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The Salinity Threshold for Safe Saline Water Irrigation of Tall Wheatgrass in Coastal Saline-Alkaline Land around Bohai Sea

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Abstract: Saline water irrigation contributes significantly to forage yield. However, the salinity threshold for safe saline water irrigation of tall wheatgrass in coastal saline-alkaline land remains unclear. In this study, 2 g L^{-1}, 3 g L^{-1}, 4 g L^{-1}, 5 g L^{-1}, and 8 g L^{-1} saline waters were used for irrigation. Two irrigations with 2+3 g L^{-1} saline waters produced the highest yield, followed by one irrigation with 4 g L^{-1} or 5 g L^{-1} saline water. After rainfall's leaching, the soil electrical conductivity ($EC_{1:5}$) reduced by 41.7%–79.3% for the saline water irrigation treatments. In combination with saline water irrigation, plastic film mulching can be used for sward establishment of seed propagated tall wheatgrass. However, irrigation with high salinity of drainage water enhanced the risk of plant death. Further, a pot experiment demonstrated that irrigation with 5 g L^{-1} saline water led to the least reduction of forage yield and the highest crude protein content in leaves. However, the plants irrigated with ≥7 g L^{-1} saline water enhanced soil salinity and reduced plant height, leaf size, and gas exchange rate. Conclusively, one irrigation with ≤5 g L^{-1} saline water at the end of April or early May could be acceptable to maximize the forage yield of tall wheatgrass and minimize soil salinization risk in the coastal saline-alkaline land around the Bohai Sea.

Keywords: saline water irrigation; saline-alkaline soil; forage yield; tall wheatgrass; coastal grass belt

1. Introduction

According to the "14th Five-Year Plan" National Forage Industry Development Plan, released by the Ministry of Agriculture and Rural Affairs of the People's Republic of China in 2022, there is an annual shortage of 5×10^7 t forage for Chinese animal husbandry. To meet the demand, marginal land has great potential to produce forage in avoidance of competition with cereal crops for arable land and water [1–3]. For instance, in 2020 Zhensheng Li, the Academician and former vice President of the Chinese Academy of Sciences, put forward a proposal to construct a Coastal Grass Belt through the cultivation of salt-alkali tolerant forage crops on coastal saline-alkaline wasteland, which is unprofitable for food crops [4–8]. Tall wheatgrass (*Elytrigia elongata* (Host) Nevski = *Thinopyrum ponticum* (Podp.) Barkworth and D. R. Dewey (2n = 10x = 70)) is a perennial cool-season grass that confers tolerance to salt-alkali [9–13], drought [14–16], and waterlogging [17–19]. It has been widely

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cultivated in America, Australia, Argentina, Canada, and other European countries as a saline pasture or energy crop for more than half a century. However, tall wheatgrass has been neglected for 40 years since its introduction to Austral in the 1950s [20]. After tall wheatgrass cultivar Largo was introduced to Austral in the mid-1950s, two cultivars Tyrrell and Dundas were released in 1963 [20] and 2000 [21], respectively. Annually 30–70 t of commercial seed of Tyrrell is produced in Australia [20], indicative of successful industrialization. The situation was even worse in China. Since its first introduction to China, tall wheatgrass has long been utilized as a disease resistance germplasm resource for wheat (*Triticum aestivum* L.) genetic improvement. Although some tall wheatgrass varieties like Jose, Largo, and Alkar were introduced to China during the 1980s and 1990s, it has been ignored for a long time and is not widely cultivated as a forage crop in China [7]. Considering its role in coastal saline-alkaline land, Zhensheng Li's group planted tall wheatgrass in Caofeidian, Haixing, and Nanpi in Hebei Province and Dongying in Shandong Province near Bohai Sea to evaluate its adaptability and utility [7]. They screened a salt-alkali tolerant and high productive line C2 [22], which is now named as Zhongyan 1 [23]. Due to its huge and complex genome, molecular breeding techniques in tall wheatgrass lagged far behind the major cereal crops such as rice (*Oryza sativa*) and wheat [24]. Now, new breeding methods in combination with genome sequencing information are under development to breed new varieties in China. Recently, Li et al. gave some suggestions for the industrialization of tall wheatgrass in China for the construction of the Coastal Grass Belt [8].

The optimum annual precipitations of tall wheatgrass range from 350 mm to 600 mm [25]. The mean annual precipitations in the Coastal Grass Belt targeted region is 500–600 mm, however, 80% of precipitation occurs from July to September. Drought stress usually occurs in spring in this region, which severely restricts forage production. Saline-alkaline drainage waters can sometimes be used as a supplementary irrigation resource for urgent purposes. For instance, recently a long-term study demonstrated that irrigation with saline water having electrical conductivity (EC_w)=3.4 dS m^{-1} produced a similar yield as that using freshwater under maize-wheat crop rotation. However, irrigation with saline water with EC_w>3.4 dS m^{-1} resulted in yield loss and soil salinity enhancement [26]. Saline-alkaline drainage waters (4 dS m^{-1} < EC_w< 30 dS m^{-1}; 10 < sodium adsorption ratio (SAR) < 40) had been recommended to be used to irrigate salt tolerant forage crops [27]. Reuse or sequential use of saline-alkaline drainage waters has the potential for the production of salt tolerant forage crops like tall wheatgrass [27–30]. The forage yield of tall wheatgrass irrigated with saline water (EC_w=10 dS m^{-1}) was 74% of those irrigated with freshwater [31]. The tall wheatgrass, grown in saline land with a salinity below 8 g kg^{-1} and irrigated with 5.84 g L^{-1} drainage water during the first year of sward establishment, produced 7.16 t ha^{-1} hay [32]. Tall wheatgrass, growing in the highly saline fields with EC of "saturation paste extract" (EC_e)=17.6–19.1 dS m^{-1}, produced 5.9–8.3 t ha^{-1} dry matter yield after irrigated with drainage water having a salinity of EC_w=10.5 dS m^{-1} [29]. Under high salinity (EC_e=21.5 dS m^{-1}), it had a relative yield of 85% whereas the relative yield of alfalfa was 43% [33]. Additionally, tall wheatgrass can stabilize the level of moderately saline groundwater and reduce the potential for soil salinization [34].

It is reasonable to irrigate tall wheatgrass with saline-alkaline drainage waters in the Coastal Grass Belt targeted region as the rainfall leaching will counteract the risk of soil salinization [35]. However, the salinity threshold for safe saline water irrigation of tall wheatgrass to maximize forage yield without soil salt accumulation remains unclear, which restricts the utilization of tall wheatgrass for the construction of the Coastal Grass Belt. Therefore, the first objective of this study was to determine the salinity threshold for safe saline water irrigation of tall wheatgrass in the Coastal Grass Belt targeted area.

Sward establishment is critical for saline forage pasture, especially for cultivation of tall wheatgrass in coastal saline-alkaline wasteland. Plastic film mulching was considered as an effective way of producing forage with high yield and good quality of forage-oriented maize in North China [36]. In addition, plastic film mulching promoted alfalfa (*Medicago sativa* L.) forage yield with phosphorus-saving, low risk of soil nitrogen loss, and improved soil water condition in a semiarid environment [37,38]. The role of plastic film on tall wheatgrass establishment in combination with saline water irrigation and fertilization was explored in this work. Finally, the photosynthetic

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responses of tall wheatgrass to saline water irrigation were also assayed to confirm the salinity threshold of safe saline water irrigation.

