sustainability

MDPI

Article

Canola Seed Priming and Its Effect on Gas Exchange, Chlorophyll Photobleaching, and Enzymatic Activities in Response to Salt Stress

Waleed Iqbal [1], Muhammad Zahir Afridi [1], Aftab Jamal [2,*], Adil Mihoub [3], Muhammad Farhan Saeed [4], Árpád Székely [5], Adil Zia [1], Muhammad Awais Khan [1], Alfredo Jarma-Orozco [6] and Marcelo F. Pompelli [6,*]

[1] Department of Agronomy, Faculty of Crop Production Sciences, Amir Muhammad Khan Campus Mardan, The University of Agriculture, Peshawar 25130, Pakistan; wiqbal@aup.edu.pk (W.I.); drmzahir@aup.edu.pk (M.Z.A.); aadil.zia00@gmail.com (A.Z.); awaiskhanzada787@gmail.com (M.A.K.)
[2] Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of the Yangtze River), Ministry of Agriculture, College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China
[3] Center for Scientific and Technical Research on Arid Regions, Biophysical Environment Station, Touggourt 30240, Algeria; adilmihoub15@yahoo.com
[4] Department of Environmental Sciences, COMSATS University Islamabad, Vehari Campus, Vehari 61100, Pakistan; farhansaeed@cuivehari.edu.pk
[5] Research Centre for Irrigation and Water Management, Institute of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Anna-Liget str. 35, 5540 Szarvas, Hungary; szekely.arpad@uni-mate.hu
[6] Faculty of Agronomy, University of Córdoba, Montería 230002, Colombia; ajarma@correo.unicordoba.edu.co
* Correspondence: aftabses98@gmail.com (A.J.); marcelo@fca.edu.co (M.F.P.)

check for updates

Citation: Iqbal, W.; Afridi, M.Z.; Jamal, A.; Mihoub, A.; Saeed, M.F.; Székely, Á.; Zia, A.; Khan, M.A.; Jarma-Orozco, A.; Pompelli, M.F. Canola Seed Priming and Its Effect on Gas Exchange, Chlorophyll Photobleaching, and Enzymatic Activities in Response to Salt Stress. *Sustainability* **2022**, *14*, 9377. https://doi.org/10.3390/su14159377

Academic Editor: Márcio Carocho

Received: 9 June 2022
Accepted: 26 July 2022
Published: 31 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Canola is the second-largest oil seed crop in the world, providing oil mainly composed of long-chain fatty acids (C14 to C20). When mixed with fossil-diesel, canola-based biofuel can be used in passenger vehicles, trucks, or even in aviation. Canola is the most productive type of biofuel due to its oil's long-chain and unbranched fatty acid composition, which makes it more fluid. However, canola yields are constrained by drought and salinity that can aggravate climate change, resulting in negative consequences. Therefore, it is becoming necessary for studies that involved the canola salt-tolerant genotypes to consider soil salinization by use of saline soil or salinized soil by a non-efficient irrigation method. This study was carried out to assess the effects of salinity on seed germination and the effect of $CaCl_2$ (ψ_s = −1.2 MPa) on the promotion of regenerated plant memory when a new cycle of stress occurs. Our experiment shows that salt-stressed canola plants resulted in a high reduction in chlorophylls and carotenoids, with a high impact on gas exchange and a reduction in the efficiency of the chloroplast electron chain transporter, producing the negative effect of reduced molecules that affect the membrane integrity. However, canola seed priming could produce a memory in the regenerated plants when the second round of salt stress was applied. This research concludes that canola genotypes appear to have a tolerance mechanism against salt stress which could be an important trait for developing high-yielding canola varieties in future breeding programs under salt stress conditions.

Keywords: multivariate analyses; antioxidant activity; climatic change; biofuel; food security and plant memory; salt tolerance; seed priming

1. Introduction

The intensity of drought, salinity, and temperature extremes are increasing with climate change and are a serious threat to many natural ecosystems [1–3], due to the implications for germination and seedling survival [4,5]. Some scholars [6–8] describe that the main effects of global climate change are to aggravate the problem of less productive areas, such as those with poor, dry and saline soil. Evaluating drought tolerance during seed germination could

assist with understanding population persistence and community assembly patterns, and how these may be affected by a changing climate. Thus, scientists are interested in studying the impact of salt damage and the mechanism of plant resistance to salts, including plant growth regulators, mineral elements, plant hormones, and antioxidants to overcome the adverse effects of salinity and develop tolerant crop plants varieties [9].

Salinity is one of the major limiting factors for crops worldwide, among other abiotic stresses. Currently, more than 20% of agricultural land is affected by salinity; however, the rapid spread of salinity is a serious threat to degradation. This degradation affects nearly 954 million hectares of the world's total land area [10]. As a result, lands are rapidly becoming unproductive, leading to large economic losses because of the decrease in crop yields [11]. Therefore, it is becoming critical to devise effective strategies to increase crop yields through salt tolerance varieties and exploring new genotypes. The accumulation of toxic salts, primarily sodium and chloride ions, is the main cause of ecophysiological damage caused by salinity, disrupting cellular metabolic processes and all ecophysiological attributes, reducing CO_2 uptake and photosynthetic capacity [12]. In addition, salt stress is associated with oxidative stress through altering enzymes such as catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) [13]. Furthermore, excess reactive oxygen species (ROS) generated under stress conditions cause changes in normal cellular metabolism via oxidative damage to some cellular components, peroxidation of membrane lipids, nucleic acids, and proteins.

Canola is the second-largest oil seed crop in the world, providing 13% of the world's supply [4], the world commerce of which is largely supplied by two species, *Brassica napus* L. and *Brassica rapa* L. Seeds of canola normally produce oils that contain less than 4% linolenic acid (18:3) and/or greater than 70% oleic acid (18:1), mainly composed of long-chain (C_{14}–C_{20}) and unsaturated fatty acid [14]; most of these are required for biofuel production. Fatty acid-derived fuels and chemicals have attracted a great deal of attention in recent decades, due to their properties of high compatibility with gasoline-based fuels and existing infrastructure for their direct utilization, storage and distribution [15]. Concerning biofuels, there are two major products of great commercial importance: ethanol and biodiesel (fatty acid alkyl ester), which currently account for roughly 90% of the biofuel market [16]. The effects of global climate change have stimulated many research groups in the synthesis of biofuels from renewable resources [17]. It is highly recommended that programs that aim to build a matrix of sustainable biofuels keep in mind that they should aim to be less dependent on fossil fuels or that they should depend on rain or groundwater, rather than using land with little or no economic or ecological value to our alternatives [17]. In this context, *B. napus* and *B. napa* are cultivated worldwide, and in 2012, the global production of canola was 61.05 Mt [18], where the largest producers were Canada (20.34 Mt), China (13.28 Mt), and India (1.18 Mt). In 2018, a new record of 69.34 Mt [18] of canola was achieved. However, the USDA (United States Department of Agriculture) estimated in April 2022 that the world rapeseed production in 2022/2023 will be around 71.18 Mt, about 0.06 million tons more than the previous month's projection. Canola production in 2021/2022 was 73.60 Mt. In the 2022/2023 period, it is expected that the production of 71.18 Mt could represent a decrease of 2.43 Mt, or 3.30%, in rapeseed production around the world.

The average canola yield in Pakistan is 839 kg ha^{-1} [19], which is very low compared to advanced countries of the world. It is mostly grown on marginal land where soil fertility is a great hindrance in attaining potential yield. Obtaining a higher yield of canola requires the luxurious application of mineral nutrients, especially nitrogen and sulfur. Canola is sensitive to salinity during the early vegetative growth stage [20] and is classified as moderately sensitive to saline conditions at the juvenile stage. However, a considerable inter-specific variation has been reported for salinity tolerance [21]. Salt tolerance is a polygenic characteristic that is controlled by the interaction of many genes. These physiological characteristics indicate genetic variation in salt tolerance. As a result, there is a need to investigate the physiological significance of specific traits in plant adaptation and productivity under salinity conditions. Studies on salt tolerance mechanisms have shown

that reduced plant growth and yield under salinity are generally attributed to the combined effects of the lower osmotic potential of soil solution, disturbances in nutrient uptake, and toxic effects of harmful ions [9].

Plants are exposed to different biotic and abiotic factors; being sessile, they must face unfavorable environmental conditions [12,22,23]. However, they have evolved in their adaptation and defense mechanisms, where signaling cascades and gene transcription networks participate [24]. Recent studies reported that the stomatal index, the ratio of stomata to other epidermal cells, is developmentally controlled by the levels of relative humidity [25]. Low relative humidity triggers the accumulation of small interfering RNAs (siRNAs) that interact with FAMA and SPEECHLESS (SPCH) loci that encode bHLH transcription factors required for stomatal development [26]. The ability to perceive, store, and recall previous stressful events are likely to be useful for efficient, quick, and cost-effective responses, but little is known about the mechanisms involved. Considering that environmental disturbances can occur frequently, it is conducive for plants to be able to "*remember*" these events and use that stored information to adapt to new challenges. Environmental stress is an important factor to consider in crop production, since it directly infers yields and important physiological processes for the plant, such as photosynthesis, stomatal regulation, nutrient absorption, development and growth. Drought stress produces epigenetic changes in DNA directly on the chromatin that surrounds genes sensitive to drought. Moreover, these changes include the epigenetic mechanisms of plants that have been studied to understand how they mitigate environmental stress [26]. Some studies indicate these mechanisms by controlling gene expression with short RNA strands, histone modification, and DNA methylation [27].

Improving salt tolerance in current canola varieties is crucial to overcoming food security under a climate-changing scenario, which could increase if the biofuel production is initiated or increased [28]. The real potential for salinity tolerance of genotypes, however, may not be represented or applicable by standard breeding methods. Considering this issue, the current study was designed to determine the degree of variability and adaptability of canola genotypes to saline environments to screen the salt-tolerant and salt-sensitive genotypes as well as the stress memory phenomenon caused by earlier priming. The five canola genotypes (NARC/GP/2020A, NARC/GP/2020B, NARC/GP/2020C, NARC/GP/2020D, and Punjab Sarson) were tested for seed germination, plants development, gas exchange, electron transport use efficiency as well as pigments and antioxidant enzyme activity under canola salt-stressed plant promoted by $CaCl_2$.

2. Materials and Methods

2.1. Study Area and Plant Material

The study took place at Amir Muhammad Khan (AMK) Research Farm, (34°15′38″ N and 72°06′36″ E, 310 m a.s.l.); District Mardan, Province Khyber Pakhtunkhwa Pakistan, during the 2020–2021 winter season. This region has intensive agricultural activities with the cultivation of various field crops (wheat, rice, maize, tobacco and sugarcane). This area has a humid climate, having an average temperature of 22 °C with annual average precipitation of 559 mm.

A set of five canola (*Brassica napus*) genotypes (Table 1) were tested for salt tolerance in a hydroponic trial. These genotypes were obtained from the National Agricultural Research Center (NARC), Pakistan.

Table 1. List of 5 canola genotypes used in the study for screening of salt tolerance.

Symbol	Genotype's Name
#1	NARC/GP/2020A
#2	NARC/GP/2020B
#3	NARC/GP/2020C
#4	NARC/GP/2020D
#5	Punjab Sarson

2.2. Experimental Design and Treatment

The experiment was carried out to screen tolerant and susceptible varieties of canola against salt treatments. Peat moss was used as a medium for plant growth. Five canola varieties were soaked in each tray, with 1.2 kg peat moss per tray; peat moss (composed of organic material with higher water (88–92%) retention, carbon (50–60%), hydrogen (5–7%), nitrogen (2–3%), phosphorus (<0–2%) and traces of micro-nutrients developed over 9 months from layers of partially decomposed plant remains) was obtained from NARC Islamabad. The experiment consisted of two osmotic potentials (0 and −1.2 MPa) caused by $CaCl_2$. All the treatments were replicated thrice, at 25 °C, and arranged in a completely randomized design (CRD).

2.3. Plant Traits Measurements

2.3.1. Seed Germination and Plant Bank

In total, fifty seeds were sown with three replicates to test the germination, and every replicate had the combination of the five canola genotypes and the two osmotic potentials. An agronomical concept was used to compute the seed germination as plant emergence. The plant's emergence was used to compute germination percentage, mean germination time (MGT) and synchrony (Syn), using a template spreadsheet available on GerminaQuant package [29]. As the seedling emerged from the soil and gained strength, saline stress was continued until the establishment of a 15-day-old canola seedling. From there, the plants germinated in both osmotic potential (ψs = 0 MPa, and −1.2 MPa) and were transplanted to bags filled with 7 kg of soil, previously described above, until the 30th day to acclimatize into a new environment, forming a seedling bank, both germinated in 0 MPa and −1.2 MPa. In this case, all plants were daily irrigated with only tap water without salt stress. After that, plants originating from both primed and non-primed plants were subdivided, forming four treatments, as pictured in Figure 1.

