agronomy

MDPI

Article

Threshold Tolerance of New Genotypes of *Pennisetum glaucum* (L.) R. Br. to Salinity and Drought

Kristina Toderich [1,2,*], Elena Shuyskaya [3], Zulfira Rakhmankulova [3], Roman Bukarev [3], Temur Khujanazarov [4], Rauan Zhapaev [5], Shoaib Ismail [2], Shashi K. Gupta [6], Norikazu Yamanaka [7] and Fazyl Boboev [8]

1 International Platform for Dryland Research and Education (IPDRE), Tottori University, Tottori 680-8550, Japan
2 International Center for Biosaline Agriculture for Central Asia and Caucasus (ICBA-CAC), 6A, Osyo Street, Tashkent 100000, Uzbekistan; shoaib_is@hotmail.com
3 K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Science, Botanicheskaya Street 35, Moscow 127276, Russia; evshuya@gmail.com (E.S.); zulfirar@mail.ru (Z.R.); bukarev_r@mail.ru (R.B.)
4 Water Resources Research Center, Kyoto University, Gokasho, Kyoto 611-0011, Japan; khu.temur@gmail.com
5 International Maize and Wheat Improvement Center for Central Asia and the Caucasus, 51, Zhandosova Street, Almaty 050035, Kazakhstan; R.Zhapayev@cgiar.org
6 International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, Patancheru, Telangana 502 324, India; S.Gupta@cgiar.org
7 Arid Land Research Center, Tottori University, Tottori 680-8550, Japan; yamanaka@alrc.tottori-u.ac.jp
8 Nukus Branch of Uzbek Corn Research and Experimental Station, 25, Kegeli Street, Nukus 230100, Uzbekistan; sarmish@mail.ru
* Correspondence: kristina@biosaline.org.ae

Received: 31 August 2018; Accepted: 16 October 2018; Published: 18 October 2018

Abstract: With continued population growth, increasing staple crop production is necessary. However, in dryland areas, this is negatively affected by various abiotic stresses, such as drought and salinity. The field screening of 10 improved genetic lines of pear millet originating from African dryland areas was conducted based on a set of agrobiological traits (i.e., germination rate, plant density, plant maturity rate, forage, and grain yields) in order to understand plant growth and its yield potential responses under saline environments. Our findings demonstrated that genotype had a significant impact on the accumulation of green biomass (64.4% based on two-way ANOVA), while salinity caused reduction in grain yield value. *HHVBC Tall* and *IP 19586* were selected as the best-performing and high-yielding genotypes. *HHVBC Tall* is a dual purpose (i.e., forage and grain) line which produced high grain yields on marginal lands, with soil salinization up to electrical conductivity (EC) 6–8 dS m^{-1} (approximately 60–80 mM NaCl). Meanwhile, *IP 19586*, grown under similar conditions, showed a rapid accumulation of green biomass with a significant decrease in grain yield. Both lines were tolerant to drought and sensitive to high salinity (above 200 mM NaCl). The threshold salinity of *HHVBC Tall* calculated at the seedling stage was lower than that of *IP 19586*. Seedling viability of these lines was affected by oxidative stress and membrane peroxidation, and they had decreased chlorophyll and carotenoid biosynthesis. This study demonstrated that ionic stress is more detrimental for the accumulation of green and dry biomass, in combination with increasing the proline and malonic dialdehyde (MDA) contents of both best-performing pearl millet lines, as compared with osmotic stress.

Keywords: salinity stress; drought; *Pennisetum glaucum* germplasm; photosynthesis; non-conventional crops

1. Introduction

The scarcity of irrigation water and increasing soil salinization, combined with climate change variables, are threatening the sustainability of forage and grain crop production in the arid zones of the Aral Sea Basin. The introduction of abiotic stress-tolerant crops to meet the increasing demand of human and livestock consumption is crucial. The adoption of new non-conventional crop germplasm that is able to produce good-quality green biomass levels and grain yields by reducing the salt accumulation in the root zone, which is generated by different international agricultural programs, is a novel agricultural approach for Aral Sea Basin countries [1,2]. Pearl millet is a dual-purpose (i.e., grain and forage) crop. Its biomass is used as a fat-based feed for many animals, and the seeds are utilized for the poultry industry and human consumption [3,4]. In recent years, the green biomass of millet has been investigated as a source of ethanol [5]. Among the warm season cereals, pearl millet is the most heat- and drought-resistant crop [6]. It is a C_4-type photosynthesis species and is characterized by intensive growth. Additionally, it is often cultivated in arid and semi-arid areas [7]. Although pearl millet is well adapted to arid environments, several abiotic stresses, such as drought, heat stress, and salinity, affect its growth. There are limited studies on the impacts of high salinity stress on pearl millet [8–10]. There is no data on the expansion of this high productive crop under new marginal environments, especially those exposed to high salinity and drought.

To understand the physiological mechanisms of salt tolerance, it is important to determine the effects of osmotic stress or ion toxicity on growth [11]. It is assumed that the plant reacts to salinity in two stages: (1) the fast osmotic phase, which inhibits the growth of young leaves, and (2) the slow ion phase, which accelerates the aging of mature leaves [11]. In mesophytes with C_3 and C_4 types of photosynthesis, PEG (polyethylene glycol)-induced drought [12,13], like salinity [14,15], had a significant effect on plant growth. The salinity effect on plant photosynthesis depends on the species and the intensity of the salt stress [16,17]. Salinity also reduces chlorophyll (Chl) concentration, leading to the disruption of the photosynthetic activity of the electron transport chain and Photosystem II (PSII), which inhibits carbon metabolism [18,19].

The amino acid proline is involved in osmoregulation, which plays an important role in redox buffering and energy transfer. Plants usually accumulate proline in response to stress [20]. Moreover, a consequence of stresses such as salinity and drought is the formation of reactive oxygen species (ROS), which are highly reactive and cause the oxidation of organic molecules, including lipids. The end product of lipid peroxidation is malondialdehyde (MDA) [21]. Thus, MDA content can be used as a marker to assess the level of oxidative stress and plant tolerance [22,23]. The accumulation of natural antioxidants in plants (in response to the increase in ROS) is of interest because they improve the nutritional quality of the forage [24].

The introduction of new salt-tolerant crop varieties/improved lines into marginal areas requires a comprehensive understanding of alternative mechanisms of cultivation and seed multiplication of highly productive and adaptive varieties to nutrient-poor and salt stress environments in smallholder farming systems. Selection and evaluation of appropriate crop germplasm to match the environment, particularly the salinity level and the type of production system for different ecological zones of Central Asia, were evaluated in this study. The purpose of this study was to evaluate the performance of internationally selected pearl millet genotypes from various agro-ecological drylands environments affected by drought and salinity. Physiological traits were used to identify the reliability of seedling abiotic-stress responses and the threshold tolerances of the two best-performing and high-yield improved genetic lines of pearl millet in terms of green biomass and grain production. Our objective was to define the relevance of physiological traits to the maintenance of forage and grain yields of new germplasm introduced from tropical areas to the extremely dry and saline environments of the Aral Sea Basin countries. Priorities regarding the involvement of these two high-performing pearl millet genotypes into local breeding and seed production schemes were considered.

