differences among cultivars (**Table 11**). Polyphenol oxidase (PPO) activity was very low under control conditions (≈0.05–0.13 U/mg protein) but increased steadily with NaCl concentration, reaching 1.59 U/mg in Bezoul Awda (BA), 2.11 U/mg in Zbarjel (ZL), 2.33 U/mg in Sbiea Bnat (SB), and a maximum of 4.13 U/mg in Krichi (KC) at 85 mM NaCl, indicating a stronger induction in KC.

Peroxidase (POX) activity showed a similar pattern, rising from baseline values of 0.16–0.22 U/mg in the control to 7.42 U/mg for BA, 9.98 U/mg for SB, 10.94 U/mg for ZL, and 4.65 U/mg in KC under high salinity. Catalase (CAT) activity exhibited the greatest increases overall, with control levels below 1 U/mg protein and reaching 23.31 U/mg in KC, 27.88 U/mg for BA, and more than 41 U/mg for both SB and ZL at 85 mM NaCl.

These findings indicate that all cultivars activated their enzymatic antioxidant systems under salt stress, but with distinct patterns: ZL and SB demonstrated the strongest CAT and POX responses, while KC showed the most pronounced increase in PPO. ANOVA confirmed highly significant effects of NaCl level, cultivar, and their interaction for all enzyme activities (**Table 12**), highlighting the genotype-dependent regulation of antioxidant responses to salinity.

Table 11. Descriptive statistics of antioxidant enzymes in four Moroccan grapevine cultivars (*Vitis vinifera* L.) under NaCl stress.

Variety	NaCl (mM)	PPO (U/mg Protein) Mean ± SD	POX (U/mg Protein) Mean ± SD	CAT (U/mg Protein) Mean ± SD
Bezoul Awda (BA)	0	0.054 ± 0.010	0.163 ± 0.010	0.275 ± 0.209
	17	0.444 ± 0.217	0.422 ± 0.259	0.643 ± 0.119
	34	0.903 ± 1.066	2.275 ± 1.214	5.058 ± 2.963
	85	1.588 ± 0.630	7.427 ± 2.593	27.880 ± 8.724
Sbiea Bnat (SB)	0	0.133 ± 0.029	0.221 ± 0.163	0.225 ± 0.092
	17	0.334 ± 0.065	1.227 ± 0.335	1.576 ± 1.386
	34	0.883 ± 0.461	1.781 ± 0.225	11.427 ± 9.789
	85	2.330 ± 0.443	9.980 ± 1.301	41.869 ± 18.060
Zbarjel (ZL)	0	0.112 ± 0.048	0.170 ± 0.024	0.735 ± 0.772
	17	0.330 ± 0.045	0.768 ± 0.158	3.631 ± 1.244
	34	0.736 ± 0.156	1.302 ± 0.214	20.581 ± 1.372
	85	2.108 ± 0.577	10.940 ± 3.841	41.587 ± 6.385
Krichi (KC)	0	0.064 ± 0.012	0.145 ± 0.036	0.698 ± 0.262
	17	0.289 ± 0.060	0.534 ± 0.223	5.065 ± 2.221
	34	0.986 ± 0.085	1.778 ± 0.463	17.844 ± 10.667
	85	4.131 ± 0.633	4.652 ± 3.261	23.316 ± 10.238

Table 12. Analysis of variance for antioxidant enzyme activities.

Source of Variation	PPO (U/mg Protein) Mean ± SD	POX (U/mg Protein) Mean ± SD	CAT (U/mg Protein) Mean ± SD
Variety	0.004	0.055	0.049
NaCl	<0.001	<0.001	<0.001
Variety × NaCl	<0.001	0.017	0.079

3.8. Correlation Analysis

The Pearson correlation (**Figure 3**) analysis established unequivocal relationships among morphological, physiological, biochemical, and oxidative stress variables in the four Moroccan cultivars exposed to salinity stress. The morphological variables, including shoot length, root length, and biomass, were strongly and positively correlated with each other, which is expected given their interdependence in plant growth and development. The morphological variables were negatively correlated with EC in the soil and NaCl concentration, which collectively show that salinity has a major inhibiting impact on vine growth. Physiological traits, particularly relative water content (RWC), had positive correlations with the other physiological measurements (shoot length, biomass, and chlorophyll). This indicates that vegetation holding water balance is tightly related to photosynthetic capacity and plant performance under salt stress. Other partnerships appeared as well, including a positive association with chlorophyll and carotenoids, which demonstrates coordinated regulation of the photosynthetic pigments, as well as a positive association of soluble sugars and proteins with growth and pigment traits, which would suggest a role in osmotic adjustment and are a way to mitigate stress.