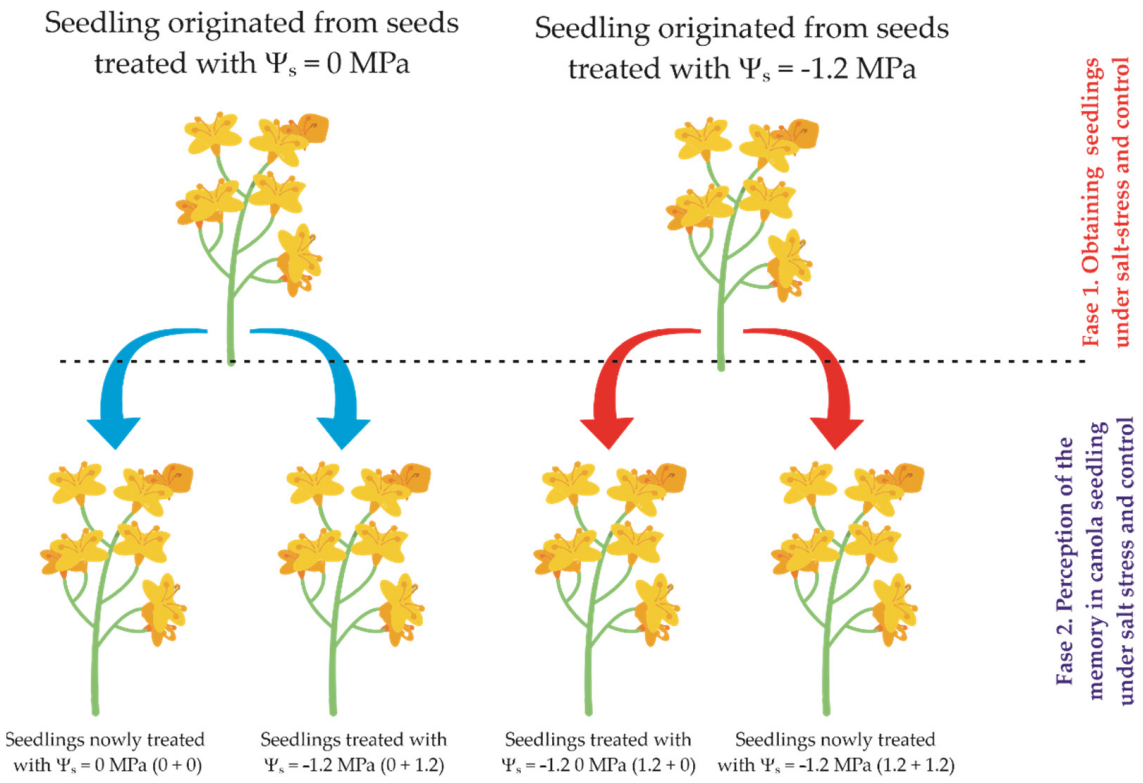

Figure 1. Schematic drawing showing the study, from phase 1 (seed germination) to phase 2 (memory perception) in plants under salt stress or control.

The respective treatments were applied in the form of sprinkle irrigation; 20 mL per application was used daily until the achievement of the required level of the respective treatment in the morning time to meet the water requirement of the crop. Only distilled water was used to meet crop water requirements after treatment completion. The plants were kept exposed to sunlight for 6 h daily.

2.3.2. Gas Exchange

Gas exchange was measured on the 3rd attached fully expanded leaf from the apex, using a portable open-flow infrared gas analyzer (LI-6400XT; LI-COR Inc., Lincoln, NE, USA) with integrated fluorescence chamber heads (LI-6400-40; LI-COR Inc.). The net photosynthesis (A_N, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), and electron transport rate were obtained on 30-day-old canola plants every 7 days at approximately 09:00–11:00 h solar time, under a clear sky, with the leaf irradiance of saturation of 900 μmol m^{-2} s^{-1} (as previously tested by light curves versus A_N), fixed CO_2 concentration of 390 μmol mol^{-1} and airflow of 400 μmol s^{-1} [30]. This experiment lasted until the canola plants were 45 days old, when the plants irrigated with salt began to show the first signs of stress, such as wilted leaves and chlorosis, with some leaves even showing necrosis on the edges. During these 15 days, the canola plants were monitored daily to avoid diseases and pathogen attacks. The objective of this second module was to test the presence of cellular memory in plants from seeds that received priming after germination and developed with or without salt stress.

2.3.3. Photosynthetic Pigments Analysis

At the stage of 45-day-old canola plants, the chlorophylls (Chl a, Chl b, and total) plus carotenoid content were determined spectrophotometrically using 200 mg FW of leaf material were immersed in 8 mL of acetone 80% solution (v/v) and were kept at −40 °C in dark for 48 h, as previously reported by Lichtenthaler [31].

2.3.4. Enzymatic Antioxidant Activity

At the stage of 45-day-old canola plants, the antioxidant activities including glutathione peroxidase (GPX, EC 1.6.4.2), ascorbate peroxidase (APX; EC 1.11.1.11), and catalase (CAT; EC 1.11.1.6) were determined by grinding 0.1 g of fresh leaves with 1 mL of 50 mM potassium phosphate buffer, as described in [32].

2.4. Data Analysis

Data were subjected to a two-factorial analysis of variance using a completely randomized design, and mean separation was performed by Newman–Keuls test at $p < 0.05$. To obtain a multivariable view of all five genotypes at different salinity levels, as well as to obtain the best tolerant canola genotypes related to salt tolerance, cluster analysis (CA) was performed using Pearson's correlation and Ward's method. Moreover, Principal Component Analysis (PCA) was performed based on the data of the observations. All statistical analyses were performed using the Sigma Plot ver. 11.0 (Systat Software, Inc., Point Richmond, CA, USA). For heatmap construction, the color was standardized by expressing values as a ratio of the value obtained for each treatment against the pairwise observed in 0 + 0 plants.

3. Results

3.1. Germination Features

Data regarding germination percentage in all canola genotypes and salt levels are presented in Table 2. In non-stressed canola seeds, the germination ranged from 32.40 ± 4.24 in Punjab Sarson genotype to 66.20 ± 0.28 in NARC/GP/2020B. However, in salt-stressed canola seed, the germination ranged from 18.10 ± 2.69 in NARC/GP/2020A to 51.30 ± 4.95 in NARC/GP/2020B. Both non-salt-stressed and salt-stressed canola seeds showed the highest mean germination time (MGT) and lowest Syn, though this genotype shows no

significant Syn with many other genotypes. The MGT has a narrow range from 8.13 ± 0.03 to 9.27 ± 0.10 in non-stressed NARC/GP/2020B and NARC/GP/2020A, respectively. However, in salt-stressed canola seed, the range between the highest and lowest value was only 0.085, being non-significant between the genotypes. The synchrony of non-stressed canola seeds was the same in all genotypes, while in salt-stressed canola seeds, the genotypes NARC/GP/2020A and NARC/GP/2020D showed a lesser value of Syn (Table 2). It highlights the importance of salt stress in Syn because NARC/GP/2020A and NARC/GP/2020D were the only ones to show a significant difference between non-stressed and salt-stressed seeds.

Table 2. Germination percentage (GRP), mean germination time (MGT), and synchrony (SYN) of five genotypes of canola germinated in 0 or −1.2 MPa. Each value denotes mean ± sd for 3 replicates. For each feature, lower case letter denotes statistical difference between the osmotic potential for each genotype and capital letter denotes statistical difference between genotypes for each osmotic potential.

Genotype	Osmotic Potential (MPa)	GRP (%)	MGT (Days)	SYN
NARC/GP/2020A	0	40.90 ± 2.40 Ca	9.27 ± 0.10 Aa	0.12 ± 0.01 Aa
	−1.2	18.10 ± 2.69 Cb	9.42 ± 0.51 Aa	0.06 ± 0.01 Db
NARC/GP/2020B	0	66.20 ± 0.28 Aa	8.13 ± 0.03 Bb	0.12 ± 0.01 Aa
	−1.2	59.10 ± 0.14 Ab	8.57 ± 0.08 Ba	0.12 ± 0.01 Aa
NARC/GP/2020C	0	51.30 ± 4.95 Ba	8.52 ± 0.12 Bb	0.11 ± 0.01 Aa
	−1.2	35.50 ± 4.95 Bb	9.42 ± 0.13 Aa	0.12 ± 0.01 Aa
NARC/GP/2020D	0	36.00 ± 3.39 Ca	8.92 ± 0.17 ABb	0.10 ± 0.01 Aa
	−1.2	21.00 ± 2.26 Cb	9.44 ± 0.12 Aa	0.08 ± 0.01 Cb
Punjab Sarson	0	32.40 ± 4.24 Ca	9.15 ± 0.04 Aa	0.10 ± 0.01 Aa
	−1.2	29.00 ± 1.98 Ca	9.02 ± 0.14 Aa	0.10 ± 0.01 Ba

3.2. Gas Exchange

At first glance, it appears that salt stress does not promote or inhibit net photosynthesis (A_N) (Figure 2). However, it is clear that genotypes #1, #4, and #5 had lower A_N than #2, and #3. Moreover, seems that the effect of genotype was overwhelmed by salt treatment. It is clear that salt stress negatively affects the A_N; however, in plants where seeds were primed with ψ_s = −1.2 MPa, the A_N was maintained or increased since it was under salt stress, as an effect of seed priming with −1.2 MPa, with an exception in genotype #3, and #4, where the A_N was abruptly (genotype #3) or slightly (genotype #4) changed. In the non-stressed canola plants, A_N of genotype #3 and #4 were 6.3-fold and 1.7-fold higher, respectively, in comparison to those of salt-stressed plants. It shows that genotype #4 was significantly impacted by the effects of salt stress where negative A_N was shown (under the dotted line in Figure 2D); however, when this value was shown as media, the effect of salt stress may be overwhelming.

The stomatic conductance (Figure 2F–J) seems to be with the same pattern as A_N. Moreover, a deep analysis of Figure 2G–I shows us that salt-stressed plants were significantly affected by salinity, with a high decrease in g_s. A part of this in Figure 2F,J the effect of salt stress was strongly different in 0 MPa plus −1.2 MPa and −1.2 MPa plus −1.2 MPa, where the difference between the treatments was the presence of osmotic seed priming in −1.2 MPa plus −1.2 MPa, as shown in Figure 2J. However, the prominent dissimilarity between the genotypes is shown in Figure 3, which shows the relationship between the stomatal opening (higher g_s) and the ratio between the electron transport rate (ETR) by the photosynthetic rate (A_N). Higher values of g_s denote plants that practically did not feel the osmotic stress since they opened their stomata to the maximum had a lower ETR: A_N ratio. This was achieved because higher g_s are normally linked to higher A_N (Figure 2) which ends up reducing the ETR:A_N ratio; thus, it is evident that the NARC/GP/2020B genotype is the best, because even under salt stress it managed to open its stomata, making the most of the electrons conducted by the ETR.

