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Establishment of a Cultivation Mode of *Glycine soja*, the Bridge of Phytoremediation and Industrial Utilization

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Abstract: Soil salinity is a growing challenge for today's agriculture. It is one of the most brutal abiotic factors limiting crop productivity globally. Millions of hectares of agricultural land throughout the world are too saline to produce economic crop yields and the area affected by salt accumulation is increasing day by day. Saline soils could however be a potential land resource with utilization value under the process of phytoremediation. Wild soybean (*Glycine soja*) is a salt-tolerant plant widely used in the cosmetic and pharmaceuticals industries as well as in crop improvement programs. This crop shows potential value for saline soil phytoremediation. However, due to its procumbent growth habit, accumulation of biomass is reduced and consequently reducing its value in phytoremediation. In this study, artificial facilities were used to make wild soybeans grow upright. Compared to the control plants, which yielded 1629.74 kg/ha of seed and 6075.76 kg/ha above ground biomass, erect wild soybean plants yielded 2608.10 kg/ha of seed and 10,286.40 kg/ha of above ground biomass (dry weight). The potential phytoremediation ability of wild soybean was also studied. The wild soybean could absorb up to 264.57 kg soluble salt/ha/year with an average of 25.72‰ salt content. The soluble salt content in the wild soybean rhizosphere was 1.50‰ higher than that in the bulk soil, suggesting that the rhizosphere of wild soybean can enrich soluble salt. The K–Na ratio of seed, leaf, shoot, and capsule were all greater than 1 suggesting that the wild soybean has a good salt tolerance capacity. Additionally, the bioaccumulation factor (BF) value of Na in roots was >1 suggesting that the root of wild soybean was the main organ for Na^+ storage and suitable for Na phytostabilization. Furthermore, wild soybean could be potentially play an important role in Ca and Mg phytostabilization due to their corresponding BF values, which were greater than 1 in different organs. In other words, the establishment of a cultivation mode of wild soybean, as demonstrated in this study, will be a bridge towards phytoremediation of saline soils and better industrial utilization of the crop.

Keywords: *Glycine soja*; cultivation mode; phytoremediation; salinity; erect growth

1. Introduction

Wild soybean (*Glycine soja*) is an annual legume wild races, which produces ellipsoid black seeds with an average length of about 2.50–4.00 mm and a diameter of about 1.80–2.50 mm. It is widely distributed in the East Asian countries such as China, Japan, Korea, and Russia [1]. It is believed that today's cultivated soybean (*Glycine max*), which is one of the most important food and feed crop in the world, was domesticated from this wild soybean about 3000–5000 years ago [2,3].

Wild soybean is a salt-tolerant plant widely used in the cosmetic and pharmaceuticals industries as well as in crop improvement programs. It carries many potentially useful alleles for crop improvement. It is therefore an important genetic resource for soybean breeding and genetic engineering since it can be easily hybridized with cultivated soybeans. What is more, there are about 9000 accessions conserved in the genebank in China making it easy to mine for useful genes or variants for soybean breeding [4]. The seeds of wild soybean are also a treasure for health care. They are known to contain saponins, isoflavonoids such as daidzein glycosides, genistein, genistein glycosides, daidzein, 6-OH daidzein, and glycitein glycosides [4,5]. A study by Deshimaru et al. (2002) reported that nine trypsin inhibitors (WSTI-I-VIII) were isolated and characterized from the seeds of wild soybean, which belonged to the soybean kunitz inhibitor and Bowman–Birk inhibitor protease families [6]. The content of α-linolenic acid (ALA), which are important compounds that prevent cardiovascular disease and cancer in humans [7], that were recorded in wild soybean were twice as high that of cultivated soybean. Apart from that, wild soybean is not only an excellent fodder plant in graziery [8], it has been reported to be a salt tolerant plant. Zhou et al. (2007) reported that isoflavonoids played an important role in the adaption of wild soybean to saline environments [9]. These finding are not surprising since wild soybean usually grows in harsh conditions such as on the edges of crop fields, roadsides, and riverbanks where it faces many biotic and abiotic stress factors including water deficiency and salinity [10] but it continues to thrive indicating a strong adaptability of wild soybean to unfavorable growth conditions.

