

Researches on the Salt Tolerance and Restraint Effects of *Radix astragali* in Saline-Alkali Soils

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In recent years, with rising bioengineering, the improving saline-alkali soils of biological measures have become the lasting, stable and effective measures in favour of water and soil conservation and ecological balance. More studies have shown that the biological measures are the most effective and basic measures to improve saline-alkali soils, especially in the inland. *Radix astragali* is classified to leguminous plant and is a kind of non-toxic and widely used Chinese herbal medicine which is used in cardiac, diuretic, anti-inflammatory, germicide and immunity improvement because of being slightly sweet and tepid. This paper mainly studied the salt tolerance and restraint effects of *Radix astragali* in saline-alkali soils. In the experiment, different cultivation conditions were applied to investigate the improving saline-alkali soils effect of *Radix astragali*, which should provide an effective and system scientific technological approach for biological salt control and developing medicinal industry in the saline-alkali soils.

1. Introduction

Soil secondary salinization is a global problem (Ali et al., 2014; Bose et al., 2014). The increasing soil salinization and secondary salinization have become two serious environmental problems in today's world (Chen et al., 2014; Ceccoli et al., 2015). About 55% lands in the world are distributed in the arid and semi-arid zones with few rainfalls (Deinlein et al., 2014; Di et al., 2014). Annual precipitations in the arid and semi-arid climate zones are less than 250mm and 250-500mm, respectively (Flowers et al., 2014). The main or unique measure for developing the agriculture is to develop irrigation in the arid and semi-arid zones (Di-Bella et al., 2014). However, unreasonable or excessive irrigation may cause secondary salinization in the irrigated area (Hasegawa 2013; Hamamoto et al., 2015); that problem is very serious in the Mediterranean region, India, Pakistan, Iran, Iraq, Syria, Azerbaijan, Uzbekistan, Ukraine, Russia, Mexico, Southwestern United States, Australia, Egypt, Africa and other countries or areas (Iqbal et al., 2014). Saline soils, also called saline-alkali soils, are collectively referred to salinized and alkalized soils (Islam et al., 2016). Saline-alkali soils is very widely spread in various countries and mainly distributed in arid and semi-arid regions in the world (Ismail and Horie, 2017). China is one of countries with the most serious secondary salinization of soils in the world (Jayakannan et al., 2013). The high content of minerals in the soil and abundant sunlight, uncultivated lands in those areas, such as Xinjiang, Gansu and Ningxia in the Northwest China, and Shanxi and Inner Mongolia in the North China, can be transformed to potential agricultural land only with help of water (Manaa et al., 2014; Mostofa et al., 2015). But, with the development and utilization of such lands and under the comprehensive functions of many factors, secondary salinization of soils inevitably occurs or will occur (Rivero, et al., 2014; Rahman, et al., 2016). Since 1970s, secondary salinization has occurred to different degrees in many new and old irrigation areas, such as Minsheng and Houtao irrigated areas in Inner Mongolia, Yinchuan Pingyuan irrigation area in Ningxia, Fenhe River irrigation area in Shanxi and Huang-Huai-Hai plain (Shrivastava and Kumar, 2015). Secondary salinization also occurs in Hexi Corridor, irrigation area along the yellow river in the middle, and Yindaruqin irrigation area in Gansu (Song and Wang, 2014).

2. Materials and methods

2.1 General situations of experimental area

The experimental area located in the Duandi village, Pandian town, Gengqiu county, Xinxiang city, Henan Province in April, 2015 to October, 2016. It is in the north latitude 34°53'~35°14' and east longitude 114°14'~114°46'. The climate belongs to warm temperate continental monsoon. Annual average temperature is 13.5°C to 14.5°C, annual rainfall is 615.1 millimeters, and frost-free period is 214 days.

2.2 Design of experiment

Experimental material: *Astragalus membranaceus* Bge (*AmB*, a species of *Radix astragali*).

Transplantation: It was conducted during soil thawing in the April of 2015 to ensure that the root length of seedling was above 25cm. Both ditch depth and row spacing were 30cm. The seedling was smoothly placed or obliquely (flatly) grew in the groove and earthed for 2~3cm. *AmB* used for the experiment was introduced from Min county of Gansu province. The five treatments of planting densities were designed that (Table 1): the control (CK): no planting; D1: plant spacing was 30cm and hectare density was 110,000 seedlings; D2: plant spacing was 25cm and hectare density was 133,500 seedlings; D3: plant spacing was 20cm and hectare density was 165,000 seedlings; D4: plant spacing was 15cm and hectare density was 222,000 seedlings; plot area is 20 m² with the 10m length and 2m width; randomized block design with three replications were used. The spacing of each plot was 40 cm.