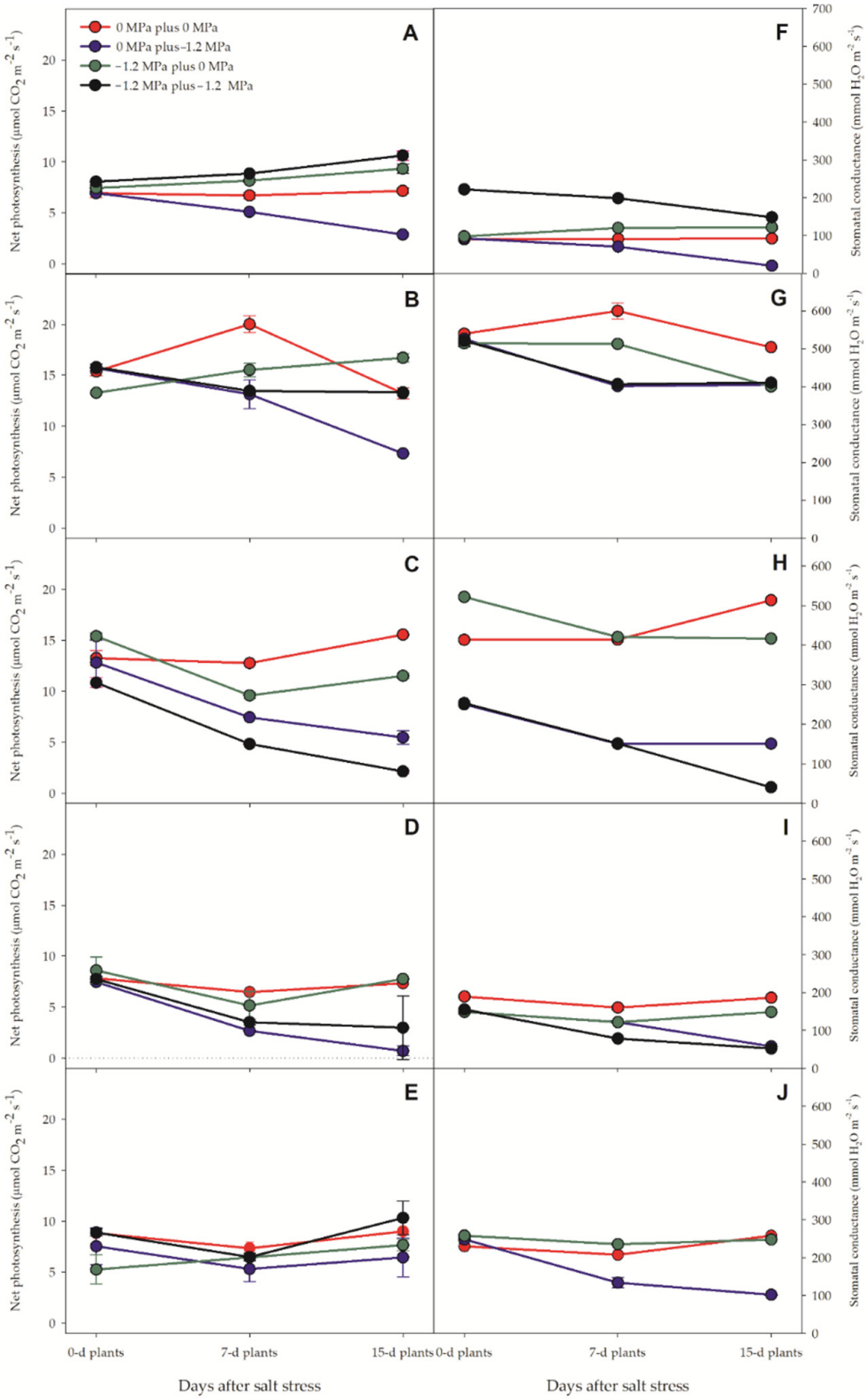

Figure 2. Gas exchange analysis in entire experiment. (**A**–**E**) denote net photosynthesis, and (**F**–**J**) denote stomatal conductance. See that in 15-day salt-stressed canola plants, the fall in treatments under salt stress was more relevant than in 0- or 7-day salt-stressed canola plants. All point denotes the means ± se. $n = 3$.

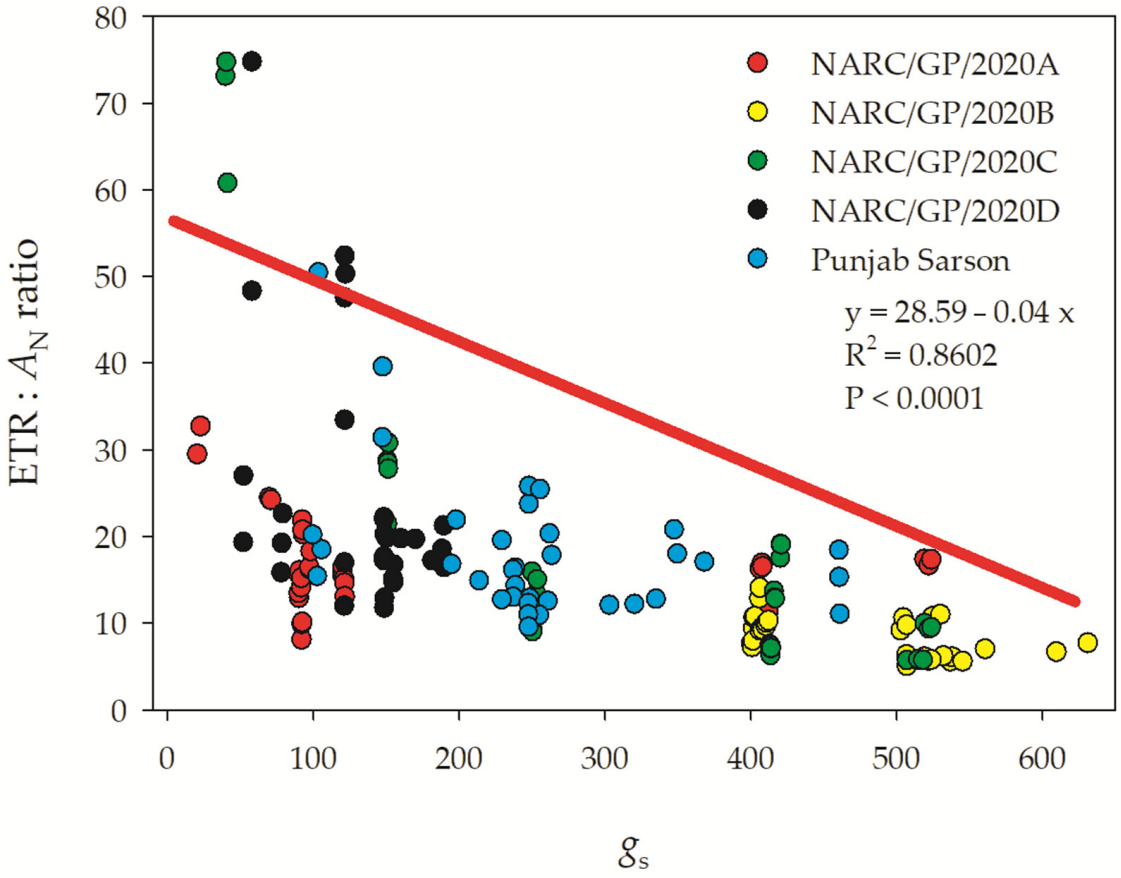

Figure 3. Relationship between the ratio of electron transport rate (ETR) and net photosynthesis (A_N) versus stomatal conductance (gs) was measured in five genotypes (NARC/GP/2020A, red symbols; NARC/GP/2020B, yellow symbols; NARC/GP/2020C, green symbols; NARC/GP/2020D, black symbols and Punjab Sarson, blue symbols) of canola under ψ_s = 0 MPa and ψ_s = −1.2. Data represent averages of five biological replicates per genotype and condition. The regression coefficient (R^2) and *p* value are shown.

3.3. Interaction of Foliar Pigments and Salt-Stressed Canola Seedlings

Salt stress leads to significant changes in chlorophyll content in all five canola genotypes. Both Chl and carotenoids were significantly decreased under salt stress. The interaction (genotype × salinity) was significant (Table 3). In the salt-stressed plants that originated from non-primed seeds, the Chl a abruptly felled in all the genotypes, ranging from 0.5- to 0.7-fold higher than non-stressed ones. Elsewhere, in the salt-stressed plants originating from primed seeds, the Chl a was increased 1.2-fold in NARC/GP/2020B, 1.3-fold in Punjab Sarson, and 1.9-fold in NARC/GP/2020D. In the NARC/GP/2020C and NARC/GP/2020A, the Chl a concentration was decreased, respectively, by 23.3% and 30.6% on salt-stressed plants when compared to non-stressed canola plants. Likewise, when comparing only salt-stressed canola plants, it was noticed that Chl a was increased in all genotypes, apart from the Punjab Sarson, where plants originated from primed seeds showed a decrease of 0.3% (p = 0.964). Another four genotypes originated from primed seeds showed an increase of 28.2% (NARC/GP/2020C), 66% (NARC/GP/2020A), 73% (NARC/GP/2020B), and 113% in NARC/GP/2020D (Figure 4). Data for Chl b follow the same pattern than Chl a, where salt-stressed plants originated from non-primed seeds showed a decrease of 0.2% (p = 0.762), 17.6%, 28.7%, 32.2%, and 37.4% in NARC/GP/2020A, NARC/GP/2020B, NARC/GP/2020C, Punjab Sarson, and NARC/GP/2020D, respectively. In another pairwise, the salt-stressed plants originating from primed seed showed an increase of 16.8%, 18%, 94%, and 102.5% in NARC/GP/2020D, NARC/GP/2020A, Punjab

Sarson, and NARC/GP/2020B, respectively. In NARC/GP/2020C the salt-stressed plants originating from primed seeds showed a decrease of 18% of Chl b, when compared to non-primed ones (Figure 4).

Table 3. Analysis of variance showing the effect of the source of variation in chlorophyll "a", chlorophyll "b", chlorophyll "a + b" and total carotenoids.

Chlorophyll "a"					
SV	**DF**	**SS**	**MS**	**F_{cal}**	***p***
Genotype	4	157.706	39.426	2513.142	≤0.001
Osmotic potential (ψ_s)	3	24.343	8.114	517.223	≤0.001
Genotype × ψ_s	12	31.775	2.648	168.787	≤0.001
Residual	40	0.628	0.016		
Total	59	214.452	3.635		
Chlorophyll "b"					
SV	**DF**	**SS**	**MS**	**F_{cal}**	***p***
Genotype	4	606.833	151.708	2984.923	≤0.001
Osmotic potential (ψ_s)	3	110.144	36.715	722.378	≤0.001
Genotype × ψ_s	12	298.910	24.909	490.099	≤0.001
Residual	40	2.033	0.051		
Total	59	1017.920	17.253		
Chlorophyll "a + b"					
SV	**DF**	**SS**	**MS**	**F_{cal}**	***p***
Genotype	4	1047.547	261.887	3966.766	≤0.001
Osmotic potential (ψ_s)	3	337.793	112.598	1705.503	≤0.001
Genotype × ψ_s	12	378.505	31.542	477.764	≤0.001
Residual	40	2.641	0.066		
Total	59	1766.486	29.940		
Carotenoids					
SV	**DF**	**SS**	**MS**	**F_{cal}**	***p***
Genotype	4	710.744	177.686	3899.852	≤0.001
Osmotic potential (ψ_s)	3	228.031	76.010	1668.271	≤0.001
Genotype × ψ_s	12	147.788	12.316	270.304	≤0.001
Residual	40	1.822	0.046		
Total	59	1088.386	18.447		

SV, Source of variation; DF, Degrees of freedom; SS, Sum of squares; MS, Mean squares; F_{cal}, calculated F; *p*, *p* value.

Usually, in higher plants, more than 95% of the chlorophylls are Chl a and Chl b. In this sense, Chl a + b should reflect the same pattern shown in Chl a and Chl b. Figure 4D shows exactly this profile with salt-stressed plants originating non-primed seed showing an average reduction of 25.4%, with genotypes NARC/GP/2020A and NARC/GP/2020B contributing a reduction of 6.3% and 19%, while for other genotypes the reduction was in the order of ~35%. Meanwhile, plants originating from primed seeds showed an increase of 33% and 50% in Punjab Sarson and NARC/GP/2020B, respectively, while other genotypes showed a moderate fall of 9.5% (NARC/GP/2020A), 10.4% (NARC/GP/2020C), and 18.9% (NARC/GP/2020D). The pool of carotenoids was decreased about 26% in salt-stressed non-primed seeds. However, salt-stressed plants originated from priming seeds showed an increase in carotenoids of 8.3%, 13.6%, 21.6%, and 22.6% in NARC/GP/2020D, NARC/GP/2020A, NARC/GP/2020B, and NARC/GP/2020C, respectively. In Punjab Sarson, the carotenoids decrease 35.2% in salt-stressed plants (Figure 4).