2. Materials and methods

2.1. Plant materials

A tall wheatgrass line C2, also named as Zhongyan 1, was used in this study, which was previously screened for salt-alkali tolerance and high productivity by Tong et al. [22]. The experiments were carried out at the Agricultural Experiment Station for Saline-Alkaline Land in the Yellow River Delta Region (118°84′03″E, 37°68′74″N), Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. The soil salinity in the 0–10 cm soil depth ranges from 2 to 10 g kg^{-1}. The soil type is coastal clay and poor drainage. The mean annual precipitation are 500–600 mm. The precipitations in 2021 and 2022 are shown in Figure S1. The highest air temperatures of 40.6℃ and 37.9℃ were observed in June 2020 and 2021, respectively, while the lowest of –11.2℃ and –16.5℃ were observed in December 2020 and January 2021, respectively (Figure S2). Both second-year transplanted and first-year seed propagated plants were included in this study.

After the small plantlets with 8–10 tillers were prepared by hand, they were transplanted into the fields with a 4-row transplanter (2ZBX-4, Chengfan Agricultural Equipment Co., LTD, Weifang, China) in autumn 2020, which had been previously described by Li et al. [23]. The rows and plants were spaced by 0.3 m and the 4-row plots were spaced by intervals of 0.9–1.0 m space. During the first establishing year, freshwater irrigations were conducted in spring. In March 2022, 187.5 kg ha^{-1} of diammonium phosphate (DAP) was applied. Subsequently, in combination with saline water irrigation treatments, 150 kg ha^{-1} of urea was also topdressed.

Additionally, the seed propagated plants were used to explore the effects of saline water irrigation and plastic mulching on sward establishment and forage yield. The seeds with the purity of 50%–60% were sown at the seeding rate of 18 kg ha^{-1} on October 23, 2021, with a drill planter. The spacing between the rows and plants was 0.3 m and less than 0.2 m, respectively. A randomized block design involving three factors such as plastic film mulching, saline water irrigation, and fertilization was performed. Plastic film mulching was started on December 5, 2021, and ended on March 4, 2022. Seven nitrogen (N) levels including NP0 (no fertilizer), NP1 (56 kg N ha^{-1}, 158 kg P_2O_5 ha^{-1}), NP2 (73 kg N ha^{-1}, 205 kg P_2O_5 ha^{-1}), NP3 (90 kg N ha^{-1}, 252 kg P_2O_5 ha^{-1}), N4 (86 kg N ha^{-1}), N5 (138 kg N ha^{-1}), N6 (190 kg N ha^{-1}) were conducted by application of DAP or urea on April 29, 2022. One irrigation with 3 g L^{-1} saline water on May 25 and two irrigations, the first irrigation with 8 g L^{-1} saline water on May 3, followed by a second irrigation with 3 g L^{-1} saline water on May 25, were carried out. The plot area for each treatment was around 20 m^2.

2.2. Irrigation treatments with saline drainage water

The flooding irrigation treatments with saline drainage waters were performed in April and May 2022. The salinities of the available drainage waters varied depending on month. Before irrigation, the salinity of drainage water was determined by using a salinity meter (EB-158DP, Japan). Usually, approximately 600 m^3 ha^{-1} drainage water was used for one irrigation. The salinities for irrigation were 2 g L^{-1} (EC_w=2.45 dS m^{-1}, pH=8.3), 3 g L^{-1} (EC_w=4.36 dS m^{-1}, pH=8.5), 4 g L^{-1} (EC_w=4.42 dS m^{-1}, pH=8.0), 5 g L^{-1} (EC_w=5.42 dS m^{-1}, pH=8.5), and 8 g L^{-1} (EC_w=7.13 dS m^{-1}, pH=8.3) (Table 1). Except that the saline water having a salinity of 2 g L^{-1} came from a mixture of freshwater and saline water while the others were from the nearby drainage ditch directly. The irrigated area for each treatment with three repeats was around 0.2 ha.

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Table 1. The soil electrical conductivity (EC) and pH as well as tall wheatgrass dry matter yield after saline water irrigation.

Water salinity ($g\ L^{-1}$)	Irrigation date (Month/day)	$EC_{1:5}$ ($dS\ m^{-1}$) Sampling dates (Month/day) 05/17	10/21	Percentage change (%)	pH Sampling dates (Month/day) 05/17	10/21	Percentage change (%)	Dry matter yield ($kg\ ha^{-1}$)
CK[a]	**-[b]**	**0.69 ± 0.36c**	**0.64 ± 0.50a**	−7.2	8.44 ± 0.14bc	8.70 ± 0.24c	3.1**	1839 ± 264d
3	05/27	0.36 ± 0.13d	0.21 ± 0.12b	−41.7**	8.74 ± 0.08a	8.90 ± 0.09b	1.8**	3823 ± 676c
4	05/02	0.58 ± 0.07c	0.12 ± 0.01b	−79.3**	8.52 ± 0.08b	9.08 ± 0.08a	6.6**	5838 ± 548b
5	04/24	1.20 ± 0.49a	0.69 ± 0.18a	−42.5**	8.28 ± 0.14d	8.68 ± 0.10c	4.8**	5627 ± 242b
2+3[c]	04/26+05/25	0.91 ± 0.35b	0.24 ± 0.12b	−73.6**	8.42 ± 0.18c	8.84 ± 0.12b	5.0**	6962 ± 196a
5+3[c]	04/26+05/28	0.76 ± 0.05b	0.24 ± 0.13b	−68.4**	8.53 ± 0.07b	9.10 ± 0.19a	6.7**	4253 ± 461c

Notes: [a]CK and [b]- indicate unirrigated. [c]Two irrigations. Data are represented as mean ± SD (n=5 for $EC_{1:5}$ and pH as well as n=3 for dry matter yield). Different letters indicate significant differences at $p < 0.05$. **, means the significant difference between two measurements ($p < 0.01$).

2.3. Determination of EC, pH, and water content

The soil samples were collected at 0–10 cm, 10–20 cm, and 20–30 cm depths following the five-point diagonal sampling method. After air drying for one week, the soil samples were grounded and passed through a 0.25 mm screen. When measuring, 3 g of soil powder sample was diluted in 15 mL of purified water. Then, the EC was measured with a conductivity meter (DDS-12DW, Shanghai LIDA Instrument Factory, China) while the pH was measured with a pH meter (pH848, Smart Sensor, China). The soil water content was determined as the ratio of moisture mass to the soil dry mass.

2.4. Evaluation of plant height, dry matter yield, and death rate

Before harvesting on June 8, the plant height from 10 plants was measured as the height from the soil surface to the highest plant tip. For each treatment, three randomly selected 2.0 m×3.5 m plots were handily harvested with 10–15 cm of stubble height left. Then, the fresh forage yield was weighed immediately. The ratio of dry to fresh weight was determined with 1.2–2.0 kg plant samples oven-dried at 65°Cfor 72 h, which was used to compute dry matter yield. Additionally, the death rate was also surveyed on five randomly selected plots which area was 3.4 m^2 (1.7 m×2 m).