2. Materials and Methods

2.1. Field Growth Conditions

On-farm multiplication trials were established to evaluate the agrobiological characteristics (i.e., period of vegetation, plant density, field germination, plant height, green biomass, dry biomass, and grain yields) of 10 improved genetic lines of pearl millet compared with local cultivar under two different eco-agro-climatic zones differing significantly in soil salinity level. Experiments were conducted within the period of 2012–2016 summer seasons in two agri-field sites in Kazakhstan that differ in climatic and edaphic conditions. The annual precipitation in these areas varies from 157 to 296 mm. The rainfall is abundant in April and contributes to an increase in soil moisture that stimulates good seed germination. Maximum annual precipitation (120.3 mm) and minimum air temperature (22.2 °C) were observed during the crop season in 2015. The average air temperature during the crop season was lower in 2014 and the precipitation higher than in 2016 (22.2 vs. 22.1 °C and 59.8 vs. 34.6 mm, respectively). The highest air relative humidity (75.59%) and plentiful precipitation (110.8 mm) was noted in April and May. The period with an average daily temperature above 10 °C was observed from 26 March to 1 November, with an average annual air temperature of 14 °C. During June and July, the maximum temperature of both air and soil were observed.

Experimental plots were irrigated three times with a total volume of 2550 m^3 ha^{-1}. Irrigation was stopped during the flowering and seed maturation stage for all evaluated cultivars. Total dissolved solids (TDS) of irrigation water was relatively low (1200 ppm) with 3.5 ± 0.5 $mmol_c/L$ of Ca^{2+}. The pre-experiment soil salinity levels (for two seasons, 2012–2016) in terms of total soluble salts (TSS) at different soil depths extending up to 1.0 m were in the range of 5910 to 8170 mg L^{-1}.

Two experimental locations were chosen with different levels of soil salinity. (1) The low salinity (electrical conductivity (EC) 1.5–3.5 dS m^{-1}) area at the Svetlana Farm (in the Almaty region) site is characterized by loamy soil with a medium to heavy texture at soil depths from 0–100 cm. (2) A medium salinity (EC 6–8 dS m^{-1}) area at Karaultubinsk experimental station in Kyzylorda region, which is comprised of old irrigated paddy fields with meadow-loamy secondary salt affected soils. The type of salt for both sites was chloride sulfate. Quantitatively, the average data for four years indicated low organic matter contents (0.50–0.84%) with low nitrogen content in soil compared to phosphorus and potassium. The depth to the water table during the crop vegetation season varied from 1.2 to 2.5 m.

2.2. Plant Material

On-farm multiplication trials to evaluate the 10 improved genetic lines of pearl millet (*Pennisetum glaucum* (L.) R. Br.), *HHVBC Tall*, *IP 19586*, *GB 8735*, *Sudan POP I*, *JBV 2*, *ICMV 155*, *Raj 171*, *IP 13150*, *IP 22269*, and *ICMS 7704* were chosen for this study and compared with the Hashaki1 local variety.

Ten genetic lines of pearl millet were developed by ICRISAT (International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India) through a breeding program for salt and drought tolerance traits from existing lines of pearl millet. After development, these improved lines were tested under extremely dry and saline conditions in CAC countries. Based on a three-year screening process, *HHVBC Tall* and *IP 19586* showed stable forage and grain yields. Therefore, we used them for laboratory experiments in order to understand the range of their salt and drought tolerance.

2.3. Laboratory Germination Conditions

Seeds of the *HHVBC Tall* and *IP 19586* pearl millet lines were germinated under laboratory conditions at 20 °C in Petri dishes (50 seeds per dish) on filter paper for 5 days. Seeds were germinated in distilled water (control) and at 100, 200, 300, and 400 mM NaCl. The germination was performed in three replications (leading to a total of 750 seeds).

2.4. Laboratory Growth Conditions

At 3 to 4 d of age, seedlings were transplanted into perlite in plastics pots (24 cm length × 20 cm width × 10 cm depth). The seedlings were grown under circadian illumination (using commercial luminescent white light tubes) in a 10 h dark/14 h light cycle, using a 200 µmol m^{-2} s^{-1} R LI-205 light meter (LI-COR Inc., Lincoln, NE, USA), at 25 ± 5 °C. Then, 15-day-old plants were transferred into the experimental solutions (irrigated perlite). Solutes of 200 and 300 mM NaCl and 15.8% and 18.8% (*m*/*v*) polyethylene glycol (PEG 6000) were used as the experimental treatments. PEG 6000 is usually used to create low osmotic potential (i.e., artificial drought) in laboratory experiments. The osmotic potentials of the experimental solutions were measured using a freezing-point osmometer (Osmomat 030; Gonotec, Berlin, Germany). The water potentials of both the 200 mM NaCl and 15.8% PEG treatment solutions were approximately −0.6 MPa, and the water potentials of both the 300 mM NaCl and 18.8% PEG treatment solutions were −0.8 MPa. For each treatment, Hoagland, salt, or PEG solutions were added to a plastic tray, and the plastic pots were then placed on the tray. After transfer, the experimental solutions in the trays were renewed every 2 days to maintain the initial concentrations. The Hoagland nutrient solution in the tray was replaced every 2 days for the control plants. The experiment lasted 6 days. For each treatment and control measurement, four different plants from four different pots were used.

2.5. Water and Proline Contents

At the end of the experiment, the water content (WC, measured in g g^{-1} dry biomass (DM)) was assessed for the shoots in all of the groups. Biomasses were estimated for fresh and dry shoots. Plant samples were dried at 80 °C for 2 days, until reaching a constant mass, to quantitatively measure the dry shoot matter. The WCs in the shoots for each treatment and control plants were calculated as WC = [fresh biomass (FM) − DM]/DM. Free proline was determined according to the procedure described in [25]. Dry shoot samples (0.2 g) from each group were homogenized in 2 mL of boiling distilled water and heated at 100 °C for 10 min in a water bath. Then, the homogenates were centrifuged. The mixtures were heated at 100 °C for 1 h in a water bath after adding acid ninhydrin and glacial acetic acid. The reaction was stopped by submerging the sample into an ice bath. The mixtures were read at 520 nm using a Genesis 10 UV scanning spectrophotometer (Thermo Scientific, Valtame, MA, USA). Proline concentrations were determined using a calibration curve and expressed as mg g^{-1} DM.

2.6. Lipid Peroxidation

The lipid peroxidation levels in plant tissues were determined by measuring the MDA contents, which is the product of lipid peroxidation [26]. Shoot samples (0.2 g) were homogenized in 4 mL of 20% trichloroacetic acid, then centrifuged at 10,000× *g* at 4 °C for 15 min. The supernatant (1 mL) was then mixed with 4 mL of 20% trichloroacetic acid containing 0.5% of 2-thiobarbituric acid, and the solution was heated for 30 min at 95 °C. The samples were cooled on ice for 5 min and centrifuged for 12 min at 10,000× *g*. The nonspecific absorbance of the supernatant, measured by a Genesis 10 UV scanning spectrophotometer (Thermo Scientific, Valtame, MA, USA) at 600 nm, was subtracted from the maximum absorbance at 532 nm. To estimate the MDA concentration, an extinction coefficient of 155 mM^{-1} cm^{-1} was used. Changes in the MDA content were expressed as percentages relative to the control plant results.

