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Analysis of Morphological, Physiological, and Biochemical Traits of Salt Stress Tolerance in Asian Rice Cultivars at Seedling and Early Vegetative Stages

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Abstract: Rice (*Oryza sativa* L.) is a primary energy food for the Asian population. One of the greatest constraints in rice production is soil salinity because rice is very susceptible to salt. Meanwhile, many agricultural lands in Asia are in saline areas. It is important to identify and develop salt-tolerant rice varieties that highly adapt to Asian climates. By combining morphological, physiological, and biochemical assessments for screening the salt tolerance of 116 Asian rice cultivars, we were able to classify them into tolerant, moderate, and sensitive rice cultivars under salinity stress conditions and also understand salt tolerance mechanisms. The rice cultivars that are salt-tolerant include Pokkali from India, TCCP 266 and IR 45427 from the Philippines, and Namyang 7 from Republic of Korea. However, salt-sensitive rice varieties like IR29 and IR58 are from the Philippines, and Daegudo and Guweoldo are from Korea. The salt-tolerant varieties showed signs of tolerance, including a lower percent reduction in germination percentage, root length, root fresh weight, shoot length, plant biomass, and chlorophyll content. In order to maintain the cellular osmotic balance under saline conditions, the salt-tolerant varieties exhibited less membrane damage, a lower Na/K ratio, high proline and sugar accumulation, and lower levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). Pokkali from India, TCCP 266 and IR 45427 from the Philippines, and Namyang 7 from Republic of Korea are recommended as valuable germplasm resources for Asian rice breeding programs in saline agricultural areas.

Keywords: Asian rice; salt stress; morphology; physiology; biochemistry

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1. Introduction

Rice (*Oryza sativa* L.) is one of the major food sources for more than half of the global population [1–4]. One of the constraints in rice production is soil salinity as it greatly affects rice production because rice is very susceptible to salt, especially at the seedling, early vegetative (three-leaf stage), and reproductive stages [5–9]. Based on history, Mesopotamian civilization (now part of Iraq) collapsed due to salinization in agricultural areas that caused crop failures [10]. Because of human activities and natural phenomena, soil salinity is increasing. Soil salinity stress generally occurs in rice field areas that have improper irrigation and drainage systems and also in coastal areas [11,12]. The effects of soil salinity stress also depend on rice genotypes and salt concentration. Indica rice is more salt-tolerant compared with japonica rice subspecies [13]. Soil salinity is a serious problem in most of the rice-growing areas of Asian countries in the tropics and temperate regions. Most of the agricultural areas in Asian regions have saline soils [14,15]. Rice is the most susceptible crop to soil salinity stress compared with barley and wheat [16]. Saline soils have an electrical conductivity (ECe) greater than 4 dSm^{-1} (~40 mM NaCl) because of the presence of salts, including sodium chloride, bicarbonates, magnesium, and calcium sulfates, and also a number of inorganic ions, such as Na^+, Ca^{2+}, Mg^{2+}, K^+, $CO_3{}^{2-}$, HCO^{3-}, $SO_4{}^{2-}$, and NO^{3-} [17,18]. Sodium chloride (NaCl) is the most abundant salt in saline soils.

Saline soil conditions make it difficult for roots to uptake nutrients and water, which induces osmotic and ionic stress in rice plants [19]. Under salinity stress conditions, there is a large amount of Na^+ influx into plant cells and an increase in the Na^+ concentrations in the cytoplasm and vacuole, which are toxic to the metabolism mechanism and lead to cell death due to osmotic and ionic stresses [16]. Excessive salt concentration in plant cells absolutely leads to reduced cell membrane stability; cell wall damage; cytoplasmic degradation; plasmolysis; endoplasmic reticulum damage; accumulation of malate, citrate, and inositol in the leaf; and increased proline concentration, which leads to a decrease in grain yield [20]. A recent study found that Pokkali is a salt-tolerant rice cultivar and IR20 is a salt-sensitive cultivar [21]. A salt-tolerant cultivar maintains a higher K^+/Na^+ ratio than a salt-sensitive cultivar. Excess salts in the soil cause high osmotic pressure outside the roots, which reduces the ability of root cells to take up water and nutrients from the soil. In order to adapt to soil salinity stress, plant cells need to accumulate osmolytes, including proline, glycine, taurine, sugars, inositols, glycerol, and sorbitol. A salt-tolerant cultivar accumulates more proline than a salt-sensitive cultivar [22,23].

Salinity stress affects the morphological, physiological, and biochemical characteristics of rice plants, which vary with the growth stages, including reductions in plant height, productive tiller number, biomass, grain yield, filled grain per panicle, grain weight, grain quality, harvest index, and photosynthetic activity and increased the uptake of Na^+ and Cl^- to the shoot [24–26]. Under salinity stress at 3.5 dSm^{-1} or 50 mM NaCl, the grain yield of rice was significantly reduced by 90% [27,28]. Meanwhile, rice seedlings die at 10 dSm^{-1} [29]. In order to cope with soil salinity stress, rice plants have developed several mechanisms, such as antioxidants for reactive oxygen species (ROS) detoxification, ion homeostasis, biosynthesis and accumulation of osmolytes for osmo-protection, and programmed cell death [22,30].

Soil salinity tolerance is a quantitative trait that is controlled by multiple genes and highly affected by environmental conditions [31–35]. The screening of rice for salinity tolerance is also complex. Thus, salinity screening under laboratory conditions is more controllable, rapid, and accurate than field screening. Under field screening, environmental conditions such as dynamic climate factors and soil heterogeneity might influence the accuracy of the determination of the effects of salinity on rice plants [36]. The potential indicators for salt tolerance screening for morphological, physiological, and biochemical characteristics are germination percentage, germination time, seedling vigor index, root length, shoot length, plant biomass, cell membrane stability, Na^+/K^+ ratio, proline content, malondialdehyde (MDA) content, hydrogen peroxide (H_2O_2) content, sugar content, ethylene content, and chlorophyll content [37,38]. The International Rice Research Institute (IRRI) released a standard evaluation score, ranging from 1 to 9, for visual determination of salinity injury [5]. Salt tolerance is indicated by a lower score (1), and salt sensitivity is denoted by a higher score (9), based on the leaf symptoms, tiller number, and growth characteristics under saline conditions. The identification of quantitative trait loci (QTLs) and cloning of genes correlated with salt tolerance in rice may accelerate the development of salt-tolerant rice varieties [39]. Meanwhile, not many genes associated with salt tolerance have been isolated and applied in rice breeding programs.

Many QTLs associated with salinity tolerance have been identified by using mapping populations derived from crosses between salt-tolerant rice varieties and salt-sensitive varieties. The most popular marker in QTL mapping is a single-nucleotide polymorphism (SNP) [39,40]. A recombinant inbred line (RIL) population derived from a cross between salt-tolerant Pokkali and salt-sensitive IR29 was used to identify a major QTL, Saltol, on chromosome 1 which is involved in regulating Na^+/K^+ homeostasis [41]. Other QTLs that have been identified are qSKC-1, qSNC-7, qSE3, and qST1, which play important roles in salt tolerance at different growth stages [22]. Genome-wide association studies (GWASs) accelerate the breeding of salt-tolerant rice varieties because of the availability of the rice reference genome and next-generation sequencing (NGS) techniques. A GWAS identified more accurate genomic locations associated with the salinity tolerance. A total of twenty-one QTLs and two candidate genes correlated with salinity tolerance were identified in a GWAS with 664 rice varieties [42]. A GWAS was also conducted to identify salt-tolerant loci during the reproductive stage [43,44]. A number of genes associated with grain yield under salinity stress conditions were also identified in a GWAS with 708 rice genotypes [45].

The analysis of genomics, transcriptomics, proteomics, and metabolomics is also important in identifying genes correlated with salinity tolerance in rice. Under salinity stress, there is a series of changes in rice plants, including changes in gene expression, protein content, and metabolite concentrations [46,47]. A number of potential genes associated with salinity tolerance can be identified by comparing the transcriptome, proteome, and metabolome characteristics of salt-tolerant rice varieties with those of salt-sensitive varieties under salinity stress versus normal conditions. The most important goal in relieving the soil salinity problem is to identify and develop rice varieties with high tolerance to salinity stress. Rice genotypes with high salinity tolerance can be identified by using effective screening methods and can provide donor alleles for salt tolerance to develop high-salinity-tolerance varieties through rice breeding programs. By understanding the mechanism of salinity tolerance in rice plants based on morphological, physiological, biochemical, and genetic effects, the development of rice varieties with high salinity tolerance by genetic engineering techniques can be accelerated [24]. Breeding for rice salt tolerance is a major goal for rice breeders in agricultural areas with saline soil conditions to ensure food sustainability. The objective of this research is to screen the salt tolerance of Asian rice cultivars based on morphological, physiological, and biochemical characteristics.

2. Results

2.1. Morphological Responses of Asian Rice Cultivars to Salt Stress at Seedling and Early Vegetative Stages

All of the Asian rice varieties showed normal growth in the control condition. The germination percentage of Asian rice cultivars under a salinity condition of 200 mM NaCl showed a reduction compared with the normal condition because the salt caused retardation in the plumule and radicle length. Under salinity stress conditions, the Asian rice cultivars had a wide range of germination percentages (Figure 1) and visual symptoms of salt injury at the seedling stage ranging from a score of 1 (salt-tolerant) to a score of 9 (salt-susceptible) (Figure 2). Based on the SES of visual salt injury at the seedling stage, 31 rice cultivars were identified as salt-tolerant, 45 cultivars were classified as moderately salt-tolerant, and 40 cultivars were classified as salt-susceptible. The most salt-tolerant were Pokkali from India, TCCP 266 from the Philippines, IR 45427 also from the Philippines, and Namyang 7 from Republic of Korea. Meanwhile, the most salt-susceptible rice varieties were IR29 from the Philippines, IR58 also from the Philippines, Daegudo from Republic of Korea, and Guweoldo also from Republic of Korea.