Field management: Weeding and ripping should be conducted immediately after emergence of seedlings. The diseases and pests and their control were conducted the same as field production.

Table 1: Design of planting density

Treatment	Plant spacing (cm)	Row spacing (cm)	Density (plant/ha)
CK	0	0	0
D1	30	30	110,000
D2	25	30	133,500
D3	20	30	165,000
D4	15	30	222,000

2.3 Sampling method

Selection of soil samples: the sampled soil layers were 0-20cm, 20-40cm, 40-60cm and 60- 80cm. Three points were randomly selected in each area on June 30th and August 10th of 2015, August 10th and October 20th of 2016. Soils in the same layer were mixed into a soil sample.

Selection of plant samples: Total 10g plant samples including the underground root in the distance of 10cm from the ground and aboveground stem in the distance of 2/3 plant height from ground and leaves were sampled on June 30th and August 10th, 2015. During dredging, 20 underground roots and five aboveground branches and leaves of *AmB* in each area were taken on June 30th, August 10th and October 20th.

Determination method: Soluble salt contents were measured with the residue drying method, K⁺ and Na⁺ contents were examined with the 6410A flame spectrophotometer method, acidity meter was determined pH value, the contents of Ca²⁺, Mg²⁺ and SO₄²⁻ were measured with the EDTA titration method, AgNO₃ titration method was detected the Cl⁻ content.

Data analyses: The analyses of variance (ANOVA) among different treatments were carried on the experimental data to use PROC GLM in SAS (Version 9.4, SAS Institute).

Table 2: pH values of 0-80cm soil layer in different plant densities of *AmB*

Soil layer	Planting density				
	CK	D1	D2	D3	D4
0~20cm	8.64	8.62	8.53	8.54	8.35
20~40cm	8.51	8.45	8.41	8.37	8.36
40~60cm	8.55	8.51	8.46	8.44	8.38
60~80cm	8.62	8.60	8.55	8.50	8.47
Average	8.58	8.55	8.49	8.46	8.39

3. Results and analyses

3.1 Changes of pH values in different plant densities of *AmB*

The pH values of 0-80cm soil layer in different plant densities of *AmB* were shown in Table 2. The results showed that the soil pH value decreased with increasing planting density of *AmB*, which meant that *Radix astragalus* had obvious alkali restraint effect that was positively correlated with planting density. The reason for that is that pH value in the soil is determined by the quantities of NaHCO_3 , CaCO_3 , MgCO_3 and K^+ and Na^+ in the soil. Decreasing salt ions in the soil would be beneficial for decreasing the pH value.

3.2 Changes of total soluble salt content in different plant densities of *AmB*

The data in Table 3 indicated that total average soluble salt content 0-80cm soil layer in four planting densities of *AmB* had greatly significantly lower than that of CK ($p < 0.05$), and those of D1, D2 and D3 or D4 had also significant differences, but there was no significant difference between D3 and D4. In the 0-80cm soil layer, total average soluble salt content of the soil in different plant densities of *AmB* is 2122.3 mg/kg for D1, 1821.6 mg/kg for D2, 1691.9 mg/kg for D3 and 1608.9 mg/kg for D4, which were reduced for 67.4%, 72.0%, 74.0% and 75.3% compared with the 6506.6 mg/kg for CK. It meant that *Radix astragalus* had the effective salt restraint effect, and its reduced degree was positively correlated with the plant density. The main reason for reducing the soil salinity was that water in the soil was absorbed by the root system of *Radix astragalus*, and soil evaporation was changed to transpiration, which could prevent gathering of salinity to the surface along with water; moreover, soil evaporation was greatly decreased due to green coverage that formed another barrier for preventing salt from moving up.

According to soil soluble salt amount in different times (Table 4), highest value appeared in the October and the lowest was in August; which was due to the more precipitation in July and August to make salt leak subsoil with the leakage of water, but precipitation after September decreased and surface evaporation severely increased to make salt to surface soil. The results in Table 4 showed that soil soluble salt amount between CK and four planting densities had significant differences ($p < 0.05$), and those of D1 and D2 had significantly higher than those of D3 and D4, but there was no significant difference between D1 and D2 or D3 and D4.