In contrast, stress-related metabolites displayed the inverse trend. The proline accumulation was negatively related to growth parameters, RWC, and pigment levels but was positively correlated with soil EC, malondialdehyde (MDA), and hydrogen peroxide (H_2O_2). The correlation suggests that proline was a better stress marker than it was a growth-promoting metabolite in these cultivars. The oxidative stress markers (MDA and H_2O_2) were positively related to one another, indicating that they are complementary indicators of oxidative damage and lipid peroxidation. Moreover, both MDA and H_2O_2 were negatively related to growth and physiological parameters and therefore contribute negatively to plant health. Indeed, the activity of antioxidant enzymes (catalase, peroxidase, and polyphenol oxidase) positively correlated with markers of oxidative stress. This supports the suggestion that their induction is part of the internal defense response to salinity. Catalase's activity also revealed a weak positive correlation with proline, which perhaps suggests a synergistic role between enzymatic and non-enzymatic antioxidants.

The overall correlation matrix exhibited two polar opposite clusters of traits. Growth parameters remained grouped (biomass, shoot/root length, RWC, pigments, proteins, and sugars) and were positively associated with each other but negatively associated with salinity and oxidative stress markers. Stress-related traits grouped together (proline, MDA, H_2O_2, and antioxidant enzymes) and were positively associated with salinity and negatively associated with growth. The two clusters, together, highlight the trade-off between maintaining growth and building a stress defense, which both correlate with the overall saline tolerance reflected in the studied grapevine cultivars.