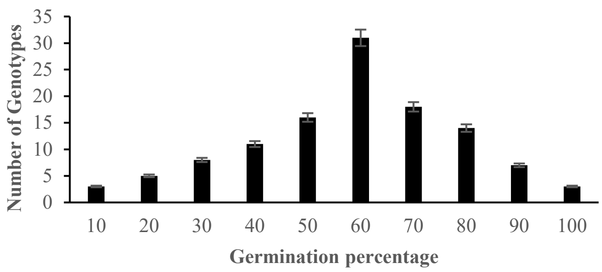

Figure 1. Germination percentage of 116 Asian rice cultivars under a salinity stress condition of 200 mM NaCl.

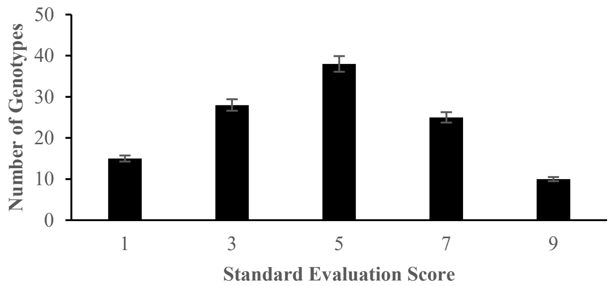

Figure 2. Standard evaluation score (SES) of visual salt injury at seedling stage of 116 Asian rice cultivars.

Other morphological responses to salinity stress conditions, including root length, root fresh weight, shoot length, and plant biomass, showed variation among the Asian rice cultivars (Figure 3). In all of the rice cultivars, root length, root fresh weight, shoot length, and plant biomass were reduced under salinity stress conditions, and the reduction was as high as 59%, 51%, 61%, and 55%, respectively. Pokkali from India, TCCP 266 from the Philippines, IR 45427 also from the Philippines, and Namyang 7 from Republic of Korea showed the lowest reduction for root length, root fresh weight, shoot length, and plant biomass. Meanwhile, IR29 from the Philippines, IR58 also from the Philippines, Daegudo from Republic of Korea, and Guweoldo also from Republic of Korea had the highest reduction for root length, root fresh weight, shoot length, and plant biomass. All of the Asian rice cultivars displayed a root length reduction ranging from 33% to 59%, the reduction for root fresh weight ranged from 35% to 51%, the shoot length had a reduction ranging from 37% to 61%, and the reduction for plant biomass ranged from 31 to 55%.

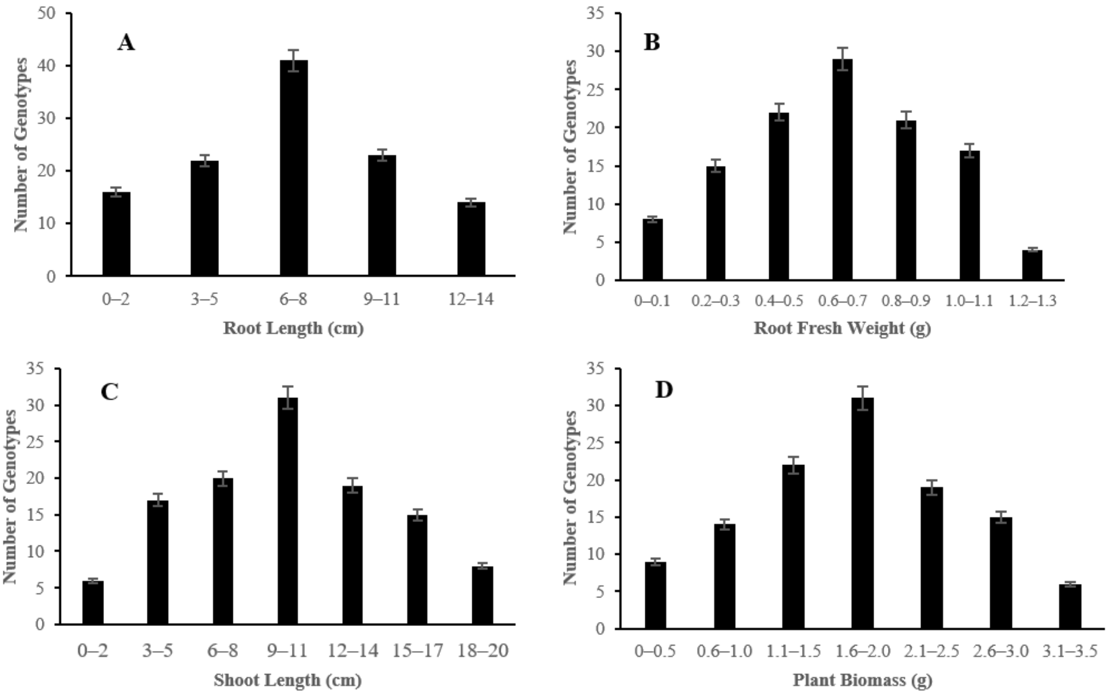

Figure 3. Effect of salinity stress conditions on root length (**A**), root fresh weight (**B**), shoot length (**C**), and plant biomass (**D**) of 116 Asian rice cultivars.

Rice plants develop leaf symptoms, such as leaf rolling, yellowing, and necrotic lesions, when rice is grown under salinity stress conditions (Figure S1). Leaf rolling leads to a minimization in the water loss by respiration caused by water deficit under salinity stress conditions. Under salinity stress at the early vegetative stage of rice plants with three leaves, leaf rolling, yellowing, necrotic lesions, drying of leaves, and other senescence symptoms were observed in all Asian rice cultivars with a wide range of variation (Figure 4). The damage to leaves under salinity stress conditions is related to the accumulation of Na^+ from root to shoot.

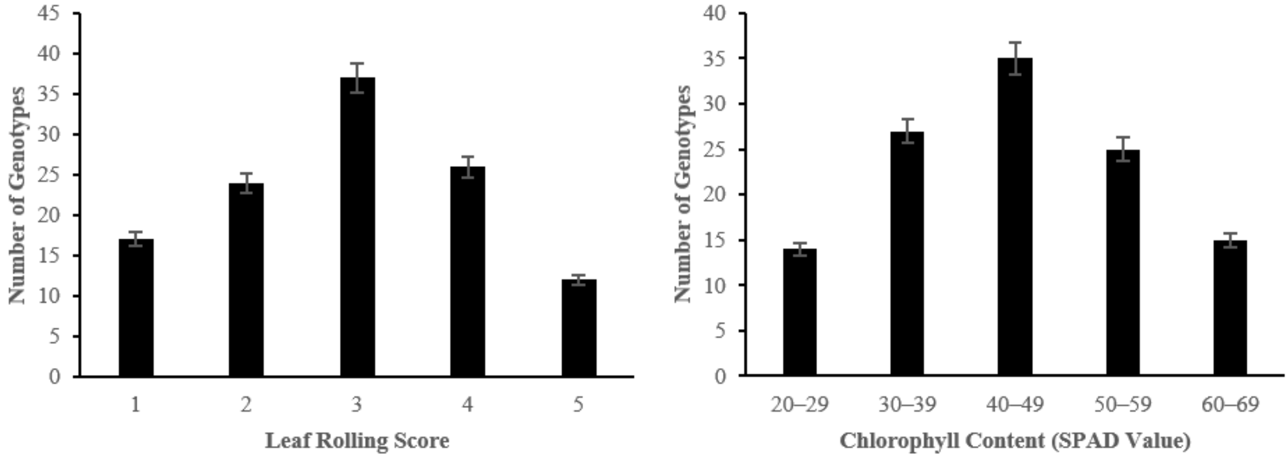

Figure 4. Leaf symptoms of leaf rolling (**left**) and chlorophyll content (**right**) from 116 Asian rice cultivars under salinity stress conditions.

Salinity stress conditions reduced the total chlorophyll content significantly in the Asian rice cultivars classified as salt-susceptible cultivars. The decrease in chlorophyll content in the 116 Asian rice cultivars showed a wide range variation (Figure 4). A reduction in chlorophyll content decreases photosynthetic activity. This result was in line with the results found for other crops, including peas [48], wheat [49], rapeseed [50], and safflower [51].

2.2. The Effect of Salinity Stress on Physiological and Biochemical Characteristics of Asian Rice Cultivars at Seedling and Early Vegetative Stages

Under salinity stress conditions, sodium (Na) content in all of the Asian rice cultivars increased significantly. The highest accumulation of sodium was found in the salt-susceptible rice varieties such as IR29 from the Philippines, IR58 also from the Philippines, Daegudo from Republic of Korea, and Guweoldo also from Republic of Korea followed by moderately salt-tolerant rice varieties including Nipponbare from Japan, Padi Tarab from Malaysia, and Ciherang from Indonesia. Meanwhile, the lowest sodium concentration was observed in the shoots of salt-tolerant varieties such as Pokkali from India, TCCP 266 from the Philippines, IR 45427 also from the Philippines, and Namyang 7 from Republic of Korea, but these varieties had higher potassium (K) accumulation than the sensitive ones. The Na/K ratio in all 116 Asian rice varieties exhibited a wide variation (Figure 5).