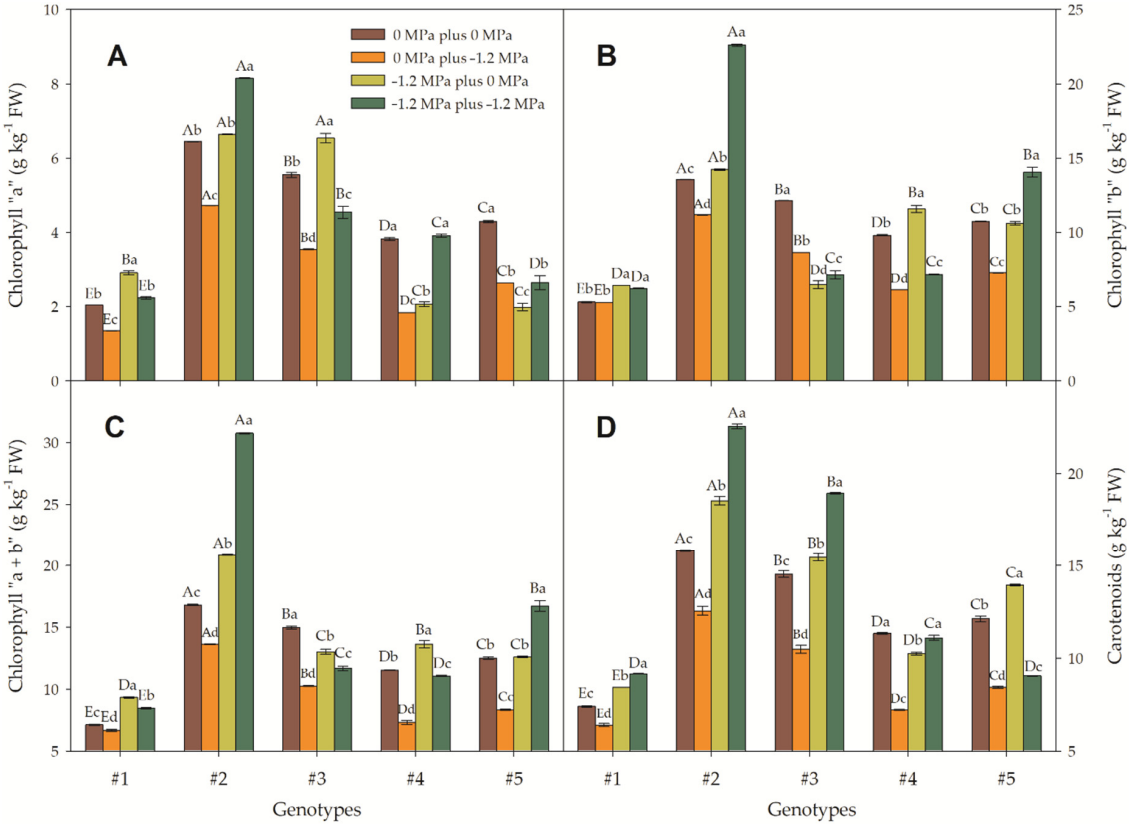

Figure 4. Chlorophyll "a" (**A**), "b" (**B**), "a + b" (**C**), and carotenoids (**D**) evaluated in five genotypes (NARC/GP/2020A, #1; NARC/GP/2020B, #2; NARC/GP/2020C, #3; NARC/GP/2020D, #4; Punjab Sarson, #5) of canola under ψ_s = 0 MPa and ψ_s = −1.2. Mean followed by capital letters denotes the statistical difference between genotypes in each feature, and small letters denote the statistical difference between treatments in each genotype. All data represent averages of five biological replicates per genotype and conditions plus standard error. $\alpha \leq 0.001$.

3.4. Enzymatic Responses in Different Canola Genotypes under Salt Stress

According to statistical analysis of the data, both canola genotypes and salt stress had a significant effect ($p \leq 0.0001$) on glutathione peroxidase activity (Figure 5A). The interactive effect of genotypes and salt stress was also found. The maximum peroxidase activity (GPX) content in leaves was found in NARC/GP/2020B genotype followed by NARC/GP/2020C, NARC/GP/2020A, NARC/GP/2020D, and Punjab Sarson. Under ψ_s = −1.2 MPa, the GPXactivity increased significantly in all canola genotypes (Figure 5). Highest increase was recorded in NARC/GP/2020C, which increased by approximately 92%, followed by 86% in both NARC/GP/2020A and NARC/GP/2020B. The Punjab Sarson genotype had the lowest increase in GPX activity ~44%. The interaction (genotype × salinity) revealed that a higher activity was found in NARC/GP/2020B under ψ_s = −1.2 MPa, whereas the lowest was observed in the NARC/GP/2020A and Punjab Sarson genotypes. Among plants that those received salt stress only in plant phase, the genotype NARC/GP/2020B showed superior sensitivity to an increase in the GPX activity, wherein the 0 MPa plus −1.2 MPa plants showed 6.9-fold higher GPX activity than control plants. In addition, among plants that received salt stress both in seed germination (priming) plus in plant phase, the genotype NARC/GP/2020C showed 28.2-fold higher GPX activity than control plants or 2.2-fold higher GPX activity when compared to plants regenerated from the seeds without priming in germination.

Here, it is revealed that different osmotic potentials had a significant effect ($p < 0.0001$) on the CAT activity of different canola genotypes (Figure 5B). Plants originated from non-primed seeds, and the CAT activity was 2.3-fold higher in genotypes NARC/GP/2020C, followed by

NARC/GP/2020A and NARC/GP/2020A, both showing 2-fold higher CAT activity as compared to the controls. However, in plants that originated from primed seeds, the CAT activity was 1.3-fold higher in NARC/GP/2020C, followed by NARC/GP/2020A (1.4-fold higher), NARC/GP/2020B (2.2-fold higher), NARC/GP/2020D (2.9-fold higher), and Punjab Sarson (3.4-fold higher). A very distinct pattern was demonstrated in the APX activity (Figure 5C). While NARC/GP/2020B showed the highest GPX and CAT activity, NARC/GP/2020D and Punjab Sarson showed the lowest in APX activity. With regard to plants originated from non-primed seeds, the NARC/GP/2020D genotype showed the highest APX activity in comparison to that of control (1.34-fold higher), followed by NARC/GP/2020A (1.33-fold higher), NARC/GP/2020B (1.28-fold higher), NARC/GP/2020C (1.21-fold higher), and Punjab Sarson (1.16-fold higher). However, in primed seeds, in decreasing order of APX activity was Punjab Sarson (4.01-fold higher), NARC/GP/2020D (2.56-fold higher), NARC/GP/2020C (2.49-fold higher), NARC/GP/2020B (1.51-fold higher) and NARC/GP/2020A (1.16-fold higher).

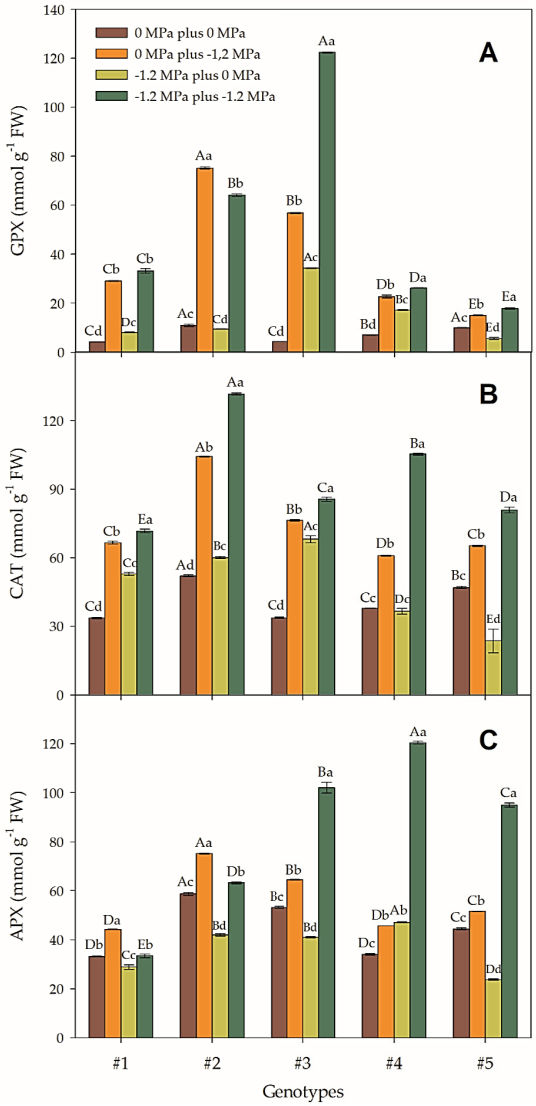

Figure 5. (**A**) Glutathione peroxidase (GPX), (**B**) catalase (CAT), and (**C**) ascorbate peroxidase (APX) evaluated in five genotypes (NARC/GP/2020A, #1; NARC/GP/2020B, #2; NARC/GP/2020C, #3; NARC/GP/2020D, #4; and Punjab Sarson, #5) of canola under ψ_s = 0 MPa and ψ_s = −1.2. Mean followed by capital letters denote the statistical difference between genotypes in each feature, and small letters denote the statistical difference between treatments in each genotype. All data represent averages of five biological replicates per genotype and conditions plus standard error $\alpha \leq 0.001$.

3.5. Salt Tolerance between Genotypes

The geospatial distribution of genotypes in both PC1 and PC2 denotes that all non-stressed genotypes are clustered in only one group (Figure 2A). Two groups were produced in a separate form that is present in the salt-stressed canola plants. The first cluster can be grouped with NARC/GP/2020B and NARC/GP/2020C, while a second cluster can be grouped with NARC/GP/2020A, NARC/GP/2020D, and Punjab Sarson genotypes (Figure 6A). The clustering of the NARC/GP/2020A, NARC/GP/2020D, and Punjab Sarson genotypes can be supported by MGT, according to the loading plot (Figure 6B), while enzymatic activity was the main feature used to group the NARC/GP/2020B and NARC/GP/2020C genotypes. These clusters were confirmed by dendrogram, such that at 60% of linkage distance, it was possible to distinguish three groups. The PC1 plus PC2 together explain 93.9% of the analyzed features.

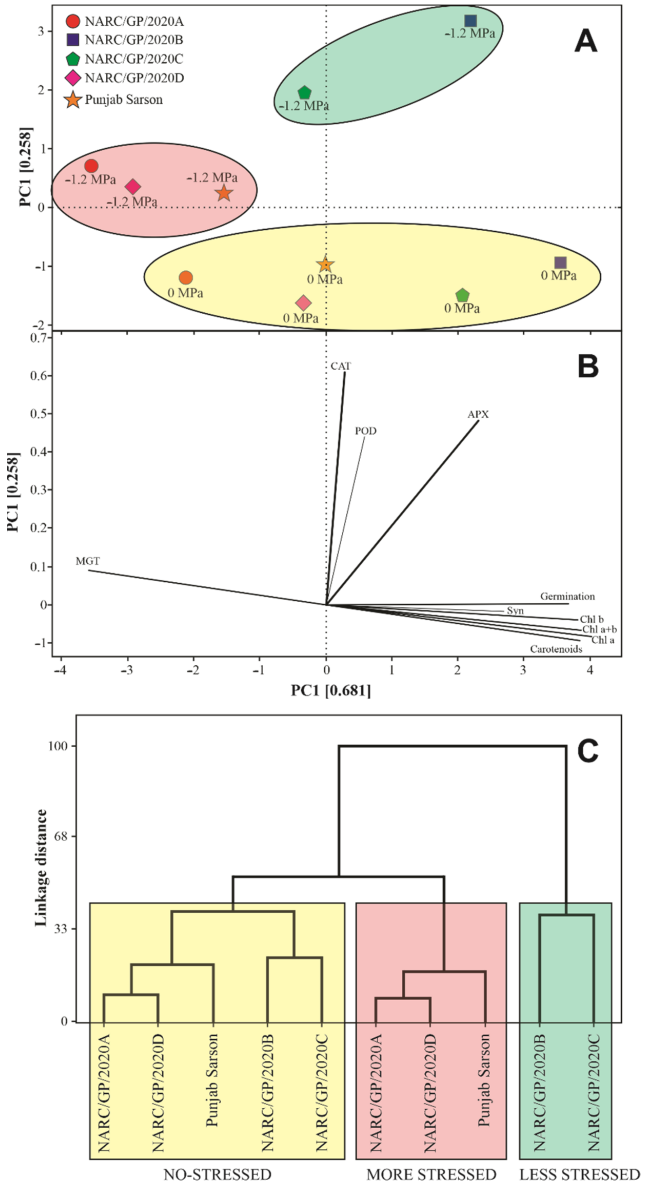

Figure 6. Multivariate analysis to assess salt tolerance among genotypes. (**A**) Spatial distribution of all canola seeds. In yellow were shown all non-stressed seeds, while in red were shown the most stressed canola plants; yellow would be an intermediary between the two groups (more details are given throughout the text). (**B**) The geospatial location of all features. (**C**) Dendrogram to show the similarity or diversity among genotypes.