2.5. Pot experiment: sufficient irrigation with saline water

A total of 25 plantlets, handily divided from a single two-year plant on November 6, 2022, were planted in plastic pots. Each plantlet was planted in one pot which size was 24 cm×26 cm×18.8 cm (upper inner diameter × height × bottom inner diameter). Each pot was filled with 2.6 kg culture medium composed of field soil: substrate (0–10 mm, Pindstrup, Denmark) =1:3. The $EC_{1:5}$ and pH in the field soil were 4.03 $dS\ m^{-1}$ and 8.4 while they were 2.13 $dS\ m^{-1}$ and 6.7 in the mixed culture medium, respectively. The content of available N and P_2O_5 in the culture medium was 744.0 $mg\ kg^{-1}$ and 907.8 $mg\ kg^{-1}$, respectively. On May 9, 2023, 5 g urea was applied to each pot, resulting in a supplement of 885 $mg\ N\ kg^{-1}$. All the plants were cultured outdoors during the whole treatment.

The pot experiment sufficiently irrigated with saline water was carried out in two phases: salt stress and leaching phase. Salt stress was performed with five saline water irrigations including the salinities of 0 $g\ L^{-1}$ (EC_w=1.02 $dS\ m^{-1}$, pH=8.0), 5 $g\ L^{-1}$, 7 $g\ L^{-1}$ (EC_w=6.31 $dS\ m^{-1}$, pH=8.1), 10 $g\ L^{-1}$ (EC_w=8.61 $dS\ m^{-1}$, pH=8.1), and 13 $g\ L^{-1}$ (EC_w=9.60 $dS\ m^{-1}$, pH=8.5). The 10 $g\ L^{-1}$ saline water was collected from a drainage ditch on March 16, 2023, which was diluted with tap-water to the 5 $g\ L^{-1}$ and 7 $g\ L^{-1}$ salinities and concentrated to 13 $g\ L^{-1}$ in sunlight. Saline water irrigation was started from April 13 to May 23, followed by irrigation with tap water until harvesting on June 26. For each pot, a volume of 10 L saline water was irrigated in the salt stress phase for 40 d, followed by luxurious leaching with 26 L tap water for 26 d. During rainy days all the pots were covered with a plastic shed in avoidance of rainfall effects. The plant height, leaf size, and gas exchange were assessed on all five pots for each treatment at the end of both two phases. The curled leaf width ratio was determined as the ratio of curled leaf width to expanded leaf width. Dry matter yield per plant was determined ultimately.

2.6. Gas exchange measurement

The gas exchange parameters such as gas exchange rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate were determined in the middle parts of leaves from 9:00 am to 11:00 am by using a gas-exchange system (GFS3000, WALZ, Germany). When measuring, the light intensity was set as 1200 μmol m^{-2} s^{-1}, CO_2 concentration was around 410 μmol mol^{-1}, and airflow rate was 750 μmol s^{-1}, accordingly. The mean ambient air temperature and relative humidity were 28°C and 35% for the penultimate leaf measurements and 30°C and 55% for the flag leaf measurements, respectively. The penultimate and flag leaves were assayed at the end of the salt stress and before harvest, respectively. All five plants for each treatment were measured.

2.7. Crude protein content determination

After oven-dried at 65°C for 72 h and grounded with a ZM200 Ultra Centrifugal Mill (Retsch, Germany), about 0.1 g dry powder samples were used to determine the total N content by using a Kjeltec Analyser (8400, FOSS, Denmark). Subsequently, the crude protein content (%) was calculated by multiplying the total N content with a factor of 6.25.

2.8. Assessment of concentrations of ions in the representative drainage waters

The concentrations of ions in the drainage waters with salinities of 4 g L^{-1}, 5 g L^{-1}, and 10 g L^{-1} were determined by Beijing Yandu Taihua Enterprise Management Group Co., Ltd. The cations were assessed with an iCAP 6000 Series ICP Emission Spectrometer (Thermo Fisher Scientific, USA) while the anions were determined by using a Dionex ICS-1000 Ion Chromatography System (Thermo Fisher Scientific, USA) according to the manufacturer's instruction.

2.9. Data summary and statistical analysis

One-way analysis of variance (ANOVA), univariate ANOVA based on the General Linear Model, multiple comparison, least significant difference (LSD) test, and an independent t-test were performed by using the SPSS software (version 19.0, IBM, Armonk, NY, USA). Data were expressed as mean ± standard deviation (SD), which was used for figures depicture.

3. Results

3.1. The effects of saline irrigation on soil salinity and forage yield of transplanted tall wheatgrass.

The soil salinity and pH at 0–10 cm depth, sampled on May 17 and October 21, and forage dry matter yield were summarized in Table 1. A total of 437 mm and 415 mm precipitations occurred between two sampling dates in 2021 and 2022 (Figure S1), respectively, which caused considerable reductions in soil salinity. For instance, the $EC_{1:5}$ of the unirrigated control decreased from 0.69 dS m^{-1} to 0.64 dS m^{-1} while for the saline water irrigation treatments, the reductions of $EC_{1:5}$ ranged from 41.7% to 79.3%. However, at the same time, the pH values increased for all the saline water irrigation treatments after rainfall's leaching (Table 1). Therefore, it appeared that irrigation with saline water in spring influenced little to the soil salinity due to rainfall's leaching.

The dry matter yields in the plants irrigated with saline waters were significantly higher than in the unirrigated control (Table 1). Two irrigations with 2+3 g L^{-1} saline waters produced the highest dry matter yield (6962 kg ha^{-1}), followed by one irrigation with 4 g L^{-1} or 5 g L^{-1} saline water. Next, the fields treated with two irrigations of 5+3 g L^{-1} saline waters produced less than those treated with one irrigation of 4 g L^{-1} or 5 g L^{-1} saline water, but slightly more than one irrigation with 3 g L^{-1} saline water. The lower yield in the plants irrigated with 3 g L^{-1} saline water on May 27 relative to those irrigated with 4 g L^{-1} (May 2) or 5 g L^{-1} (April 24) saline water indicated that irrigation time played a key role in forage yield formation. The forage yield of tall wheatgrass irrigated with 5+3 g L^{-1} saline

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waters was significantly lower than that irrigated with 2+3 g L^{-1}, 5 g L^{-1} or 4 g L^{-1} saline water, demonstrating that high water salinity exerted a negative effect on forage yield. Taken together, it can be deduced that irrigation with ≤5 g L^{-1} saline waters may be acceptable to maximize the forage yield of tall wheatgrass and have a low risk of soil salt accumulation.

3.2. The effects of saline water irrigation on forage yield and death rate of seed propagated tall wheatgrass.

Three factors including plastic film mulching, fertilization, and saline water irrigation were considered to determine the key factor for the sward establishment of seed propagated tall wheatgrass. ANOVA analysis showed that the plant height and forage yield in all three single factor treatments differed significantly (p <0.01). In addition, the interactions of mulching × saline water irrigation as well as mulching × fertilization also differed significantly (Table 2). To explore the effects of saline water irrigation on soil salinization, the $EC_{1:5}$ in the soil depths of 0–10, 10–20, and 20–30 cm were determined on May 17, June 21, October 21, and December 10 in 2022, and February 23 in 2023 (Tables S1 and S2). Inconsistent difference of $EC_{1:5}$ between the mulched and unmulched treatment suggested that mulching with plastic film for 68 d appeared to have a marginal impact on soil salinization due to saline water irrigation (Table S1). The $EC_{1:5}$ peaked on June 21 but declined drastically after the rainy season's leaching, resulting in reductions of 15.9%–75.8%, 23.5%–69.4%, and 21.2%–61.3% for two irrigations and 34.5%–71.7%, 43.1%–65.5%, and 39.7%–48.7% for one irrigation on October 21 and December 10 in 2022, and February 23 in 2023, accordingly (Table S2).

