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Original Research

Responses of Soil Microorganisms and Enzymatic Activities to Alkaline Stress in Sugar Beet Rhizosphere

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Abstract

The objective of this study was to evaluate the effects of alkaline stress (0, 5, 7 and 9 g/kg $NaHCO_3$ and Na_2CO_3, identified as A0, A5, A7 and A9) on sugar beet seedlings root growth, rhizosphere soil microbial population, and soil enzyme activities to better understand their functions and relationships The present results total root length (TRL), total root surface area (TRSA) and root volume (RV) with the increase of alkaline levels, whereas the above items were significantly improved in A7 at S2 and S3 stages. The root activity (RA), quantity of rhizosphere microorganisms (bacteria, fungi, actinomycete and total microbial) and soil enzyme (urease, alkaline phosphatase and catalase) activities were decreased at A0, A5, and A9 but significantly increasedat A7. Moreover, the value of all indexes except rhizosphere fungi quantity of tolerant cultivar under the same treatment was higher than sensitive cultivar. These results demonstrated that alkaline stress inhibited root growth and reduced whole plant biomass, however, the suitable concentration of alkaline could stimulate the growth of sugar beet seedlings and increase the activities of rhizosphere microorganisms and soil enzymes.

Keywords: *Beta vulgaris* L., alkaline soils, microbial biomass, soil enzyme activities

Introduction

Soil saline-alkalization is a major abiotic stress to agriculture worldwide, causing considerable damage to crop growth and loss of crop productivity [1]. More than 954 million hectares of land in the world is made up of sodic soil [2]. Saline-alkali land is the result of natural properties such as arid climate, neotectonic movement and human activities [3-4]. In northeastern China, approximately 3.78 million hectares of land are threatened by soil salinization and alkalization [5]. Sodic soils with high salt concentration and pH restrict plant growth and limit agricultural production in this area, in which Na_2CO_3 and $NaHCO_3$ are the major limiting factors [6]. Enhanced salinity tolerance will enable more productive use of saline soil, and hence

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mechanisms involved in this ability are important areas of plant research [7].

The rhizosphere is characterized by high microbial abundance and activity which is involved in the nutrient acquisition and/or stress reduction strategies of plants [8-9]. Microorganisms are the principal drivers of all nutrient cycles, and especially for the decomposition of soil organic matter (SOM), thereby regenerating plant nutrients. Therefore, any effects by salt on microbial processes will have large connotations for SOM dynamics, ecosystem biogeochemical cycling, and plant nutrition [10-11]. Soil enzymes originate from a variety of organisms, especially fungi and bacteria, and represent the activity and diversity of microbial communities [12] because they are related to soil physiochemical characters, microbial community structure and vegetation [13-14]. Their activity reflects the functional diversity and activity of the microorganisms involved in decomposition processes, which are essential for soil functioning and soil ecosystem services [15-16]. Under the condition of soil pollution, the abundance and diversity of microbes decreased, but it was not clear how the rhizosphere microorganisms changed.

Sugar beet (*Beta vulgaris* L.) is used not only in the sugar industry but also in the production of bioethanol as a source of renewable energy [17]. It is breeding for adaptation to many abiotic stresses, including drought and salinity, and has been cultivated successfully in a wide range of climates on many different soils in temperate areas of the world [18]. Extensive studies have been conducted to elucidate the mechanisms by which plants themselves respond and adapt to salinity resulting from increases of salts in soils [19]. However, the linkage between rhizosphere processes and roots under salinity stress is not well understood. Therefore, in order to examine the effect of alkaline levels on microorganism and enzyme activity changes in the rhizosphere of sugar beets and to compare the effect of cultivars and triadic relationships under sodic stress has been defined as an important goal for this study and the study aimed at exploring the microorganism and soil enzyme changes of seedling rhizosphere and whether there were significant correlations among roots, microorganisms and soil enzyme changes under alkaline stress.

Materials and Methods

Experimental Site, Soil and Plant Material

The pot experiment was conducted at Northeast Agricultural University (126°63′E, 45°44′N, Harbin, China) in 2015. Physicochemical properties of the soil are shown in Table 1. The research site is in the northern temperate zone and continental monsoon area (rainy and hot during the summer; cold and arid during the winter) and the highest mean temperature was 23ºC and the lowest was 12ºC during the experiment. The average available accumulated temperature (≥10ºC) is 2709ºC.