Soil salinity significantly reduces crop yields worldwide and it is predicted to become more of a problem in the future [11,12]. It is estimated that 831 million hectares of saline land exist worldwide [13,14]. In the past decades, considerable efforts have been made to remediate saline soil, including the implementation of chemical, physical, and mechanical remediation [15]. Although these methods are effective, they are expensive and not feasible methods for most farmers, especially, for those small-holder farmers lacking of resources, and, moreover, are not ecofriendly at all. It is therefore clear that a more sustainable and cost-effective technology, which can help in the reclamation of saline soil, will be highly appreciated. Phytoremediation is one of such technologies that can be used to address salinity in a sustainable and cost-effective way. In this context, phytoremediation can be achieved by growing salt-tolerant plants that are able to absorb salt from the soil and thus to reduce soil salinity [16]. The two main advantages of phytoremediation are (1) no financial outlay needed for the purchase of chemicals and (2) salt-resistant crops can generate high-value by-products [17]. Excellent phytoremediation efficacy depends on the ability of the plants to accumulate salt and biomass production [18]. Zhao et al. (2005) reported that the hyperaccumulators halophyte plants such as *Suaeda salsa* (L.) Pall and *Kalidium folium* (Pall.) Moq could absorb about 2300 kg and 2800 kg NaCl ha^{-1} in one year from saline soils, and can produce dry biomass of up to 7700 and 8700 kg ha^{-1}, respectively. The salt content of these plants is 150 to 180-fold higher than in glycophytic plants and is also 4–6-fold higher than in other halophytes such as *Lyceum ruthenium* Murr and *Korelinia caspica* (Pall.) Less [19].

When used in phytoremediation, hyperaccumulators should be able to absorb and accumulate salt within their organs thus reducing the salt content of saline soils. If such plants can have extensive applications and uses, the use of hyperaccumulators in phytoremediation will also bring great social and environmental benefits. Wild soybean is one such plant with a wide range of social, economic, and environmental benefits, which shows great potential for use in phytoremediation. Based on previous studies, the mechanism through which wild soybean tolerates salt includes four main aspects. The first one is the, accumulation of low molecular weight metabolites termed compatible solutes in the cytoplasm to reduce the water potential in the cytoplasm by balancing the decreased water potential associated with Na^+ accumulation [20]. The second aspect involves the elevation of the contents and activities of various antioxidative components for ROS (reactive oxygen species) scavenging to restore the oxidative balance [20] while the third aspect involves the release of unsaturated fatty acids from membrane lipids to keep the plasma membrane intact thus avoiding the leakage of electrolytes and organic compounds from the cells [21]. The last one involves increasing the level of ABA to alleviate the inhibitory effect of salt on photosynthesis, plant growth, and translocation of solutes [22]. Although a lot of studies have been done on wild soybean, they mainly focused on its agronomic, biochemical, pest, and disease resistance properties described above. Other studies on wild soybean reported that the shoot and root lengths and biomass of wild soybean was significantly higher than semi-wild or cultivated soybean species under salt stress [23] while its ability to tolerate salinity has been reported better than cultivated soybean species [24,25]. A relative high salt tolerance combined with its important uses could be one of the strengths for using wild soybean for phytoremediation. However, the procumbent growth characteristic will seriously reduce the yield of wild soybean (both biomass and seed yield).

In this study, wild soybean's ability to absorb salt from the soil was studied. Furthermore, to improve the biomass yield of wild soybean, we also developed a wild soybean cultivation model, which was regarded as the bridge of phytoremediation and industrial utilization. We hope that the cultivation model will support the balance between biomass for the utilization of wild soybean and phytoremediation.

2. Materials and Methods

2.1. Study Site

The study was carried out in Guangrao District, Dongying City (37°17′53.45′′ N, 118°37′15.04′′ E), Shandong Province, China (Figure 1). Based on the FAO World Reference Base for Soil Resources, the soil was cinnamon soil, and the soil data were digitized using 1:200,000 scale soil maps developed by the Land Resource Investigation Office (LRIO) of Dongying Municipality [26]. During the study period (May to October 2019) the area had an average temperature of 24 °C and a total rainfall of 2740 mm.

Figure 1. Display of the study site on the map. The red triangle indicated the study site, which was located in Guangrao District, Dongying City (37°17′53.45′′ N, 118°37′15.04′′ E), Shandong Province, China.

