

Analysis of the relationship between rusty root incidences and soil properties in *Panax ginseng*

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Abstract. Rusty root is a serious problem in ginseng cultivation that limits the production and quality of ginseng worldwide. The Changbai Mountains are the most famous area for ginseng cultivation in China. To clarify the relationship between rusty root and soil characteristics, physico-chemical properties and enzymatic activities of soil collected from five different fields in the Changbai Mountains were analyzed and a controlled experiment carried out by increasing the concentration of Fe (II). Soil bulk density, moisture, total iron (Fe) and total manganese (Mn) concentrations and polyphenol oxidase (PPO) activity were significantly higher in rusty root than healthy root groups (two-sample test, $P < 0.05$ or $P < 0.01$), respectively. Pearson test showed that there was a significant positive correlation between rusty root index and pH, N, Fe, Mn, Al, Zn and Ca of soil samples collected from fields ($P < 0.05$ or $P < 0.01$), and a significant positive correlation also occurred between rusty root index and Fe (II) added to soil in Fe (II) inducing rusty root ($P < 0.01$). Physiological factors may be very important roles giving rise to ginseng rusty root. Fe (III) reduction and Fe (II) oxidation could be important in increasing the incidence of rusty root. Soil moisture and bulk density of non-rhizosphere soil not attached to the root surface, and pH, N and PPO content of rhizosphere soils attached to the root surface were heavily involved in the reduction, oxidation and sequestration of metal ions.

1. Introduction

Panax ginseng C.A.Mey is a valuable herbal root with multifunctional properties, including anti-cancer, anti-emetic, anti-oxidative, anti-angiogenic, anti-proliferation and apoptosis properties [1-4]. During cultivation, pharmacological/bioactive constituents of root in *P. ginseng* tend to increase with plant age [5]. But a widespread problem with ginseng roots such as rusty root is



caused by continuous cultivation over years [6,7]. Ginseng roots are commercially graded primarily on their size, shape and overall appearance. Roots with an off white to beige color and smooth surface without blemishes are highly valued [8,9]. It is the development of reddish-brown superficial discolored areas that eventually become dry and slough-off^[10]. When roots are dried, the affected areas are dark and sunken; a root shape that is distorted limits the production and quality of ginseng worldwide [11].

The pathogenesis of rusty root has always been controversial, it having been suggested that it arises due to physiological stresses [12-14]. It has inferred that the production of phenolic compounds and their transportation to the outside skin of ginseng is the cause, and iron compounds deposited on the root surface may be another rusty root inducing factor [8,15,16]. Most studies have focused on element content and the production of phenolic compounds in ginseng roots, whereas only a few have explored soil characteristics. The rhizosphere is immensely important to terrestrial life on Earth, being very closely related to plant growth. Thus, the rhizosphere, the component of soil adherent to the root, is where most of the important soil-plant relationships occur [17]. There may be a very close relationship between the incidence of rusty root and ginseng rhizosphere soil properties. Ferrous ions in the soil might be the leading factor related to rusty root. Li et al. [18] and Wang et al. [19] suggested that active reducing organic substances formed in soil under high moisture conditions could promote the activation of iron oxides and enhanced the accumulation of its divalent iron, and rusty root could be attributed to the oxidizing and depositing of ferrous iron (Fe (II)) in epidermis of ginseng root.

The object of this study was characterization and comparison of the physico-chemical properties and enzymatic activities of soil collected from Changbai Mountains to identify any possible relationship between rusty root of ginseng and soil characteristics. A controlled experiment was carried out by increasing the concentration of Fe (II) to see if this induced rusty root.

2. Materials and methods

2.1 Soil sampling and analysis

The sampling areas were located in Fusong country, which is one of the most well-known areas for ginseng cultivation in the Changbai Mountains. Rhizosphere soils of ginseng roots were sampled from five ginseng cultivation fields (A to E in Table 1) suggested by FuSong ginseng research institute. The 20 × 20 m plots were sampled in autumn 2014 during the ginseng harvesting. The middle of each field was divided into 4-6 plots (1×1m) to avoid border undesired effects on experiment accuracy. Ten ginseng roots were harvested randomly from each plot, and 40-60 ginseng roots in each field were used for rusty root index statistics. Four to six soil samples were collected in each field consisting of soils from 10 ginseng roots of the same rusty-root grade. The rusty root index statistics were calculated according to Liu et al. [14] and Li et al. [18] as follow:

$$\text{Rusty root index} = \frac{\sum(n \times \text{rusty root grade})}{N \times \text{the highest rusty root grade}}$$

where n equals the number of ginsengs with different rusty root grade, N equals the number of total ginsengs. The rusty-root grades ranged from 0 to 4, which were defined by healthy ginseng root without rust, and a rust area <10%, 10-25%, 25-50%, and >50% (Table 1).