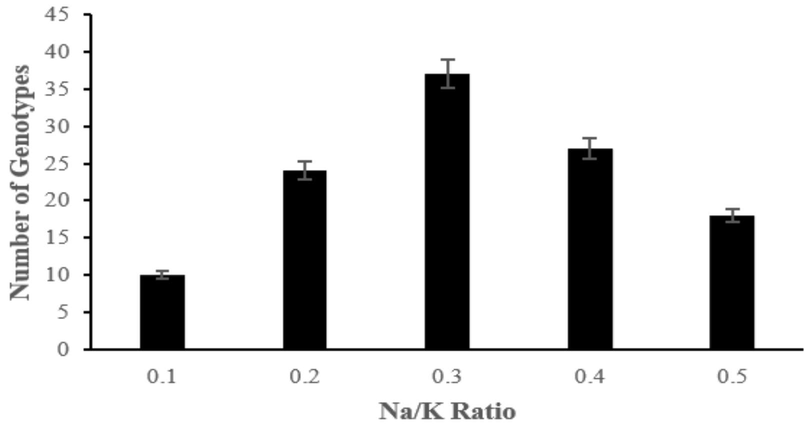

Figure 5. Na/K ratio of 116 Asian rice varieties under salinity stress conditions.

The cell membrane stability of the rice plants under salinity stress was influenced by osmotic adjustment. With the increase in Na^+ concentration in the cells, the water potential inside of the cells is reduced, which ultimately affects the cell membrane stability. Salt-sensitive rice varieties like IR29 from the Philippines, IR58 also from the Philippines, Daegudo from Republic of Korea, and Guweoldo also from Republic of Korea accumulated high concentrations of Na^+ which cause toxicity and cell damage (Figure 6).

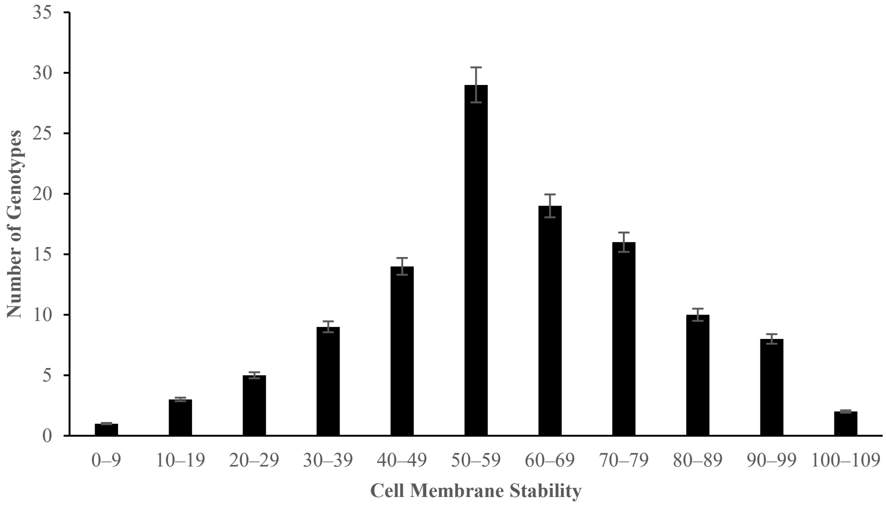

Figure 6. Effect of salinity stress conditions on cell membrane stability of 116 Asian rice varieties.

In order to maintain the cellular osmotic balance under saline conditions, rice plant cells accumulate compatible solutes or metabolites such as proline, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and sugar. All of the 116 Asian rice cultivars showed fluctuating levels of compatible solutes under salinity stress conditions (Figure 7). The salt-tolerant rice varieties such as Pokkali from India, TCCP 266 from the Philippines, IR 45427 also from the Philippines, and Namyang 7 from Republic of Korea accumulated higher amounts of proline and sugar than the salt-sensitive varieties. Meanwhile, the salt-tolerant varieties accumulated lower concentrations of MDA and H_2O_2 compared to the sensitive ones.

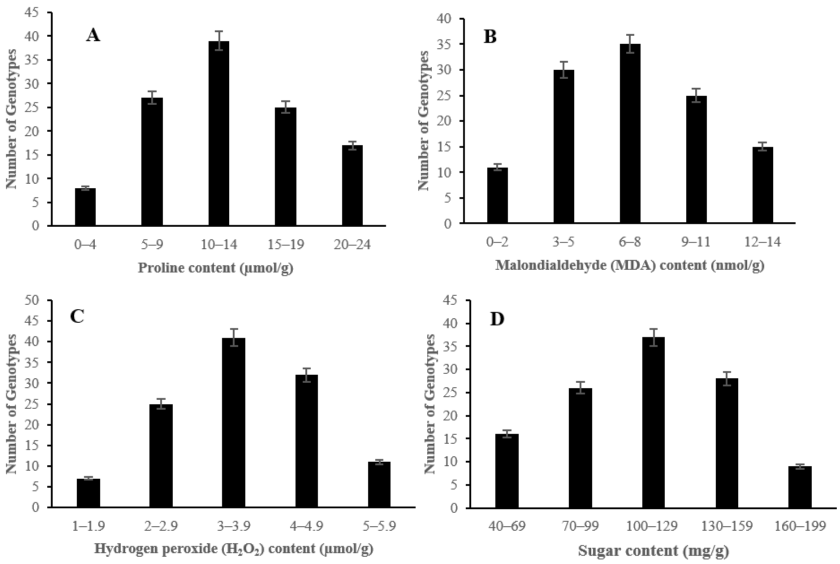

Figure 7. Accumulation of proline (**A**), malondialdehyde (MDA) (**B**), hydrogen peroxide (H_2O_2) (**C**), and sugar (**D**) in 116 Asian rice varieties under salinity stress conditions.

2.3. Correlation among Morphological, Physiological, and Biochemical Traits under Salt Stress

The study of the correlation of all of the traits allowed us to understand the relationships among traits under salinity stress tolerance in Asian rice cultivars. Instead of considering only morphological traits such as salinity score, germination percentage, root length, root fresh weight, shoot length, plant biomass, plant height, leaf rolling, and chlorophyll content, physiological and biochemical traits, including the Na/K ratio, cell membrane stability, proline, MDA, hydrogen peroxide, and sugar could be potential parameters for assessing salinity tolerance mechanisms.

Pearson correlations were calculated among 15 traits of morphological, physiological, and biochemical parameters under salinity stress conditions at the seedling and early vegetative stages (Table 1). Based on the Pearson correlations, there are 20 correlation pairs that reached significant levels under the 200 mM NaCl condition. Salt-tolerant rice cultivars showed higher germination percentage, root length, root fresh weight, shoot length, plant biomass, plant height, and chlorophyll content (r = 0.87 *, r = 0.91 *, r = 0.93 *, r = 0.92 *, r = 0.85 *, r = 0.89 *, and r = 87 *, respectively). Meanwhile, salt-sensitive rice cultivars with higher salinity scores had higher leaf rolling, Na/K ratio, and H_2O_2 content (r = 0.95 *, r = 0.92 *, r = 0.91 *, and r = 0.91 *, respectively) under salinity stress conditions. Meanwhile, salt-tolerant rice cultivars had high proline (r = 90 *) and sugar contents (0.91 *). High root length, root fresh weight, and shoot length were associated with a high plant biomass (r = 0.87 *, r = 0.91 *, and r = 0.92 *, respectively). Asian rice cultivars with a high Na/K ratio under salinity stress conditions were associated with high concentrations of MDA and H_2O_2 (r = 0.97 * and r = 0.96, respectively). * Significant at $p < 0.05$.

Table 1. Correlation coefficients among morphological, physiological, and biochemical parameters from 116 Asian rice cultivars under 200 mM NaCl at seedling and early vegetative stages.

	SS	PG	RL	RFW	SL	PB	PH	LR	CC	CMS	Na/K	Proline	MDA	H_2O_2	Sugar
SS	1														
PG	−0.87 *	1													
RL	−0.91 *	0.61	1												
RFW	−0.93 *	0.57	0.89 *	1											
SL	−0.92 *	0.68	0.83	0.75	1										
PB	−0.85 *	0.73	0.87 *	0.91 *	0.92 *	1									
PH	−0.89 *	0.67	0.88 *	0.68	0.79	0.92 *	1								
LR	0.95 *	0.53	0.51	0.42	0.39	0.41	0.37	1							
CC	−0.87 *	0.56	−0.73	0.83	0.74	0.86	0.79	−0.59	1						
CMS	−0.14	0.51	0.81	0.69	0.51	0.62	0.84	0.68	0.36	1					
Na/K	0.92 *	−0.47	−0.61	−0.47	−0.68	−0.57	−0.62	0.55	−0.27	−0.35	1				
Proline	−0.90 *	−0.67	−0.58	−0.53	−0.53	−0.41	−0.68	0.61	−0.22	−0.29	−0.47	1			
MDA	0.57	−0.61	−0.51	−0.42	−0.41	−0.47	−0.79	0.69	−0.22	−0.41	0.97 *	−0.39	1		
H_2O_2	0.91 *	−0.73	−0.62	−0.39	−0.36	−0.58	−0.61	0.54	−0.27	−0.32	0.96 *	0.42	0.85	1	
Sugar	−0.91 *	−0.64	−0.64	−0.54	−0.31	−0.64	−0.57	0.62	−3.48	−0.37	−0.51	0.38	0.41	0.25	1

* Significant at $p < 0.05$. SS: salinity score; PG: germination percentage; RL: root length; RFW: root fresh weight; SL: shoot length; PB: plant biomass; PH: plant height; LR: leaf rolling; CC: chlorophyll content; CMS: cell membrane stability; Na/K: Na^+/K^+ ratio; Proline: proline content; MDA: malondialdehyde content; H_2O_2: hydrogen peroxide content; Sugar: sugar content.