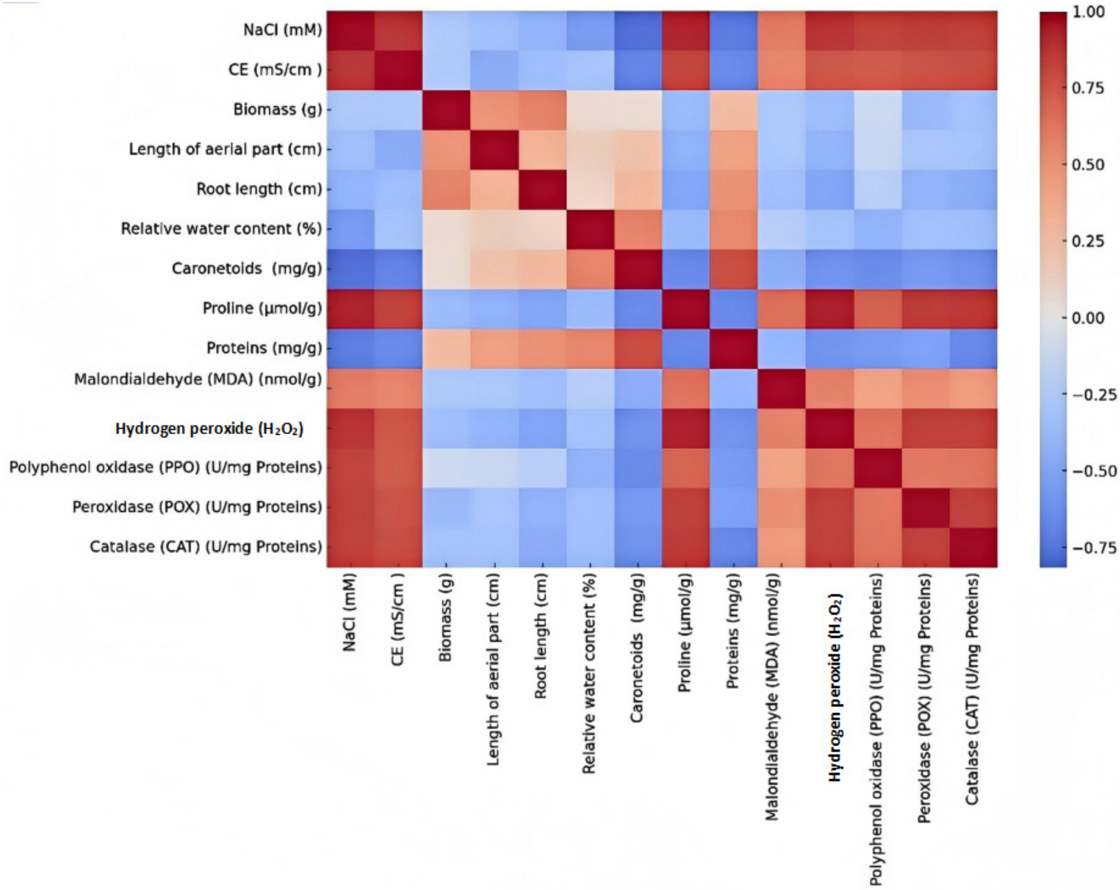

Figure 3. Pearson correlation matrix of morphological, physiological, biochemical, and oxidative stress parameters in four Moroccan grapevine cultivars under salinity stress.

3.9. Principal Component Analysis

The principal component analysis (PCA) carried out on all morphological, physiological, and biochemical parameters measured in four Moroccan grapevine varieties that dealt with varying levels of salt stress distinctly identified two primary axes that explained a large proportion of the variance observed (**Figure 4**). The first axis (PC1) explained 60.1% of the total variance and was the most distinct and composed primarily of growth and physiological traits (shoot and root length, biomass, relative water content, and chlorophyll and carotenoid pigments) versus dunc Determined based on the types of biochemical variables denoting oxidative stress (H_2O_2, MDA, proline, electrical conductivity, and antioxidant enzyme activities). Accordingly, this first axis (60.1%), representing salt stress tolerance, primarily reflected the relationship between salt stress and plant responses. Individuals positioned on the negative side of the axis were associated with better growth and water balance, whereas those on the positive side exhibited strong biochemical defense responses. The second axis (PC2) explained 11.8% of the variance and was purely discriminating for total sugars and some enzyme activities (PPO), with a clear indication of secondary differences in metabolic strategies among the varieties assessed.

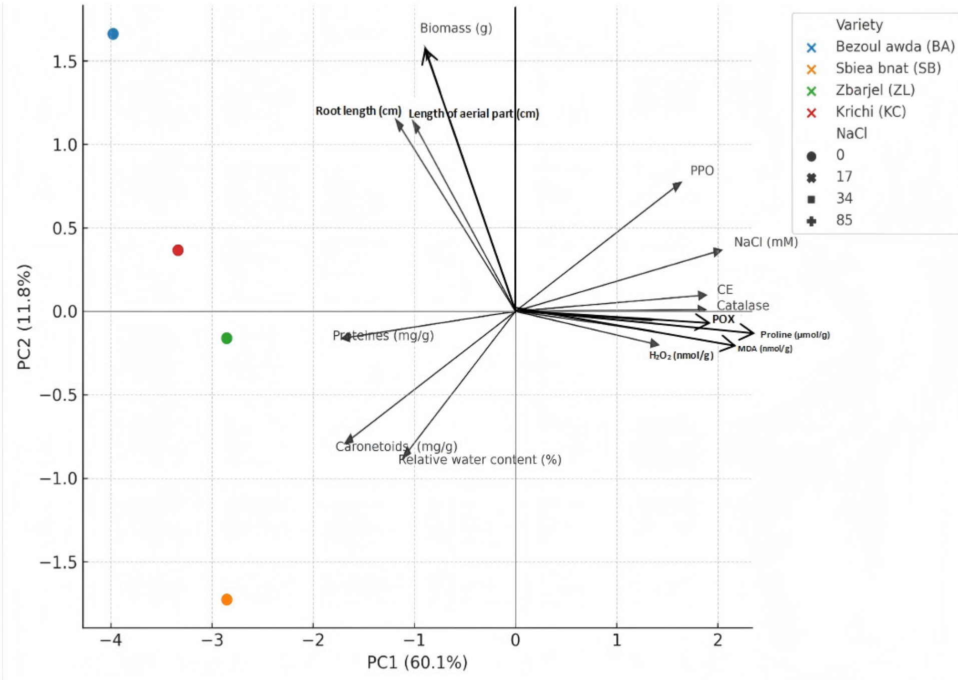

Figure 4. Principal Component Analysis (PCA) of morphological, physiological, and biochemical traits in four Moroccan grapevine varieties under different salt stress levels.