Table 3: Soluble Salt Amounts of 0-80cm Soil Layer in Different Densities of *AmB* (Unit: mg/kg)

Soil layer	Planting density				
	CK	D1	D2	D3	D4
0~20cm	9412.9a	2011.4b	1593.3c	1502.9c	1444.7c
20~40cm	5600.0a	2116.8b	2263.2b	2116.8b	2093.6b
40~60cm	5152.8a	1641.6b	1586.2b	1549.4b	1390.8c
60~80cm	5860.8a	2719.5b	1843.7c	1598.4d	1506.3d
Average	6506.6a	2122.3b	1821.6c	1691.9d	1608.9d

Note: Significance between different letters in a row at 0.05 level. The same as below.

Table 4: Soluble Salt Amount of different time in Different Densities of *AmB* (Unit: mg/kg)

Time	CK	D1	D2	D3	D4
June 30th	6020.3a	1800.4b	1418.0b	1380.2b	1335.1b
August 10th	4810.5a	1720.1bc	1816.2b	1650.4c	1586.2c
October 20th	7984.2a	1912.7b	2052.8b	1736.8c	1712.5c

3.3 Effects of different plant densities of *AmB* on $(\text{K}^+ + \text{Na}^+)/(\text{Ca}^{2+} + \text{Mg}^{2+})$ in the soil

After *AmB* was planted, $(\text{K}^+ + \text{Na}^+)/(\text{Ca}^{2+} + \text{Mg}^{2+})$ in soils was very changed, the order of that was D1 (1.40) > D2 (1.27) > D3 (1.25) > D4 (1.22) and were respectively decreased for 27.5%, 34.2%, 35.2% and 36.8% compared to 1.93 of CK (Table 5). The reduction degree of $(\text{K}^+ + \text{Na}^+)/(\text{Ca}^{2+} + \text{Mg}^{2+})$ was positively correlated with planting density of *AmB*. Poor soil structure is one of main characteristics in saline-alkali soils, such as poor water stability. The water-stable aggregate content of soil is negatively correlated with $(\text{K}^+ + \text{Na}^+)/(\text{Ca}^{2+} + \text{Mg}^{2+})$, the reason is that increasing relative content of $(\text{K}^+ + \text{Na}^+)/(\text{Ca}^{2+} + \text{Mg}^{2+})$ improves the replacement of Na^+ in the soil, which is beneficial for coagulating soil colloid and forming soil aggregates.

Table 5: Change of $(K^+ + Na^+) / (Ca^{2+} + Mg^{2+})$ in different plant densities of AmB (weight ratio)

Soil layer	Planting density					
	CK	D1	D2	D3	D4	
0~20cm	1.91	1.30	1.40	1.27	1.26	
20~40cm	1.94	1.29	1.15	1.15	1.18	
40~60cm	1.90	1.47	1.11	1.20	1.09	
60~80cm	1.95	1.52	1.41	1.39	1.33	
Average	1.93	1.40	1.27	1.25	1.22	

3.4 Yield and growth in different densities of AmB

The plant spacing of D1, D2, D3 and D4 was 30cm, 25cm, 20cm and 15cm, respectively. The yield and upper branch of AmB were negatively correlated with plant spacing (Table 6); Lower branches in different densities were almost same (Table 6); whereas there were no regularity for other indexes, such as diameter of main root, main root length and lateral root length. Those in the D3 with plant spacing 20cm had the best comprehensive individual growth index and its yield was higher than those in the D4 with plant spacing 15cm. It further illustrated that planting density of AmB in the D3 with plant spacing 20cm was the best.

Table 6: Yield and Growth in Different Densities of AmB Unit: (kg/hm², 10⁻²cm, 10⁻³kg, cm, g)

Treatment	Yield	Diameter	Main root length	Side root length	Upper branch	Lower branch
D1	7296.2c	186.1a	38.0a	38.1a	6.6c	7.8a
D2	8534.7b	188.6 a	33.8b	33.4b	6.7c	7.7a
D3	9154.9a	182.0 a	34.4b	34.5b	7.6b	7.6a
D4	8991.5ab	165.4 b	31.0c	30.5c	8.7a	7.6a
Average	8494.33	180.53	34.30	34.13	7.40	7.68

3.5 Membrane permeability and proline content of leaves in AmB

The data in Table 7 showed that the leaves permeability of AmB planted in saline-alkali soil is 18.8% -25.4%; membrane permeability increased with the increase of plant density, the reason might be that more *Radix astragali* plants competed to cause insufficient nutrition and easily be suffered by salt, alkali and other adversities.