4. Discussion

Significant reductions in germination percentage were observed among all five canola genotypes under salt stress. A wide range of literature covers the effect of NaCl to promote salt stress on germination [33–36]. Whereas the effect of $CaCl_2$ on seed germination has received minor attention [37–40], this salt is preferred in germination analysis using saline priming to induce the fastest and most efficient response to a new stress cycle [39–42]. Seed priming can be understood as "seed hardening", a process that involves previous exposure to a biotic or abiotic stress factor, making the plant more resistant to further exposure [43]. This characteristic, in higher plants, indicates some capacity of *"memory"* different from the memory of the animals. The seed priming process is not fully known, but it is known that accumulation of intermediates in cellular compartments, modification of key regulatory proteins, epigenetic mechanisms, signaling cascades and gene transcription networks are involved [26]. Epigenetic mechanisms control the gene expression of various processes without altering the DNA sequence, these changes modify the response of the plant to various environmental stimuli such as the response to vernalization, defense against parasites and environmental stress [24]. Chromatin modification can act as a memory for systemic acquired resistance in the plant stress response [44]. A small-RNA (siRNA)-based mechanism was implicated in the transgenerational seed priming for enhanced herbivory defense as described by Rasmann et al. [45] and in their findings. In addition, in plants, the phloem mobile siRNAs suggested that small RNAs could provide the signal that was passed [27]. Due to the sessile pattern, plants are capable of photosynthesis; however, they cannot escape environmental stresses; therefore, they have developed a sophisticated, dynamic, highly responsive physiology [46].

Our results are in line with Jacob et al. [47], who also reported a decrease in germination percentages due to the osmotic action of salts in the growth medium. However, the low germination percentage shown in this paper is due to the applied methodology, where only seeds can regenerate a seedling that emerges from soil which is computed as germinated. Frequently, that seed germination occurs under the soil, but the seedling does not have the strength to grow above the soil top and dies after its seedling emergence. Some scholars describe this occurrence as a seed bank [48–51], which is very important in ecology but is usually ignored in agronomy and botany concepts. For clarity, botanical seed germination was defined as the protrusion of a radicle by $\geq$2 mm through the external integument, as recommended by the International Seed Society [52], while in the agronomy concept, the seed germination was computed as being only when the seedling breaks the soil surface, giving rise to a seedling with normally open cotyledons [53]. For the same reason, the MGT was high and Syn was low. A recent study of five canola genotype germination demonstrated that the MGT ranged from 1.12 to 2.68 days to germination. This same study also demonstrated that the salt stress increases the MGT from 1.46 to 2.20 days under a range of NaCl, from 0 mM NaCl to 200 mM [54]. MGT evaluated in other five canola cultivars seed germination ranged from 1.11 to 2.4 days, with the lowest value attributed to salt stress and the higher to control [54]. Canola may increase or decrease its MGT from 1.1 to 2.0 when primed with heavy metals [55]. However, when seed germination is computed using the agronomic concept, the MGT will be very high, increasing with an increase in the salt concentration [56], where the values were described from 10 to 20 days, with results ranging from 1.1- to 2.5-fold higher than our results.

In terms of synchrony, it was reported that canola seed germination inoculated with bacteria, fungi or both increased the Syn by about 1.3-, 1.5-, and 1.8-fold higher when compared to non-inoculated canola seeds [57]. Similarly to that reported above, the lowest value of Syn on germination evaluation is a problem to solve, because canola is a plant species for which the synchrony on seed germination is very important. It is worth noting that results showed [57] have no unit; however, a comparison with our result is impossible. Furthermore, a very recent study showed that osmotic stress sometimes does not affect the embryo, instead reducing its metabolism at a very low rate, thus delaying the respiration

reserves consumption and protecting the embryo that germinated with a high level after stress relief [51].

The wide range of literature shows that $CaCl_2$ attenuates the effect of NaCl salt stress [44,58,59]. Canola plants were exposed to 30% seawater and showed growth inhibition, decrease in leaf area, and concentration of photosynthetic pigments, in addition to decreasing APX, CAT, and GPX activity [60]. Meanwhile, the seeds soaked in 200 mM of $CaCl_2$ and exposed to the same saline condition showed the greatest leaf area and greatest activities of all the enzyme activity (APX, CAT, GPX). This improvement in the growth of canola plants was attributed to reverse stress, imprinting seed priming, or by ameliorative effect [60]. In our study, the effect of salt-stressed plant promoted by $CaCl_2$ on net photosynthesis (A_N) was genotype dependent. Whereas with non-primed canola seeds, the A_N was reduced by 14.4% in 15-day, in Punjab Sarson canola plants, the decrease in A_N was 53.3%, 57.3%, 58.6%, and 90.6% in NARC/GP/2020B, NARC/GP/2020C, NARC/GP/2020A, and NARC/GP/2020D, respectively. However, in primed canola seed, the A_N was increased by 16.1% in Punjab Sarson and 31.7 in NARC/GP/2020A. With regard to NARC/GP/2020B, NARC/GP/2020D, and NARC/GP/2020C, there was a slight decrease in A_N in NARC/GP/2020B (15.6%), and a strong decrease in NARC/GP/2020D (61.6%) and NARC/GP/2020C (80.4%). A deep analysis of these data demonstrated that the seed-primed $CaCl_2$ canola shows a delta (Δ) A_N (Δ_{AN} in seed-primed minus Δ_{AN} in non-seed-primed) that was positive in Punjab Sarson (0.3 µmol CO_2 m^{-2} s^{-1}) and NARC/GP/2020C (1.4 µmol CO_2 m^{-2} s^{-1}) and negative in NARC/GP/2020A (−1.5 µmol CO_2 m^{-2} s^{-1}), NARC/GP/2020D (−2.0 µmol CO_2 m^{-2} s^{-1}), and NARC/GP/2020D (−3.9 µmol CO_2 m^{-2} s^{-1}); i.e., the genotype Punjab Sarson demonstrated a greater benefit of seed priming in 15-day salt-stressed canola plants, followed by NARC/GP/2020C.

In terms of stomatal conductance (g_s), the pattern was similar to those presented to A_N, where non-primed canola seeds showed a decrease in A_N of 22.9% (NARC/GP/2020B), 39.7% (NARC/GP/2020C), 58.9% (Punjab Sarson), 61.1% (NARC/GP/2020D), and 78.1% (NARC/GP/2020A). However, in seed-primed canola plants under 15 days of salt stress, there was a decrease in g_s of 21.2% (NARC/GP/2020B), 33.2% (NARC/GP/2020A), 66.4% (NARC/GP/2020D), and 84.4% (NARC/GP/2020C). In the Punjab Sarson genotype, foliar spray of $CaCl_2$ promoted an increase of 44.3% in A_N. So, the Δ_{gs} was negative in NARC/GP/2020B (−10.2 µmol CO_2 m^{-2} s^{-1}) and Punjab Sarson (−106.7 µmol CO_2 m^{-2} s^{-1}), and it was positive in NARC/GP/2020A (1.3 µmol CO_2 m^{-2} s^{-1}), NARC/GP/2020D (12.2 µmol CO_2 m^{-2} s^{-1}), and NARC/GP/2020C (113 µmol CO_2 m^{-2} s^{-1}).

Our results were in consonance with [60], where salt stress affects photosynthesis per unit leaf area indirectly through stomatal closure [14], and to a smaller extent through direct interference with the photosynthetic apparatus [13,54,61] or directly by inhibiting some Calvin–Benson enzymes, such as RuBisCo, fructose 1, 6-bisphosphatase, transketolase, sedoheptulose 1, 7-bisphosphatase, and rubisco activase. At the same time, salt stress promotes the oxygenase activity of RuBisCo (reviewed by [13]). However, as demonstrated in this study, foliar spray application of $CaCl_2$ can improve the A_N in NARC/GP/2020A and Punjab Sarson genotypes, which corroborates a similar study [60] with canola seedings. In a similar manner, [62] describes how exogenous foliar application of glycine betaine improved A_N in salt-stressed canola plants.

Photosynthetic pigments such as chlorophyll and carotenoids [61,63,64] were chosen as an excellent predictor of photosynthesis function. Salt toxicity mostly causes photobleaching [32,65] and sometimes burning [12,30] of leaves or other succulent parts and degradation of other pigments. Photobleaching due to salinity stress is very common in salt-sensitive plant species [12,13,65–69]. However, saline-tolerant species can protect themselves from such salinity stress deterioration [70]. Photobleaching was also reported in shade plants when exposed to a gap [71] in the forest or to a light that is more intense than the usual exposure [65] or in drought stress [32]. Our results corroborated the findings of Cha-Um and Kirdmanee [72], which stated that concentrations of Chl "a", "b" or both, significantly dropped in salt-stressed maize. Our findings are also supported by

Shin et al. [73] who reported that major photosynthetic pigments Chl b were affected by salt stress during the progressive treatments. Salt stress also increased response time and decreased chlorophyll "a" fluorescence parameters [63,74]. Salt stress reduces carotenoid contents [75].

To further elucidate the physiological performance of the genotypes to salinity, we compared the relationship between the electron transport rate (ETR) and A_N versus g_s (Figure 3). We observed that in 15-day salt-stressed canola plants originated from primed seeds, the A_N decreased by 15.6%, 61.6% and 80.4% in NARC/GP/2020B, NARC/GP/2020D, and NARC/GP/2020C, respectively, while it increased by 16.1% in Punjab Sarson and 31.7% in NARC/GP/2020A. In addition, ETR decreased by 1.2% in NARC/GP/2020C and 7.2% in NARC/GP/2020A, while in NARC/GP/2020D, Punjab Sarson, and NARC/GP/2020B the ETR increased by 14.1%, 34%, and 44.8%, respectively. With a stronger reduction in A_N than ETR, the ETR/A_N ratio increases. Thus, we also observed that the ETR/A_N ratio was reduced by 29.1% in NARC/GP/2020A and 93.9% in NARC/GP/2020D. However, this ratio was slightly increased in Punjab Sarson (20.8%) and moderately increased in NARC/GP/2020B (71.5%), while the strongest increase was in NARC/GP/2020C (406%).

Reduced A_N, as seen in the current study, may be related to dysfunctions in the metabolic processes that fix CO_2, presumably as a result of ATP deficiency-related restrictions on RuBP synthesis, as suggested to occur under severe drought conditions [76]. Net photosynthesis is the preferred pathway for electrons under optimal conditions; however, A_N is very sensitive and decreases more rapidly than ETR under stress conditions, thus promoting an increase in the ETR/A_N ratio, which indicates that an energy compromise takes place, and thus, it can be linked to the ROS production [77]. The ETR/A_N ratio reflects the energy transfer to A_N, and an increase in this ratio [77], as noted here in the NARC/GP/2020B and NARC/GP/2020C genotypes, indicates that the main electron sinks increased A_N in the salt-stressed canola plants. This pattern could be translated in less-stressed canola plants, as we show in Figure 6A, where both NARC/GP/2020B and NARC/GP/2020C genotypes are clustered in a unique group, which we herein call low-stressed plants, in comparison with NARC/GP/2020A, NARC/GP/2020D, Punjab Sarson, herein called high-stressed plants.

In our study, both canola genotypes and osmotic potentials had a significant effect ($p \leq 0.0001$) on all enzyme activities. In non-primed plus salt-stressed canola seed, the activity of GPX was 1.5-, 3.2-, 6.9-, 7.1-, and 13.1-fold higher than those controls in Punjab Sarson, NARCGP2020D, NARCGP2020B, NARCGP2020A, NARCGP2020C, respectively. Apart from this, in the seed-primed salt-stressed canola plants, the GPX activity was 1.5-, 3.2-, 3.6-, 4.1- and 6.8-fold higher than those controls in NARCGP2020D, Punjab Sarson, NARCGP2020C, NARCGP2020A, and NARCGP2020B, respectively. These data show that NARCGP2020B does not change its activities in salt-stressed canola plants. In addition, Punjab Sarson saw a two-fold increase in GPX activity, while NARCGP2020A, NARCGP2020D, and NARCGP2020C saw a fall in GPX activity of 41.9%, 52.2%, and 72.7%, respectively. These data permit us to infer that NARCGP2020B does not modulate GPX activity when compared to non-primed and primed canola seeds. Punjab Sarson shows that 18.5% higher GPX activity in seed-primed seems to reduce tolerance to salt stress, while NARCGP2020A (+14.3%), NARCGP2020D (+16%), and NARCGP2020C (+115%) positively modulated GPX activity, thus promoting seed priming and showing a more tolerant genotype. Our results agree with [78], which reported that GPX and CAT activity were found to be increased in canola cultivars at all salinity levels. Similarly, Xu et al. [79] stated that salt stress increased CAT and APX activities.