The biplot projection of the variables shows morphological and physiological parameters clustering together on the negative-half of PC1, indicating their association with tolerance to salinity and maintenance of growth under saline conditions. On the other hand, biochemical variables with correlations with stronger positive values on PC1—proline, hydrogen peroxide, malondialdehyde, and antioxidant enzyme activities (catalase, peroxidase, and PPO)—were primarily correlated with the response and progression of salinity-induced oxidative stress. Additionally, total sugars and PPO activity had very similar trajectories with regard to PC2, suggesting leadership and importance in exhibiting metabolic differentiation among the varieties.

The representation of the grapevine varieties on the factor plane shares two opposite groups. The Bezoul Awda (BA) and Krichi (KC) varieties are mainly on the negative side of PC1, which is associated with the traits for growth and water maintenance. Therefore, the BA and KC varieties should be regarded as the most tolerant of salt stress, which demonstrates their ability to reduce the physiological harmful effects of salt stress while maintaining their morpho-physiological performance. The Sbiea Bnat (SB) and Zbarjel (ZL) varieties were projected more on the positive side of the first axis, which correlates with the accumulation of oxidative stress markers due to the activation of antioxidant systems, which signifies greater sensitivity to salinity and requires a greater amount of the biochemical defense systems be mobilized to reduce cellular damage.

In general, the PCA results illustrate two different adaptive strategies in the studied Moroccan grapevine varieties. Tolerant varieties prefer to maintain growth, water balance and photosynthetic integrity as a preventive strategy, while sensitive varieties are more reliant on a curative response based on the accumulation of osmolytes and the activation of antioxidant systems to counteract oxidative stress. This distinction highlights the importance of looking at both morpho-physiological and biochemical responses in evaluating salt tolerance, in order to identify genotypes that are the most suited to challenging new environmental conditions.

3.10. Hierarchical Classification Analysis

Hierarchical cluster analysis (HCA), with Ward's method on standardized physiological and biochemical variables, allowed for the grouping of the four studied grapevine cultivars into class-one (SB and KC) and class-two (ZL and BA) clusters (**Figure 5**). This clustering captures a divergence in their responses to salt stress whereby the SB and KC are more tolerant and the ZL and BA are sensitive.

The clustering of SB and KC can be explained in terms of their similarities in adaptive response. These varieties maintain a more stable biomass and relative water content under salinity stress; thus, they are better able to preserve overall water balance. Additionally, there is a substantial amount of proline accumulation in these cultivars, which is an osmolyte involved in osmoprotection and cellular stabilization. Their antioxidant system (CAT, POX, PPO) is also activated to a high degree, and as such, their accumulation of reactive oxygen species like MDA and H_2O_2 is limited. As these mechanisms work together, this enables them to possess a higher tolerance to salinity constraints. By contrast, the two cultivars in the second cluster, ZL and BA, have a notably more sensitive profile. With less biomass and relative water content reduction, less proline accumulation and less effective antioxidant enzyme activation, it is unsurprising that the MDA and H_2O_2 are greater, showing damage due to severe stress. This biochemical and physiological profile shows that the capacity to establish efficient defence mechanisms against stress is substantially lower.

The results obtained from HCA were in full agreement with those obtained from the principal component analysis (PCA), which had already produced a clear separation the tolerant, SB and KC, and sensitive cultivars, BA and ZL. The ranking of cultivars from the composite salt tolerance index also reviewed the same order, SB > KC > ZL > BA, confirming the typology observed. However, the HCA indicates two clear functional profiles. SB and KC represented a homogeneous group of tolerant cultivars, able to maintain their physiological and biochemical integrity under salt stress significantly better than the others, whilst ZL and BA represented a group of sensitive cultivars that are more susceptible to water imbalance and oxidative damage. The cross-validation, which PCA confirmed with the composite index, provide a strong rationale for breeding and genetic improvement for salinity tolerance in grapevine.