2.7. Pigment Extraction and Quantification

Chlorophyll (Chl) and carotenoids were extracted in 96% ethanol using purified glass sand for sample homogenization. After centrifugation at 4 °C, the Chl *a* and Chl *b* contents were determined at 665 and 649 nm, respectively, and the carotenoids were determined at 470 nm, spectrophotometrically, using a Genesis 10 UV scanning spectrophotometer (Thermo Scientific, Valtame, MA, USA). The concentrations were calculated according to the procedure in [27].

2.8. Chl Fluorescence

Chl fluorescence was measured with a PAM 101 pulse amplitude-modulated fluorometer, (Walz, Effeltrich, Germany), following the recommendations of the manufacturer [28]. The Chl fluorescence of the leaf in the leaf chamber was excited and directed to the fluorometer through a 101 F flexible fiber-optic light guide (Walz). The minimal (F_0) and maximal (F_m) fluorescence values from the shoots in a dark-adapted (20 min) state were determined. The maximum quantum efficiency (yield) of photosystem II (PSII) (F_v/F_m) in a dark-adapted shoot was calculated using the equation $(F_m - F_0)/F_m$.

2.9. Statistical Analysis

All of the physiological measurements were assessed four times, and the means and standard errors (SEs) were calculated using the Sigma Plot 12.0 statistical program. Comparisons of the parameters were made between treatments using an analysis of variance with a post hoc Tukey test. Differences were considered significant at $p < 0.05$. Two-way ANOVA was performed by using the Statistica 10 program.

3. Results

3.1. Field Trials

Field screening of 10 pearl millet accessions/improved genetic lines (ICRISAT) and one local variety were evaluated based on agrobiological characteristics: time of planting, seed germination, seedling emergence, plant density, plant maturity rate, green and dry biomass, and grain yield. As seen in Table 1, the investigated pearl millet, grown under low soil salinity level, showed insignificant difference in field germination (from 56.8 to 61.5%), while the screened cultivars highly differed in plant density (from 137.8 to 177.6/1000 plants ha^{-1}), plant height (2.02–2.54 m), duration of seasonal vegetation (i.e., pearl millet Hashaki1 and *HHVBC Tall* were early maturing lines, followed by IP19586 and ISMV 155, which were late maturing), accumulation of green biomass (27.7–62.26 t ha^{-1}), and yield of produced grain (1.89–4.47 t ha^{-1}). The remaining samples showed intermediate growth habits.

HHVBC Tall pearl millet line was the top performing in grain production (4.47 t ha^{-1}), while the IP 19856 pearl millet line was distinguished by having the highest value of green and dry forage production. A significant increase in the yield of seeds of *HHVBC Tall* was associated with panicle size, the weight of the seeds/panicle, and the weight of 1000 seeds. Both these two selected pearl millet germplasms showed about 30% more dry fodder yield and 25% more seeds compared to the Hashaki1 local variety. Pearl millet line performance in response to different soil salinity levels varied. Both the best-performing *HHVBC Tall* and IP 586 pearl millet lines had a gradually decreasing yield productivity with increases in salinity. However, *HHVBC Tall* showed high grain yield despite this. The *IP 19586* pearl millet line was distinguished by its high biomass yield compared with other investigated cultivars.

Table 1. Impact of soil salinity on agrobiological characteristics, green/dry biomass and grain yield production of introduced of pearl millet *Pennisetum glaucum* improved genetic lines (average of filed data, Kazakhstan). Low salinity—EC 1.5–3.5 dS m^{-1}; medium salinity—EC 6–8 dS m^{-1}. The values are means ± SEs. Different letters above the bars represent significant differences at the $p < 0.05$ level (Tukey's pairwise comparison).