2.2. Planting Method

The wild soybean seeds used in this study were the self-pollinated progeny of a single wild soybean plant collected in the field near the study site. Planting of wild soybean was done using artificial facilities (Figure S1). Each artificial holder was 6.00 m long and 2.50 m high and had two rows of wild soybeans, with a spacing of 60 cm between rows. In this experiment, a total of six artificial holders were used. A total of five seeds were sown in each planting hole with a spacing of 40 cm between holes. The wild soybeans were tied to the artificial holder at the V3 to V5 growth stage [27]. Wild soybeans without artificial facilities were grown as the control using the same spacing. Neither irrigation nor fertilizers were applied during the entire growth period. Only appropriate weeding was carried out. At the end of the growing season, seed yield and biomass (both the above ground and below ground biomass) were harvested and evaluated.

2.3. Soil and Plant Sampling

To evaluate the phytoremediation ability of wild soybean at the harvest stage, soil and plant samples were collected one week before the harvest for further analysis. The composite rhizosphere and bulk soil samples were collected from five individual plants, which were randomly selected from the field. The bulk soil was obtained by shaking plants vigorously to remove loose soil from the roots. The soil that remained attached to the roots after shaking was considered to be part of the rhizosphere [15]. Different plant organs (roots, shoots, leaves, seeds, and capsules) were collected from the same plants. The soil samples were air-dried to a constant weight in the laboratory and then passed through a 2 mm sieve to remove plant debris, roots, and stones for further analysis. The plant samples were oven dried at 60 °C to a constant weight for further analysis.

2.4. Measurement of Na^+, K^+, Ca^{2+}, and Mg^{2+} Concentration

Measurement of major cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) was done according to previously described methods with minor modifications [28,29]. The air- or oven-dried soil and plant samples were ground to pass through a 0.50 mm sieve. Each 1 g sample of soil and 0.25 g sample of plant tissue was digested in 5 mL HNO3 at 110 °C for 6 h until a colorless liquid was obtained. After cooling, the solution was adjusted to 10 mL by adding deionized water, rendering it ready for analysis with a Perkin-Elmer Model 360 atomic absorption spectrophotometer. Three replicates were prepared for each plant or soil samples.

2.5. Soluble Salt Content Measurement

To measure the soluble salt content of the plants, plant tissue samples were pretreated according to a previously describe method with minor modifications [19]. In brief, each 5 g dried plant tissue samples were put in a muffle furnace and burnt to ashes. The ashes were dissolved in 5 mL of distilled water. The mixture was thoroughly shaken for 30 min with a mechanical stirrer and then filtered through qualitative filter paper. The supernatant was stored in a preweighted 25-mL beaker. Similarly, each 5 g sieved soil samples was added 25 mL distilled water and also shaken for 30 min. The filtered supernatant was stored in a preweighted 50-mL beaker. The total soluble salt content was measured using a previously describe method with minor modifications [30]. In brief, aliquots of 30% *v/v* H_2O_2 were added into the supernatant obtained from plant and soil samples to digest the organic matter. The beakers with supernatant were heated to evaporate all the liquid on an electrothermal furnace and then weighed. Three replicates were prepared for each plant or soil samples.

2.6. Bioaccumulation and Translocation Factor Calculation

The bioaccumulation factor (BF) was defined according to a previous method with some modifications [31,32]. BF was calculated as the ratio of the content of a salt ion in plant tissue (mg kg^{-1} dry matter) to that in soil (mg kg^{-1} dry matter). The translocation factor (TF) was calculated as the ratio of the content of a salt ion in shoots (mg kg^{-1} dry matter) to that in roots (mg kg^{-1} dry matter) [33].

2.7. Statistical Analysis

Each data point was provided as the average ± SD of three independent replicates. All experiments were repeated a minimum of three times. An analysis of variance (ANOVA) was done using the SPSS v.17.0 software (SPSS, Inc., Chicago, IL, USA) and, at $p < 0.05$, differences were considered statistically significant.

3. Results

3.1. Growth, Yield, and Biomass Estimation of Wild Soybean with Artificial Facilities

The wild soybeans were sown on May 15 and harvested on September 30, (that is a total growth period of 135 days divided into about 70 days of vegetative growth and 65 days of reproductive growth). It grew slowly during the early stages of the vegetative growth and then rapidly during the later stages of the vegetative growth stage and the beginning of the reproductive growth stage. In the first 50 days of the vegetative growth stage, the average height of wild soybean was 64.80 cm with a thin plant morphology and few branches (Figure 2A). At 70 days, the plants showed a cone-shape with a large number of branches and an average plant height of 115.60 cm. It was around this same time when the first flowers opened (Figure 2B). At 90 and 110 days, the whole artificial facilities (250 cm height) were completely covered (Figure 2C,D). It was around the same time that the seeds were filled (Figure 2D). At 130 days, the plants showed the characteristics of senescence with leaves turning yellow while the capsules turned black-brown (Figure 2E).