Two sugar beet cultivars (*Beta vulgaris* L.) with contrasting salt tolerance were selected as tolerant (KWS0143) and as sensitive (Beta464).

Experimental Design

Each pot was filled with 3 kg of soil and placed into a rain-proof shelter during the experiment. Nitrogen, phosphorus, and potassium were applied to each pot at planting with 113.41 $mg \cdot kg^{-1}N$, 85.06 $mg \cdot kg^{-1}$ P_2O_5 and 56.71 $mg \cdot kg^{-1}K_2O$. Alkaline composites ($NaHCO_3$:Na_2CO_3, molar ratio 2:1) with different mass concentrations, including 0 g/kg (A0), 5 g/kg (A5), 7 g/kg (A7) and 9 g/kg (A9), were mixed with soil, and each treatment preserved ablank control with no plant species. All the fertilizer or alkaline salt were evenly mixed into soils for each treatment.

The sowing date was April 30. Two sugar beet cultivars were used in the pot experiment. The soil pH values of the A0, A5, A7 and A9 treatments were 7.22, 9.18, 9.40, and 9.56, respectively. The soil EC values of A0, A5, A7 and A9 treatments were 127.67 us/cm, 559.33, 782.67, and 981.67, respectively. 7 plants remained in each pot after emergence. 276 pots were prepared, and 800 ml of water was watered in each pot. The pots were arranged in a randomized complete block (RCB) design.

Sampling and Preparation

Sugar beet seedlings were sampled from 08:00 to 10:00 h at 0 day (June 2, S1), 14 days (June 16, S2), and 28 days (June 30, S3) after the first pair of true leaves unfolded and morphological and physiological parameters were measured at each stage. At the same time, rhizosphere and bulk soil samples were collected and divided into two portions. The first portion wasused for determining soiling soil enzyme activities, and the second portion was stored at 4ºC for analysis of microbial biomass. Dry matter weight of the whole seedling and root morphological parameters, root activity microbial biomass and soil enzyme activities were determined.

Table 1. Physico-chemical properties of soil used in the experiment.

Organic matter ($g \cdot kg^{-1}$)	Available nitrogen ($mg \cdot kg^{-1}$)	Available phosphorus ($mg \cdot kg^{-1}$)	Available potassium ($mg \cdot kg^{-1}$)	Soil moisture (%)	pH	EC (μs/cm)
51.20	120.03	171.20	117.01	10.70	7.22	126.70

Determining Seedling Dry Matter Weight and Root Parameters

Roots were washed with distilled water and blotted dry on filter paper. Subsequently, roots were arranged and floated on shallow water in a glass tray (20 cm × 30 cm), scanned (on a Microtek Scan Maker i800), and analyzed with an image analyzer (Plant Root Analyzer Type LA-S; Wseen, China). Afterward, total root length (TRL, cm), total root surface area (TRSA, cm^2) and total root volume (TRV, cm^3) were determined.

Root activity (RA, $\mu g \cdot g^{-1} \cdot h^{-1}$) was measured according to the triphenyl tetrazolium chloride (TTC) method [20]. The dehydrogenase activity is regarded as an index of root activity. Root activity = amount of TTC reduction (μg)/ fresh root weight (g) × time (h).

Five whole seedlings for each treatment were sampled to measure dry matter weight. The seedlings were heated for 15 min at 105ºC and then dried to a constant weight at 80ºC, after which dry plant weight was determined.

Determining Dry Matter Weight of Soil Microbial Population and Enzymatic Activities

Enumeration of cultivable microbial populations was determined with traditional plate-dilution frequency technique on agar media in Petri plates [21]. Well mixed 0.1 mL samples of dilutions (Bacteria 10^{-6}–10^{-4}, Actinomycete 10^{-5}–10^{-3} and fungi 10^{-3}–10^{-1}; 3 repeats per concentration) with sterile deionized water were spread on the following media for cultivable microbe enumerations.