3. Discussion

Seedlings of 116 Asian rice cultivars grown under salinity stress conditions exhibited different visual symptoms of salt injury. Saline conditions decreased the germination percentage of Asian rice cultivars due to the osmotic and ionic stresses causing insufficient water absorption, leading to toxic effects on the seed embryo. Based on the interaction between saline conditions and rice cultivars, among the Asian rice cultivars, there is a wide difference in response to salinity stress conditions. Salt-tolerant seedlings were distinguished from salt-sensitive seedlings under salinity stress conditions. Our results are consistent with previous studies which mentioned that different genotypes of barley, cabbage, and *Suaeda* species showed different responses to saline conditions with regard to germination percentage [52–55]. Saline conditions decreased the growth of the radicle and plumule. The retardation of the radicle and plumule length was because of the reduction in cell turgor.

Visual salt injury begins with a reduction in effective leaf area. Salt-sensitive rice cultivars had high scores for leaf rolling, which led to a decrease in photosynthetic activity. The distribution for root length, root fresh weight, shoot length, and plant biomass across 116 Asian rice cultivars under salinity stress conditions showed wide fluctuations. Root length and shoot length were shorter in saline conditions compared to the normal condition. The root fresh weight and plant biomass of salt-susceptible cultivars showed a higher percent reduction than salt-tolerant cultivars. Lower percent reductions in root length, root fresh weight, shoot length, and plant biomass were recorded in Pokkali from India, followed by TCCP 266 from the Philippines, IR 45427 also from the Philippines, and Namyang 7 from Republic of Korea. On the other hand, higher percent reductions in root length, root fresh weight, shoot length, and plant biomass were exhibited by IR29 from the Philippines, IR58 also from the Philippines, Daegudo from Republic of Korea, and Guweoldo also from Republic of Korea. Salt-tolerant rice cultivars displayed less growth reduction than salt-sensitive cultivars under salinity stress conditions.

The cell membrane stability of 116 Asian rice cultivars was affected by salinity stress conditions. In the salt-sensitive rice cultivars, the cell membrane structure was damaged by salt, which increased the membrane permeability and destroyed the plasma membrane; as a result, the plant growth was reduced [56]. In order to maintain the Na/K balance in the shoot, plants absorb more K and exclude the toxic Na [34,57]. In this study, salt-tolerant rice cultivars showed the ability to absorb more K than Na in order to maintain the Na/K balance in the shoot. According to Ponnamperuma [58], the K concentration in the shoot has a positive correlation with salinity tolerance because K is important in stomatal functions. Gregorio and Senadhira [34] also reported that salt-tolerant rice cultivars had a higher K concentration and lower Na content in the shoot. This Na/K balance and the maintenance of a low Na/K ratio are part of the salt tolerance mechanisms and could be promising criteria for the selection of salt-tolerant varieties [59,60].

The variation in chlorophyll content in the 116 Asian rice varieties studied can be used as a potential salinity stress indicator because chlorophyll content is reduced in salt-sensitive rice varieties under salinity stress conditions. The salt-sensitive rice varieties, including IR29 from the Philippines, IR58 also from the Philippines, Daegudo from Republic of Korea, and Guweoldo also from Republic of Korea, showed lower chlorophyll content compared to the salt-tolerant varieties. Salinity stress conditions subject chloroplasts to oxidative stress that reduces the size and number of chloroplasts in the leaves by inhibiting chloroplast synthesis [61–63]. The activity of the chlorophyllase enzyme, which degrades chlorophyll, increases under salinity stress conditions, leading to a decrease in photosynthetic activity [14].

Salt-tolerant rice varieties accumulated high levels of proline under salinity stress conditions [64]. In this study, salt-tolerant varieties such as Pokkali from India followed by TCCP 266 from the Philippines, IR 45427 also from the Philippines, and Namyang 7 from Republic of Korea also showed higher proline concentrations compared to the salt-sensitive varieties. These results were consistent with those of Ghosh et al. [65], who found that salt-tolerant rice cultivars like Nonabokra and Pokkali under salinity stress conditions displayed a high proline concentration in the seedling stage. Under salinity stress conditions, proline plays an important role in protecting proteins against denaturation by regulating redox potential and acts as a source of nitrogen and carbon for recovery after salt stress in rice plants [66,67].

Under salinity stress conditions, malondialdehyde (MDA) increased in the salt-sensitive rice cultivars such as IR29 from the Philippines, IR58 also from the Philippines, Daegudo from Republic of Korea, and Guweoldo also from Republic of Korea. MDA represents biological membrane damage because MDA is the primary product of the decomposition process from unsaturated fatty acids due to oxidative stress under 200 mM NaCl [67–72]. The amount of hydrogen peroxide (H_2O_2) showed a wide variation in the 116 Asian rice cultivars studied under salinity stress conditions. Salt-tolerant rice varieties like Pokkali from India followed by TCCP 266 from the Philippines, IR 45427 also from the Philippines, and Namyang 7 from Republic of Korea exhibited lower H_2O_2 levels than the salt-sensitive ones. The sugar content of salt-tolerant rice varieties showed a significant increase under salinity stress conditions. These results are consistent with a previous study by Chang et al. [73] that found many sugars like raffinose, glucose, fructose, sucrose, galactose, mannose, ribose, xylose, melibiose, galactitol, mannitol, rhamnose, ribose, and erythritol increased in the leaves of salt-tolerant rice cultivars such as Fatmawati and Dendang under salinity stress conditions. Under salinity stress conditions, sugars are accumulated to avoid osmotic stress [74,75].

At the seedling and early vegetative stages, salt-tolerant rice varieties showed higher values for morphological characteristics such as germination percentage, root length, root fresh weight, shoot length, plant biomass, plant height, and chlorophyll content and also higher values for physiological and biochemical traits like cell membrane stability, proline, and sugar content. Meanwhile, salt-sensitive ones had high values for leaf rolling, Na/K ratio, H_2O_2, and MDA content. These results were supported by previous studies such as those of Peng et al. [76], Zhang et al. [77], Dwiningsih et al. [78], and Bhowmik et al. [9]. Many studies indicated that all of the growth stages in rice plants under salinity stress conditions showed different responses to salt [79]. The most sensitive stage to salt is fertilization and flowering, followed by the early vegetative stage, germination, and maturity [80,81]. The tolerance responses of rice cultivars to the salinity stress conditions may become the foundation of breeding tolerant rice cultivars in saline agricultural areas.

Since genomic locations that control the abiotic stress tolerance are pleiotropic, the potential candidate genes correlated with salt tolerance also were shown to be associated with heat [82], drought [83], and flooding stresses. Some of the genes that are responsible for salt tolerance were directly linked to heat-responsive pathways, such as germination and seedling development (glycosyl-hydrolase family), the abscisic acid (ABA) signaling pathway (histone-like transcription factors NFYB) [84], and cell wall stability (GAE6) [82]. Salt tolerance also showed a correlation with ABA stress response (*Asr)* genes [84], ERECTA-mediated drought tolerance [85], and DREB-based ABA-independent drought tolerance response [86]. Each gene that was correlated with salt tolerance was associated with a genotype × environment association, showing that allelic diversity might allow each Asian rice genotype to have diverse adaptation abilities under a salt stress environment [87].

Genome-wide diversity in Asian rice cultivars showed a high genetic variability that allowed them to adapt to various stress conditions, including salinity stress environments. The adaptation of Asian rice cultivars under salt stress conditions is observed in morphological, physiological, and biochemical properties, such as germination percentage; root length; root fresh weight; shoot length; plant biomass; leaf rolling; chlorophyll content; Na/K ratio; cell membrane stability; and proline, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and sugar contents. These genome–environment associations are supported by Cortes and Blair [88].

Each gene that correlated with salt tolerance was associated with a genotype × environment association, showing that allelic diversity might allow each Asian rice genotype to have diverse adaptation abilities under a salt stress environment [87]. Genome-wide association, genomic prediction, machine learning, big data approaches, and gene editing techniques (such as CRISPR-Cas9) might assist the identification and functional analysis of genes associated with salt tolerance in order to speed up rice adaptability under saline conditions [89–91]. In order to validate the salt stress mechanisms in Asian rice cultivars, a combination of several abiotic stress conditions, such as drought, heat, and flooding, needs to be completed. And also, more Asian rice cultivars, about 500 cultivars, need to be involved.

4. Materials and Methods

4.1. Plant Materials

A total of 116 rice genotypes originally from Asian countries (Table 2) were screened for salinity tolerance at the seedling stage and early vegetative stage (3-leaf stage). Seeds were sterilized with 10% (*v*/*v*) NaClO for 15 min and washed with distilled water. Pokkali was used as the salt-tolerant standard check, and IR29 was used as the salt-sensitive check [92].

Table 2. Rice varieties for salt stress tolerance screening.