Under mulching conditions, the plant height of tall wheatgrass treated with two irrigations of 8+3 g L^{-1} saline waters was significantly higher than that irrigated once with 3 g L^{-1} saline water independent of fertilization levels (Figure 1a). It was similar for plant height under no mulching condition (Figure 1c) and for dry matter yield under mulching condition (Figure 1b), although the difference was not significant at NP0 level. Under no mulching condition, the differences in dry matter yield between the two irrigation treatments were significant only at NP0 and NP1 levels (p < 0.05).

Under two irrigations and mulching conditions, the plant height and dry matter yield increased significantly from the NP1 level in comparison with the NP0 control. However, under one irrigation and mulching conditions, no significant increment was found for dry matter yield due to fertilization (Figure 1b). It seemed that the effects of irrigation and fertilization on plant height and forage yield were negligible under no mulching conditions (Figure 1c,d). Collectively, plastic film mulching, followed by saline water irrigation determined sward establishment and dry matter yield to a large extent. Relatively, fertilization appeared to play a marginal role in sward establishment and the first year's forage yield of seed propagated tall wheatgrass.

Table 2. ANOVA analysis of plant height and forage yield.

Variation Source	df	Plant height		Dry matter yield	
		Mean Square	F	Mean Square	F
Corrected Model	27	2674.8	44.2**	6238649	57.8**
Intercept	1	1428151.6	23600.2**	227600000	2110.5**
Saline water irrigation	1	16417.7	271.3**	13137343	121.8**
Mulching	1	41883.0	692.1**	126500000	1173**
Fertilization	6	610.6	10.1**	672596	6.2**
Saline water irrigation × mulching	1	496.5	8.2**	8854228	82.1**
Saline water irrigation × fertilization	6	157.1	2.6*	83811	0.8
Mulching × fertilization	6	173.8	2.9*	959185	8.9**
Saline water irrigation × mulching × fertilization	6	45.2	0.7	160269	1.5

Notes: *, ** denote significant differences at $p < 0.05$ and $p < 0.01$, respectively.

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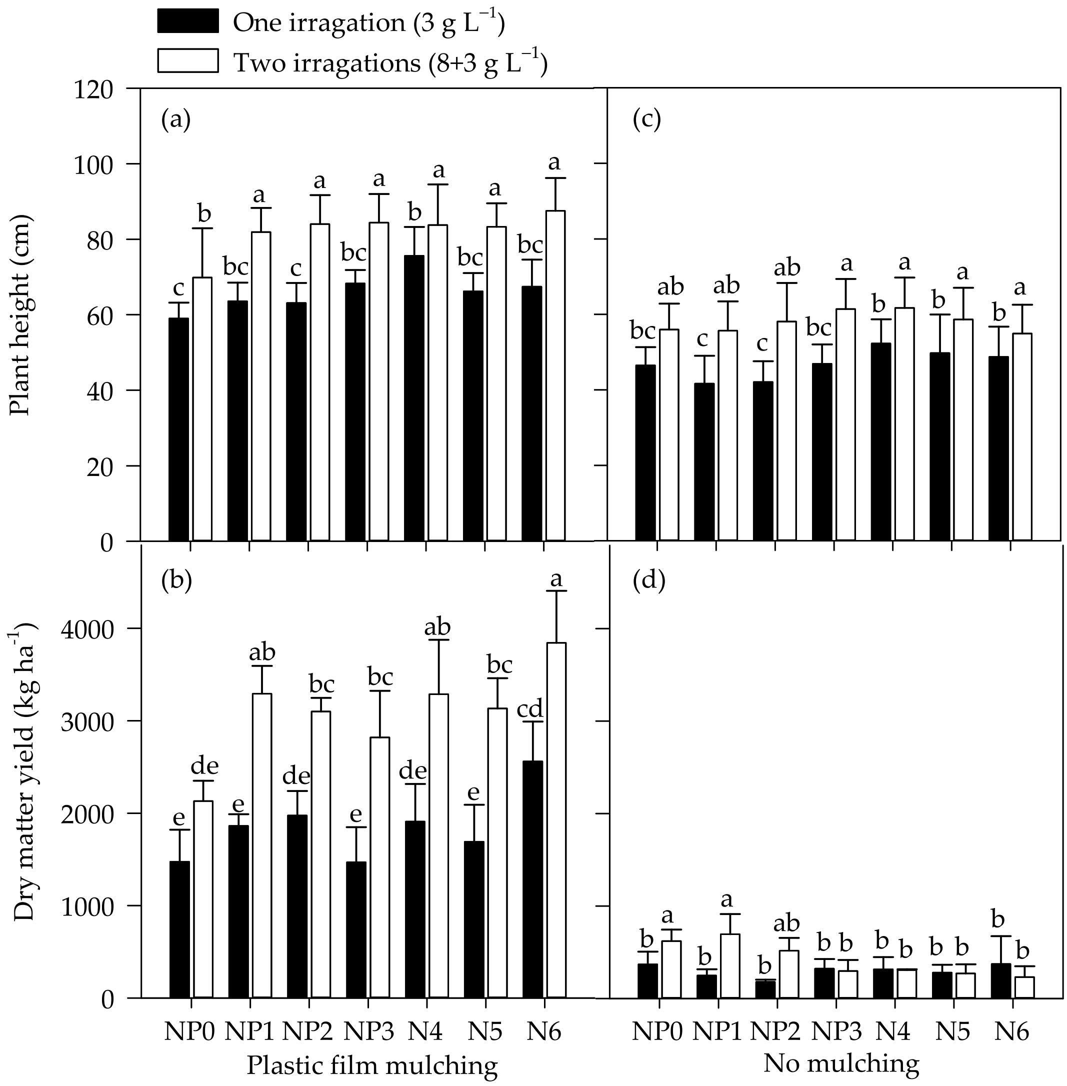

Figure 1. The effects of saline water irrigation, plastic film mulching, and fertilization on plant height and forage yield of tall wheatgrass. Two irrigations with 8+3 g L⁻¹ saline water were performed on May 3 and 25, respectively, while one irrigation with 3 g L⁻¹ saline water was carried out on May 25. Data are represented as mean ± SD (n=10 for plant height and n=3 for dry matter yield). Different letters indicate significant differences at $p < 0.05$.

For the unmulched control, 16% of plants were dead when treated with two irrigations of 8+3 g L^{-1} saline waters, which was significantly higher than those irrigated once with 3 g L^{-1} saline water. In addition, for plastic film mulching treatment, no dead plant was observed for one irrigation of 3 g L^{-1} saline water while 3.2% of plants were dead when two irrigations of 8+3 g L^{-1} saline waters were applied (Figure 2). Therefore, one irrigation with low salinity water is favorable for the sward establishment of tall wheatgrass.