The number of bacteria was determined in the culture medium of beef-cream and peptone for 2-3 days. Actinomycete was determined in the culture medium of improved Gao 1 for 3-5 days, and fungi was determined in that of Martin's agar for 5-7 days [21].

Table 2. Effect of alkaline stress on dry matter weight and root activity in sugar beet seedlings.

Stages	Treatments	DW (g)		RA ($\mu g \cdot g\ h^{-1}$)	
		KWS0143	Beta464	KWS0143	Beta464
S1	A0	0.133a	0.095a	79.24b	70.83b
	A5	0.117b	0.082b	83.44b	76.44b
	A7	0.108c	0.072c	104.9a	100.25a
	A9	0.048d	0.032d	52.63c	40.96c
	ANOVA effect				
	Alkaline (A)	***	***	***	***
	Cultivar (C)	***		***	
	A × C	***		ns	
S2	A0	0.408c	0.360c	134.79b	118.92b
	A5	0.463b	0.402b	141.33b	121.72b
	A7	0.632a	0.575a	158.14a	145.07a
	A9	0.187d	0.145d	82.51c	70.83c
	ANOVA effect				
	Alkaline (A)	***	***	***	***
	Cultivar (C)	***		***	
	A × C	ns		ns	
S3	A0	1.034b	0.854b	153.00c	125.92c
	A5	1.043b	0.869b	170.28b	132.93b
	A7	1.147a	0.991a	189.42a	156.74a
	A9	0.370c	0.257c	86.71d	72.70d
	ANOVA effect				
	Alkaline (A)	***	***	***	***
	Cultivar (C)	***		***	
	A × C	ns		***	

Different lower-case letters mean significant differences at 0.05 level (P<0.05). ***, *, and ns denote significance at 0.001, 0.05 and not significant (P>0.05), respectively. The same below.

All microbes were cultured at 30ºC. Soil urease, catalase, and alkaline phosphatase activities were determined by Tabatabai (1994) [22].

Statistical Analysis

Differences between treatments were calculated by one-way ANOVA and Duncan's multiple range tests at 0.05 level of probability. The least significant difference (LSD) multiple comparisons were conducted when there was significant difference (P<0.05). All the data were statistically analyzed using SPSS software (Statistical Product and Service Solutions, Version 22.0, SPSS Inc. Chicago, IL, USA). Alkaline stress effects were considered fixed and cultivars were treated as random effects. Mean comparisons were made under Fisher's protected LSD Test at the 0.05 level of probability. Pearson correlation analysis wasalso performed to evaluate the degree and significance of the correlation.

Results and Discussion

Changes in Plant Biomass and Root Growth in Seedlings

As shown in Table 2, dry matter weight of seedling and root activity of sugar beet were influenced significantly by alkaline stress at each stage. There was significant difference between cultivars regarding dry matter weight and root activity at the S3 stage. For dry matter weight and root activity, significant interaction between alkaline concentration and cultivar was observed at the S1 stage. The dry matter weight for both sugar beet cultivars decreased with an increase in alkaline concentration at S1 stage and in A9 significantly decreased ateach stage (P<0.05). These were consistent with most studies [23-25]. At S2 and S3 stages, however, dry matter weight first increased, and then decreased with an increase in alkaline concentration.

Table 3. Effect of alkaline stress on root morphology characteristics in sugar beet seedlings.

	Treatments	TRL (cm)		TRSA (cm^2)		RV (cm^3)	
		KWS0143	Beta464	KWS0143	Beta464	KWS0143	Beta464
S1	A0	14.96a	13.36a	3.29a	2.59ab	0.09a	0.07ab
	A5	14.05ab	10.85b	2.80b	2.31b	0.08ab	0.07b
	A7	12.12c	8.15c	2.37c	1.71c	0.07c	0.05c
	A9	3.53d	3.41d	1.02d	0.74d	0.03d	0.02d
	ANOVA effect						
	Alkaline (A)	***	***	***	***	***	***
	Cultivar (C)	***		***		***	
	A × C	***		ns		*	
S2	A0	55.87b	44.20b	19.49b	17.09b	1.25b	0.87b
	A5	56.29b	47.46b	20.27b	18.17b	1.34b	0.97b
	A7	76.42a	57.68a	29.36a	25.54a	1.69a	1.31a
	A9	12.81c	5.57c	3.01c	1.77c	0.24c	0.14c
	ANOVA effect						
	Alkaline (A)	***	***	***	***	***	***
	Cultivar (C)	***		***		***	
	A × C	*		*		*	
S3	A0	114.20b	95.62b	60.10b	47.27b	2.60b	2.14b
	A5	104.81c	85.48c	55.05b	45.76b	2.49c	2.01c
	A7	124.88a	104.73a	67.34a	54.76a	2.90a	2.46a
	A9	23.89d	8.55d	5.70c	4.06c	0.47d	0.27d
	ANOVA effect						
	Alkaline (A)	***	***	***	***	***	***
	Cultivar (C)	***		***		***	
	A × C	ns		*		**	