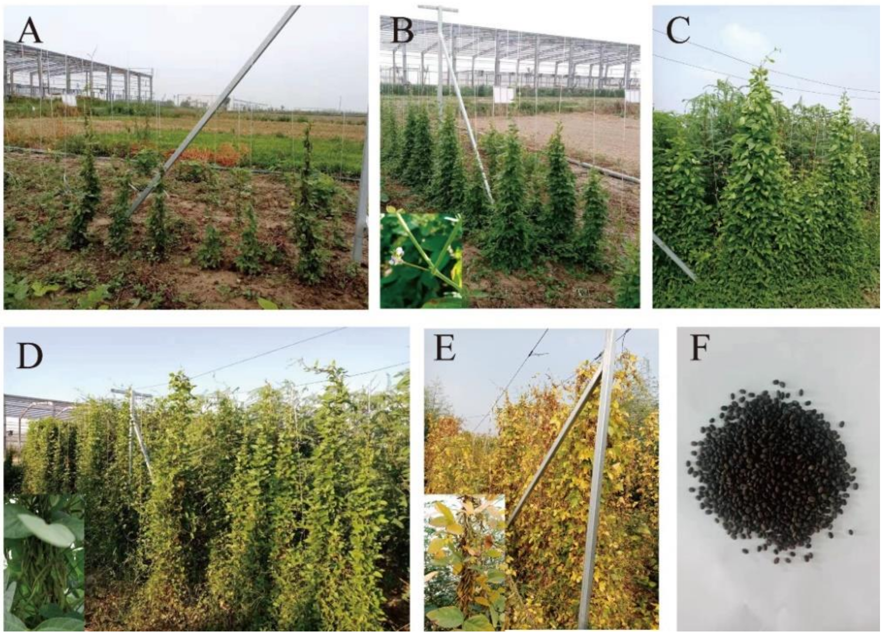

Figure 2. Growth status of wild soybean in different growth periods. (**A**): Vegetative growth stage in the 50th; (**B**): the first opened flower occurred in the 70th; (**C**): boom stage in the 90th; (**D**): the "wild soybean wall" stage in the 110th; (**E**): senescence stage in the 130th; and (**F**): matured seeds.

After weighing the dried seed, it was observed that the one thousand seed weight for the wild soybean grown on artificial facilities was 13.56 g, and dry weight (DW) of biomass was 256.87 g per hole while the control recorded a one thousand seed weight of 12.60 g and a dry weight biomass of 145.75 g per hole (Table 1). The use of artificial facilities cultivation mode yielded 2608.10 kg seeds and 10,286.40 kg biomass (DW) ha^{-1} (Table 2) on the saline land without irrigation or fertilizer application while the control recorded significantly lower yields of 1629.74 kg and 6075.76 kg respectively ha^{-1} under similar conditions (Table 2). Thus, compared to the control, the artificial facilities cultivation mode recorded an increase of 60.03% and 69.30% in seed and DW biomass yield respectively.

Table 1. Statistics of biomass and seeds yield of wild soybean with artificial facilities and control conditions.

Cultivation Mode	Holes NO.	Plants per Hole	Biomass (Dry Weight, g)	Pods NO.	Average Seeds NO. per Pod	Total Seeds	Thousand Seed Weight (g)	Total Seeds Weight (g)
Artificial facilities	1	3	309.44	1862	3.06	5698	13.61	77.55
	2	3	319.32	1639	3.42	5605	14.59	81.78
	3	2	184.05	1150	3.20	3680	12.92	47.55
	4	2	219.40	1240	3.20	3968	15.13	60.02
	5	3	272.88	1590	3.28	5215	12.51	65.24
	6	2	176.14	1120	3.08	3450	12.59	43.43
	Average	**2.50**	**246.87**	**1433.50**	**3.21**	**4603**	**13.56**	**62.59**
Control conditions	1	3	151.99	817	3.23	2635	12.00	31.62
	2	2	168.92	985	3.07	3024	12.88	38.95
	3	3	111.47	788	3.15	2479	12.35	30.62
	4	3	155.49	976	3.25	3168	13.30	42.13
	5	2	140.88	1334	3.20	4264	12.48	53.21
	Average	**2.60**	**145.75**	**980.10**	**3.18**	**3104**	**12.60**	**39.11**

Table 2. Comparison of biomass and seed yield of wild soybean under artificial facilities and wild conditions.