In saline-alkali soil, the leaves proline content of AmB was 0.13-0.50 µg/ml (Table 7). The leaves proline content of AmB basically increased with the increase of planting density, which suggested that indicating that saline-alkali soil could induce to enhance the leaves proline content of *Radix astragali* to resist saline-alkali damage.

Table 7: Membrane permeability and proline content of leaves in AmB

Planting density	Membrane permeability (%)			Proline (ug/ml)		
	June 30th	August 10th	Average	June 30th	August 10th	Average
CK	15.8	30.5	23.2	0.13	0.19	0.16
D1	13.2	24.3	18.8	0.21	0.27	0.24
D2	15.7	26.4	21.1	0.35	0.33	0.34
D3	16.4	28.7	22.6	0.48	0.31	0.40
D4	18.6	32.1	25.4	0.50	0.36	0.43

3.6 Salt Restraint effect of AmB in different years

It could be seen from Table 8 that the soluble salt, Na⁺, Cl⁻ and pH values of annual and biennial AmB were very different. The soluble salts, Cl⁻ and pH values of AmB were significantly decreased and but Na⁺ increased in two years. After planting AmB, the harmful salt ions decreased obviously. Those should be beneficial to improve the soil K⁺/Na⁺, which is very important for the growth of *Radix astragali* in saline-alkali soil. In general, saline-alkali K⁺/Na⁺ are not enough. The cultivation of *Radix astragali* regulates K⁺/Na⁺, thereby improving the nutritional status of soil to achieve the purpose of improving saline-alkali soil.

Table 8: Salt ions of *AmB* at different years (Unit: mg/kg)

Years	Soil layer	pH	Cl ⁻	Na ⁺	Total salt content
Annual	0-20cm	8.69	217.61	152.38	2563.02
	20-40cm	8.65	231.50	156.09	2694.60
	40-60cm	8.60	266.89	154.38	2625.34
	60-80cm	8.57	238.93	151.31	2443.85
	Average	8.63	238.73	153.54	2581.70
Biennial	0-20cm	8.51	212.80	167.46	2248.72
	20-40cm	8.55	227.94	166.69	2355.16
	40-60cm	8.46	213.16	170.15	2450.35
	60-80cm	8.45	209.92	165.37	2272.29
	Average	8.57	215.96	164.92	2331.63

4. Conclusions

The pH value of soil was decreased with increasing planting density of *AmB*, planting *AmB* could reduce the average soluble salt in 0–80cm soil layer for 67.4%-75.3%, those meant that *Radix astragali* had obvious alkali restraint function. Total average soluble salt content 0-80cm soil layer in four planting densities of *AmB* had greatly significantly lower than that of the control (CK) ($p < 0.05$). Meanwhile, the alkali restraint was positively correlated with planting density. Among different soil layers, *AmB* in the 0–20cm soil layer had the best salt restraint effect. The salt in 0–20cm soil layer was decreased to improve the growth of *AmB*. Highest soil soluble salt amount in different times appeared in October and the lowest was in August. The soluble salts, Cl⁻ and pH values of *AmB* were significantly decreased and but Na⁺ slightly increased in two years. *AmB* with plant spacing 20cm had the best individual growth index and the highest yield, which suggested that planting density with plant spacing 20cm and rowing spacing 30 cm of *AmB* was the best. Leaves membrane permeability and proline content of *AmB* increased with the increase of plant density; the soluble salts, Cl⁻ and pH values were significantly decreased and but Na⁺ increased in two years. So, planting *Radix astragali* in saline-alkali soils could accomplish salt restraint effects, improve saline-alkali soil and acquire the high crop yield, which should play very important role in exploiting industry, developing economy and improving ecology environment in saline-alkali land.