	Pearl Millet Lines										
	Hashaki 1	***HHVBC Tall***	***IP 19586***	**GB 8735**	**Sudan POP I**	**JBV 2**	**ICMV 155**	**Raj 171**	**IP 13150**	**IP 22269**	**ICMS 7704**
						Low salinity					
Period of vegetation (days)	90 ± 1.6 ^{d}	97 ± 1.9 ^{c}	113 ± 2.3 ^{a,b}	97 ± 2.9 ^{c}	100 ± 1.8 ^{b,c}	103 ± 3.2 ^{b}	103 ± 2.6 ^{b}	103 ± 3.6 ^{b}	118 ± 2.6 ^{a}	115 ± 1.1 ^{a}	110 ± 3.5 ^{b}
Plant density (1000 plants ha^{-1})	148.4 ± 33.4 ^{a,b}	168.8 ± 27.7 ^{a}	137.9 ± 13.5 ^{a,b}	160.8 ± 26.7	177.8 ± 30.8 ^{a}	137.8 ± 27.7 ^{a,b}	146.6 ± 23.1 ^{a,b}	166.6 ± 10.2 ^{a}	110.2 ± 18.9 ^{a}	191.2 ± 13.3 ^{a}	152.8 ± 24.8 ^{a,b}
Field germination (%)	50.2 ± 0.52 ^{b}	59.8 ± 0.60 ^{a}	58.5 ± 1.91 ^{a}	57.6 ± 2.66 ^{a}	61.0 ± 2.62 ^{a}	59.6 ± 2.81 ^{a}	53.8 ± 2.60 ^{b}	59.8 ± 1.68 ^{a}	57.3 ± 0.98 ^{a}	53.1 ± 0.45 ^{b}	59.5 ± 0.79 ^{a}
Plant height (m)	2.02 ± 0.20 ^{a}	2.47 ± 0.10 ^{a}	2.54 ± 0.23 ^{a}	1.72 ± 0.07 ^{b}	2.04 ± 0.21 ^{a}	2.32 ± 0.36 ^{a}	2.23 ± 0.03 ^{a}	2.24 ± 0.44 ^{a}	2.37 ± 0.45 ^{a}	2.51 ± 0.52 ^{a}	2.19 ± 0.15 ^{a}
Green biomass (t ha^{-1})	27.7 ± 3.1 ^{d}	43.60 ± 2.9 ^{c}	62.26 ± 5.3 ^{a}	50.7 ± 4.6 ^{b}	40.2 ± 3.4 ^{c}	47.4 ± 5.1 ^{b}	36.6 ± 4.0 ^{c}	48.9 ± 2.7 ^{b}	36.4 ± 2.1 ^{c}	44.8 ± 2.7 ^{c}	49.4 ± 2.8 ^{b}
Dry biomass (t ha^{-1})	11.1 ± 1.7 ^{d}	16.1 ± 1.4 ^{c}	27.0 ± 1.7 ^{a}	20.3 ± 1.4 ^{b}	13.8 ± 1.2 ^{c}	20.1 ± 0.7 ^{b}	12.8 ± 2.5 ^{c}	18.4 ± 0.4 ^{b}	15.1 ± 1.9 ^{c}	17.4 ± 1.9 ^{c}	15.0 ± 2.6 ^{c}
Grain yields (t ha^{-1})	2.51 ± 0.46 ^{b}	4.37 ± 0.57 ^{a}	2.14 ± 0.29 ^{b,c}	2.85 ± 0.41 ^{b}	2.93 ± 0.31 ^{b}	2.88 ± 0.56 ^{b}	1.89 ± 0.32 ^{c}	2.84 ± 0.23 ^{b}	0.79 ± 0.18 ^{d}	1.17 ± 0.37 ^{c,d}	2.75 ± 0.42 ^{b}
						Medium salinity					
Period of vegetation (days)	93.2 ± 1.9 ^{d}	102.4 ± 2.6 ^{c}	121.4 ± 1.5 ^{b}	109.4 ± 2.2 ^{c}	136.3 ± 2.8 ^{a}	105.4 ± 1.4 ^{c}	103.3 ± 0.8 ^{c}	116.5 ± 0.9 ^{b}	117.0 ± 2.4 ^{b}	136.1 ± 0.9 ^{a}	133.4 ± 1.5 ^{a}
Plant density (1000 plants ha^{-1})	133.9 ± 5.4 ^{b}	159.1 ± 4.6 ^{a}	155.6 ± 5.3 ^{a}	152.5 ± 5.9 ^{a}	162.5 ± 5.6 ^{a}	152.2 ± 6.0 ^{a}	143.8 ± 14.3 ^{a,b}	157.8 ± 10.2 ^{a}	110.2 ± 9.2 ^{c}	160.0 ± 13.3 ^{a}	148.4 ± 7.8 ^{a}
Field germination (%)	37.1 ± 8.4 ^{a}	42.2 ± 6.9 ^{a}	36.7 ± 3.4 ^{a}	40.2 ± 6.7 ^{a}	44.4 ± 7.7 ^{a}	34.4 ± 6.9 ^{a}	36.7 ± 5.8 ^{a}	39.4 ± 2.5 ^{a}	27.6 ± 4.8 ^{b}	40.0 ± 3.3 ^{a}	37.1 ± 6.2 ^{a}
Plant height (m)	2.29 ± 0.09 ^{b}	2.35 ± 0.06 ^{b}	2.61 ± 0.10 ^{a}	2.32 ± 0.11 ^{b}	2.55 ± 0.12 ^{a}	2.57 ± 0.10 ^{a}	2.35 ± 0.06 ^{a,b}	2.37 ± 0.10 ^{a}	2.65 ± 0.07 ^{a}	2.45 ± 0.18 ^{a}	2.17 ± 0.09 ^{c}
Green biomass (t ha^{-1})	25.4 ± 0.8 ^{d}	40.4 ± 1.2 ^{b}	50.5 ± 0.8 ^{a}	42.6 ± 1.8 ^{b}	37.4 ± 3.6 ^{b,c}	26.7 ± 1.7 ^{d}	25.1 ± 1.7 ^{d}	44.9 ± 1.3 ^{b}	32.9 ± 1.7 ^{c}	36.4 ± 0.7 ^{c}	39.0 ± 1.2 ^{b,c}
Dry biomass (t ha^{-1})	7.3 ± 0.7 ^{c}	12.8 ± 1.2 ^{b}	17.0 ± 1.1 ^{a}	10.7 ± 0.7 ^{b}	10.3 ± 1.8 ^{b}	7.1 ± 1.8 ^{c}	7.5 ± 0.8 ^{c}	10.7 ± 0.9 ^{b}	10.1 ± 0.9 ^{b}	9.5 ± 0.5 ^{b}	8.4 ± 0.7 ^{b,c}
Grain yields (t ha^{-1})	1.29 ± 0.07 ^{b}	2.23 ± 0.09 ^{a}	1.38 ± 0.15 ^{b}	1.51 ± 0.08 ^{b}	1.15 ± 0.21 ^{b,c}	1.30 ± 0.10 ^{b}	1.12 ± 0.15 ^{b,c}	1.01 ± 0.20 ^{c}	1.41 ± 0.09 ^{b}	0.83 ± 0.07 ^{c}	0.92 ± 0.09 ^{c}

Results of two-way ANOVA (Figure 1, Table 2) on the effect of salt concentration on the green biomass and grain yield values of 11 pearl millet improved lines showed that the genotype (A) had a significant impact of 64.4% on the yield of green biomass (Figure 1a), but only had a 33.3% for the grain yields (Figure 1b). Soil salinity (B) had a significant impact of 41.3% on the grain yields (Figure 1b). The interaction of genotype and salinity (AB) slightly affected the crop yield production.

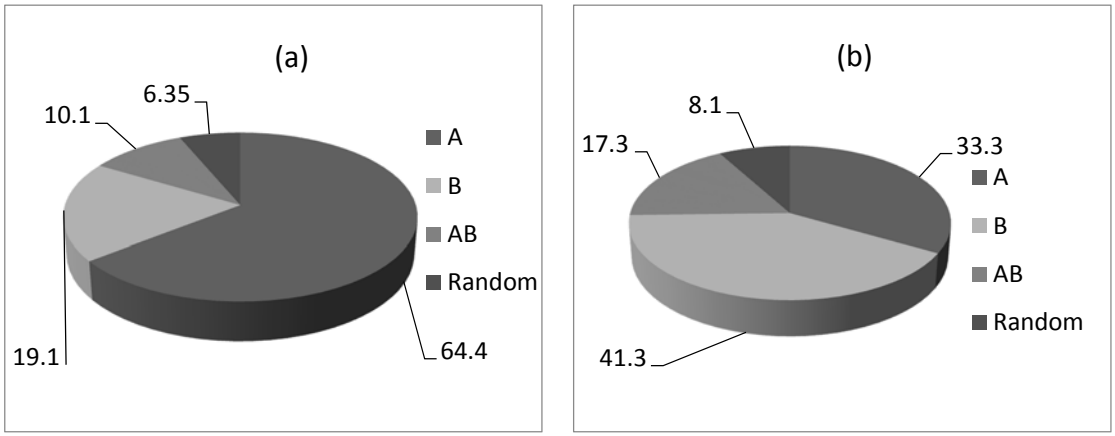

Figure 1. R of two-way ANOVA of 11 lines of pearl millet *Pennisetum glaucum*: (**a**) green biomass (%), (**b**) grainyields (%), where A—Genotype, B—Salinity level, AB—Genotype–salinity level.

Table 2. Results of two-way ANOVA on effect of salinity level and genotype on green biomass and grain yields of 11 lines of pearl millet *Pennisetum glaucum.* F—Fisher distribution, df—degree of freedom, Sig.—significance level. Minimum value of Fisher statistics is 7.008 and 9.351.

Source	Green Biomass			Grain Yields		
	df	F	Sig.	df	F	Sig.
Genotype	10	44.631	<0.0001	10	18.041	<0.0001
Salinity level	2	132.168	<0.0001	2	223.707	<0.0001
Genotype and salinity level	10	7.008	<0.0001	10	9.351	<0.0001
Error	44			44		

Thus, based on field evaluation data, *HHVBC Tall* and *IP 19586* were chosen from among the other pearl millet accessions/improved genetic lines from the ICRISAT germplasm for the investigation of salinity and drought thresholds (in the model experiment).