	Rice Variety	Country	Sub-Population *
1	Karang Serang	Indonesia	TRJ
2	Rojolele	Indonesia	TRJ
3	Cempo Ireng	Indonesia	TRJ
4	Ciherang	Indonesia	IND
5	Mayang Khang	Indonesia	IND
6	Sipirasikkam	Indonesia	TRJ
7	Mitak	Indonesia	TRJ
8	Dara	Indonesia	AUS
9	B805D-Mr-16-8-3	Indonesia	IND
10	Tia Bura	Indonesia	TRJ
11	C 5560	Thailand	TEJ/TRJ
12	Nam Dawk Mai	Thailand	IND
13	Bkn 6987-68-14	Thailand	IND
14	Td 70	Thailand	IND
15	Cntlr80076-44-1-1-1	Thailand	IND
16	Nahng Sawn	Thailand	IND
17	Quinimpol	Philippines	TRJ
18	TCCP 266	Philippines	IND
19	IR 4482-5-3-9-5	Philippines	IND
20	IR 45427	Philippines	IND
21	IR 9660-48-1-1-2	Philippines	IND
22	IR 238	Philippines	IND
23	IR 2061-214-2-3	Philippines	IND
24	IR 2462	Philippines	IND
25	IR 58614-B-B-8-2	Philippines	IND
26	Pakkali	Philippines	ARO
27	IR64	Philippines	IND
28	IR58	Philippines	IND
29	IR29	Philippines	IND
30	Taichu Mochi 59	Taiwan	TRJ
31	Ai Chueh Ta Pai Ku	Taiwan	IND
32	Ragasu	Taiwan	TEJ/TRJ
33	Tobura	Taiwan	TEJ/TRJ
34	Kao Chio Lin Chou	Taiwan	IND
35	Taino 38	Taiwan	IND/AUS

Table 2. *Cont.*

	Rice Variety	Country	Sub-Population *
36	Nanton No. 131	Taiwan	TRJ/(admix)
37	Hsin Hsing Pai Ku	Taiwan	IND
38	Tainung 45	Taiwan	IND
39	Ao Chiu 2 Hao	China	IND
40	Chun 118-33	China	IND
41	Kin Shan Zim	China	IND
42	Pan Ju	China	IND
43	Kechengnuo No. 4	China	IND
44	4484	China	IND
45	4595	China	IND
46	You-I B	China	IND
47	Chunjiangzao No. 1	China	TEJ
48	Shimizu Mochi	Japan	TEJ
49	Norin 11	Japan	TEJ
50	Tamanishiki	Japan	TEJ
51	Niwahutaw Mochi	Japan	TEJ
52	Somewake	Japan	TEJ
53	A 5	Japan	TEJ
54	C.B. Ii	Japan	AUS
55	Fujisaka 5	Japan	IND
56	Nipponbare	Japan	TEJ
57	Khao Phoi	Laos	TEJ/TRJ
58	Khao Luang	Laos	TRJ/(admix)
59	Padi Pohon Batu	Malaysia	TRJ
60	Acheh	Malaysia	IND
61	Mahsuri	Malaysia	IND
62	Padi Tarab Arab	Malaysia	TRJ
63	Nc 1/536	Pakistan	AUS
64	Red	Pakistan	AUS/(Admix)
65	Santhi 990	Pakistan	IND/AUS
66	Daudzai Field Mix	Pakistan	AUS
67	Jp 5	Pakistan	IND/AUS
68	Won Son Zo No. 11	Republic of Korea	IND
69	Daegudo	Republic of Korea	TEJ
70	Guweoldo	Republic of Korea	TEJ
71	Namyang 7	Republic of Korea	TEJ
72	Yong Chal Byo	Republic of Korea	TEJ
73	Thang 10	Vietnam	IND
74	Cm1_ Haipong	Vietnam	IND
75	Nang Bang Bentre	Vietnam	AUS
76	Lua Chua Chan	Vietnam	TRJ

Table 2. *Cont.*

	Rice Variety	Country	Sub-Population *
77	Soc Nau	Vietnam	IND
78	Heo Trang	Vietnam	IND
79	Pd 46	Sri Lanka	IND
80	Bakiella 1	Sri Lanka	IND
81	Gallawa	Sri Lanka	AUS
82	Ittikulama	Sri Lanka	AUS
83	Karayal	Sri Lanka	AUS
84	Amane	Sri Lanka	IND
85	Thavalu	Sri Lanka	AUS
86	Patnai 6	Myanmar	AUS
87	Buphopa	Myanmar	TEJ/TRJ
88	Kaukkyi Ani	Myanmar	TRJ
89	A100943-R	Myanmar	AUS
90	Nsgc 5953	Myanmar	IND
91	A 36-3	Myanmar	IND
92	Jumli Dhan	Nepal	TEJ/TRJ
93	N-2703	Nepal	AUS
94	Bhim Dhan	Nepal	TEJ/TRJ
95	Juppa	Nepal	IND
96	Tauli	Nepal	AUS
97	Darmali	Nepal	TEJ/ARO
98	Dhan	Nepal	IND
99	Tchampa	Iran	AUS
100	Phudugey	Bhutan	AUS
101	Jyanak	Bhutan	TEJ/TRJ
102	Wir 3039	Tajikistan	TEJ
103	Ak Tokhum	Azerbaijan	ARO
104	Gasym Hany	Azerbaijan	ARO
105	Celiaj	Azerbaijan	TEJ
106	Shimla Early	Iraq	IND/AUS
107	A 152	Bangladesh	IND/TRJ
108	Dnj 179	Bangladesh	AUS
109	Dj 24	Bangladesh	AUS
110	Dj 102	Bangladesh	AUS
111	Dnj 121	Bangladesh	AUS
112	Aswina 330	Bangladesh	AUS
113	Tranoeup Beykher	Cambodia	IND
114	Srav Prapay	Cambodia	IND
115	Simpor	Brunei	TRJ
116	Pokkali	India	IND

* IND = indica; TEJ = temperate japonica; TRJ = tropical japonica; ARO = aromatic; AUS = aus.

4.2. Salinity Screening at Seedling and Early Vegetative Stage

The concentration of salt (NaCl) used in this experiment was 200 mM. Ten seeds of each rice genotype were germinated in Petri dishes containing blotting paper and were treated with 200 mM NaCl and kept in an incubator at 30 °C. For the control condition, seeds were germinated with distilled water. After 12 days of salt treatment, germinated seedlings were transferred to a hydroponic system containing Yoshida's medium [93] with 200 mM NaCl. Rice plants were grown in a growth chamber at a temperature of 28 °C/24 °C for day/night with 65% humidity and a light intensity of 500 $\mu Em^{-2}s^{-1}$ until the early vegetative stage (3-leaf stage). Morphological, physiological, and biochemical responses, including root length, root fresh weight, shoot length, plant biomass, leaf rolling, chlorophyll content, Na^+/K^+ ratio, cell membrane stability, proline content, malondialdehyde (MDA) content, hydrogen peroxide (H_2O_2) content, and sugar content, were measured at early vegetative stage (3-leaf stage).

4.3. Measurement of Morphological, Physiological, and Biochemical Traits

4.3.1. Determination of Germination Percentage and Standard Evaluation Score for Salt Injury

The indicator for the germination of seeds was when the radicle had protruded through the seed coat, the hypocotyl was extended, and the cotyledon was unfolded. The germination percentage and visual symptoms of salt injury at the seedling stage were evaluated based on the standard evaluation score at 12 days of salt treatment (Table 3) [5].

Table 3. Standard evaluation score (SES) of visual salt injury at seedling stage [5].

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

4.3.2. Measurement of Root Length, Root Fresh Weight, Shoot Length, and Plant Biomass

Root length was measured as the maximum length of the root for each rice plant at the early vegetative stage (3-leaf stage). The whole weight of the roots of each rice plant was determined as the root fresh weight. Shoot length was measured from the ground surface to the tallest leaf tip with a ruler. The roots and shoot of each rice plant were weighed fresh and determined as plant biomass.

4.3.3. Determination of Leaf Rolling

The leaf rolling score on the three leaves of each plant was identified at the early vegetative stage (3-leaf stage) based on the standard evaluation system for rice [5]. The range of the score is from 1 to 5, with 1 indicating unrolled and fully turgid leaves, 2 indicating leaves are folded (deep-V-shaped), 3 indicating leaves are fully cupped (U-shaped), 4 indicating leaf margins touching (O-shaped), and 5 indicating completely rolled leaves.

4.3.4. Analysis of Chlorophyll Content

The chlorophyll content of the fully expanded leaves on the top of each plant was measured by using a Soil and Plant Analyzer Development (SPAD)-502 Plus Chlorophyll Meter (Spectrum Technologies, Aurora, IL, USA). Each leaf was inserted into the sample slot of the SPAD in such a way as to avoid the midrib, and five readings were measured for each leaf.

4.3.5. Measurement of Na/K Ratio

The ratio of sodium (Na) and potassium (K) concentrations in the root and shoot was measured for each rice genotype grown under a salinity condition of 200 mM at the early vegetative stage (3-leaf stage). Each rice plant was rinsed with distilled water and then dried at 65 °C for 2 days. A dried tissue sample for each rice genotype was ground using mortar and pestle. A total of 100 mg of a ground sample was digested in 3 mL of hydrogen peroxide and 5 mL of nitric acid for 3 h at 152–155 °C. Then, the digested sample was diluted to a final volume of 12.5 mL. The concentrations of Na and K were quantified by using a flame photometer (ANA-135, Tokyokoden, Tokyo, Japan). The estimated concentrations of Na and K were calculated based on a standard curve [14].

4.3.6. Measurement of Cell Membrane Stability

The leaf cell membrane stability (CMS) of each rice genotype under saline conditions was determined by using the following equation:

$$\text{CMS (\%)} = 1 - (\text{E1}/\text{E2}) \times 100$$

Leaf samples were washed with distilled water and then placed in 10 mL of deionized water at 10 °C for 18 h. Next, the samples were heated at 52 °C for 1 h in a water bath. In order to diffuse the electrolytes from the leaf tissue to aqueous media, the samples were incubated at 10 °C for 24 h. The samples were shaken, and the initial conductance (E1) was measured for each sample. All of the samples were then autoclaved at 121 °C and 0.10 MPa for 15 min in order to kill the plant tissue and release the electrolytes. The samples were placed in an incubator at 25 °C for cooling down; then, the samples were shaken, and the final conductance (E2) was measured [14].