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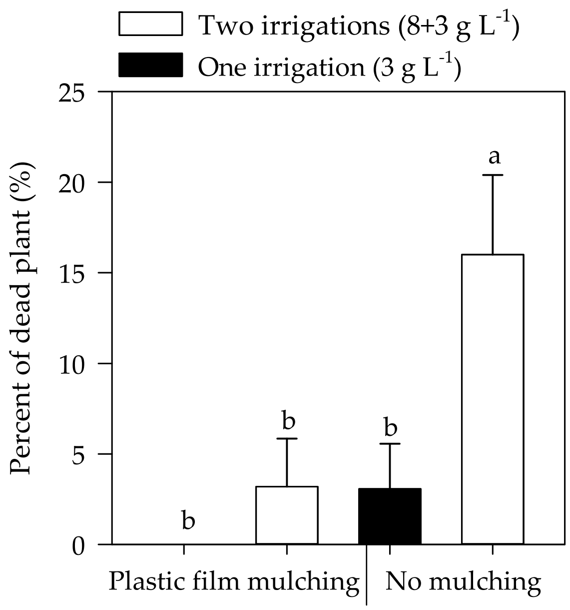

Figure 2. The percentage of dead plants irrigated with saline waters. Two irrigations with 8+3 g L⁻¹ saline waters were performed on May 3 and 25, respectively, while one irrigation with 3 g L⁻¹ saline water was carried out on May 25. Data are represented as mean ± SD (n=5). Different letters indicate significant differences at $p < 0.05$.

3.3. The effects of sufficient irrigation with saline water on soil salinity and forage yield.

To confirm the field saline water irrigation results, a pot experiment was carried out. Both the salt stress phase, no irrigation water leaked, and the leaching phase, nearly half of the irrigation water leaked, were conducted as a consecutive process. At the end of salt stress, the soil $EC_{1:5}$ in the culture medium increased from 2.13 dS m⁻¹ to 5.15 dS m⁻¹, 8.60 dS m⁻¹, 10.64 dS m⁻¹, 11.97 dS m⁻¹, and 12.06 dS m⁻¹ for irrigation with salinities of 0 g L⁻¹, 5 g L⁻¹, 7 g L⁻¹, 10 g L⁻¹ and 13 g L⁻¹, accordingly. Then, at the end of the leaching phase, the soil $EC_{1:5}$ was reduced to 1.29–1.52 dS m⁻¹, which was lower than the pretreatment level (Table 3). Meantime, the soil pH decreased from 6.7 to 6.1–6.3 under salt stress but increased to 7.1–7.3 at the leaching phase. The soil water content elevated significantly when irrigated with saline waters at the salt stress phase. However, the difference was not significant at the leaching phase. Comparatively, the irrigation with 5 g L⁻¹ salinity water had the lowest soil water content (81.9%) among all the four saline water irrigation treatments at the salt stress phase.

Table 3. The soil electrical conductivity (EC), pH, and water content as well as dry matter yield and crude protein content of tall wheatgrass after saline water irrigation and tap water leaching.

Water Salinity (g L⁻¹)	Salt stress phase			Leaching phase			Dry matter yield (g plant⁻¹)	Crude protein content (%)	
	$EC_{1:5}$ (dS m⁻¹)	pH	Water content (%)	$EC_{1:5}$ (dS m⁻¹)	pH	Water content (%)		Leaf	Stem
0	5.15 ± 1.69d	6.1 ± 0.2a	64.1 ± 10.1c	1.29 ± 0.28b	7.24 ± 0.15ab	120.2 ± 5.7a	44.74 ± 9.03a	13.58 ± 0.64b	9.32 ± 0.48a
5	8.60 ± 0.54c	6.2 ± 0.1a	81.9 ± 7.6b	1.46 ± 0.34ab	7.14 ± 0.09c	120.8 ± 8.8a	31.93 ± 6.85b	15.45 ± 1.52a	9.09 ± 0.59a
7	10.64 ± 0.51b	6.2 ± 0.2a	104.4 ± 9.0a	1.45 ± 0.21ab	7.16 ± 0.08bc	123.9 ± 7.4a	23.12 ± 5.29bc	14.31 ± 1.00a	8.99 ± 0.43a
10	11.97 ± 0.77a	6.3 ± 0.3a	98.7 ± 11.5a	1.44 ± 0.22ab	7.26 ± 0.15a	112.3 ± 6.7b	24.78 ± 8.13bc	14.11 ± 0.71a	9.10 ± 0.56a
13	12.06 ± 1.22a	6.1 ± 0.0a	104.9 ± 5.0a	1.52 ± 0.25a	7.30 ± 0.15a	120.1 ± 7.7a	22.00 ± 2.51c	14.25 ± 1.38a	8.80 ± 0.20b

Notes: Data are represented as mean ± SD (n=5). Different letters indicate significant differences at $p < 0.05$.

The dry matter yield per plant reached the highest value (44.74 g) when irrigated with tap water. However, 28.6%, 48.3%, 44.6%, and 50.8% of yield reductions occurred in plants irrigated with 5 g L⁻¹, 7 g L⁻¹, 10 g L⁻¹, and 13 g L⁻¹ saline waters, accordingly. Saline water irrigation appeared to improve the crude protein content in leaves of tall wheatgrass (Table 3). For instance, the crude protein content in the leaves of plants irrigated with 5 g L⁻¹ saline water reached the highest value (15.45%). However, no significant difference was observed in the stems of tall wheatgrass. Comparatively, irrigation with 5 g L^{-1} saline water resulted in the least yield reduction and enhancement of crude protein content in leaves.

3.4. The effects of sufficient irrigation with saline water on plant height and tiller number.

As shown in Figure 3a, the plant statue reduced with the increase of the water salinity for irrigation. Additionally, more curled and yellow leaves were found in the plants irrigated with a salinity of ≥7 g L^{-1} saline water. The plant height and tiller number were surveyed at the start and end of salt stress as well as after tap water leaching. No significant difference was observed for both plant height and tiller number after saline water irritation for 6 d. However, the plant height was reduced significantly by irrigation with ≥7 g L^{-1} saline waters for 40 d. It was significantly lower in plants irrigated with saline water than those irrigated with tap water (Figure 3b). Interestingly, no significant difference was observed for the tiller number at both the start and end of saline water treatment. However, at the end of the leaching phase, the plants irrigated with ≥7 g L^{-1} saline waters had a slightly less tiller number (Figure 3c).