Cultivation Mode	Biomass (Dry Weight, kg/ha)	Total Seeds Weight (kg/ha)
Wild conditions (control)	6075.76	1629.74
Artificial facility	10,286.40	2608.10

3.2. Relationships of Na^+, K^+, Ca^{2+}, and Mg^{2+} in Soil and Wild Soybean Organs under Saline Soil

The concentration of K^+, Ca^{2+}, and Mg^{2+} showed no significant differences between the bulk and rhizosphere saline soil of wild soybean. However, the Na^+ concentration was significantly decreased in the rhizosphere soil ($p < 0.05$). Ca^{2+} was the most abundant ion in the saline soil, then Mg^{2+}, and Na^+ were the least (Table 3). In the different plant organs, the roots contained the most Na^+, which was significantly higher than that in bulk or rhizosphere soil. Compared to the roots, the concentrations of Na^+ distributed in the leaves, shoots, capsules, and seeds were significantly lower ($p < 0.05$). On the contrary, the concentration of K^+ in the root was the least, which was significantly lower than that in the soil. However, the seeds contained the most abundant of K^+. On the other hand, the concentrations of K^+ in the leaves, shoots, capsules, and seeds were all significantly higher than that in the soil ($p < 0.05$). The contents of Ca^{2+} and Mg^{2+} in all the organs of wild soybean were all significantly lower than that in the soil, respectively ($p < 0.05$). The contents of both Ca^{2+} and Mg^{2+} in leaves were the highest compared with other organs. According to our results, the distribution of K^+ and Ca^{2+} in the organs of wild soybean was relatively balanced, however, the Na^+ and Mg^{2+} showed great fluctuation (Table 3).

Table 3. Mean content of Na^+, K^+, Ca^{2+}, and Mg^{2+} in saline soil and accumulations in organs of wild soybean.

Ion Content (mg g^{-1})	Na^+	K^+	Ca^{2+}	Mg^{2+}
Bulk soil	10.34 ± 0.97^{a}	10.47 ± 0.46^{a}	17.51 ± 2.31^{a}	11.63 ± 0.40^{a}
Rhizosphere soil	8.96 ± 0.19^{b}	10.81 ± 0.28^{a}	18.48 ± 1.65^{a}	11.53 ± 0.19^{a}
Root	13.08 ± 1.68^{c}	5.63 ± 0.82^{b}	10.12 ± 1.25^{b}	1.89 ± 0.09^{b}
Leaf	1.38 ± 0.27^{d}	16.26 ± 3.76^{c}	14.24 ± 2.58^{c}	10.04 ± 2.44^{a}
Shoot	0.97 ± 0.17^{e}	24.70 ± 3.33^{d}	5.95 ± 0.77^{d}	1.98 ± 0.08^{b}
Capsule	0.47 ± 0.11^{f}	15.64 ± 1.11^{c}	7.26 ± 1.10^{e}	0.46 ± 0.04^{c}
Seed	0.17 ± 0.03^{g}	27.36 ± 2.96^{d}	0.23 ± 0.01^{f}	0.66 ± 0.13^{c}

Values are the means of three replicates ± SD. Values within a column followed by different lowercase letters are significantly different ($p < 0.05$).

3.3. K–Na Ratio in Wild Soybean Different Organs

Among the different organs of wild soybean, the seed showed the highest K–Na ratio (166). The K–Na ratio of leaf, shoot, and the capsule was 12.24, 25.92, and 34.34, respectively. However, root showed the lowest K–Na ratio with 0.43 (Figure 3).