Non-rhizosphere soils not attached to the root surface for measuring bulk density and moisture were collected from a depth of 5-10 cm using a 100 cm³ stainless steel cylinder, and dried at 105°C. Rhizosphere soil samples for chemical and enzymatic activity analysis of adhering soil were collected by gently shaking roots into sterile bags, which were sealed and placed in ice [20]. Samples were air-dried and passed through a 0.15 mm sieve for metal and organic matter content analysis, and 2 mm sieve for other properties, respectively. Soil pH was determined with a glass electrode pH-meter (PHS-3C; Shanghai, China) in a suspension of 1:2.5 soil/water ratio (w/v) [21]. Organic matter content was determined by the Walkley and Black method [22]. Available nitrogen was detected by alkaline hydrolysis; available phosphorous was determined by the sodium hydroxide-molybdenum stibium anti-color method, and available potassium was measured by the ammonium acetate-flame photometer method [23].

For analysis of metal content (total Ca, Mg, Al, Fe, Mn, Cu, Ni), samples were digested with 15 mL HNO₃ and 20 mL HClO₄ (70%), and analyzed by ICP-AES [24]. And the measured metals in our study were selected according to Rahman and Punja [11].

Table 1. Samples from five fields.

Field name	Cultivation years	Latitude (N)	Longitude (E)	Soil sample	Rusty-root grade	Rusty root index
A	5	41°58'40"	127°31'38"	A-1	4(>50%)	
				A-2	4(>50%)	
				A-3	4(>50%)	
				A-4	1(<10%)	
				A-5	1(<10%)	
				A-6	1(<10%)	
B	9	41°58'54"	127°31'37"	B-1	4(>50%)	0.50
				B-2	4(>50%)	
				B-3	4(>50%)	
				B-4	0	
				B-5	0	
				B-6	0	
C	5	42°14'31"	127°45'22"	C-1	2(10%-25%)	0.69
				C-2	4(>50%)	
				C-3	3(25%-50%)	
				C-4	2(10%-25%)	
D	5	41°18'15"	127°24'8"	D-1	3(25%-50%)	0.33
				D-2	0	
				D-3	0	
				D-4	1(<10%)	
E	2	42°25'27"	127°20'56"	E-1	4(>50%)	0.80
				E-2	3(25%-50%)	
				E-3	4(>50%)	
				E-4	4(>50%)	

Samples for enzymatic activity were stored at 4°C and analyzed within a few days after collection [23]. Soil enzymatic activities (urease, acid phosphatase, catalase and polyphenol oxidase) were assayed according to Guan et al. [25]. Each assay was repeated independently at least three times and averaged.

2.2 Fe (II) induction of rusty root

Rusty root could be attributed to the oxidation and deposition of ferrous iron (Fe (II)) in epidermis of ginseng root [18, 19]. Rusty root was induced through a controlled experiment by increasing the concentration of Fe (II) at a time to elucidate the cause in our study. Four-year-old fresh ginseng roots were collected in April from Fusong country. They were placed in sand at 23°C. Six days after germination, the roots of uniform seedlings were washed with deionized water, and transferred in batches of 5 to separate pots containing 2.5 kg soil. To induce rusty root in ginseng by Fe (II), different amounts of Fe(II)EDTA were added to the soils (pH 6.36, 3.60g/kg iron, and 0.245 g/kg manganese) to reach the Fe (II) concentration as follows: 0.67, 1.34, 2.68, 5.36, and 10.72 g/kg. No Fe (II) was added to the control treatment, and the different treatments were referred to as Fe0 (0), Fe1 (0.67 g/kg), Fe2 (1.34 g/kg), Fe3 (2.68 g/kg), Fe4 (5.36 g/kg) and Fe5 (10.72 g/kg) based on the amount of Fe (II). No external nutrients were supplied during the experiment but the plants were watered. After 28-day, plants were harvested and visual inspection of root symptoms were recorded for statistical analysis.