The CAT activity was very similar to that previously reported to GPX, where NARCGP2020B does not modulate GPX activity when compared to non-primed and primed canola seeds. The genotypes NARCGP2020A, NARCGP2020C, Punjab Sarson, and NARCGP2020D showed an increase in CAT activity in primed seeds of 7.5%, 12.3%, 24.1%, 72.9%, respectively, in comparison with non-primed canola seeds. Our findings are consistent with [75], which stated that salt stress or seawater decreased photosynthetic capacity

while increasing CAT and POD activity. Similarly, Zhao, Liang, Chu, Sun, Wei, Yang and Zheng [74] reported that CAT activity increased in *Ginkgo biloba* under salt stress promoted by NaCl. The study of Jaleel et al. [80] confirmed that CAT increases under salt stress, and further, however, it found that the addition of $CaCl_2$ lead to a reduction in the proline concentration by increasing the level of proline oxidase and decreasing the γ-glutamyl kinase activities. Calcium ions also increase the glycine betaine concentration. Thus, $CaCl_2$ appears to confer greater osmoprotectant activity by the additive role with NaCl.

The first two enzymes showed a similar pattern, which cannot be translated to APX. In non-primed seeds, the salt stress led to a 1.2-, 1.2-, 1.3-, 1.3-, and 1.3-fold increase in APX activity in NARCGP2020C, Punjab Sarson, NARCGP2020A, NARCGP2020B, and NARCGP2020D, respectively. However, in seed-primed canola plants, the APX was increased 1.2-, 1.5-, 2.5-, 2.6-, and 4.0-fold in salt-stressed canola plants NARCGP2020A, NARCGP2020B, NARCGP2020C, NARCGP2020D, and Punjab Sarson, respectively. Our results are consistent with those shown in *Triticum aestivum*B under 2 to 16 $dS{\cdot}m^{-1}$ [81] of NaCl or 100 to 200 mM NaCl [82]. In totum, we show that genotypes NARCGP2020B and NARCGP2020C share the same pattern, with higher A_N and g_s, chloroplast pigments, similar pattern ETR/$A_N \times g_s$, higher GPX and CAT activity, and low APX activity. Based on these features, we have arguments to speculate that these two genotypes have more robust machinery than others, facts that are in agreement with other research studies [83–85].

Ascorbate peroxidase belongs to the class I heme-peroxidases that are found in higher plants, present in cytosol, mitochondria, chloroplast stroma and other membrane-bound isoforms including peroxisome and glyoxisome (mAPX) and chloroplast thylakoids (tAPX) [86]. The APX mechanism action is a complex process [87]. Its action occurs, like CAT, under H_2O_2. However, unlike the latter, APX uses reduced ascorbate for the conversion of H_2O_2 to $H_2O + \frac{1}{2}\ O_2$, forming monodehydroascorbate as a by-product, which needs to be reduced again to dehydroascorbate at the expense of 2 moles of reduced glutathione, producing 2 moles of oxidized glutathione, which needs to be reduced again at the expense of 1 mol of NADPH. Summarizing, to convert one molecule of H_2O_2 into H_2O, APX needs 1 mol of reduced ascorbate, 2 mol of reduced glutathione, and 1 mol of NADPH to feed back the glutathione reductase enzyme that requires 1 FAD as cofactor. So, plant enzyme machinery cannot afford to use APX under any kind of stress, because it is expensive to cells. Thus, plants with high APX activity are plants that have been exposed to severe stress, so APX activity is another criterion for selecting elite genotypes [87,88].

With a fine understanding of Figure 6A,B, we perceive that all non-stressed plants could be grouped in only one group, while salt-stressed canola plant NARCGP2020B and NARCGP2020C genotypes grouped and NARCGP2020A, NARCGP2020D, and NARCGP2020E were grouped in another cluster. With a fine-tuning of Figure 6B, we noticed that mean germination time (MGT) was crucial to clustered NARCGP2020A, NARCGP2020D, and NARCGP2020E, herein called highly stressed, while a high activity of antioxidative enzymes (CAT, GPX, and APX) was crucial to group NARCGP2020B and NARCGP2020C, herein called less stressed. In a single image, Figure 7 summarizes the pattern of genotype reactions to all treatments. In the basal part of the figure, it is important to make clear that genotypes NARCGP2020B and NARCGP2020C show higher antioxidative enzymes and efficient use of electrons through the chloroplast chain transport rate. Additionally, plants grown from priming seed were stronger and better equipped to withstand salt stress, which has been extensively discussed in the literature as cellular stressful memories [24,89,90].

The catalytic activity of canola oil is higher for esterification, higher for transesterification, and at its peak during processes. It also has improved fuel properties when combined with biodiesel and glycerol ether [91]. The biodiesel produced worldwide is currently produced from recycled oils and animal fats. Canola and soybean oil will also be required to offer sufficient feedstocks if biodiesel is to account for 5% of diesel consumption. In accordance with Baroi, Mahto, Niu and Dalay [91], the energy ratio was similar for canola and soybean; however, for a given weight of seed, soybean required fewer energy inputs and produced less oil than canola. Thus, canola oil is one type of biodiesel, and it

has an advantage in oil production per unit area compared with other biodiesels. It cost 7,146,537 kcal to create 600 kg of canola oil per hectare [92]. It is estimated that 1500 kg ha^{-1} of seeds were produced, yielding an estimated 9,930,000 kcal in energy per hectare, resulting in an energy balance of 1.39. Results indicated the viability of biofuel production from canola but also showed the need to improve the technology used to increase the energy and economic balance ratios.

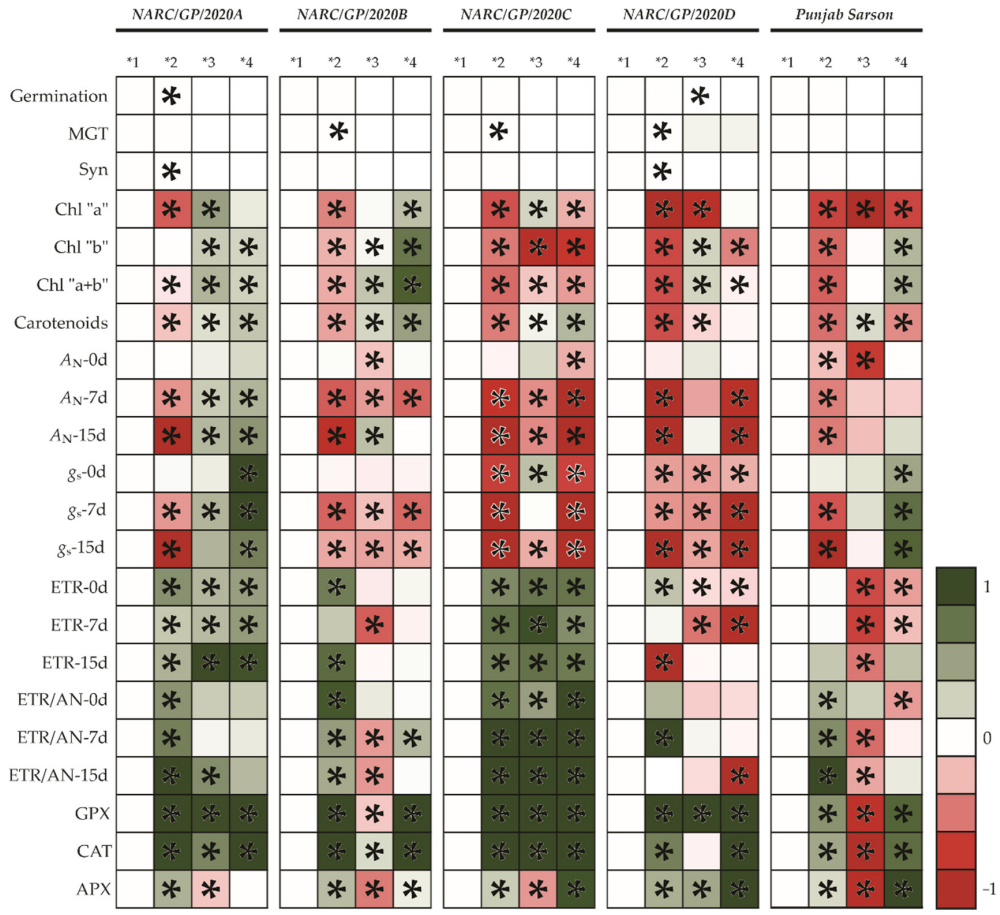

Figure 7. Heatmap was constructed to summarize all analyzed features in each genotype shown at the top of the figure. In each genotype, each column denotes one different treatment, being 0 MPa plus 0 MPa (*1), 0 MPa plus −1.2 MPa (*2), −1.2 MPa plus 0 MPa (*3), and −1.2 MPa plus −1.2 MPa (*4). Each square was constructed by a ratio of the mean of each analyzed feature to the mean of the control (0 MPa plus 0 MPa) in each analyzed feature. The false color was performed by $\log_2$, using a color scale. The asterisks (*) in each square denote a significant difference between each treatment and control (0 MPa plus 0 MPa). $\alpha \leq 0.05$.

Food security has become a very strong focus with new FAO statistics. Food security has been identified as the agrifood sector's next major challenge, due to the rapid increase in the global population. FAO estimates that in 2050, the planet will have 9.7 billion habitats, considering 1.1% annual as the mean. This crucial global issue is even stronger in developing countries such as Pakistan. Achieving this 35-year target will require sustained increases in agricultural productivity. More investment in agriculture will be needed as a result. If there are underlying factors hindering agriculture, they should be investigated (Smyth et al. [93]). Globally, over two billion tonnes of grains are produced annually. The grains are stored at different stages of the grain distribution chain, in defined units such as bags, silos, warehouses, containers and even in piles on the ground [94]. However, most of these grains are lost during transportation from the field where they are harvested and occasionally kept to the end location, which may be a port region where grains are

transported on large export ships. Due to this, figures for post-harvest losses of grains are unavailable, although they can range from 1–2% in industrialized nations where grain is stored in facilities that are properly maintained and 20–50% in less developed nations with poorly managed storage systems.

To have a more sustainable world with a more equal distribution among people, we should have more efficient harvesters, improvement in the transportation and storage system, as well as improvements in packaging and selling the bulk product [95]. One cannot speak of sustainability without thinking about its bottlenecks and reviewing our lifestyles.

5. Conclusions

The development and identification of salt-tolerant canola genotypes are immediate requests to overcome the challenges of canola production in arid regions where complex limiting factors such as freshwater scarcity, salinity, and heat stress exist. In this study, we tested five canola genotypes for resistance to salinity stress. According to the findings of the study, a negative effect was generally observed in all genotypes. In terms of germination, gas exchange, chloroplast pigments, and antioxidant enzymatic activities, the genotypes NARC/GP/2020B and NARC/GP/2020C were found to be tolerant to high salt stress (ψ_s = −1.2 MPa) promoted by $CaCl_2$. Based on a statistical analysis of all analyzed traits, genotype NARC/GP/2020B followed by NARC/GP/2020C could tolerate salt stress through better utilization of electrons to produce photosynthesis, produced more chloroplast pigments and had a very well-equipped antioxidant system. So, these genotypes appear to have an interesting tolerance mechanism against salt stress and could be an important salt stress-resistant resource for developing high-yielding canola varieties in future breeding programs under salt stress conditions.