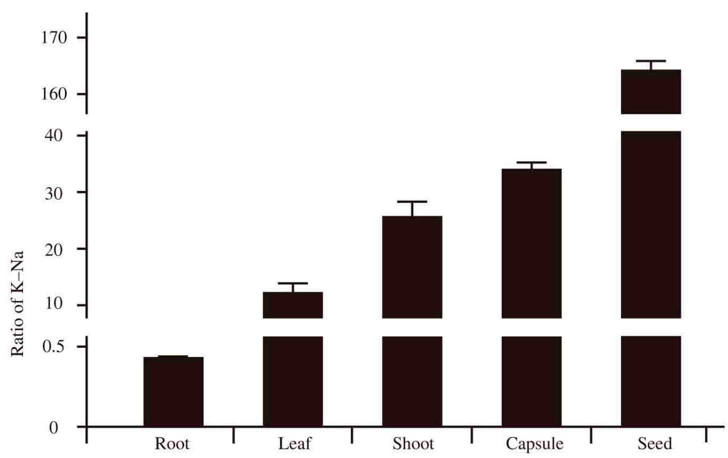

Figure 3. The K–Na ratio in different organs of wild soybean.

3.4. Ion Translocation Capacity in Wild Soybean Organs

The BF value of Na was significantly greater in the root (1.46) than other organs, which were all less than 1 ($p < 0.05$). On the contrary, the least BF value of K occurred in the root (0.52), which was <1. The BF value of K in the leaf, shoot, capsule, and seed was 1.50, 2.29, 1.45, and 2.53, respectively, which were all significantly higher than that in the root ($p < 0.05$). The BF value of Ca in leaf and capsule were both >1, which was significantly higher than that in root, shoot and seed that all showed values <1 ($p < 0.05$). The BF value of Mg in these organs were all <1 with the highest value recorded in leaf (0.87; Table 4). The TF value of Na in the leaf, shoot, capsule, and seed were all <1 with the highest value that observed in the leaf (0.11). However, the TF value of K in these organs were all >1. The shoot and seed showed a similar level, which was significantly higher than that in leaf and capsule ($p < 0.05$). The TF value of Ca was >1 in the leaf only, which was significantly higher than that in other organs ($p < 0.05$). The highest TF value of Mg occurred in leaf with 5.18, which was significantly higher than that in seed (1.24), capsule (0.24), and shoot (0.19; $p < 0.05$; Table 4).

Table 4. Bioaccumulation factor and translocation factor in different organs of wild soybean.

Organs	Bioaccumulation Factor (BF)				Translocation Factor (TF)			
	Na	**K**	**Ca**	**Mg**	**Na**	**K**	**Ca**	**Mg**
Root	1.46 ± 0.19 [a]	0.52 ± 0.09 [a]	0.59 ± 0.07 [a]	0.16 ± 0.01 [a]	-	-	-	-
Leaf	0.15 ± 0.03 [b]	1.50 ± 0.33 [b]	1.59 ± 0.29 [b]	0.87 ± 0.21 [b]	0.11 ± 0.03 [a]	2.94 ± 1.02 [a]	1.42 ± 0.32 [a]	5.18 ± 1.26 [a]
Shoot	0.11 ± 0.02 [b]	2.29 ± 0.31 [c]	0.55 ± 0.07 [a]	0.17 ± 0.01 [a]	0.07 ± 0.01 [b]	4.58 ± 0.59 [b]	0.59 ± 0.06 [b]	0.19 ± 0.01 [b]
Capsule	0.05 ± 0.01 [c]	1.45 ± 0.11 [b]	1.27 ± 0.19 [b]	0.04 ± ND [c]	0.04 ± 0.01 [c]	2.78 ± 0.44 [a]	0.71 ± 0.15 [b]	0.24 ± 0.02 [b]
Seed	0.02 ± ND [c]	2.53 ± 0.25 [c]	0.03 ± ND [c]	0.06 ± 0.01 [c]	0.01 ± ND [d]	4.87 ± 1.09 [b]	0.02 ± ND [c]	1.24 ± 0.23 [c]

Values are the means of three replicates ± SD. Values within a column followed by different lowercase letters are significantly different ($p < 0.05$). ND, not detected.

3.5. Total Content of Soluble Salt in Soils and Wild Soybean Organs

The total soluble salt content of bulk soil in this study was 3.50‰, which shows that the soil was moderately saline. However, the content of soluble salt in the rhizosphere soil of wild soybean was 5.07‰, which was significantly higher than that in bulk soil ($p < 0.05$; Table 5). Amongst the different plant organs, the seed showed the highest soluble salt content (32.51‰), which was significantly higher than that in other organs, which ranged from 21.36‰ to 23.52‰ ($p < 0.05$). The entire plant showed a soluble salt content of 25.72‰ (Table 5). The ash content of wild soybean organs ranged between 5.00‰ and 6.20‰. The capsule showed the highest ash content, which was significantly higher than what was recorded in other organs ($p < 0.05$) while the leaf recorded the least (5.01‰; Table 5).