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References

- Ali S., Charles T.C., Glick B.R., 2014, Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase, *Plant Physiology and Biochemistry*, 80, 160-167, DOI: 10.1016/j.plaphy.2014.04.003
- Bose J., Rodrigo-Moreno A., Shabala S., 2014, ROS homeostasis in halophytes in the context of salinity stress tolerance, *Journal of Experimental Botany*, 65(5), 1241-1257, DOI: 10.1093/jxb/ert430
- Chen S., Hawighorst P., Sun J., Polle A., 2014, Salt tolerance in *Populus*: significance of stress signaling networks, mycorrhization, and soil amendments for cellular and whole-plant nutrition, *Environmental and experimental botany*, 107, 113-124, DOI: 10.1016/j.envexpbot.2014.06.001
- Ceccoli G., Ramos J., Pilatti V., Dellaferrera., Tivano J.C., Taleisnik E., Vegetti A.C., 2015, Salt glands in the Poaceae family and their relationship to salinity tolerance, *The Botanical Review*, 81(2), 162-178, DOI: 10.1007/s12229-015-9153-7
- Deinlein U., Stephan A.B., Horie T., Luo W., Xu G., Schroeder J.I., 2014, Plant salt-tolerance mechanisms, *Trends in plant science*, 19(6), 371-379, DOI: 10.1016/j.tplants.2014.02.001
- Di-Bella C.E., Jacobo E., Golluscio R.A., Rodríguez A.M., 2014, Effect of cattle grazing on soil salinity and vegetation composition along an elevation gradient in a temperate coastal salt marsh of Samborombón Bay (Argentina), *Wetlands ecology and management*, 22(1), 1-13, DOI: 10.1007/s11273-013-9317-3
- Flowers T.J., Munns R., Colmer T.D., 2014, Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes, *Annals of botany*, 115(3), 419-431, DOI: 10.1093/aob/mcu217
- Hamamoto S., Horie T., Hauser F., Deinlein U., Schroeder J.I., Uozumi N., 2015, HKT transporters mediate salt stress resistance in plants: from structure and function to the field, *Current opinion in biotechnology*, 32, 113-120, DOI: 10.1016/j.copbio.2014.11.025

- Hasegawa P.M., 2013, Sodium (Na⁺) homeostasis and salt tolerance of plants, *Environmental and Experimental Botany*, 92, 19-31, DOI: 10.1016/j.envexpbot.2013.03.001
- Iqbal N., Umar S., Khan N.A., Khan M.I.R., 2014, A new perspective of phytohormones in salinity tolerance: regulation of proline metabolism, *Environmental and Experimental Botany*, 100, 34-42, DOI: 10.1016/j.envexpbot.2013.12.006
- Islam F., Yasmeen T., Arif M.S., Ali S., Ali B., Hameed S., Zhou W., 2016, Plant growth promoting bacteria confer salt tolerance in *Vigna radiata* by up-regulating antioxidant defense and biological soil fertility, *Plant growth regulation*, 80(1), 23-36, DOI: 10.1007/s10725-015-0142-y
- Ismail A.M., Horie T., 2017, Genomics, Physiology, and Molecular Breeding Approaches for Improving Salt Tolerance, *Annual Review of Plant Biology*, 68, 405-434, DOI: 10.1146/annurev-arplant-042916-040936
- Jayakannan M., Bose J., Babourina O., Rengel Z., Shabala S., 2013, Salicylic acid improves salinity tolerance in *Arabidopsis* by restoring membrane potential and preventing salt-induced K⁺ loss via a GORK channel, *Journal of Experimental Botany*, 64(8), 2255-2268, DOI: 10.1093/jxb/ert085
- Mostofa M.G., Hossain M.A., Fujita M., 2015, Trehalose pretreatment induces salt tolerance in rice (*Oryza sativa* L.) seedlings: oxidative damage and co-induction of antioxidant defense and glyoxalase systems, *Protoplasma*, 252(2), 461-475, DOI: 10.1007/s00709-014-0691-3
- Manaa A., Gharbi E., Mimouni H., Wasti S., Aschi-Smiti S., Lutts S., Ahmed H.B., 2014, Simultaneous application of salicylic acid and calcium improves salt tolerance in two contrasting tomato (*Solanum lycopersicum*) cultivars, *South African Journal of Botany*, 95, 32-39, DOI: 10.1016/j.sajb.2014.07.015
- Rivero R.M., Mestre T.C., Mittler R.O.N., Rubio F., GARCIA- SANCHEZ., FRANCISCO., Martinez V., 2014, The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants, *Plant, cell & environment*, 37(5), 1059-1073, DOI: 10.1111/pce.12199
- Rahman M.A., Thomson M.J., Shah-E-Alam M., de Ocampo M., Egdane J., Ismail A.M., 2016, Exploring novel genetic sources of salinity tolerance in rice through molecular and physiological characterization, *Annals of botany*, 117(6), 1083-1097, DOI: 10.1093/aob/mcw030
- Shrivastava P., Kumar R., 2015, Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation, *Saudi journal of biological sciences*, 22(2), 123-131, DOI: 10.1016/j.sjbs.2014.12.001
- Song J., Wang B., 2014, Using euhalophytes to understand salt tolerance and to develop saline agriculture: *Suaeda salsa* as a promising model, *Annals of botany*, 115(3), 541-553, DOI: 10.1093/aob/mcu194