3.2. Laboratory Germination

The *HHVBC Tall* line showed a high salinity tolerance for this species, with a germination rate that was not significantly different from the control plants after 5 d at 100–300 mM NaCl. However, when the salinity increased to 400 mM NaCl, the seed germination rate decreased almost twofold (Figure 2a). For the *IP 19586* line, the germination rate gradually decreased from 98 to 67% as the salinity level increased from 0 to 400 mM NaCl (Figure 2b). At 400 mM NaCl, the seed germination rate decreased by 50% in *HHVBC Tall* and by 33% in *IP 19586* compared with the control plants.

There were no significant differences in the effects of different NaCl and PEG concentrations on the growth parameters (Figure 2c–e) of the shoots of two pearl millet lines under experimental conditions. Both genetic lines showed similar tolerance levels of growth parameters to great osmotic stress (15.8% PEG or PEG (1)) (Figure 2c,d). Differences between lines were revealed in WCs of seedlings at 300 NaCl (NaCl (2)) (Figure 2f). The sharp (two- to three-fold) reductions in fresh biomass (FM) and DM and the dehydration of seedlings were observed in both lines of pearl millet under extremely osmotic and high/extremely high-salt stress conditions (Figure 2c,d,f).

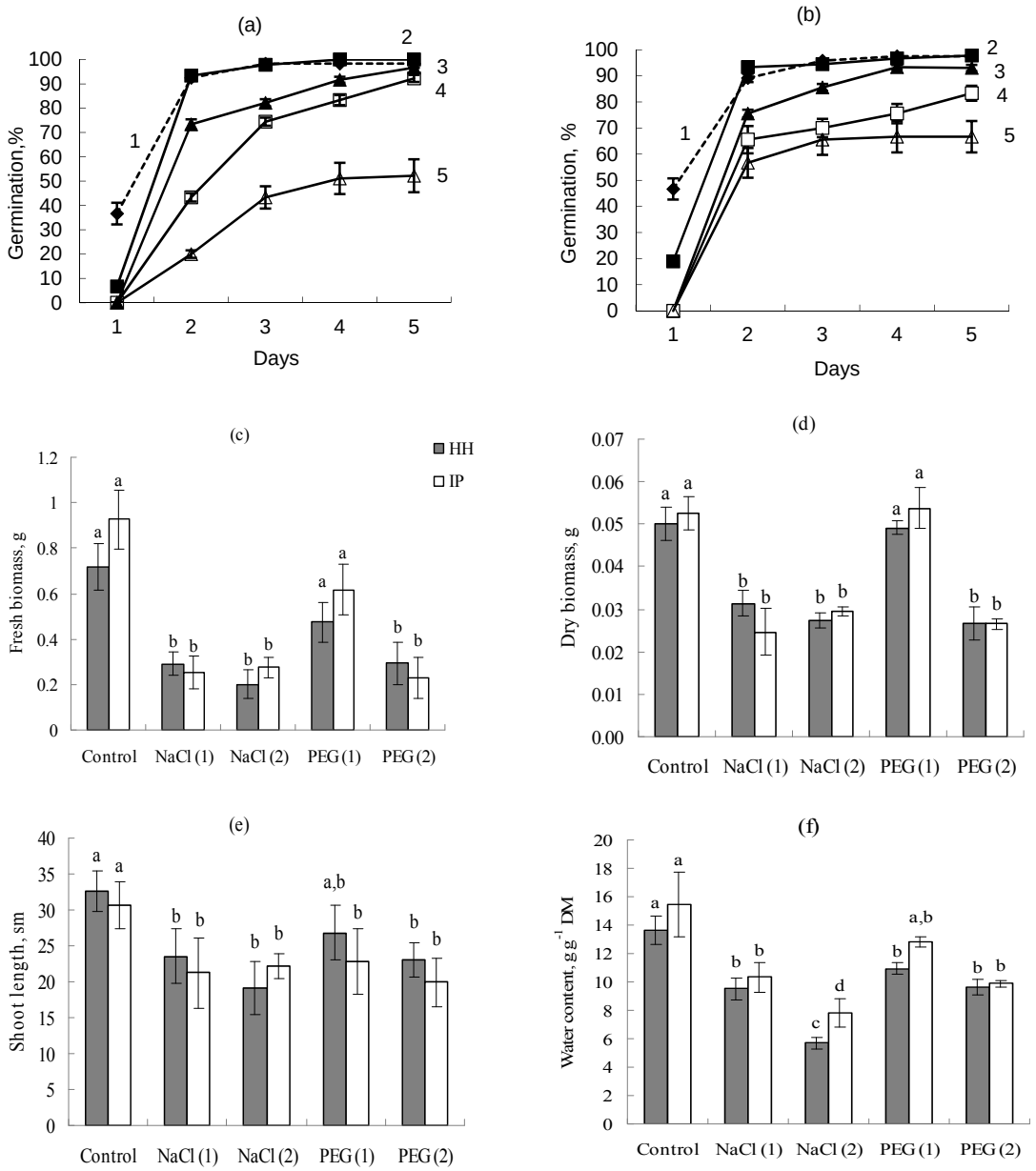

Figure 2. Seed germination rates and agrobiological characteristics of two genetic lines of pearl millet *Pennisetum glaucum* under different stresses. (**a**,**b**), '*HHVBC-Tall*' (**a**) and '*IP 19586*' (**b**), under different salinity levels; 1, control (distilled water); 2, 100 mM NaCl; 3, 200 mM NaCl; 4, 300 mM NaCl; 5, 400 mM NaCl. (**c**–**f**), Effects of salt (NaCl) and osmotic (polyethylene glycol (PEG)) stresses on the fresh (**c**) and dry (**d**) biomasses, shoot lengths (**e**) and water contents (**f**) of leaves from these lines. HH, '*HHVBC-Tall*' line; IP, '*IP 19586*' line; NaCl (1), 200 mM NaCl; NaCl (2), 300 mM NaCl; PEG (1), 15.8% (*m*/*v*) PEG 6000; and PEG (2), 18.8% (*m*/*v*) PEG 6000. The values are means ± SEs. Different letters above the bars represent significant differences at the $p < 0.05$ level (Tukey's pairwise comparison). SEs—Standard Errors.

3.3. Model Experiment

The differences in Chl *a* contents between lines were found under extremely high salinity conditions (NaCl (2)), whereas there were no differences in Chl *b* contents (Figure 3a,b). Also, a twofold reduction in carotenoid content was observed in the *HHVBC Tall* line compared with *IP 19586* seedlings under extremely high salinity conditions (NaCl (2)) (Figure 3c). A significant change in photosystem II (F_v/F_m) was observed in *HHVBC Tall* seedlings under high and extremely high

salinity conditions, but there is no difference observed in *IP 19586* seedlings (Figure 3d). The highest proline concentration in *HHVBC Tall* seedlings, which was 50-fold greater than in the control seedlings, occurred under extremely high salinity conditions (NaCl (2)). Meanwhile, in *IP 19586* seedlings, the proline concentration increased only approximately 30-fold compared with the control seedlings under extremely high salinity and osmotic stress conditions (Figure 3e). The MDA concentration was the highest in *HHVBC Tall* seedlings under extremely high salinity (NaCl (2)) and osmotic stress (PEG (2)) conditions, with these values increasing approximately 8.6-fold over those of the control seedlings (Figure 3f).