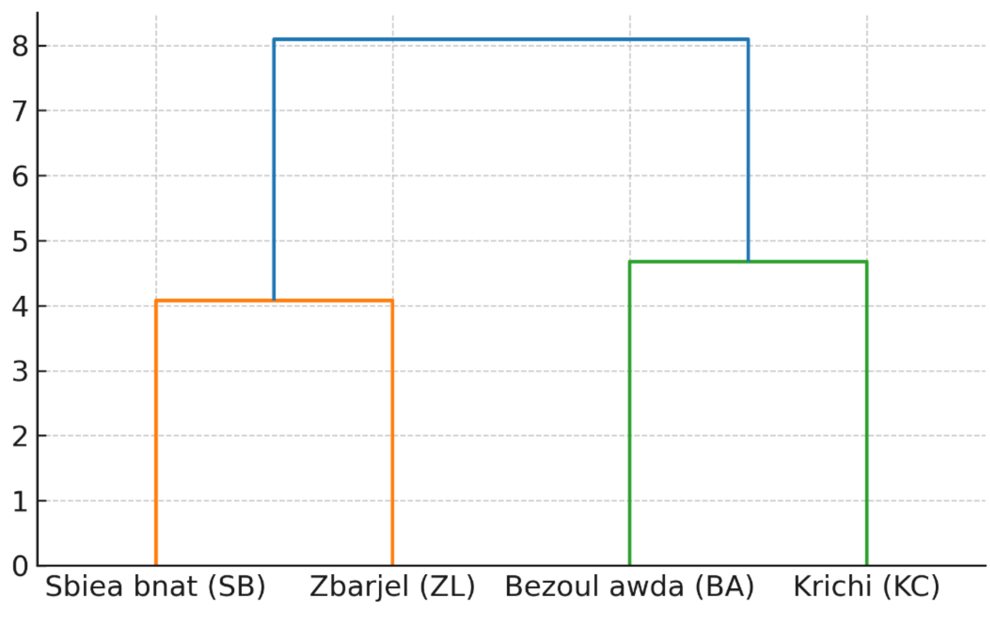

Figure 5. Hierarchical classification dendrogram.

4. Discussion

Salinity stress has grown to be one of the most important environmental constraints limiting plant productivity, mainly in arid and semi-arid environments where climate change, soil degradation, and unsustainable irrigation result in secondary salinization. This study showed that the four indigenous Moroccan grapevine cultivars displayed unique responses to increased levels of NaCl, demonstrating the variability of adaptive mechanisms between and among species of the genus *Vitis vinifera* L. and their possible significance for the area of sustainable viticulture within saline environments.

Morphological and growth parameters showed a strong effect of salinity on each, as demonstrated by the significant decrease in stem and root length and biomass. Consistent with many previous studies, salinity limits cell elongation and division, and subsequently, growth [42,43]. There were differences in the degree of reduction between cultivars, where Bezoul Awda and Krichi were able to remain relatively higher in growth than Sbiea Bnat and Zbarjel. Growers can select cultivars based on their ability to maintain metabolic and structural integrity under salt stress, which is an important trait for growers in arid climate zones, as soil salinity will likely increase with increased water uptake through evapotranspiration and salinity of irrigation water.

Physiological responses show varietal differences. The reduction in relative water content (RWC) under salinity stress cannot only indicate osmotic imbalance caused by salt accumulation. However, BA and KC held higher RWC, indicating their efficiency in osmotic adjustment and water use efficiency. Similarly, chlorophyll content decreased under salinity treatment, as reported in grapevine and other crops, due to pigment breakdown and instability of the photosystems. The smaller reduction in BA and KC would suggest photosynthetic photoprotection and stability to photosynthesis, which might support their greater tolerance. These results also support previous research [44] on grapevine ecotypes from Mediterranean regions that identify photosynthetic stability as a critical factor for salt tolerance.