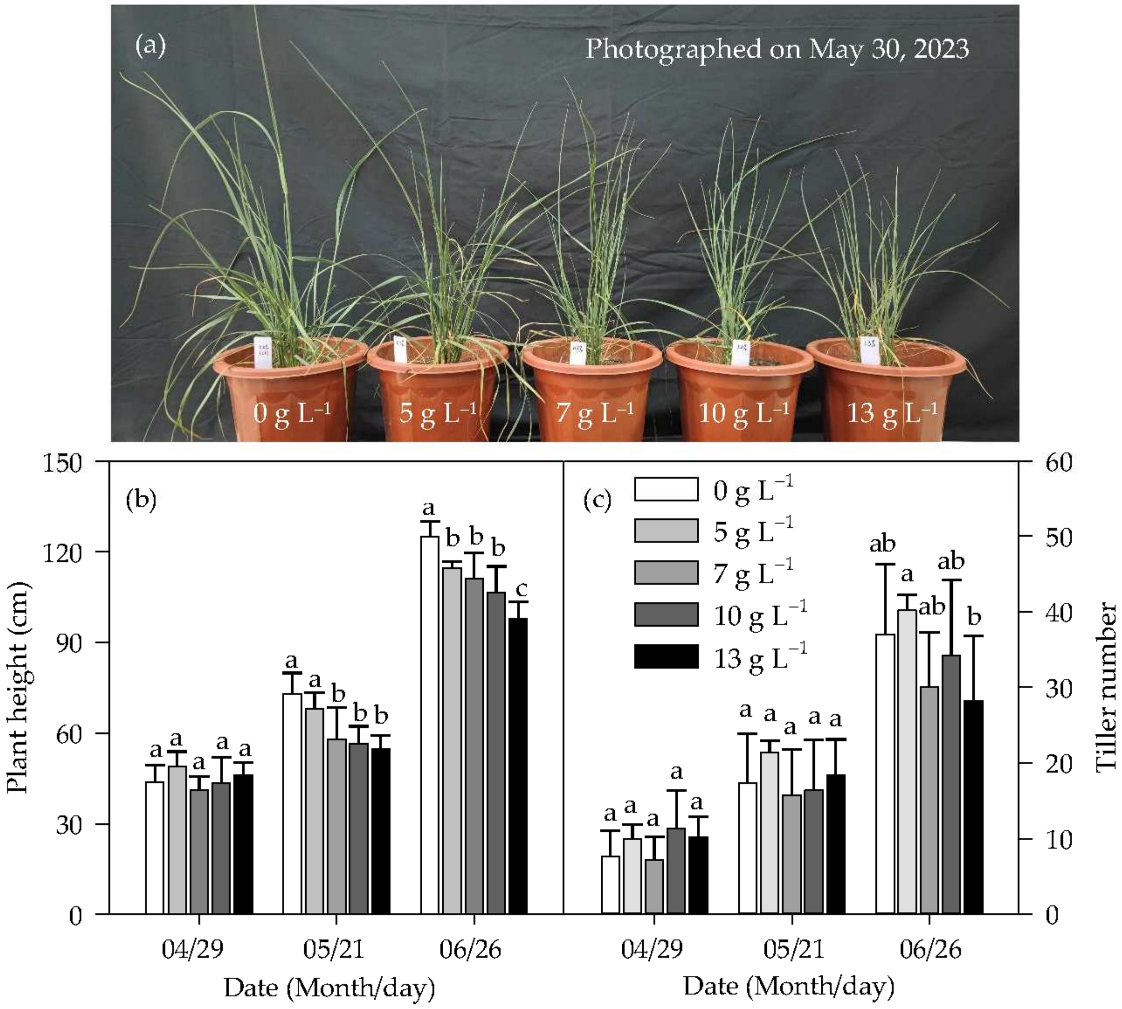

Figure 3. The photograph, plant height, and tiller number of tall wheatgrass irrigated with saline (salt stress, before May 21) and tap water (leaching phase, before June 26). Data are represented as mean ± SD (n=5). Different letters indicate significant differences at $p < 0.05$.

3.5. The effect of sufficient irrigation with saline water on leaf size.

The leaf width and length of the penultimate leaves reduced significantly in plants irrigated with ≥5 g L^{-1} and ≥7 g L^{-1} saline waters under salt stress (Figure 4a), respectively. Even at the leaching

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phase, the sizes of flag leaves also reduced in plants irrigated with ≥5 g L^{-1} saline water for leaf length and ≥7 g L^{-1} saline water for leaf width (Figure 4b). The curled leaf width ratio declined in the penultimate leaves in plants irrigated with ≥10 g L^{-1} saline water while it changed little in the flag leaves after leaching treatment.

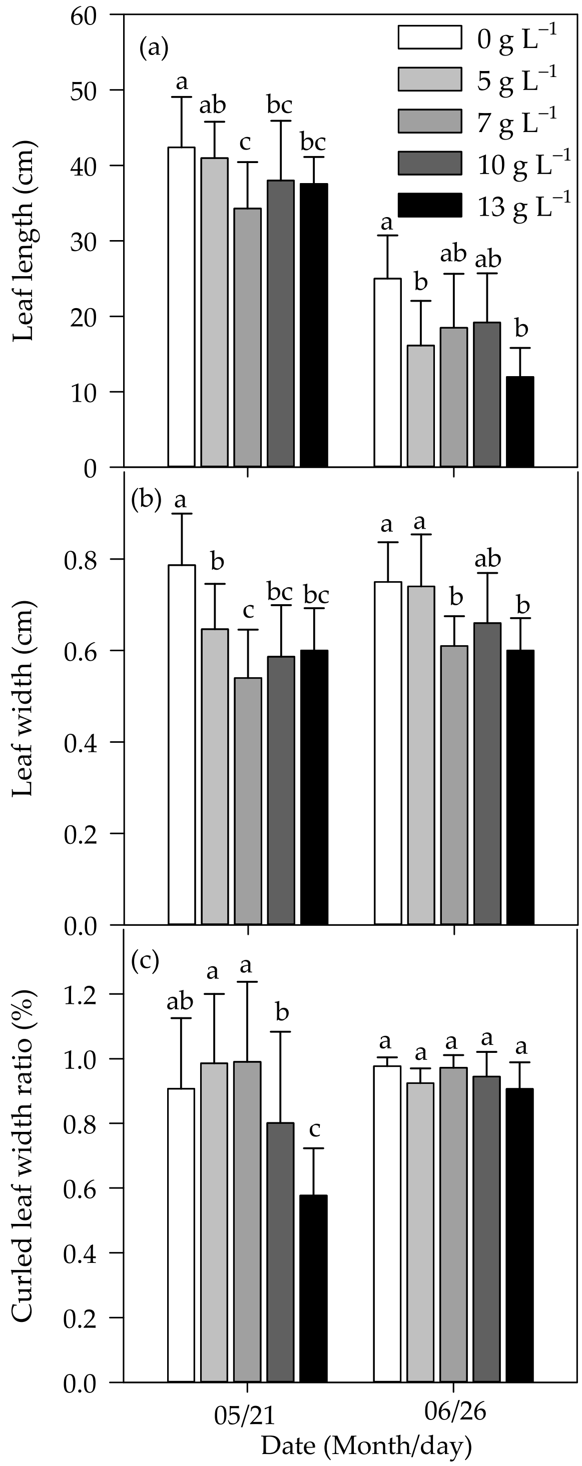

Figure 4. Leaf length, width, and curled width ratio of tall wheatgrass irrigated with saline water. The data, collected on May 21 and June 26, were from the penultimate and flag leaves, respectively. Data are represented as mean ± SD (n=10). Different letters indicate significant differences at $p < 0.05$.

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3.6. The effect of sufficient irrigation with saline water on gas exchange.

Further gas exchange analysis demonstrated that at the end of salt stress, the gas exchange rate in the penultimate leaves declined consistently with the increase of irrigation water salinity (Figure 5a). The stomatal conductance and transpiration rate showed a similar trend as the gas exchange rate (Figure 5b,d). The intercellular CO_2 concentrations in the plants irrigated with 5 g L^{-1} and 7 g L^{-1} saline waters were significantly lower than the control at the salt stress phase (Figure 5c), suggesting that the lower gas exchange rate was majorly determined by stomatal closure. The intercellular CO_2 concentrations in the plants irrigated with 10 g L^{-1} and 13 g L^{-1} saline water were not significantly different from the control (Figure 5c), indicating that the decline of the gas exchange rate was not only due to the closure of the stomata but also due to the restriction of carbon fixation enzymes' activity. At the end of the leaching phase, the gas exchange rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate in flag leaves of plants irrigated with saline water were all significantly lower than those irrigated with tap water, demonstrating that saline exerted profound impacts on gas exchange. The effects of saline water irrigation on gas exchange in this study appeared cannot be eliminated after leaching treatment. Especially, the stomatal conductance and transpiration rate seemed to be more sensitive to saline water irrigation with salinity ≥10 g L^{-1} (Figure 5b,d).