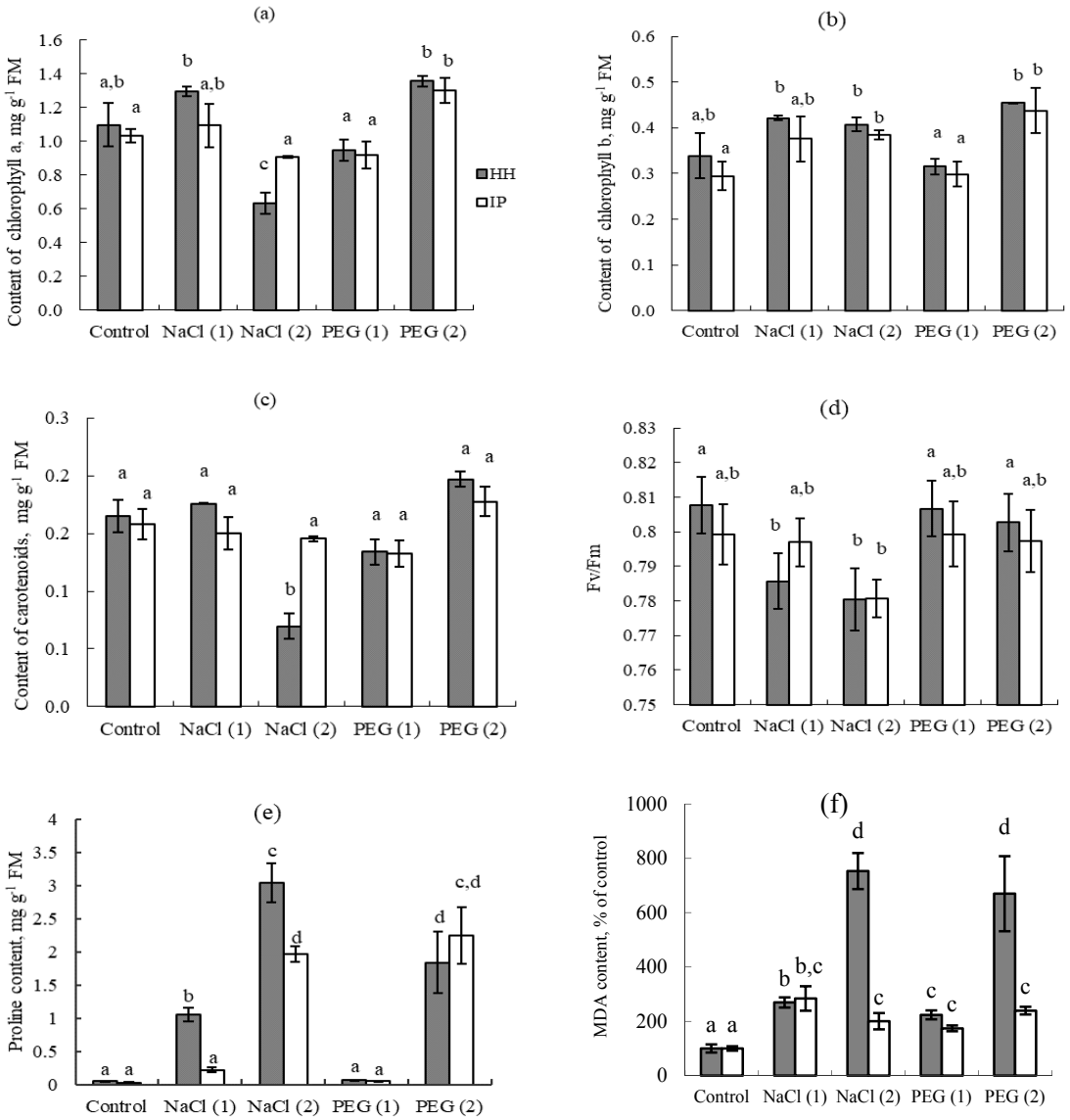

Figure 3. Effects of salt (NaCl) and osmotic (PEG) stresses on the photosynthesis-related characteristics of two improved genetic lines of pearl millet *Pennisetum glaucum*. The chlorophyll *a* (**a**), chlorophyll *b* (**b**), and carotenoid (**c**) contents, the maximum quantum yield of photosystem II (**d**), as well as the proline (**e**) and MDA (**f**) contents, in the leaves from the two pearl millet lines. HH, '*HHVBC-Tall*' line; IP, '*IP 19586*' line; NaCl (1), 200 mM NaCl; NaCl (2), 300 mM NaCl; PEG (1), 15.8% (*m*/*v*) PEG 6000; and PEG (2), 18.8% (*m*/*v*) PEG 6000. The values are means ± SEs. Different letters above the bars represent significant differences at the $p < 0.05$ level (Tukey's pairwise comparison).

4. Discussion

Crop growth is strongly affected by drought and salinity stresses, especially during the early stages of ontogenesis, at seed germination, and during seedling growth [29]. Drought and salinity stresses cause adverse physiological and biochemical changes as a result of complex factors, including osmotic stress, toxic ion effects, and oxidative stress [30]. In Africa and India, pearl millet is the dominant crop in soils with high salt and low humus contents [31]. Pearl millet grows well under drought conditions and in the saline environment, although it is less salt tolerant than *Cynodon* spp. and *Panicum* [32]. The increase in soil salinity negatively affects grain yield. The harvest index (i.e., the proportion of total shoot biomass to total produced grain) varies from 0.2 to 0.5, depending on the timing and severity of the salt treatment [33]. At the same time, low salinity levels may not reduce grain yield, even though the numbers of leaves, leaf area, and stover biomass are reduced. This may reflect a harvest index that increases with salinity or grain yield that does not decrease until a given ("threshold") salinity is reached.

Our findings indicated that the dry biomass yield can be used as a screening and selection criterion for evaluating salt-tolerance behaviors among a large collection of plant accessions. Based on their relative dry biomasses (DMs), it is possible to select the optimum genotypes for each salinity level. From the field screening of 10 pearl millet germplasms of new breeding materials from ICRISAT, we selected *IP 19586* and *HHVBC Tall*, which showed good growth and yield productivity under different ranges of soil salinity. The top-performing *IP 19586* improved pearl millet germplasm line produced an average dry matter production of 27.6–35.0 t ha^{-1}. Among the investigated pearl millet germplasms, the *HHVBC Tall* line was distinguished by its having the highest value of seed production, at up to 4.3–4.7 t ha^{-1}. Results of two-way ANOVA on the effect of salt concentration on green biomass and grain yields for 11 investigated pearl millet lines showed that the genotype (A) had a significant impact of 64.4% on the yield of green biomass and only a 33.3% impact for the grain yields (Figure 1). A significant increase in the yield of seeds of *HHVBC Tall* was associated with the panicle size, weight of the seeds/panicle, and weights of 1000 seeds. These two pearl millet germplasms selected by us showed about 30% more dry fodder yield and 25% seeds compared to the Hashaki1 local variety.