Table 5. The content of soluble salt in different soil and wild soybean tissue samples.

Samples	Ash Content (% of DW)	Soluble Salt Content (‰ DW)
Bulk soil	-	3.50 ± 0.88 [a]
Rhizosphere soil	-	5.07 ± 0.32 [b]
Root	5.84 ± 0.18 [a]	22.24 ± 0.86 [c]
Leaf	5.01 ± 0.13 [b]	23.52 ± 1.79 [c]
Shoot	5.18 ± 0.09 [b]	22.45 ± 1.41 [c]
Capsule	6.19 ± 0.12 [c]	21.36 ± 1.82 [c]
Seed	5.45 ± 0.14 [d]	32.51 ± 0.21 [d]
Total Plant	5.39 ± 0.11 [d]	25.72 ± 2.11 [c]

Values are the means of three replicates ± SD. Values within a column followed by different lowercase letters are significantly different ($p < 0.05$).

4. Discussion

Soil salinity is a global environmental problem seriously limiting the productivity of cultivated crops, fruits, and vegetables. Saline soil exists worldwide in over 100 countries, with a salinization area increasing by 10%–16% every year [29]. What is worse, 20%–50% of irrigated lands were reported to be threatened by salt stress [34]. Over the past few decades, soil salinization has resulted in a reduction of more than 1/2 of the production of major global crops [35–37]. Salt stress is a combination of ionic stress due to the chaotropic effects of incoming Na^+ and Cl^- and osmotic stress resulting from a decrease in water potential [38]. To make matters worse, most main crops and vegetables such as wheat, rice, maize, and tomato are glycophytic species, and they are vulnerable to salt stress [39]. Thus, soil salinity is a very serious problem affecting agricultural production.

Phytoremediation can be seen as an effective, low-cost, and environmental friendly technology for the improvement of plant health when grown on saline land [40]. It is a plant-based technology that reduces soil salinity and increases/improves the land productivity potential. Hence, the adoption of this green technology, saline soils can be reclaimed and considered as an important land resource that can be utilized for socioeconomic development of those countries, which suffer from this serious agricultural land problem. Planting salt-tolerant crops and vegetables with industrial and agricultural value on saline land will not only make saline land resources useful but could also bring social or economic benefits to communities. Wild soybean (*Glycine soja* Siebold and Zucc) was reported as a salt-tolerant plant with multiple use values and widely distributed in China [41]. A high ratio of K to Na was reported as a vital determinant of salt tolerance in plants. It is considered as one of the important indicators to measure the salt tolerance capacity of plants [42]. For instance, when the K–Na ratio is >1, the plant can tolerate salt stress due to the capacity of the plant to maintain a high cytosolic K–Na ratio. [43]. In this study, the wild soybean showed a high K–Na ratio in the leaf, shoot, capsule, and seed (all >1), which is consistent with a previous study [23]. The ratio of K to Na in seeds even reached the value of 166.0 (Figure 3). Additionally, according to the soluble salt content result in this study, the rhizosphere soil contained more salt than the bulk soil (Table 5) suggesting that salt had accumulated in the rhizosphere of wild soybean. This is consistent with the result that wild soybean plant contained 25.72‰ soluble salt. This means that wild soybean promoted salt accumulation in the rhizosphere, absorbed salt and stored it in some of its organs. These characteristics enable wild soybean to be used in the phytoremediation of saline soils. It has been reported that a plant can be efficient in the phytoremediation of salinity-affected soils when the BF value is >1 [44]. In this study, the BF value of Na in the root was >1 (Table 4) suggesting that the root of wild soybean was the main organ for Na^+ storage and suitable for Na phyto-stabilization. The TF > 1 indicates that ions can transfer from roots to other organs [44]. In this study, the TF value of Na in each of the organs were all <1, which means that the ions were not translocated from the roots to other organs of the plants.