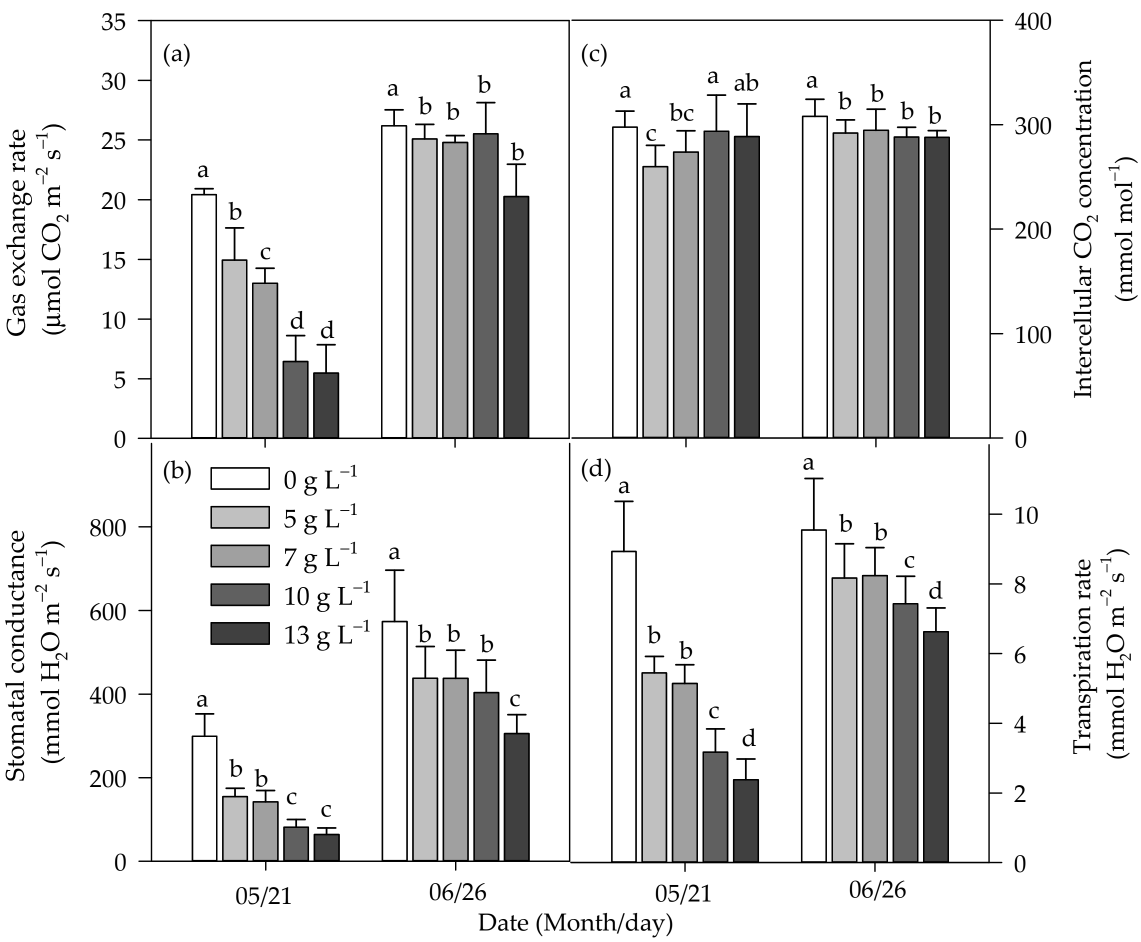

Figure 5. Gas exchange parameters in the penultimate and flag leaves after saline water irrigation on May 21, followed by tap water leaching on June 26, respectively. Data are represented as mean ± SD (n=5). Different letters indicate significant differences at $p < 0.05$.

4. Discussion

Saline drainage water is a potential resource for salt tolerant forage crops for urgent purposes. It is important to balance forage production and soil salinization which may in turn reduce forage yield for long-term saline water irrigation. Tall wheatgrass is salt-alkali tolerant grass with a

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relatively greater salt accumulator and accumulates a high content of Na, K, Mg, and Ca ions [39]. In this study, once and twice irrigation with various salinities of saline-alkaline drainage waters was carried out in the field in the Yellow River Delta Region to determine the salinity threshold for safe saline water irrigation of tall wheatgrass. The field experiments revealed that tall wheatgrass irrigated once with 5 g L^{-1} saline water produced significantly higher forage yield (5627 kg ha^{-1}) than the unirrigated control. Irrigation with lower salinity water increased forage yield. For instance, one irrigation with 4 g L^{-1} saline water produced 5838 kg ha^{-1} dry matter, which was higher than that irrigated with 5 g L^{-1} saline water. However, two irrigations with 5+3 g L^{-1} saline waters produced a lower yield (4253 kg ha^{-1}). The highest dry matter yield was observed in two irrigations with 2+3 g L^{-1} saline waters (6862 kg ha^{-1}). A previous study showed that tall wheatgrass, growing in the highly saline fields with EC_e=17.6–19.1 dS m^{-1}, produced 5.9–8.3 t ha^{-1} dry matter yield after irrigation with EC_w=10.5 dS m^{-1} drainage water [29]. The lower dry matter yield in this study may be due to harvest time, sampling method, or environmental factors. Highly saline waters not only reduce dry matter yield but also enhance death rate of the first year seed propagated tall wheatgrass. For instance, two irrigations with 8+3 g L^{-1} saline waters of the first year seed propagated tall wheatgrass resulted in 3.2% and 16.0% of dead plants under mulching and no mulching conditions, respectively.

Sward establishment of seed propagated tall wheatgrass is critical for saline pasture and forage production. In combination with saline water irrigation, the roles of fertilization and mulching on sward establishment were also assayed in this work. Comparatively, plastic film mulching, followed by saline water irrigation determined sward establishment and dry matter yield to a large extent. Relatively, fertilization appeared to play a marginal role in sward establishment and the first year forage yield. Plastic film mulching in winter increased temperature and protected soil moisture, which promoted plant growth of tall wheatgrass in winter in coastal regions around the Bohai Sea. It is an effective technique for sward establishment of tall wheatgrass in this region to mulch plastic film in mid-late November and uncover at the end of February or the start of March. Plastic film mulching can increase one times tillers and one to two times forage yield in comparison with the unmulched control (unpublished data), which was consistent with the forage-oriented maize [36] and alfalfa [37,38]. This is the first report on the role of plastic film mulching on the sward establishment of tall wheatgrass, which can guide the cultivation of tall wheatgrass on highly saline-alkaline soils. Although the forage yield increment for plastic film mulching is significant, it is not recommended to be performed on tall wheatgrass from second year when considering mulching costs. The marginal effects of fertilization on the sward establishment of tall wheatgrass may be due to the fertile fields used in this work, which are sufficient to support the first year's sward establishment. Hence, fertilization is possibly essential for the sward establishment of tall wheatgrass in barren and highly saline-alkaline soils. Although two irrigations with 8+3 g L^{-1} saline waters enhanced the death rate, such treatment produced more dry matter than one irrigation with 3 g L^{-1} saline water. Therefore, saline water irrigation can contribute a positive effect on the forage yield of seed propagated tall wheatgrass in its first year's establishment. Irrigation with high drainage water salinity should be avoided to minimize the risk of enhancing the percentage of dead plants.