The investigated pearl millet lines had different salt tolerance strategies at the seed germination stage. The *HHVBC Tall* line showed tolerance up to a high salinity level (300 mM NaCl), but it was sensitive to extra-high salinity (400 mM NaCl). For the *IP 19586* line, the germination rate gradually decreased from 0 to 400 mM NaCl (Figure 2). For other pearl millet varieties, approximately 60% decreases in seed germination rates occurred with 1.5% NaCl treatment [10]. Controlled experiments allow for the investigation of plant resistance-related physiological and biochemical characteristics under ionic and osmotic stresses. Both lines of pearl millet showed similar resistance levels (as assessed by growth parameters) to high PEGinduced osmotic stress (PEG (1)) in our experiments (Figures 2 and 3). However, under these conditions, 1.5–2-fold increases in MDA contents were observed in both lines, which indirectly indicates the presence of low-level oxidative stress [22,23]. At the same time, the photosynthetic pigment contents and functional characteristics of PSII were not affected (Figure 2), which indicates only minor damage to the photosynthetic apparatus [19]. The pearl millet lines were sensitive to extremely high PEGinduced osmotic stress (PEG (2)). There was a significant reduction in growth parameters and water content (Figure 2d,f). Increased proline content indicates significant osmotic stresses [20] in both lines (Figure 3e). However, there are differences in oxidative stress levels between lines under high drought conditions, as assessed from the MDA concentrations (Figure 3f).

Our study provides evidence of the salt tolerance thresholds of the investigated pearl millet lines. Salinity (combined effects of osmotic and ionic stresses) at 200 mM NaCl (NaCl (1)) caused a significant twofold reduction in the growth parameters (Figure 2c,d). Because a PEG-induced osmotic stress with a similar osmotic potential caused no decrease in these growth parameters, we believe the toxic actions of ions resulted in the growth reduction [11,34]. The *HHVBC Tall* line was more sensitive to ionic stress, having significantly higher proline content (Figure 3e). Under extremely high salinity conditions, the most significant differences between the studied genetic lines were revealed.

Under these conditions, there was significant tissue dehydration in both lines, but especially in *HHVBC Tall* plants (Figure 2f). Furthermore, the Chl *a* and carotenoid contents significantly decreased in *HHVBC Tall* plants at 300 mM NaCl (NaCl (2)) (Figure 3a,c). For other *P. glaucum* varieties, significant reductions in Chl contents occurred at 100–200 mM NaCl [9]. The high proline and MDA contents (Figure 3e,f) indicate the presence of high stress levels in *HHVBC Tall* plants under these conditions [20,22,23].

Both lines of pearl millet had similar physiological parameters under normal conditions; they were tolerant to −0.6 MPa osmotic stresses but were sensitive to high salinity. The reduction in growth parameters was primarily the result of toxic ion effects. Differences between lines appeared only under extreme stress conditions. The *HHVBC Tall* line was more sensitive to ionic stress. In these plants, severe dehydration was accompanied by high osmotic and oxidative stresses, which led to the disruption of photosynthetic pigment biosynthesis and membrane peroxidation.

The *IP 19586* pearl millet germplasm line, which has a high biomass yield potential, is of interest as a forage resource for livestock in salt-affected agro-landscapes. Since soil salinization is negatively reflected in the grain production, the seed multiplication of the high-grain *HHVBC Tall* pearl millet line would guarantee stable seed production only in soils with low and medium levels of salinity. This research has allowed us to formulate the following recommendations. The *IP 19586* line of pearl millet should be used for maximal green biomass production under drought stress (−0.6 MPa), while the HHVBC-Tall line should be used to produce high grain yields under low and medium salinity and drought (−0.6 MPa) conditions. High and extremely high salinity conditions severely limit plant growth and development, as well as agrobiological characteristics, including the forage and grain yields of the two investigated pearl millet lines. Thus, physiological and biochemical traits related to the severity of the stress load appear to be crucial in selecting the most suitable breeding strategy and in the improvement of drought and salinity tolerance levels in the cultivation of pearl millet under the extremely hot and saline desert conditions in the Aral Sea Basin countries.

5. Conclusions

Our results in field screening of 11 investigated pearl millet lines have demonstrated that the genotype had a significant impact on the yield of green biomass (i.e., 64.4% impact based on a two-way ANOVA analysis) and less on grain (i.e., 33.3% impact) yields. Salinity caused more reduction in grain yield production (i.e., 43% impact). Two of the best-performing and most productive lines selected for local breeding program, *IP 19586* and *HHVBC Tall*, showed different strategies of adaptation to salinity at the seed germination stage. Seeds of *IP 19586*, germinating more gradually, decreased from 98 to 67% as saline treatment increased from 0 to 400 mM NaCl. In contrast, there was almost no effect caused by salt concentration on seed germination rates for *HHVBC Tall* line at 100–300 mM NaCl. However, when the salinity increased to 400 mM NaCl, the seed germination rate for this line decreased almost twofold. Both lines are tolerant to osmotic stress (e.g., PEGinduced drought) but sensitive to ionic stress at the seedling emergence stage. The salinity treatment affected *HHVBC Tall* more than *IP 19586*, based on proline and MDA content, confirming the greater salt tolerance potential of *IP 19586*.

Author Contributions: Conceptualization, K.T., E.S. and Z.R.; Field investigation, K.T., R.Z., T.K., F.B., Model Experiment, E.S., Z.R. and R.B.; Funding acquisition K.T.; Writing—original draft, K.T., E.S., Z.R., S.I. and S.K.G.; Writing—review & editing, K.T., E.S., S.I., S.K.G. and N.Y.

Funding: Parts of this study were conducted within the project NAS Sub-Grant Award Number: AID-OAA-A-11-00012 "Use of Non-Conventional Agricultural Water Resources to Strengthen Water and Food Security in Transboundary Watersheds of the Amu Darya River Basin (UNCAWR)", which was financially supported by the United States Agency for International Development, and within the framework of the National State Program "Scientific-based knowledge for sustainable use of water resources and promotion of technologies of improvement productivity of agricultural landscapes", which was financially supported by the Ministry of Agriculture of the Republic of Kazakhstan.

Acknowledgments: We thank Lesley Benyon, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