Biomass is a key factor to determine whether plants can be used for phytoremediation or not [18]. The halophyte plants, for example, *T. tetragonoides* (Pall.) Kuntze can produce between 40,000 and 50,000 kg DW biomass ha^{-1} with a density of 75,000 plants ha^{-1} when grown on saline soils (the range of EC was 0.90–10.80 dS/m) [45]. *S. portulacastrum* L. (Aizoaceae) was reported to produce 6636 kg DW ha^{-1} [46]. The DW biomass of other salt-tolerant plants for phytoremediation such as *S. maritima* Dum, *S. portulacastrum* L., *E. agallocha* L., and *I. pes-caprae* Sweet, ranges between 2500 and 4000 kg ha^{-1} [47]. Although wild soybean gives a better yield than these plants, its procumbent growth habit hinders its optimal utilization of sunlight thereby limiting its yield potential and thus making it less ideal for utilization in phytoremediation. The use of artificial facilities in this study solved the problem of procumbent growth of wild soybean and made it possible to grow them upright and improve its yield (Figure 2), which is beneficial for phytoremediation and industrial or agricultural utilization of wild soybean. The DW biomass of wild soybean was 10,286.40 kg ha^{-1} (Table 2), which is higher than that observed in other halophytes. However, productivity is dependent on plant density. Hansi et al. reported that increasing density can result in decreased productivity, but may also increase salt accumulation in plant organs [48]. With the planting density we adopted in this experimentation, the soluble salt content of wild soybean was 25.72‰ and could absorb up to 264.57 kg salt ha^{-1} (Tables 2 and 5), which is however lower than that of some hyperaccumulators halophyte plants, which can range between 2000 and 3000 kg ha^{-1} [19,46]. It is however noteworthy that the range of salt absorption by common halophytes is generally between 300 and 700 kg ha^{-1} [45,47], which is slightly above that of wild soybean. Nonetheless, the phytoremediation plant *Lotus corniculatus* has been reported to absorb only 15–33 kg total dissolved salt ha^{-1} every year [29], which is extremely lower than what we recorded with wild soybean. This shows that salt absorption is highly dependent on the plant species utilized. Although the amount of salt that a plant species can absorb from the soil is an important indicator of phytoremediation capacity, a compromise among species chosen, planting density, biomass yield, and phytostabilization goals are all important and there is need for carrying out further studies on this agronomical aspect. However, we must point out that a shortcoming of this study was that it was only conducted in one site for one year. As we known, for field experiments, the results need to be validated over years and locations. Hence, the result of this study may be deviated from the actual values according to different environments and years.

Even though we have established a cultivation model for growing wild soybean in an upright position, which improves its yield and even though it is resistant to biotic and abiotic stresses, with multiple uses, at least three problems need to be solved before it can be grown on a large-scale. These are (1) pod dehiscence; (2) time of pods maturity, currently they mature at different times, and (3) hard seediness. A previous study reported that the degradation of leaf litter of salt accumulating plants eventually increases the surface soil salt content [49]. Hence, the fall of the seeds to the ground caused by different times of pod maturity does not only reduce the effectiveness of phytoremediation but also reduces the subsequent economic and utilization value of wild soybean seeds. Previous studies reported that a *NAC* gene and *GmHs1-1* were associated with pod shattering and hard seediness, respectively [50,51]. Thus molecular biology tools may be employed to solve them, for example, knocking out some genes positively related to these traits with CRISPR/Cas9 technique, or overexpressing some genes that inhibit these traits. However, due to the characteristics of wild races, it is unlikely that wild soybeans can be manipulated and grows to a height of several meters using molecular biology tools only. This validates the importance of the rapid and effective method of artificial facilities-assisted cultivation mode for wild soybean described in this study: the establishment of the cultivation mode of wild soybean will be a bridge of phytoremediation and industry or agriculture utilization.

5. Conclusions

Although wild soybean has the potential to play a crucial role in phytoremediation, it is however limited by its inability to yield reasonable quantities of biomass and this is mainly attributed to its procumbent growth characteristics. To overcome this problem, artificial facilities that promoted erect growth and thus higher biomass accumulation were used in this study. Further, the phytoremediation ability of wild soybean for saline soil was also validated. High biomass production is an important index of a species for phytoremediation. Hence, the establishment of a cultivation mode of wild soybean will act as a bridge for phytoremediation and its industrial utilization.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/4/595/s1, Sketch of artificial facilities was shown in Supplementary File 1 (Figure S1).

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