Table 4. Effect of alkaline stress on cultivable microbial numbers in sugar beetrhizosphere and bulk soil.

	Treatments	Bacteria ($\times10^5$ CFU·g^{-1})			Fungi ($\times10^3$ CFU·g^{-1})			Actinomycete ($\times10^5$ CFU·g^{-1})			Total microbes ($\times10^5$ CFU·g^{-1})		
		Bulk soils	KWS0143	Beta464	Bulk soils	KWS0143	Beta464	Bulk soils	KWS0143	Beta464	Bulk soils	KWS0143	Beta464
S1	A0	29.85b	35.36c	33.52c	49.75a	91.50b	98.09b	12.90a	17.34b	16.24b	43.25b	53.61c	50.73c
	A5	28.03b	47.46b	42.38b	1.00b	70.84c	66.46c	6.44b	17.57b	14.33c	34.48c	65.74b	57.37b
	A7	45.33a	86.43a	81.61a	2.03b	184.57a	188.15a	4.89c	20.68a	16.87a	50.24a	108.96a	100.36a
	A9	12.48c	21.63d	16.44d	0.54b	22.36d	24.32d	2.31d	6.52c	4.93d	14.80d	28.38d	21.61d
	ANOVA effect												
	Alkaline (A)	***	***	***	***	***	***		***	***		***	***
	Cultivar (C)		***		ns		***		***				
	A × C		ns		ns		***		ns				
S2	A0	104.04a	148.69c	123.45c	18.36a	20.43d	41.15c	23.75a	28.26a	25.59a	127.97a	177.16c	149.45c
	A5	66.97c	185.92b	145.50b	0.76b	58.36b	67.55b	10.70b	22.80b	21.59b	77.68c	209.31b	167.77b
	A7	82.33b	239.50a	200.47a	1.61b	77.19a	88.44a	6.03c	28.83a	26.65a	88.37b	269.10a	228.01a
	A9	32.97d	67.31d	52.84d	0.38b	28.85c	20.48d	3.97d	11.68c	9.31c	36.95d	79.27d	62.36d
	ANOVA effect												
	Alkaline (A)	***	***	***	***	***	***		***	***		***	***
	Cultivar (C)		***		***		***		***				
	A × C		*		***		ns		*				
S3	A0	58.86b	93.80c	80.07c	19.18a	53.11c	63.36b	25.79a	35.18a	31.96a	84.85a	129.51c	112.67c
	A5	54.97b	118.43b	97.67b	0.73b	62.37b	71.85b	12.08b	26.91c	23.30c	67.05b	145.96b	121.68b
	A7	75.57a	155.48a	141.16a	1.52b	625.69a	838.97a	7.09c	33.56b	30.30b	82.67a	195.29a	179.85a
	A9	31.93c	47.05d	40.97d	0.34b	15.45d	31.46c	5.08d	14.47d	11.85d	37.02c	61.68d	53.13d
	ANOVA effect												
	Alkaline (A)	***	***	***	***	***	***		***	***		***	***
	Cultivar (C)		***		***		***		***				
	A × C		ns		***		ns		ns				

Table 5. Effect of alkaline stress on soil enzyme activities in sugar beet rhizosphere and bulk soil.