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

References

1. Massino, A.I.; Edenbaev, D.; Khujanazarov, T.M.; Azizov, K.; Boboev, F.; Shuyskaya, E.V.; Massino, I.V.; Toderich, K.N. Comparative performance of corn, sorghum and pearl millet growing under saline soil and water environments in Aral Sea Basin. *J. Arid Land Stud.* **2015**, *25*, 269–272.
2. Rao, N.K.; McCann, I.; Shahid, S.A.; Butt, K.U.; Al Araj, B.; Ismail, S. Sustainable use of salt-degraded and abandoned farms for forage production using halophytic grasses. *Crop Pasture Sci.* **2017**, *68*, 483–492. [CrossRef]
3. Baltensperger, D.D. *Progress with Proso, Pearl and Other Millets Reprinted from: Trends in New Crops and New Uses*; Janick, J., Whipkey, A., Eds.; ASHS Press: Alexandria, VA, USA, 2002.
4. Toderich, K.; Khalikulov, Z.; Popova, V.; Boboev, F.; Azizov, K.; Rafiev, B.; Akinshina, N.; Yuldashev, T.; Kuliev, T.; Kurbanbaev, A.; et al. Sorghum and Pearl Millet for Crop Diversification, Improved Crop-Livestock Productivity and Farmers' Livelihood in Central Asia. ICBA. 2013. p. 4. Available online: www.cac-program.org/download/file/93 (accessed on 4 August 2018).
5. Rose, D.J.; Santra, D.K. Proso millet (*Panicum miliaceum* L.) fermentation for fuel ethanol production. *Ind. Crop. Prod.* **2013**, *43*, 602–605. [CrossRef]
6. Gupta, S.K.; Rai, K.N.; Singh, P.; Ameta, V.L.; Gupta, S.K.; Jayalekha, A.K.; Mahala, R.S.; Pareek, S.; Swami, M.L.; Verma, Y.S. Seed set variability under high temperatures during flowering period in pearl millet (*Pennisetum glaucum* L. (R.) Br.). *Field Crop. Res.* **2015**, *171*, 41–53. [CrossRef]
7. Lyon, D.J.; Burgener, P.A.; DeBoer, K.L.; Harveson, R.M.; Hein, G.L.; Hergert, G.W.; Holman, T.L.; Nelson, L.A.; Johnson, J.J.; Nleya, T.; et al. *Proso Millet in the Great Plains*; Publication # EC137; University of Nebraska Extension Serv.: Lincoln, NB, USA, 2008.
8. Borde, M.; Dudhane, M.; Jite, P. Growth photosynthetic activity and antioxidant responses of mycorrhizal and non-mycorrhizal bajra (*Pennisetum glaucum*) crop under salinity stress condition. *Crop Prot.* **2011**, *30*, 265–271. [CrossRef]
9. Sneha, S.; Rishi, A.; Chandra, S. Effect of short term salt stress on chlorophyll content, protein and activities of catalase and ascorbate peroxidase enzymes in pearl millet. *Am. J. Plant Physiol.* **2014**, *9*, 32–37. [CrossRef]
10. Ali, S.A.M.; Idris, A.Y. Germination and Seedling Growth of Pearl Millet (*Pennisetum glaucum* L.) Cultivars under Salinity Conditions. *Int. J. Plant Sci. Ecol.* **2015**, *1*, 1–5.
11. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681. [CrossRef] [PubMed]
12. Mokhberdoran, F.; Nabavi Kalat, S.M.; Sadrabadi, R. Effect of temperature, iso-osmotic concentrations of NaCl and PEG agents on germination and some seedling growth yield components in rice (*Oryza sativa* L.). *Asian J. Plant Sci.* **2009**, *8*, 409. [CrossRef]
13. Patade, V.Y.; Bhargava, S.; Suprasanna, P. Effects of NaCl and iso-osmotic PEG stress on growth, osmolytes accumulation and antioxidant defense in cultured sugarcane cells. *Plant Cell Tissue Organ Cult.* **2012**, *108*, 279–286. [CrossRef]
14. Farsiani, A.; Ghobadi, M.E. Effects of PEG and NaCl stress on two cultivars of corn (*Zea mays* L.) at germination and early seedling stages. *World Acad. Sci. Eng. Technol.* **2009**, *57*, 382–385.
15. Gholamin, R.; Khayatnezhad, M. Effects of polyethylene glycol and NaCl stress on two cultivars of wheat (*Triticum durum*) at germination and early seedling stages. *Am.-Eurasian J. Agric. Environ. Sci.* **2010**, *9*, 86–90.
16. Eshghizaden, H.R.; Kafi, M.; Nezami, A. The mechanisms of salinity tolerance in the xero-halophyte Blue Panicgrass (*Panicum antidotale* Retz). *Notulae Sci. Biol.* **2012**, *4*, 59–64.
17. Ashraf, M.; Harris, P.J.C. Photosynthesis under stressful environments: An overview. *Photosynthetica* **2013**, *51*, 163–190. [CrossRef]
18. Sudhir, P.; Murthy, S.D.S. Effects of salt stress on basic processes of photosynthesis. *Photosynthetica* **2004**, *42*, 481–486. [CrossRef]
19. Allakhverdiev, S.I.; Murata, N. Salt stress inhibits photosystems II and I in cyanobacteria. *Photosynth. Res.* **2008**, *98*, 529–539. [CrossRef] [PubMed]
20. Szabados, L.; Savoure, A. Proline: A multifunctional amino acid. *Trends Plant Sci.* **2010**, *15*, 89–97. [CrossRef] [PubMed]
21. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909–930. [CrossRef] [PubMed]

22. Pérez-López, U.; Robredo, A.; Lacuesta, M.; Sgherri, C.; Muñoz-Rueda, A.; Navari-Izzo, F.; Mena-Petite, A. The oxidative stress caused by salinity in two barley cultivars is mitigated by elevated CO_2. *Physiol. Plant* **2009**, *135*, 29–42. [CrossRef] [PubMed]
23. Liu, Y.; Wang, Q.; Zhang, Y.; Cui, J.; Chen, G.; Xie, B.; Wu, C.; Liu, H. Synergistic and antagonistic effects of salinity and pH on germination in switchgrass (*Panicum virgatum* L.). *PLoS ONE* **2014**, *9*, e85282. [CrossRef] [PubMed]
24. Sulas, L.; Re, G.A.; Bullitta, S.; Piluzza, G. Chemical and productive properties of two Sardinian milk thistle (*Silybum marianum* (L.) Gaertn.) populations as sources of nutrients and antioxidants. *Genet. Resour. Crop Evol.* **2016**, *63*, 315–326. [CrossRef]
25. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
26. Heath, R.L.; Pasker, L. Photoperoxidation in isolated chloroplasts. *Arch. Biochem. Biophys.* **1968**, *125*, 180–198. [CrossRef]
27. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Method Enzymol.* **1987**, *148*, 350–382.
28. Schreiber, U. *Chlorophyll Fluorescence and Photosynthetic Energy Conversion: Simple Introductory Experiments with the TEACHING-PAM Chlorophyll Fluorometer*; Heinz Walz GmbH: Effeltrich, Germany, 1997; p. 73.
29. Orlovsky, N.S.; Japakova, U.N.; Zhang, H.F.; Volis, S. Effect of salinity on seed germination, growth and ion content in dimorphic seeds of *Salicornia europaea* L. (Chenopodiaceae). *Plant Divers.* **2016**, *38*, 183–189. [CrossRef] [PubMed]
30. Ibrahim, E.A. Seed priming to alleviate salinity stress in germinating seeds. *J. Plant Physiol.* **2015**, *92*, 38–46. [CrossRef] [PubMed]
31. Khairwal, I.S.; Rai, K.N.; Diwakar, B.; Sharma, Y.K.; Rajpurohit, B.S.; Nirwan, B.; Bhattacharjee, R. *Pearl Millet Crop Management and Seed Production*; Anual Manual; International Crops Research Institute for the Semi-Arid Tropics: Patancheru, Andhra Pradesh, India, 2007.
32. Al-Dakheel, A.J.; Iftikhar, M. Hussain Genotypic Variation for Salinity Tolerance in *Cenchrus ciliaris* L. *Front. Plant Sci.* **2016**, *7*. [CrossRef] [PubMed]
33. Munns, R.; James, R.A.; Läuchli, A. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* **2006**, *57*, 1025–1043. [CrossRef] [PubMed]
34. Aslam, R.; Bostan, N.; Nabgha-e-Amen Maria, M.; Safdar, W. A critical review on halophytes: Salt tolerant plants. *J. Med. Plants Res.* **2011**, *5*, 7108–7118. [CrossRef]