4.3.7. Measurement of Proline Content

A total of 0.5 g of a fresh leaf sample of each rice genotype was diluted in 10 mL of 3% aqueous sulfosalicylic acid and centrifuged for 1 min at 3000 rpm. Then, 2 mL of the supernatant was reacted with 2 mL of glacial acetic acid and 2 mL of ninhydrin acid at 100 °C for 1 h. Exactly 2 mL of toluene was used to extract the chromophore. The absorbance of the chromophore was measured at 520 nm by using a Genesys 10-s UV/Vis Spectrophotometer (Thermo Spectronic, Waltham, MA, USA) with toluene as the blank. A standard curve for proline content was quantified by using purified proline (Sigma Aldrich, Melbourne, VIC, Australia) [14]. The proline content of each rice genotype was calculated by using the following formula:

$$((\mu\text{g proline/mL} \times \text{mL toluene})/115.5\ \mu\text{g}/\mu\text{mole}) \times (\text{g sample}/5) = \mu\text{moles proline gram FW} - 1$$

4.3.8. Measurement of Malondialdehyde (MDA) Content

Exactly 0.5 g of rice leaf from each rice genotype was cut and homogenized with 1.5 mL of a 0.5% (*w*/*v*) thiobarbituric acid solution consisting of 20% (*w*/*v*) trichloroacetic acid and 1.5 mL of distilled water. The solution was heated for 25 min at 95 °C, and then the reaction was stopped by placing the samples on ice. Next, the solution was centrifuged, and the absorbance of the supernatant was measured at 532 and 600 nm. The extinction coefficient of mM^{-1} cm^{-1} was used to calculate the MDA content and expressed as nanomoles per gram (nmol/g) of fresh weight [14].

4.3.9. Measurement of Hydrogen Peroxide (H_2O_2) Content

About 0.1 g of leaf from each rice genotype was diluted in 3 mL of 5% (*w*/*v*) trichloroacetic acid and incubated for 3 h at 4 °C. Next, 1 mL of FOX reagent was added to 0.2 mL of the supernatant of the sample and then mixed and incubated for 15 min at 25 °C. The absorbance of the solution was read at 560 nm. The H_2O_2 content was expressed as micromoles per gram (µmol/g) of fresh weight [14].

4.3.10. Measurement of Sugar Content

A leaf sample from each rice genotype was ground, and 1.0 g of the ground sample was added to 1 mL of distilled water. Then, 1 mL of anthrone reagent was added to the suspension and incubated for 8 min at 25 °C. The absorbance of the solution was read at 630 nm. The content of soluble sugar in each rice genotype was calculated by using a standard graph and expressed in milligrams per gram (mg/g) of fresh weight [14].

4.4. Statistical Analysis

The experiment of salinity screening was conducted in a randomized complete block design (RCBD) with five replications. The salinity treatment and control conditions were compared using the least significant difference (LSD) test at a 0.05 probability level.

5. Conclusions

The combination of morphological, physiological, and biochemical assessments for screening the salt tolerance of 116 Asian rice cultivars enabled the classification of these cultivars into tolerant, moderate, and sensitive rice cultivars under salinity stress conditions and the understanding of salt tolerance mechanisms. The rice cultivars classified as salt-tolerant included Pokkali from India, followed by TCCP 266 from the Philippines, IR 45427 also from the Philippines, and Namyang 7 from Republic of Korea. Salt-sensitive rice varieties included IR29 from the Philippines, IR58 also from the Philippines, Daegudo from Republic of Korea, and Guweoldo also from Republic of Korea. The identified salt-tolerant rice varieties may provide a valuable germplasm resource in breeding programs for developing salt-tolerant rice.

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References

1. Gopalan, C.; Rama Sastri, B.V.; Balasubramanian, S. Nutritive Value of Indian Foods. In *National Institute of Nutrition (NIN)*; ICMR: New Delhi, India, 2007.
2. Khush, G.S.; Virk, P.S. Rice Breeding: Achievements and Future Strategies. *Crop Improv.* **2000**, *27*, 115–144.
3. GRiSP. *Rice Almanac*, 4th ed.; International Rice Research Institute: Los Banos, Philippines, 2013.
4. ANU. Technological Transformation of Productivity, Profitability and Sustainability: Rice. In *The First Ten K R Narayanan Orations: Essays by Eminent Persons on the Rapidly Transforming Indian Economy*; Australia South Asia Research Centre the Australian National University: Canberra, Australia, 2006; Available online: http://epress.anu.edu.au/narayanan/mobile_devices/pr01.html (accessed on 11 February 2023).
5. Gregorio, G.B. Tagging Salinity Tolerant Genes in Rice Using Amplified Fragment Length Polymorphism (AFLP). Ph.D. Thesis, University of the Philippines Los Baños College, Los Baños, Philippines, 1997.
6. Abdullah, Z.; Khan, M.A.; Flowers, T.J. Causes of Sterility in Seed Set of Rice under Salinity Stress. *J. Agron. Crop Sci.* **2001**, *187*, 25–32. [CrossRef]
7. Dwiningsih, Y. *Molecular Genetic Analysis of Drought Resistance and Productivity Traits of Rice Genotypes*; University of Arkansas: Fayetteville, NC, USA, 2020.
8. Aref, F.; Rad, H.E. Physiological Characterization of Rice under Salinity Stress during Vegetative and Reproductive Stages. *Indian J. Sci. Technol.* **2012**, *5*, 2578–2586. [CrossRef]
9. Bhowmik, S.K.; Titov, S.; Islam, M.M.; Siddika, A.; Sultana, S.; Haque, M. Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance. *Afr. J. Biotechnol.* **2009**, *8*, 6490–6494.

10. Bado, S.; Forster, B.P.; Ghanim, A.; Jankowicz-Cieslak, J.; Berthold, G.; Luxiang, L. *Protocols for Pre-Field Screening of Mutants for Salt Tolerance in Rice, Wheat and Barley*; Springer: Berlin/Heidelberg, Germany, 2016. [CrossRef]
11. Ueda, A.; Yahagi, H.; Fujikawa, Y.; Nagaoka, T.; Esaka, M.; Calcaño, M.; González, M.; Martich, J.; Saneoka, H. Comparative physiological analysis of salinity tolerance in rice. *Soil Sci. Plant Nutr.* **2013**, *59*, 896–903. [CrossRef]
12. Ge, X.; Khan, Z.I.; Chen, F.; Akhtar, M.; Ahmad, K.; Ejaz, A.; Ashraf, M.A.; Nadeem, M.; Akhtar, S.; Alkahtani, J.; et al. A study on the contamination assessment, health risk and mobility of two heavy metals in the soil-plants-ruminants system of a typical agricultural region in the semi-arid environment. *Environ. Sci. Pollut. Res.* **2022**, *29*, 14584–14594. [CrossRef] [PubMed]
13. Lee, K.S.; Choi, W.Y.; Ko, J.C.; Kim, T.S.; Gregorio, G.B. Salinity tolerance of japonica and indica rice (*Oryza sativa* L.) at the seedling stage. *Planta* **2003**, *216*, 1043–1046. [CrossRef] [PubMed]
14. Kordrostami, M.; Rabiei, B.; Kumleh, H. Biochemical, physiological and molecular evaluation of rice cultivars differing in salt tolerance at the seedling stage. *Physiol. Mol. Biol. Plants* **2017**, *23*, 529–544. [CrossRef]
15. Maqsood, A.; Khan, Z.I.; Ahmad, K.; Akhtar, S.; Ashfaq, A.; Malik, I.S.; Sultana, R.; Nadeem, M.; Alkahtani, J.; Dwiningsih, Y.; et al. Quantitative evaluation of zinc metal in meadows and ruminants for health assessment: Implications for humans. *Environ. Sci. Pollut. Res.* **2022**, *29*, 21634–21641. [CrossRef]
16. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681. [CrossRef] [PubMed]
17. Hoang, T.M.L.; Williams, B.; Khanna, H.; Dale, J.; Mundree, S.G. Physiological basis of salt stress tolerance in rice expressing the antiapoptotic gene SfIAP. *Funct. Plant Biol.* **2014**, *41*, 1168–1177. [CrossRef]
18. Marschner, H. *Mineral Nutrition of Higher Plants*; Academic Press: London, UK, 1995.
19. Hasegawa, P.M.; Bressan, R.A.; Zhu, J.K.; Bohnert, H.J. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2000**, *51*, 463–499. [CrossRef] [PubMed]
20. Sahi, C.; Singh, A.; Kumar, K. Salt stress response in rice: Genetics, molecular biology and comparative genomics. *Funct. Integr. Genom.* **2006**, *6*, 263–284. [CrossRef] [PubMed]
21. Kavitha, P.G.; Miller, A.J.; Mathew, M.K.; Maathuis, F.J.M. Rice cultivars with differing salt tolerance contain similar cation channels in their root cells. *J. Exp. Bot.* **2012**, *63*, 3289–3296. [CrossRef]
22. Hoang, T.; Tran, T.; Nguyen, T.; Williams, B.; Wurm, P.; Bellairs, S.; Mundree, S. Improvement of Salinity Stress Tolerance in Rice: Challenges and Opportunities. *Agronomy* **2016**, *6*, 54. [CrossRef]
23. Sitrarasi, R.; Nallal, U.M.; Razia, M.; Chung, W.J.; Shim, J.; Chandrasekaran, M.; Dwiningsih, Y.; Rasheed, R.A.; Alkahtani, J.; Elshikh, M.S.; et al. Inhibition of multi-drug resistant microbial pathogens using an ecofriendly root extract of *Furcraea foetida* silver nanoparticles. *J. King Saud Univ.-Sci.* **2022**, *34*, 101794. [CrossRef]
24. De Leon, T.B.; Linscombe, S.; Gregorio, G.; Subudhi, P.K. Genetic variation in Southern USA rice genotypes for seedling salinity tolerance. *Front. Plant Sci.* **2015**, *6*, 374. [CrossRef] [PubMed]
25. Mohammadi-Nejad, G.; Singh, R.K.; Arzanic, A.; Rezaiec, A.M.; Sabourid, H.; Gregorio, G.B. Evaluation of Salinity Tolerance in Rice Genotypes. *Int. J. Plant Prod.* **2010**, *4*, 199–207.
26. Morales, S.G.; Tellez, L.I.T.; Merino, F.C.G.; Caldana, C.; Victoria, D.E.; Cabrera, B.E.H. Growth, Photosynthetic Activity, and Potassium and Sodium Concentration in Rice Plants under Salt Stress. *Acta Sci. Agron.* **2012**, *34*, 317–324. [CrossRef]
27. Asch, F.; Dingkuhn, M.; Dorffling, K.; Miezan, K. Leaf K/Na ratio predicts salinity induced yield loss in irrigated rice. *Euphytica* **2000**, *113*, 109–118. [CrossRef]
28. Yeo, A.R.; Flowers, T.J. Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. *Aust. J. Plant Physiol.* **1986**, *13*, 161–173. [CrossRef]
29. Munns, R.; James, R.A.; Lauchli, A. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* **2006**, *57*, 1025–1043. [CrossRef]
30. Dwiningsih, Y.; Alkahtani, J. Genetics, Biochemistry and Biophysical Analysis of Anthocyanin in Rice (*Oryza sativa* L.). *Adv. Sustain. Sci. Eng. Technol.* **2022**, *4*, 1. [CrossRef]
31. Ferreira, L.J.; Azevedo, V.; Maroco, J.; Oliveira, M.M.; Santos, A.P. Salt Tolerant and Sensitive Rice Varieties Display Differential Methylome Flexibility under Salt Stress. *PLoS ONE* **2015**, *10*, e0124060. [CrossRef] [PubMed]
32. Flowers, T.J. Improving crop salt tolerance. *J. Exp. Bot.* **2004**, *55*, 307–319. [CrossRef] [PubMed]
33. Koyama, M.L.; Levesley, A.; Koebner, R.; Flowers, T.J.; Yeo, A.R. Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiol.* **2001**, *125*, 406–422. [CrossRef]
34. Gregorio, G.B.; Senadhira, D. Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **1993**, *86*, 333–338. [CrossRef]
35. Bashir, S.; Gulshan, A.B.; Iqbal, J.; Husain, A.; Alwahibi, M.S.; Alkahtani, J.; Dwiningsih, Y.; Bakhsh, A.; Ahmed, N.; Khan, M.J.; et al. Comparative role of animal manure and vegetable waste induced compost for polluted soil restoration and maize growth. *Saudi J. Biol. Sci.* **2021**, *28*, 2534–2539. [CrossRef]
36. Ali, M.; Yeasmin, L.; Gantait, S.; Goswami, R.; Chakraborty, S. Screening of rice landraces for salinity tolerance at seedling stage through morphological and molecular markers. *Physiol. Mol. Biol. Plants* **2014**, *20*, 411–423. [CrossRef] [PubMed]
37. Flowers, T.J.; Yeo, A.R. Breeding for salinity resistance in crop plants: Where next? *Aust. J. Plant Physiol.* **1995**, *22*, 875–884. [CrossRef]