Author Contributions: Conceptualization, W.I. and A.J.; methodology, W.I.; software, A.Z., A.J.-O. and M.F.P.; validation, A.J., M.F.S. and A.M.; formal analysis, M.A.K. and M.F.P.; investigation, W.I.; resources, A.Z.; data curation, A.J. and M.F.P.; writing—original draft preparation, W.I., M.F.P. and A.J.-O.; writing—review and editing, A.J., A.M., M.F.S., A.J.-O. and M.F.P.; visualization, A.M.; supervision, M.Z.A.; project administration, M.Z.A.; funding acquisition, Á.S., M.F.S. and M.Z.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research is supported by the New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund (UNKP-21–3).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We are thankful to the laboratory staff of Department of Agronomy, Amir Muhammad Khan Campus Mardan, Faculty of Crop Production Sciences, The University of Agriculture, Peshawar (Pakistan) for assistance and technical support in this work. We gratefully acknowledge Muhammad Fawad (Department of Weed Science and Botany, Faculty of Crop Protection Sciences, The University of Agriculture, Peshawar 25130, Pakistan) for his technical help throughout the writing of this manuscript. The authors also thank the Higher Education Commission (HEC) of Pakistan for financial support. This study was also financially assisted by HEC, Pakistan, under the HEC Project. Ref No. 20-15833/NRPU/R&D/HEC.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Smith, M.D. The ecological role of climate extremes: Current understanding and future prospects. *J. Ecol.* **2011**, *99*, 651–655. [CrossRef]
2. Cardinale, B.J.; Duffy, J.E.; Gonzalez, A.; Hooper, D.U.; Perrings, C.; Venail, P.; Narwani, A.; Mace, G.M.; Tilman, D.; Wardle, D.A.; et al. Biodiversity loss and its impact on humanity. *Nature* **2012**, *486*, 59–67. [CrossRef] [PubMed]
3. Čanak, P.; Jeromela, A.M.; Vujošević, B.; Kiprovski, B.; Mitrović, B.; Alberghini, B.; Zanetti, F. Is drought stress tolerance affected by biotypes and seed size in the emerging oilseed crop Camelina? *Agronomy* **2020**, *10*, 1856. [CrossRef]

4. Walck, J.L.; Hidayati, S.N.; Dixon, K.W.; Thompson, K.E.N.; Poschlod, P. Climate change and plant regeneration from seed. *Glob. Change Biol.* **2011**, *17*, 2145–2161. [CrossRef]
5. Fernández-Pascual, E.; Mattana, E.; Pritchard, H.W. Seeds of future past: Climate change and the thermal memory of plant reproductive traits. *Biol. Rev.* **2019**, *94*, 439–456. [CrossRef] [PubMed]
6. Tol, R.S.J. The economic impacts of climate change. *Rev. Environ. Econ. Policy* **2018**, *12*, 1. [CrossRef]
7. Wheeler, T.; Braum, J.V. Climate change impacts on global food security. *Science* **2013**, *341*, 508–513. [CrossRef] [PubMed]
8. Mcnutt, M. Climate change impacts. *Science* **2013**, *341*, 435. [CrossRef]
9. Aymen, E.M.; Zhani, K.; Meriem, B.F.; Hannachi, C. Seed priming for better growth and yield of safflower (*Carthamus tinctorius*) under saline condition. *J. Stress Physiol. Biochem.* **2012**, *8*, 135–143.
10. Hafeez, M.B.; Raza, A.; Zahra, N.; Shaukat, K.; Akram, M.Z.; Iqbal, S.; Basra, S.M.A. Gene regulation in halophytes in conferring salt tolerance. In *Handbook of Bioremediation*; Hasanuzzaman, M., Prasad, M.N.V., Eds.; Elsevier: London, UK, 2021; pp. 341–370.
11. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681. [CrossRef]
12. Pompelli, M.F.; Ferreira, P.P.B.; Chaves, A.R.M.; Figueiredo, R.C.Q.Q.; Martins, A.O.; Jarma-Orozco, A.; Batista-Silva, W.; Endres, L.; Araújo, W.L. Physiological, metabolic, and stomatal adjustments in response to salt stress in *Jatropha curcas*. *Plant Physiol. Biochem.* **2021**, *168*, 116–127. [CrossRef] [PubMed]
13. Pompelli, M.F.; Jarma-Orozco, A.; Rodrígues-Páez, L.A. Salinity in *Jatropha curcas*: A review of physiological, biochemical, and molecular factors involved. *Agriculture* **2022**, *12*, 594. [CrossRef]
14. Raymer, P.L. Canola: An emerging oilseed crop. In *Trends in New Crops and New Uses*; Janick, J., Whipkey, A., Eds.; ASHS Press: Alexandria, VA, USA, 2002; Volume 1, pp. 122–126.
15. Tang, X.; Lee, J.; Chen, W.N. Engineering the fatty acid metabolic pathway in *Saccharomyces cerevisiae* for advanced biofuel production. *Metab. Eng. Commun.* **2015**, *2*, 58–66. [CrossRef] [PubMed]
16. Janßen, H.J.; Steinbüchel, A. Fatty acid synthesis in *Escherichia coli* and its applications towards the production of fatty acid based biofuels. *Biotechnol. Biofuels.* **2014**, *4*, 7. [CrossRef] [PubMed]
17. Atsumi, S.; Hanai, T.; Liao, J. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. *Nature* **2008**, *451*, 86–89. [CrossRef]
18. FAOSTAT. Crops and Livestock Products. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 22 July 2022).
19. MINFAL. Agricultural Statistic of Pakistan. Available online: https://www.pbs.gov.pk/content/agriculture-statistics (accessed on 22 July 2022).
20. Steppuhn, H.; Volkmar, K.; Miller, P. Comparing canola, field pea, dry bean, and durum wheat crops grown in saline media. *Crop Sci.* **2001**, *41*, 1827–1833. [CrossRef]
21. Ashraf, M.; Foolad, M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **2007**, *59*, 206–216. [CrossRef]
22. Sen, A.; Puthur, J.T. Seed priming-induced physiochemical and molecular events in plants coupled to abiotic stress tolerance: An overview. In *Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants*; Hossain, M.A., Liu, F., Burritt, D., Fujita, M., Huang, B., Eds.; Elsevier: London, UK, 2020; pp. 303–316.
23. Saddhe, A.A.; Saddhe, R.; Penna, S. Plant sugars: Homeostasis and transport under abiotic stress in plants. *Physiol. Plant* **2021**, *171*, 739–755. [CrossRef] [PubMed]
24. Martinez-Medina, A.; Flors, V.; Heil, M.; Mauch-Mani, B.; Pieterse, C.M.J.; Pozo, M.J.; Ton, T.; van Dam, N.M.; Conrath, U. Recognizing plant defense priming. *Trends Plant Sci.* **2016**, *21*, 818–822. [CrossRef]
25. Sarath, N.G.; Manzil, S.A.; Ali, S.; Alsahli, A.B.; Puthur, J.T. Physio-anatomical modifications and elemental allocation pattern in *Acanthus ilicifolius* L. subjected to zinc stress. *PLoS ONE* **2022**, *17*, e0263753. [CrossRef]
26. Kinoshita, T.; Sekil, M. Epigenetic memory for stress response and adaptation in plants. *Plant Cell Physiol.* **2014**, *55*, 1859–1863. [CrossRef] [PubMed]
27. Villagómez-Aranda, A.L.; Feregrino-Pérez, A.A.; García-Ortega, L.F.; González-Chavira, M.M.; Torres-Pacheco, I.; Guevara-González, R.G. Activating stress memory: Eustressors as potential tools for plant breeding. *Plant Cell Rep.* **2022**, *41*, 1481–1498. [CrossRef] [PubMed]
28. Sharma, R.V.; Somidi, A.K.R.; Dalai, A.K. Preparation and properties evaluation of biolubricants derived from canola oil and canola biodiesel. *J. Agric. Food Chem.* **2015**, *63*, 3235–3242. [CrossRef] [PubMed]
29. Lozano-Isla, F.; Alfaro, O.B.; Pompelli, M.F. GerminaR: An R package for germination analysis with the interactive web application 'GerminaQuant for R'. *Ecol. Res.* **2019**, *34*, 339–346. [CrossRef]
30. Corte-Real, N.; Miranda, P.V.V.C.; Endres, L.; Souza, E.R.; Pompelli, M.F. Tolerance to salinity in *Jatropha curcas* are genotype-dependent. *Braz. J. Dev.* **2019**, *5*, 22169–22199. [CrossRef]
31. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.* **1987**, *148*, 350–382.
32. Pompelli, M.F.; Barata-Luís, R.M.; Vitorino, H.S.; Gonçalves, E.R.; Rolim, E.V.; Santos, M.G.; Almeida-Cortez, J.S.; Endres, L. Photosynthesis, photoprotection and antioxidant activity of purging nut under drought deficit and recovery. *Biomass. Bioenerg.* **2010**, *34*, 1207–1215. [CrossRef]

33. Santo, A.; Mattana, E.; Hugot, L.; Spinosi, P.; Bacchetta, G. Seed germination and survival of the endangered psammophilous *Rouya polygama* (Apiaceae) in different light, temperature and NaCl conditions. *Seed Sci. Res.* **2014**, *24*, 331–339. [CrossRef]
34. Prado, F.E.; Boero, C.; Gallardo, M.; Gonzalez, J.A. Effect of NaCl on germination, growth and soluble sugars content in *Chenopodium quinoa* Wild. seed. *Bot. Bull. Acad. Sin.* **2000**, *41*, 27–34.
35. Khan, M.A.; Gul, B.; Weber, D.J. Germination responses of *Salicornia rubra* to temperature and salinity. *J. Arid. Environ.* **2000**, *45*, 207–214. [CrossRef]
36. El-Keblawy, A.; Al-Rawai, A. Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw.) D.C. *J. Arid. Environ.* **2005**, *61*, 555–565. [CrossRef]
37. Johnston, S.K.; Crowley, R.H.; Murray, D.S. Separating seed by species with $CaCl_2$ solutions. *Weed Sci.* **2017**, *26*, 213–215. [CrossRef]
38. Darrudi, R.; Hassandokht, M.R.; Nazer, V. Effects of KNO_3 and $CaCl_2$ on seed germination of *Rheum khorasanicum* B. Baradaran & A. Jafari. *J. Appl. Sci. Res.* **2014**, *10*, 171–175.
39. Islam, R.; Mukherjee, A.; Hossin, M. Effect of osmopriming on rice seed germination and seedling growth. *J. Bangladesh Agril. Univ.* **2012**, *10*, 15–20. [CrossRef]
40. Jam, B.J.; Shekari, F.; Azimi, M.R.; Zangani, E. Effect of priming by salicylic acid on germination and seedling growth of safflower seeds under $CaCl_2$ stress. *Int. J. Agric.* **2012**, *2*, 1097–1105.
41. Harmeet, S.; Kaur, J.R.; Kaur, J.S.; Sandhu, S.S.; Harrajdeep, K.; Kamaljit, G. Seed priming techniques in field crops—A review. *Agric. Rev.* **2015**, *36*, 251–264.
42. Afzal, I.; Butt, A.; Rehman, H.U.; Basra, A.; Afzal, A. Alleviation of salt stress in fine aromatic rice by seed priming. *Aust. J. Crop. Sci.* **2012**, *6*, 1401–1407.
43. Reimer-Michalski, E.V.; Conrath, U. Innate immune memory in plants. *Semin. Immunol.* **2016**, *28*, 319–327. [CrossRef] [PubMed]
44. Carvajal, M.; Cerdá, A.; Martínez, V. Does calcium ameliorate the negative effect of NaCl on melon root water transport by regulating aquaporin activity? *New Phytol.* **2000**, *145*, 439–447. [CrossRef]
45. Rasmann, S.; De Vos, M.; Casteel, C.L.; Tian, D.; Halitschke, R.; Sun, J.Y.; Agraval, A.A.; Felton, F.W.; Jander, G. Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiol.* **2012**, *158*, 854–863. [CrossRef] [PubMed]
46. Karpinski, S.; Szechyńska-Hebda, M. Secret life of plants: From memory to intelligence. *Plant Signal. Behav.* **2010**, *5*, 1391–1394. [CrossRef] [PubMed]
47. Jacob, P.T.; Siddiqui, S.A.; Rathore, M.S. Seed germination, seedling growth and seedling development associated physiochemical changes in Salicornia brachiata (Roxb.) under salinity and osmotic stress. *Aquat. Bot.* **2020**, *166*, 103272. [CrossRef]
48. Bhatt, A.; Phondani, P.C.; Pompelli, M.F. Seed maturation time influences the germination requirements of perennial grasses in desert climate of Arabian Gulf. *Saudi J. Biol. Sci.* **2018**, *25*, 1562–1567. [CrossRef] [PubMed]
49. Bhatt, A.; Bhat, N.R.; Lozano-Isla, F.; Gallacher, D.; Santo, A.; Batista-Silva, W.; Fernandes, D.; Pompelli, M.F. Germination asynchrony is increased by dual seed bank presence in two desert perennial halophytes. *Botany* **2019**, *97*, 639–649. [CrossRef]
50. Bhatt, A.; Gallacher, D.; Jarma-Orozco, A.; Pompelli, M.F. Seed mass, dormancy and germinability variation among maternal plants of four Arabian halophytes. *Seed Sci. Res.* **2022**, *32*, 53–61. [CrossRef]
51. Bhatt, A.; Daibes, L.F.; Gallacher, D.; Jarma-Orozco, A.; Pompelli, M.F. Water stress inhibits germination while maintaining embryo viability of subtropical wetland seeds: A functional approach with phylogenetic contrasts. *Front Plant Sci.* **2022**, *13*, 906771. [CrossRef]
52. Allen, E.; Alvarez, S. *International Rules for Seed Testing 2020*; The International Seed Testing Association: Bassersdorf, Switzerland, 2020.
53. Futrell, M.C. New concepts in chemical seed treatment of agronomic crops. *J. Environ. Qual.* **1972**, *1*, 240–243. [CrossRef]
54. Bybordi, A. The influence of salt stress on seed germination, growth and yield of canola cultivars. *Not. Bot. Hort. Agrobot. Cluj* **2010**, *38*, 128–133.
55. Moosavi, S.A.; Gharineh, M.H.; Afshari, R.T.; Ebrahimi, A. Effects of some heavy metals on seed germination characteristics of canola (*Brassica napus*), wheat (*Triticum aestivum*) and safflower (*Carthamus tinctorious*) to evaluate phytoremediation potential of these crops. *J. Agric. Sci.* **2012**, *4*, 11–19.
56. Zheng, G.H.; Gao, Y.P.; Willen, R.W.; Gusta, L.V. Canola seed germination and seedling emergence from pre-hydrated and re-dried seeds subjected to salt and water stresses at low temperatures. *Ann. Appl. Biol.* **1998**, *132*, 339–348. [CrossRef]
57. Somagh, J.A.; Mousav, S.M.; Omidi, H.; Mohammadian, E.; Hemmati, M. Canola seed germination and seedling growth in response to saline condition and bio-priming. *Iran J. Plant Physiol.* **2017**, *7*, 2149–2156.
58. Silva, J.V.; Lacerda, C.F.; Costa, P.H.A.; Enéas Filho, J.; Gomes Filho, E.; Prisco, J.T. Physiological responses of NaCl stressed cowpea plants grown in nutrient solution supplemented with $CaCl_2$. *Braz. J. Plant Physiol.* **2003**, *15*, 99–105. [CrossRef]
59. Nedjimi, B.; Daoud, Y. Ameliorative effect of $CaCl_2$ on growth, membrane permeability and nutrient uptake in *Atriplex halimus* subsp. schweinfurthii grown at high (NaCl) salinity. *Desalination* **2009**, *249*, 163–666. [CrossRef]
60. Latef, A.A.H.A. Ameliorative effect of calcium chloride on growth, antioxidant enzymes, protein patterns and some metabolic activities of canola (*Brassica napus* L.) under seawater stress. *J. Plant Nutr.* **2011**, *34*, 1303–1320. [CrossRef]
61. Bybordi, A. Effect of ascorbic acid and silicium on photosynthesis, antioxidant enzyme activity, and fatty acid contents in canola exposure to salt stress. *J. Integr. Agric.* **2012**, *11*, 1610–1620. [CrossRef]