Stages	Treatments	Urease (mg NH_4^+-N·g^{-1}·h^{-1})						Catalase(mL 0.1mol·L^{-1} $KMnO_4$·g^{-1})		
		Bulk soils	KWS0143	Beta464	Bulk soils	KWS0143	Beta464	Bulk soils	KWS0143	Beta464
S1	A0	0.67b	0.88b	0.80b	0.28c	0.43a	0.48a	2.28a	2.72a	2.60a
	A5	0.67b	0.93b	0.89b	0.31b	0.35c	0.35c	1.28b	2.20b	2.04c
	A7	0.76a	1.44a	1.24a	0.35a	0.39b	0.39b	1.08bc	2.76a	2.36ab
	A9	0.46c	0.50c	0.50c	0.18d	0.22d	0.20d	0.92c	1.12c	0.96d
	ANOVA effect									
	Alkaline (A)	**	***	***	***	***	***	***	***	***
	Cultivar (C)		**		ns		**			
	A × C		ns		**		ns			
S2	A0	0.97a	1.42b	1.28b	0.22c	0.40b	0.39b	1.84a	2.08a	2.16a
	A5	0.83b	1.39b	1.29b	0.26b	0.43b	0.34c	0.92b	1.68c	1.56c
	A7	0.99a	1.96a	1.80a	0.29a	0.51a	0.47a	0.72c	1.92b	1.68b
	A9	0.53c	0.74c	0.58c	0.14d	0.29c	0.23d	0.52d	1.00d	0.64d
	ANOVA effect									
	Alkaline (A)	**	***	***	**	***	***	***	***	***
	Cultivar (C)		***		***		***			
	A × C		ns		*		**			
S3	A0	0.86ab	1.15c	1.06b	0.24b	0.36c	0.33b	2.24a	2.72a	2.48a
	A5	0.75b	1.28b	1.06b	0.26b	0.41b	0.34b	1.28b	2.36c	2.08c
	A7	0.93a	1.82a	1.60a	0.29a	0.58a	0.55a	1.08c	2.60b	2.36b
	A9	0.42c	0.58d	0.49c	0.13c	0.20d	0.16c	0.92c	1.56d	1.20d
	ANOVA effect									
	Alkaline (A)	**	***	***	***	***	***	***	***	***
	Cultivar (C)		***		***		***			
	A × C		ns		ns		ns			

The A7 treatment significantly increased dry matter weight per plant of two cultivars (P<0.05). The same conclusion has been reported in *Chenopodiaceae*, such as *Kochia sieversiana* [26], *Salsolanitraria Pall*, *Haloxylonammodendron* [27], etc. Zou et al. (2017) reported that low levels of alkaline stress significantly promoted the growth of sugar beet due to the strong salt tolerance [28]. Interestingly, the root activities of both sugar beet cultivars first increased, and then declined withan increase inalkaline concentration at each stage in our study. This probably could be caused by the activation of Na^+ transporters by salinity and the enhancement of root viability to adapt to the stress environment [29].

The total root length, surface area and root volume decreased in alkaline conditions at S1 stage (Table 3), which indicated that the growth of new roots was hampered. A similar result was also found in *Arachishypogaea* plants [30]. However, it increased obviously under A7 alkaline stress compared to A0 at the S3 stage, which suggested that beet roots adapted to A7 alkaline stress [31]. Moreover, the total root length, surface area,root volume,dry matter weight and root activities values of KWS 0143 in A5, A7 and A9 were higher than those of Beta 464 at each stage.

Changes in Rhizosphere Microorganisms of Seedlings

As shown in Table 4, the number of microbes in the rhizosphere soil were significantly influenced by alkaline stress at each stage. There was significant difference between cultivars for microbial populations at stages S2 and S3. However, there was no significant interaction between alkaline concentration and cultivar

Table 6. Correlation coefficients between root parameters and other environmental factors under alkaline stress of KWS0143.