In addition, the pot experiment demonstrated that sufficient irrigation with ≥7 g L^{-1} saline water doubled soil salinity and reduced plant height, leaf size, and leaf gas exchange rate compared to tap water irrigation. Also, significant salt-induced damage such as leaf yellow and necrosis was observed in plants irrigated with ≥7 g L^{-1} saline water. Relatively, among the four saline water treatments (5–13 g L^{-1}), the irrigation with 5 g L^{-1} had the least reduction of forage yield. Therefore, 5 g L^{-1} (EC_w=5.42 dS m^{-1}) appeared to be the salinity threshold for safe irrigation of tall wheatgrass in the coastal region around the Bohai Sea. Bazzigalupi et al. recommended that the NaCl solution with EC_w=18 dS m^{-1} be used to screen and breed salt tolerant tall wheatgrass [40]. Bennett et al. reported that the tall wheatgrass plants cultured in nutrient solution with EC_w=10 dS m^{-1} resulted in a 50% reduction of dry matter yield [17], which was consistent with the 13 g L^{-1} (EC_w=9.6 dS m^{-1}) saline water irrigation in this study. However, Riedell found that the nutrient cultivation with EC_w=10 dS m^{-1} did not reduce growth but that with EC_w=30.2 dS m^{-1} resulted in a 50% reduction of dry matter yield in tall wheatgrass plants [41]. These above results were from laboratory or pot experiment factors, which

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might be inconsistent with the field situation. Considering land sustainability and forage yield persistence, 5 g L^{-1} was recommended as the salinity threshold for safe saline water irrigation of tall wheatgrass in the Coastal Grass Belt targeted area. Interestingly, a recent study demonstrated that 5 g L^{-1} desalinated saline water is the salinity threshold for drip irrigation to avoid emitter clogging [42]. Additionally, Wu et al. predicted that the highest soil salt content of 4.97 g kg^{-1} is the salinity threshold of the cotton root zone in arid areas [43]. In addition to the effects of saline water irrigation on plant growth and forage yield, saline water irrigation also enhanced leaf crude protein content, which was consistent with Temel et al. [44].

Usually, the composition of drainage water is complex and may contain potentially toxic trace elements. For instance, in California's San Joaquin Valley, the drainage eluents contain high concentrations of selenium (Se), boron (B), and molybdenum (Mo) [27,31,33,45,46]. Trace element concentrations indicate that Se toxicity is of little concern, but high concentrations of both Mo and sulfur (S) in the herbage may lead to copper (Cu) deficiency in ruminants. Se is accumulated in forage hay of tall wheatgrass when grown in Se-enriched soils or irrigated with saline-alkaline drainage waters with high concentrations of Se, which can be used as a value added in the base diet [46]. In addition, the Na_2SO_4 dominated drainage waters will probably increase S concentrations in forage products to high levels, which may result in excessive accumulation of S in the rumen and potentially cause serious animal neurological diseases [45]. Therefore, for safe irrigation with saline-alkaline drainage waters, in addition to salinity, the concentrations of toxic trace elements should be considered to produce high-quality commercial forage products. According to the concentrations of ions in the drainage waters used in this study, the water salinity is NaCl dominated. The SAR was 30.46, 36.31, and 40.39 for the salinities of 4 g L^{-1}, 5 g L^{-1}, and 10 g L^{-1} drainage waters, accordingly. The concentrations of the trace element of B (<1 mg L^{-1}), Mo (<0.01 mg L^{-1}), and Se (<0.01 mg L^{-1}) in the drainage waters were very low, which means low risk of toxicity to plant growth and forage quality when irrigated tall wheatgrass. However, the ratios of Mg/Ca were 1.93, 2.06, and 1.72 for the salinities of 4 g L^{-1}, 5 g L^{-1}, and 10 g L^{-1} drainage waters, accordingly, indicative of high Mg concentrations. Mg is important for plant growth because of its roles as an activation agent of enzymes and a component of chlorophyll. However, similar to Na^{+}, high levels of Mg^{2+} may cause deleterious effects on soil structure, reduced soil permeability, and significantly declined crop productivity [47]. When the ratios of Mg/Ca in irrigation waters were >1, the risk of soil structure deterioration increased [48]. Therefore, irrigation with a high ratio of Mg/Ca of drainage waters in the Yellow River Delta Region perhaps may have a risk of deteriorating soil structure.

It is possible to use saline drainage water as a supplementary irrigation resource for urgent purposes as the rainy season's leaching will counteract soil salinization due to saline water irrigation. In this study, the soil $EC_{1:5}$ declined drastically after the rainy season's leaching, and 415 mm precipitations occurred, for every irrigation treatment, suggesting that irrigation with saline water seemed to be acceptable for forage production of tall wheatgrass. However, long-term continuous irrigation with saline water should be carefully handled. Especially for the highly saline-alkaline soils, the risk of irrigation with saline water should be considered. The inconsistent differences in the soil $EC_{1:5}$ among different irrigation treatments may be due to the differential background salinities of the plots and sampling errors. In addition, it is noticeable that the field study was carried out for only one year, and further long-term research is needed. The salinity of the plots investigated in this study was relatively low, 0.36 dS $m^{-1} \geq EC_{1:5} \leq$ 1.20 dS m^{-1} or equivalently 2.88 dS $m^{-1} \geq EC_e \leq$ 9.6 dS m^{-1} according to the ratio of $EC_{1:5}/EC_e \approx 8$ [49]. The impacts of saline water irrigation on soil salinization in highly saline soils in the Coastal Grass Belt targeted region need further confirmation.

5. Conclusions

Irrigation with saline waters increased the forage yield of tall wheatgrass compared with the unirrigated control. Two irrigations with 2+3 g L^{-1} saline waters produced the highest dry matter yield, followed by one irrigation with 4 g L^{-1} or 5 g L^{-1} saline water. Two irrigations with 5+3 g L^{-1} saline waters produced less than one irrigation with 4 g L^{-1} or 5 g L^{-1} saline water, suggesting that cumulative irrigation salinity accumulation may reduce yield. Additionally, two irrigations with 8+3 g L^{-1} drainage water resulted in 16% and 3.2% of death rates of the seed propagated tall wheatgrass under no mulching and mulching conditions, respectively. Sufficient irrigations with saline water conferring salinity over 7 g L^{-1} for 40 d reduced plant height, leaf size, gas exchange rate, and dry matter yield to a large extent while irrigation with a salinity of 5 g L^{-1} has a least negative effects on dry matter yield-related traits. Irrigation with saline-alkaline drainage waters having various salinities, followed by rainfall or freshwater leaching, the soil salinity restored to the unirrigated levels. Taken together, irrigation with drainage water having a salinity of ≤5 g L^{-1} or EC_w≤5.42 dS m^{-1}, SAR≤36.31 may be acceptable to maximize forage yield of tall wheatgrass and minimize risk for soil salinization in coastal saline-alkaline land.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Investigation, Formal analysis, Methodology, Writing—original draft, W.L.; Investigation, Data curation, Formal analysis, J.Y.; Data curation, Formal analysis, Writing—review and editing, D.M.; Investigation, Formal analysis, Writing—review and editing, Q.Z.; Funding acquisition, Conceptualization, Writing—review and editing, H.L.; Investigation, J.W. and M.Z.; Conceptualization, Writing—review and editing, X.L.; Conceptualization, Supervision, Z.L. All authors have read and agreed to the published version of the manuscript.

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