38. Ali, M.H.; Khan, M.I.; Bashir, S.; Azam, M.; Naveed, M.; Qadri, R.; Bashir, S.; Mehmood, F.; Shoukat, M.A.; Li, Y.; et al. Biochar and *Bacillus* sp. MN54 Assisted Phytoremediation of Diesel and Plant Growth Promotion of Maize in Hydrocarbons Contaminated Soil. *Agronomy* **2021**, *11*, 1795. [CrossRef]
39. Qin, H.; Li, Y.; Huang, R. Advances and Challenges in the Breeding of Salt-Tolerant Rice. *Int. J. Mol. Sci.* **2020**, *21*, 8385. [CrossRef]
40. Dwiningsih, Y.; Rahmaningsih, M.; Alkahtani, J. Development of single nucleotide polymorphism (SNP) markers in tropical crops. *Adv. Sustain. Sci. Eng. Technol.* **2020**, *2*, 2. [CrossRef]
41. Thomson, M.J.; de Ocampo, M.; Egdane, J.; Rahman, M.A.; Sajise, A.G.; Adorada, D.L.; Tumimbang-Raiz, E.; Blumwald, E.; Seraj, Z.I.; Singh, R.K.; et al. Characterizing the saltol quantitative trait locus for salinity tolerance in Rice. *Rice* **2010**, *3*, 148–160. [CrossRef]
42. Yuan, J.; Wang, X.; Zhao, Y.; Khan, N.U.; Zhao, Z.; Zhang, Y.; Wen, X.; Tang, F.; Wang, F.; Li, Z. Genetic basis and identification of candidate genes for salt tolerance in rice by GWAS. *Sci. Rep.* **2020**, *10*, 9958. [CrossRef] [PubMed]
43. Lekklar, C.; Pongpanich, M.; Suriya-arunroj, D.; Chinpongpanich, A.; Tsai, H.; Comai, L.; Chadchawan, S.; Buaboocha, T. Genome-wide association study for salinity tolerance at the flowering stage in a panel of rice accessions from Thailand. *BMC Genom.* **2019**, *20*, 76. [CrossRef] [PubMed]
44. Adil, M.; Bashir, S.; Bashir, S.; Aslam, Z.; Ahmad, N.; Younas, T.; Asghar, R.M.A.; Alkahtani, J.; Dwiningsih, Y.; Elshikh, M.S. Zinc oxide nanoparticles improved chlorophyll contents, physical parameters, and wheat yield under salt stress. *Front. Plant Sci.* **2022**, *13*, 932861. [CrossRef]
45. Liu, C.; Chen, K.; Zhao, X.Q.; Wang, X.Q.; Shen, C.C.; Zhu, Y.J.; Dai, M.L.; Qiu, X.J.; Yang, R.W.; Xing, D.Y.; et al. Identification of genes for salt tolerance and yield-related traits in rice plants grown hydroponically and under saline field conditions by genome-wide association study. *Rice* **2019**, *12*, 88. [CrossRef]
46. Mansuri, R.M.; Shobbar, Z.S.; Jelodar, N.B.; Ghaffari, M.R.; Nematzadeh, G.A.; Asari, S. Dissecting molecular mechanisms underlying salt tolerance in rice: A comparative transcriptional profiling of the contrasting genotypes. *Rice* **2019**, *12*, 13. [CrossRef]
47. Alkahtani, J.; Elshikh, M.S.; Dwiningsih, Y.; Rathi, M.A.; Sathya, R.; Vijayaraghavan, P. In-vitro antidepressant property of methanol extract of *Bacopa monnieri*. *J. King Saud Univ.-Sci.* **2022**, *34*, 102299. [CrossRef]
48. Yildirim, B.; Yaser, F.; Ozpay, T.; Ozpay, D.; Turkozu, D.; Terziodlu, O.; Tamkoc, A. Variations in response to salt stress among field pea genotypes (*Pisum sativum* sp. *arvense* L.). *J. Anim. Vet. Adv.* **2008**, *7*, 907–910.
49. Raza, S.H.; Athar, H.U.R.; Ashraf, M. Influence of exogenously applied glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot.* **2006**, *38*, 341–351.
50. Mukhtar, E.; Siddiqi, K.; Bhatti, K.H.; Nawaz, K.; Hussain, K. Gas exchange attributes can be valuable selection criteria for salinity tolerance in canola cultivars (*Brassica napus* L.). *Pak. J. Bot.* **2013**, *45*, 35–40.
51. Siddiqi, E.H.; Ashraf, M.; Hussain, M.; Jamil, A. Assessment of intercultivar variation for salt tolerance in safflower (*Carthamus tinctorius* L.) using gas exchange characteristics as selection criteria. *Pak. J. Bot.* **2009**, *41*, 2251–2259.
52. Mano, Y.; Takeda, K. Diallel analysis of salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). *Breed. Sci.* **1997**, *47*, 203–209. [CrossRef]
53. Jamil, M.; Lee, K.B.; Jung, K.Y.; Lee, D.B.; Han, M.S.; Rha, E.S. Salt stress inhibits germination and early seedling growth in cabbage (*Brassica oleracea capitata* L.). *Pak. J. Biol. Sci.* **2007**, *10*, 910–914. [CrossRef] [PubMed]
54. Guan, B.; Yu, J.; Lu, Z.; Japhet, W.; Chen, X.; Xie, W. Salt tolerance in two *Suaeda* species: Seed germination and physiological response. *Asian J. Plant Sci.* **2010**, *9*, 194–199. [CrossRef]
55. Dwiningsih, Y.; Alkahtani, J. Phenotypic Variations, Environmental Effects and Genetic Basis Analysis of Grain Elemental Concentrations in Rice (*Oryza sativa* L.) for Improving Human Nutrition. *Preprints* **2022**, 2022090263. [CrossRef]
56. Zhang, R.; Hussain, S.; Wang, Y.; Liu, Y.; Li, Q.; Chen, Y.; Wei, H.; Gao, P.; Dai, Q. Comprehensive Evaluation of Salt Tolerance in Rice (*Oryza sativa* L.) Germplasm at the Germination Stage. *Agronomy* **2021**, *11*, 1569. [CrossRef]
57. Dwiningsih, Y.; Alkahtani, J. Rojolele: A Premium Aromatic Rice Variety in Indonesia. *Preprints* **2022**, 2022100373. [CrossRef]
58. Ponnamperuma, F.N. Role of cultivar tolerance in increasing rice production in saline lands. In *Salinity Tolerance in Plants. Strategies for Crop Improvement*; Staples, R.C., Toenniessen, G.H., Eds.; Wiley-Interscience: New York, NY, USA, 1984; pp. 255–271.
59. Dwiningsih, Y.; Al-Kahtani, J. Genome-Wide Association Study of Complex Traits in Maize Detects Genomic Regions and Genes for Increasing Grain Yield and Grain Quality. *Adv. Sustain. Sci. Eng. Technol.* **2022**, *4*, 0220209. [CrossRef]
60. Ashraf, M.; Harris, P. Photosynthesis under stressful environments: An overview. *Photosynthetica* **2013**, *51*, 163–190. [CrossRef]
61. Khafagy, M.A.; Arafa, A.A.; El-Banna, M.F. Glycinebetaine and ascorbic acid can alleviate the harmful effects of NaCl salinity in sweet pepper. *Aust. J. Crop Sci.* **2009**, *3*, 257–267.
62. Santos, V.C. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Sci. Hortic.* **2004**, *130*, 93–99. [CrossRef]
63. Lone, J.; Shikari, A.; Sofi, N.; Ganie, S.; Sharma, M.; Sharma, M.; Kumar, M.; Saleem, M.H.; Almaary, K.S.; Elshikh, M.S.; et al. Screening technique based on seed and early seedling parameters for cold tolerance of selected F2-derived F3 rice genotypes under controlled conditions. *Sustainability* **2022**, *14*, 8447. [CrossRef]
64. Kishor, P.B.; Sangam, S.; Amrutha, R.N. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr. Sci.* **2005**, *88*, 424–438.