Stages	Parameter	Root activity	Bacteria quantity	Fungi quantity	Actinomycete quantity	Total microbial quantity
S1	Dry weight	0.695	0.407	0.526	0.877	0.509
	Root activity	1	0.94	0.946	0.954*	0.973*
	Total root length	0.713	0.431	0.523	0.89	0.531
	Surface area	0.601	0.291	0.425	0.811	0.399
	Root volume	0.642	0.342	0.444	0.842	0.447
	Urease activity	0.985*	0.978*	0.981*	0.892	0.994**
	Phosphatase activity	0.766	0.519	0.685	0.899	0.609
	Catalase activity	0.877	0.68	0.814	0.955*	0.755
	Total microbial quantity	0.973*	0.993**	0.963*	0.859	1
S2	Dry weight	0.972*	0.996**	0.789	0.868	0.997**
	Root activity	1	0.971*	0.665	0.93	0.981*
	Total root length	0.995**	0.970*	0.657	0.946	0.981*
	Surface area	0.991**	0.980*	0.695	0.929	0.989*
	Root volume	1.000**	0.969*	0.657	0.937	0.980*
	Urease activity	0.962*	0.971*	0.715	0.909	0.979*
	Phosphatase activity	0.974*	0.997**	0.791	0.867	0.999**
	Catalase activity	0.878	0.751	0.238	0.986*	0.784
	Total microbial quantity	0.981*	0.999**	0.789	0.866	1
S3	Dry weight	0.978*	0.883	0.532	0.929	0.876
	Root activity	1	0.957*	0.646	0.852	0.950*
	Total root length	0.961*	0.860	0.534	0.960*	0.855
	Surface area	0.964*	0.865	0.540	0.957*	0.86
	Root volume	0.969*	0.869	0.53	0.947	0.863
	Urease activity	0.913	0.981*	0.901	0.745	0.987*
	Phosphatase activity	0.946	0.994**	0.858	0.766	0.996**
	Catalase activity	0.875	0.736	0.426	0.994**	0.734
	Total microbial quantity	0.950*	0.999**	0.839	0.732	1

Data in the table are r-values. * and ** represent correlation at 0.05 and 0.01, respectively.

for bacteria and total microbial quantity at S1 or S3. The rhizosphere soil showed significantly higher the number of the bacteria and fungi than the bulk soil, particularly at stages S2 and S3. The bacteria and total microbial quantity in the rhizosphere soil of both cultivars first increased, and then decreased with increasing alkaline concentration. Several studies have reported pH to be a major factor influencing community structures across soil habitats [32]. According to our results, the quantities of both cultivars rhizosphere bacteria in A7 were 61.07-144.44% higher than A0 treatment at each stage. It may be that plants release root exudate to the rhizosphere in order to absorb the large amounts of PGPR to feed themselves under alkaline stress [33]. Moreover, research suggests that *Arbuscular Mycorrhizae* (AM) could increase total root surface area and make plants absorb water and nutrients more effectively, thus improving plant stress resistance [34]. The quantity of both cultivars rhizosphere fungi in A7 were 91.80~1224.09% higher than A0 at all stages in this study. This may be caused by the combination of beet roots and symbiotic fungi. The bacteria, actinomycete and total microbial quantity for KWS0143 with A9 were higher than Beta464 at each stage, but that was the opposite for fungi quantity.

Table 7. Correlation coefficients between root parameters and other environmental factors under alkaline stress of Beta464.

	Parameter	Root activity	Bacteria quantity	Fungi quantity	Actinomycete quantity	Total microbial quantity
S1	Dry weight	0.633	0.373	0.456	0.908	0.481
	Root activity	1	0.953*	0.924	0.892	0.981*
	Total root length	0.490	0.208	0.311	0.824	0.324
	Surface area	0.529	0.248	0.320	0.839	0.360
	Root volume	0.548	0.269	0.325	0.844	0.379
	Urease activity	0.992**	0.983*	0.942	0.831	0.997**
	Phosphatase activity	0.660	0.443	0.606	0.918	0.546
	Catalase activity	0.792	0.598	0.714	0.978*	0.690
	Total microbial quantity	0.981*	0.993**	0.961*	0.801	1
S2	Dry weight	0.985*	0.999**	0.961*	0.892	0.999**
	Root activity	1	0.982*	0.919	0.951*	0.991**
	Total root length	0.990**	0.954*	0.889	0.963*	0.967*
	Surface area	1.000**	0.982*	0.922	0.950	0.991**
	Root volume	0.999**	0.986*	0.934	0.939	0.993**
	Urease activity	0.992**	0.990*	0.929	0.927	0.995**
	Phosphatase activity	0.961*	0.938	0.835	0.945	0.951*
	Catalase activity	0.782	0.649	0.482	0.933	0.69
	Total microbial quantity	0.991**	0.998**	0.960*	0.903	1
S3	Dry weight	0.982*	0.887	0.541	0.912	0.874
	Root activity	1	0.959*	0.685	0.865	0.951*
	Surface area	0.974*	0.870	0.525	0.932	0.857
	Root volume	0.974*	0.872	0.537	0.941	0.86
	Total microbial quantity	0.951*	0.999**	0.867	0.727	1
	Urease activity	0.945	0.984*	0.882	0.787	0.989*
	Phosphatase activity	0.949	0.988*	0.878	0.782	0.992**
	Catalase activity	0.889	0.742	0.416	0.991**	0.732
	Total root length	0.961*	0.847	0.508	0.954*	0.835