62. Athar, H.U.R.; Zafar, Z.U.; Ashraf, M. Glycinebetaine improved photosynthesis in canola under salt stress: Evaluation of chlorophyll fluorescence parameters as potential indicators. *J. Agron. Crop. Sci.* **2015**, *201*, 428–442. [CrossRef]
63. Nazarbeygi, E.; Yazdi, H.L.; Naseri, R.; Soleimani, R. The effects of different levels of salinity on proline and A-, B- Chlorophylls in canola. *Am. Eurasian J. Agric. Environ. Sci.* **2011**, *10*, 70–74.
64. Mukhtar, E.; Siddiqi, E.H.; Bhatti, K.H. Gas exchange attributes can be valuable selection criteria for salinity tolerance in canola cultivars (*Brassica napus* L.). *Pak. J. Bot.* **2013**, *45*, 35–40.
65. Pompelli, M.F.; Martins, S.C.V.; Antunes, W.C.; Chaves, A.R.M.; DaMatta, F.M. Photosynthesis and photoprotection in coffee leaves is affected by nitrogen and light availabilities in winter conditions. *J. Plant Physiol.* **2010**, *167*, 1052–1060. [CrossRef]
66. Warren, C.R.; Adams, M.A. What determines rates of photosynthesis per unit nitrogen in *Eucalyptus* seedlings? *Funct. Plant Biol.* **2004**, *31*, 1169–1178. [CrossRef]
67. Tuba, Z.; Lichtenthaler, H.; Csintalan, Z.; Nagy, Z.; Szente, K. Loss of chlorophylls, cessation of photosynthesis CO_2 assimilation and respiration in the poikilochlorophyllous plant *Xerophyta scabrida* during desiccation. *Physiol. Plant* **1996**, *96*, 383–388. [CrossRef]
68. Terashima, I.; Fujita, T.; Inoue, T.; Chow, W.S.; Oguchi, R. Green light drives leaf photosynthesis more efficiently than red light in strong white light: Revisiting the enigmatic question of qhy leaves are green. *Plant Cell Physiol.* **2009**, *50*, 684–697. [CrossRef] [PubMed]
69. Bezerra-Neto, E.; Coelho, J.B.M.; Jarma-Orozco, A.; Rodríguez-Páez, L.A.; Pompelli, M.F. Modulation of photosynthesis under salinity and the role of mineral nutrients in *Jatropha curcas* L. *J. Agron. Crop. Sci.* **2021**, *208*, 314–334. [CrossRef]
70. Alam, M.; Juraimi, A.S.; Rafii, M.; Abdul Hamid, A. Effect of salinity on biomass yield and physiological and stem-root anatomical characteristics of purslane (*Portulaca oleracea* L.) accessions. *Biomed. Res. Int.* **2015**, *2015*, 105695. [CrossRef] [PubMed]
71. Dos Santos, O.O.; Mendes, K.R.; Martins, S.V.C.; Batista-Silva, W.; dos Santos, M.A.; Figueirôa, J.M.; Souza, E.R.; Fernandes, D.; Araújo, W.L.; Pompelli, M.F. Physiological parameters and plasticity as key factors to understand pioneer and late successional species in the Atlantic Rainforest. *Acta Physiol. Plant* **2019**, *41*, 145. [CrossRef]
72. Cha-Um, S.; Kirdmanee, C. Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pak. J. Bot.* **2009**, *41*, 87–98.
73. Shin, Y.K.; Bhandari, S.R.; Jo, J.S.; Song, J.W.; Cho, M.C.; Yang, E.Y.; Lee, J.G. Response to salt stress in lettuce: Changes in chlorophyll fluorescence parameters, phytochemical contents, and antioxidant activities. *Agronomy* **2020**, *10*, 1627. [CrossRef]
74. Zhao, H.; Liang, H.; Chu, Y.; Sun, C.; Wei, N.; Yang, M.; Zheng, C. Effects of salt stress on chlorophyll fluorescence and the antioxidant system in *Ginkgo biloba* L. seedlings. *Hort. Sci.* **2019**, *54*, 2125–2133. [CrossRef]
75. Sali, A.; Rusinovci, I.; Fetahu, S.; Gashi, B.; Simeonovska, E.; Rozman, L. The effect of salt stress on the germination of maize (*Zea mays* L.) seeds and photosynthetic pigments. *Acta Agric. Slov.* **2015**, *105*, 85–94.
76. Lawlor, D.W.; Tezara, W. Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: A critical evaluation of mechanisms and integration of processes. *Ann. Bot.* **2009**, *103*, 561–579. [CrossRef]
77. Fonseca-Pereira, P.; Daloso, D.M.; Gago, J.; Nunes-Nesi, A.; Araújo, W.L. On the role of the plant mitochondrial thioredoxin system during abiotic stress. *Plant Signal. Behav.* **2019**, *14*, 1592536. [CrossRef] [PubMed]
78. Farhoudi, R.; Hussain, M.; Dong-Jin, L. Modulation of enzymatic antioxidants improves the salinity resistance in canola (*Brassica napus*). *Int. J. Agric. Biol.* **2012**, *14*, 465–468.
79. Xu, D.; Wang, W.; Gao, T.; Fang, X.; Gao, X.; Li, J.; Bu, H.; Mu, J. Calcium alleviates decreases in photosynthesis under salt stress by enhancing antioxidant metabolism and adjusting solute accumulation in *Calligonum mongolicum*. *Conserv. Physiol.* **2017**, *5*, cox060. [CrossRef]
80. Jaleel, C.A.; Manivannan, P.; Sankar, B.; Kishorekumar, A.; Panneerselvam, R. Calcium chloride effects on salinity-induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus*. *C. R. Biol.* **2007**, *330*, 674–683. [CrossRef] [PubMed]
81. Rao, A.; Ahmad, S.D.; Sabir, S.M.; Awan, S.I.; Shah, A.H.; Abbas, S.R.; Shafique, S.; Khan, F.; Chaudhary, A. Potential antioxidant activities improve salt tolerance in ten varieties of wheat (*Triticum aestivum* L.). *Am. J. Plant Sci.* **2013**, *4*, 69–76. [CrossRef]
82. Sairam, R.; Srivastava, G.; Agarwal, S.; Meena, R. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biol. Plant* **2005**, *49*, 85–91. [CrossRef]
83. Campos, M.L.O.; Hsie, B.S.; Granja, J.A.A.; Correia, R.M.; Silva, S.R.S.; Almeida-Cortez, J.S.; Pompelli, M.F. Photosynthesis and antioxidant activity mechanisms in *Jatropha curcas* L. under salt stress. *Braz. J. Plant Physiol.* **2012**, *24*, 55–67. [CrossRef]
84. Silva, E.N.; Silveira, J.A.G.; Ribeiro, R.V.; Vieira, S.A. Photoprotective function of energy dissipation by thermal processes and photorespiratory mechanisms in *Jatropha curcas* plants during different intensities of drought and after recovery. *Environ. Exp. Bot.* **2015**, *110*, 36–45. [CrossRef]
85. Corte-Real, N.; Oliveira, M.S.; Jarma-Orozco, A.; Fernandes, D.; dos Santos, M.A.; Endres, L.; Calsa, T., Jr.; Pompelli, M.F. Comparative analysis of salt-induced changes in the leaves proteome of two contrasting *Jatropha curcas* genotypes. *Braz. J. Dev.* **2020**, *6*, 39845–39872. [CrossRef]
86. Caverzan, A.; Passaia, G.; Rosa, S.B.; Ribeiro, C.W.; Lazzarotto, F.; Margis-Pinheiro, M. Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. *Genet. Mol. Biol.* **2012**, *35*, 1011–1019. [CrossRef]
87. Pandey, S.; Fartyal, D.; Agarwal, A.; Shukla, T.; James, D.; Kaul, T.; Negi, Y.K.; Arora, S.; Reddy, M.K. Abiotic stress tolerance in plants: Myriad roles of ascorbate peroxidase. *Front. Plant Sci.* **2017**, *8*, 581. [CrossRef] [PubMed]

88. Ulfat, M.; Athar, H.U.R.; Ashraf, M.; Akram, N.A.; Jamil, A. Appraisal of physiological and biochemical selection criteria for evaluation of salt tolerance in canola (*Brassica napus* L.). *Pak. J. Bot.* **2007**, *39*, 1593–1608.
89. Bruce, T.J.A.; Matthes, M.C.; Napier, J.A.; Pickett, J.A. Stressful "memories" of plants: Evidence and possible mechanisms. *Plant Sci.* **2007**, *173*, 603–608. [CrossRef]
90. Jaskiewicz, M.; Conrath, U.; Peterhänsel, C. Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Rep.* **2011**, *12*, 50–55. [CrossRef]
91. Baroi, C.; Mahto, S.; Niu, C.; Dalay, A.K. Biofuel production from green seed canola oil using zeolites. *Appl. Catal. A Gen.* **2014**, *469*, 18–32. [CrossRef]
92. Silva, L.F.L.; Gonçalves, W.M.; Maluf, W.R.; Resende, L.V.; Sarmiento, C.M.; Licursi, V.; Moretto, P. Energy balance of biodiesel production from canola. *Cienc. Rural* **2017**, *47*, e20151084. [CrossRef]
93. Smyth, S.J.; Phillips, P.W.B.; Keer, W.A. Food security and the evaluation of risk. *Glob. Food Sec.* **2015**, *4*, 16–23. [CrossRef]
94. Jayas, D.S. Storing grains for food security and sustainability. *Agric. Res.* **2002**, *1*, 21–24. [CrossRef]
95. Saeed, M.F.; Jamal, A.; Ahmad, I.; Ali, S.; Shah, G.M.; Husnain, S.K.; Farooq, A.; Wang, J. Storage cnditions deteriorate cotton and wheat seeds quality: An assessment of farmers' awareness in Pakistan. *Agronomy* **2020**, *10*, 1246. [CrossRef]