65. Ghosh, N.; Adak, M.K.; Ghosh, P.D.; Gupta, S.; Sen Gupta, D.N.; Mandal, C. Differential responses of two rice varieties to salt stress. *Plant Biotechnol. Rep.* **2011**, *5*, 89–103. [CrossRef]
66. Fariduddin, Q.; Khalil, R.R.A.E.; Mir, B.A.; Yusuf, M.; Ahmad, A. 24-Epibrassinolide regulates photosynthesis, antioxidant enzyme activities and proline content of *Cucumis sativus* under salt and/or copper stress. *Environ. Monit. Assess.* **2013**, *185*, 7845–7856. [CrossRef]
67. Saha, P.; Chatterjee, P.; Biswas, A.K. NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek). *Indian J. Exp. Biol.* **2010**, *48*, 593–600.
68. Abu-Muriefah, S.S. Effect of sitosterol on growth, metabolism and protein pattern of pepper (*Capsicum Annuum* L.) plants grown under salt stress conditions. *Int. J. Agric. Crop Sci.* **2015**, *8*, 94–106.
69. Azuma, R.; Ito, N.; Nakayama, N.; Suwa, R.; Nguyen, N.T.; Larrinaga Mayoral, J.A.; Esaka, M.; Fujiyama, H.; Saneoka, H. Fruits are more sensitive to salinity than leaves and stems in pepper plants (*Capsicum annuum* L.). *Sci. Hortic.* **2010**, *125*, 171–178. [CrossRef]
70. Stepien, P.; Klobus, G. Antioxidant defense in the leaves of C3 and C4 plants under salinity stress. *Physiol. Plantarum.* **2005**, *125*, 31–40. [CrossRef]
71. Sudhakar, C.; Lakshmi, A.; Giridarakumar, S. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci.* **2001**, *161*, 613–619. [CrossRef]
72. Meloni, D.A.; Oliva, M.A.; Martinez, C.A.; Cambraia, J. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.* **2003**, *49*, 69–76. [CrossRef]
73. Chang, J.; Cheong, B.; Natera, S.; Roessner, U. Morphological and metabolic responses to salt stress of rice (*Oryza sativa* L.) cultivars which differ in salinity tolerance. *Plant Physiol. Biochem.* **2019**, *144*, 427–435. [CrossRef] [PubMed]
74. Guo, R.; Yang, Z.Z.; Li, F.; Yan, C.R.; Zhong, X.L.; Liu, Q.; Xia, X.; Li, H.R.; Zhao, L. Comparative metabolic responses and adaptive strategies of wheat (*Triticum aestivum*) to salt and alkali stress. *BMC Plant Biol.* **2015**, *15*, 170. [CrossRef] [PubMed]
75. Ismanto, A.; Hadibarata, T.; Widada, S.; Indrayanti, E.; Ismunarti, D.; Safinatunnajah, N.; Kusumastuti, W.; Dwiningsih, Y.; Alkahtani, J. Groundwater contamination status in Malaysia: Level of heavy metal, source, health impact, and remediation technologies. *Bioprocess Biosyst. Eng.* **2022**, *46*, 467–482. [CrossRef]
76. Peng, S.; Cassman, K.G.; Virmani, S.S.; Sheehy, J.; Khush, G.S. Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. *Crop Sci.* **1999**, *39*, 1552–1559. [CrossRef]
77. Zhang, Z.H.; Li, P.; Wang, L.X.; Hu, Z.L.; Zhu, L.H.; Zhu, Y.G. Genetic dissection of the relationships of biomass production and partitioning with yield and yield related traits in rice. *Plant Sci.* **2004**, *167*, 1–8. [CrossRef]
78. Dwiningsih, Y.; Alkahtani, J. Agronomics, Genomics, Breeding and Intensive Cultivation of Ciherang Rice Variety. *Preprints* **2022**, 2022110489. [CrossRef]
79. Dwiningsih, Y.; Alkahtani, J. Potential of Pigmented Rice Variety Cempo Ireng in Rice Breeding Program for Improving Food Sustainability. *Preprints* **2023**, 2023020039. [CrossRef]
80. IRRI [International Rice Research Institute]. *Annual Report for 1967*; International Rice Research Institute: Manila, Philippines, 1967; Volume 308.
81. Pearson, G.A.; Ayers, S.D.; Eberhard, D.L. Relative salt tolerance of rice during germination and early seedling development. *Soil Sci.* **1966**, *102*, 151–156. [CrossRef]
82. López-Hernández, F.; Cortés, A.J. Last Generation Genome–Environment Associations Reveal the Genetic Basis of Heat Tolerance in Common Bean (*Phaseolus vulgaris* L.). *Front. Genet.* **2019**, *10*, 954. [CrossRef] [PubMed]
83. Cortes, A.J.; Monserrate, F.A.; Ramı'rez-Villegas, J.; Madrinan, S.; Blair, M.W. Drought Tolerance in Wild Plant Populations: The Case of Common Beans (*Phaseolus vulgaris* L.). *PLoS ONE* **2013**, *8*, e62898. [CrossRef] [PubMed]
84. Cortes, A.J.; Chavaro, M.; Madrinan, S.; This, D.; Blair, M. Molecular ecology and selection in the drought-related *Asr* gene polymorphisms in wild and cultivated common bean (*Phaseolus vulgaris* L.). *BMC Genet.* **2012**, *13*, 58. [CrossRef] [PubMed]
85. Blair, M.W.; Cortés, A.J.; This, D. Identification of an *ERECTA* gene and its drought adaptation associations with wild and cultivated common bean. *Plant Sci.* **2016**, *242*, 250–259. [CrossRef]
86. Cortes, A.J.; This, D.; Chavarro, C.; Madrinan; Blair, M. Nucleotide diversity patterns at the drought-related *DREB2* encoding genes in wild and cultivated common bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.* **2012**, *125*, 1069–1085. [CrossRef]
87. Buitrago-Bitar, M.A.; Cortés, A.J.; López-Hernández, F.; Londoño-Caicedo, J.M.; Muñoz-Florez, J.E.; Muñoz, L.C.; Blair, M.W. Allelic Diversity at Abiotic Stress Responsive Genes in Relationship to Ecological Drought Indices for Cultivated Tepary Bean, *Phaseolus acutifolius* A. Gray, and Its Wild Relatives. *Genes* **2021**, *12*, 556. [CrossRef]
88. Cortés, A.J.; Blair, M.W. Genotyping by Sequencing and Genome–Environment Associations in Wild Common Bean Predict Widespread Divergent Adaptation to Drought. *Front. Plant Sci.* **2018**, *9*, 128. [CrossRef]
89. Cortés, A.J.; López-Hernández, F. Harnessing Crop Wild Diversity for Climate Change Adaptation. *Genes* **2021**, *12*, 783. [CrossRef]
90. Cortés, A.J.; López-Hernández, F.; Osorio-Rodriguez, D. Predicting Thermal Adaptation by Looking into Populations' Genomic Past. *Front. Genet.* **2020**, *11*, 564515. [CrossRef] [PubMed]
91. Burbano-Erazo, E.; León-Pacheco, R.I.; Cordero-Cordero, C.C.; López-Hernández, F.; Cortés, A.J.; Tofiño-Rivera, A.P. Multi-Environment Yield Components in Advanced Common Bean (*Phaseolus vulgaris* L.) × Tepary Bean (*P. acutifolius* A. Gray) Interspecific Lines for Heat and Drought Tolerance. *Agronomy* **2021**, *11*, 1978. [CrossRef]

92. Pathan, M.S.; Nguyen, H.T.; Subudhi, P.K.; Courtois, B. Molecular Dissection of Abiotic Stress Tolerance in Sorghum and Rice. In *Physiology and Biotechnology Integration for Plant Breeding*; Nguyen, H.T., Blum, A., Eds.; Marcel Dekker Inc.: New York, NY, USA, 2004; pp. 525–570.
93. Yoshida, S.; Forno, D.A.; Cock, J.H.; Gomez, K.A. *Laboratory Manual for Physiological Studies of Rice*; IRRI: Las Banos, Philippines, 1976; Volume 83.