Changes of Rhizosphere Soil Enzyme Activities of Seedlings

Soil enzymes are the mediator and catalysts of most soil transformation processes. As shown in Table 5, soil enzyme activities varied significantly in response to alkaline stress at each stage. Significant interactions between alkaline concentration and cultivar are observed for urease at each stage, alkaline phosphatase at S3 stage and catalase activity at stages S1 or S3. Results from our experiment indicated that high pH decreased activities of soil enzymes. Hendriksen (2016) [16] also found that the activity of the enzymes depend on pH. The A7 treatment produced the highest values for soil enzyme activities of both cultivars at each stage, while A9 treatment gave the lowest value for the soil enzymes. The urease, alkaline phosphatase and catalase activities showed that similar changes among treatments and activitiesof KWS0143 were higher at later stages (S2 and S3).

Relationships between Root Parameters and other Indexes

The correlation among traits of two varieties was shown in Tables 6 and 7, respectively. Significantly positive correlation among bacteria quantity, total microbial quantity and urease activity was observed at all stages. Similarly, Eriksson found that enzyme is mainly derived from microbial population, in particular

bacteria, and they concluded that the phenomenon might have been due to the key role of both bacteria and urease in nutrient cycling [35]. Soil enzymes are synthetized and secreted by soil microorganisms, and are the proximate agents of organic matter formation and decomposition [36].

Soil enzymes may also be affected indirectly by plant microbe interactions. There was positively significant relationship between root activity and dry weight at stages S2 and S3. Moreover, there was positive relationship between root activity and total microbial quantity at S1, S2, and S3. With the increase of alkaline concentration, dry matter of seedling and root growth decreased at the S1 stage, whereas it increased and then decreased at the later seedling stage, while root activity kept increasing and then decreasing at the whole seedling stage. These results indicate that succinate dehydrogenase, which represents the activity of beet root, is an important factor in the formation of rhizosphere microbial communities [37]. The beet roots could release exudates to attract probiotics in the adaptive phase under alkaline stress [38].

Under suitable alkaline concentration, the number of rhizospheric microorganisms is increased. Among them, fungi increased the order of magnitude, but the number of bacteria was still the largest, dominating the whole microbial community. From the point of view of time, the effect of anaphase is more relevant, which may be related to root growth and elongation [29]. The cause of the correlation between the root activity of the beet and the enzyme is probably the role of the bacteria, but the function of fungi could not be ignored. Studies have also shown that fungi play an important role in alkaline conditions [39-41]. Urease activity comes from microbial population and the increased urease also provides nutrients for plants. Further research is required to study in detail the interactions between the root environment and microorganisms, and how such interactions affect the activities of soil enzymes [42].

Conclusions

In the present study, we evaluated the effects of different levels of alkaline on growth of sugar beet and soil enzyme activity in the rhizosphere soil. Our results indicate that a high level (A9) of alkaline stress significantly decreased dry matter accumulation, root morphology, root activity, soil microbe quantity and enzyme activity at late seedling stage, whereas middle level (A7) of alkaline stress increased all of these parameters at late seedling stage, especially root activity, rhizosphere bacteria, fungi and urease activity. Dry matter weightand root activity of KWS0143 under alkaline stress were higher compared with Beta464. The results can provide theoretical reference for improving sugar beet saline alkali soil.

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Conflict of Interest

The authors declare no conflict of interest.

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