On the biochemical level, proline accumulation was significantly induced by salinity in accordance with its role as an osmolyte and ROS scavenger. The greatest increases in proline were observed in SB and ZL, suggesting that their dependence on osmotic adjustment is higher than that of the other cultivars as a method to compensate for ionic toxicity. Proline accumulation that is excessive, however, may indicate perception of stress rather than tolerance. Oxidative stress markers, including MDA and H_2O_2, displayed slight increases under salinity. ZL exhibited the largest increase, which supports the conclusion that these cultivars are experiencing significant lipid peroxidation and oxidative damage. The findings of this study support the concept that salt-sensitive genotypes experience greater levels of oxidative stress than tolerant genotypes, which are capable of maintaining better redox homeostasis [45].

The increase in the activity of antioxidant enzymes (catalase, peroxidase, and polyphenol oxidase) with increasing salinity suggests that there are increased levels of activation of the defence mechanisms to eliminate reactive oxygen species. By contrast, the moderate but nonetheless sufficient levels of enzymatic activity in BA and KC indicate that they do not produce significant amounts of ROS and therefore do not produce any detoxifying activity against the ROS that they may produce.

Correlation analysis showed a strong positive correlation of growth-related traits (stem length, root length, biomass, RWC, plant pigments) and a negative correlation with similar indicators of stress (proline, MDA, H_2O_2, enzymatic activities). This dual clustering provides insight into the trade-offs between maintenance of growth and biochemical defense under salinity.

The electrical conductivity (EC) of the soil strongly increased with increasing NaCl concentrations at 85 mM NaCl, demonstrating the strong osmotic and ionic stress imposed by higher salinity levels; however, the response across cultivars varied widely. In an environmental light, the discovery and utilization of such tolerant cultivars provides a biological tool to limit the effects of salinization of soils that is worsening because of climate change, irrigation, and the increased anthropogenic pressure on agricultural land.

By examining salinity stress responses of grapevine cultivars in a particular region, this study is also part of a continuing effort to improve the resilience of agro-ecosystems, so that they may better withstand the effects of environmental stresses. If grape rocket uses the same principles as other agricultural species regarding the ability to sustain physiological performance/yield under different salinity levels, then these same principles can be used for the design of new grapevine cultivars to support viticulture/edaphic changes in geographic areas that lack access to expensive and resource-depleting remediation methods. The genetic variability among grapevine cultivars offers potential ways to develop naturally resilient adaptations to water and soil stresses. Grape rocket has become a primary crop for rural development and economic development, so it will be critical to develop and promote local grapevine varietals with tolerance, to maintain production in fragile environments. This research has identified new avenues toward developing sustainable food production systems in salt-prone areas through the development and incorporation of tolerant grapevine cultivars into viticulture systems capable of supporting long-term food security and resource management.

5. Conclusions

The importance of conserving indigenous grapevine varieties affected by climate-related soil salinization is underlined by this study of the variation and tolerance of four indigenous Moroccan Grape (*Vitis*) cultivars to salt stress. There are clear cultivar differences between the four cultivars tested with respect to the: electrical conductivity of the soil; physiological responses; and biomass distribution within the individual cultivar. Bezoul awda (BA) had the highest level of tolerance to salt stress, while Krichi (KC) also had a high level of tolerance. On the other hand, Sbiea Bnat (SB) and Zbarjel (ZL) had a much lower level of tolerance. The results of this study further enhance the knowledge related to how grapevines adapt to adverse environmental conditions and what the agricultural value will be with respect to preservation of landrace cultivars within Morocco, and the Mediterranean Region in general. Breeding programmes that incorporate the use of salt-tolerant grape cultivars, coupled with the development of better tools to monitor soil salinity levels, will be an essential element of future efforts to improve the resiliency and sustainability of viticulture in the Mediterranean.

Author Contributions

K.H. and Y.M. contributed equally to this work. They designed the study, carried out the physiological and biochemical experiments, performed data analysis, and drafted the initial manuscript. N.E.-K. assisted with laboratory work, biochemical assays, and data organization. N.S. and A.M. provided supervision in abiotic stress physiology, contributed to the optimization of enzymatic protocols, and critically revised the manuscript. F.E.A. and Y.H. contributed to statistical analysis, methodological support, and manuscript revision. A.E.O. supported field sampling, varietal characterization, and manuscript editing. D.H. supervised the project, validated the methodology and results, and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

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

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Conflicts of Interest

The authors declare no conflict of interest.

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