2.3 Statistical analysis

The soil samples from the five fields were divided into 2 groups: healthy root group (the corresponding rusty-root grades were 0 and 1) and rusty root group (the corresponding grades were from 2 to 4). Differences between the means in the 2 groups and relationship between rusty root index and soil properties were analyzed by the two-sample t-test and Pearson correlation test according to SPASS 20.0 software, with significant being $P < 0.05$ or less.

3. Results

3.1 Analysis of rhizosphere soil properties

Soil physico-chemical properties including bulk density (0.98 vs $0.90 \text{ g}\cdot\text{cm}^{-3}$), moisture (24.27 vs 21.01%), total iron (Fe) (7.37 vs 5.62 g/kg) and total manganese (Mn) (0.58 vs 0.35 g/kg) concentrations were significantly higher in rusty root groups than healthy root groups ($P < 0.05$ or $P < 0.01$) (Table 2 and 3). Polyphenol oxidase (PPO) activity of rusty root groups was 67.17% higher than the healthy groups ($P < 0.01$; Table 4). No significant differences in other soil properties between rusty root groups and healthy groups were observed ($p > 0.05$).

Soil samples were acidic with $\text{pH}_{\text{H}_2\text{O}}$ ranging from 4.29 to 6.56 . The soil samples from fields B (4.55) and E (6.26) had lowest and highest $\text{pH}_{\text{H}_2\text{O}}$ values in the five fields (Table 2). Pearson test showed a significant positive correlation between rusty root index and pH, Al, Zn, Ca and Ni ($P < 0.05$), and a very significant positive correlation between rusty root index and N, Fe and Mn

($P < 0.01$; Tables 2 and 3). There was no significant correlation between rusty root index and other environment factors and cultivation years ($p > 0.05$).

3.2 Fe-inducing rusty root

After 28-days, reddish-brown spots appeared at the root surface, and the ginseng root showed symptoms of rust (Figure 1). There was a very significant positive correlation between rusty root index and the amount of Fe added to the soil ($P < 0.01$; Table 5). The extensive of rusty root was Fe(II) concentration-dependent.

Figure 1. Rusty roots of *P. ginseng* inducing by the Fe (II) concentrations of 5.36 and 10.72 g/kg.



Table 2. Soil moisture, bulk density, organic matter, alkaline solved N, effective P, effective K and pH of soil samples from five fields.

Soil sample	non-rhizosphere soil		rhizosphere soil				
	Soil moisture (%)	Bulk density (g.cm^{-3})	Organic matter (g.kg^{-1})	Alkaline solved N (mg.kg^{-1})	Effective P (mg.kg^{-1})	Effective K (mg.kg^{-1})	pH
A-1	23.82±0.09	1.0492±0.0074	166.21±4.46	541.56±4.91	43.96±0.50	294.68±2.41	5.13±0.0033
A-2	22.98±0.39	1.0234±0.0052	159.24±7.58	536.94±40.0	42.08±0.54	254.45±5.72	4.86±0.0057
A-3	23.46±0.46	1.0721±0.0186	110.70±8.30	383.83±91.4	37.15±0.66	294.67±8.23	5.05±0.0088
A-4	17.36±0.20	0.7518±0.0141	136.02±3.12	482.21±34.6	32.61±0.48	316.14±3.10	5.68±0.0067
A-5	18.06±0.19	0.8641±0.0059	85.48±21.97	401.36±5.46	34.17±2.67	261.62±4.25	5.31±0.0100
A-6	17.64±0.08	0.8567±0.0072	146.87±1.67	536.42±21.9	58.61±0.50	377.47±4.81	5.31±0.0088
B-1	26.36±0.38	0.9048±0.0040	105.98±1.15	318.73±36.2	15.48±0.16	247.94±1.08	4.65±0.0133
B-2	27.14±0.17	0.9751±0.0020	104.52±5.49	352.75±31.9	14.17±0.17	361.85±3.62	4.29±0.0100
B-3	28.03±0.28	0.9963±0.0050	127.96±16.4	375.59±42.1	6.89±0.30	291.87±6.63	4.33±0.0033
B-4	21.27±0.15	0.9110±0.0239	79.40±2.69	234.35±21.7	10.33±0.47	280.06±2.83	4.65±0.0033
B-5	20.87±0.22	0.9012±0.0036	85.91±8.06	238.41±13.3	16.89±6.96	350.98±2.73	4.72±0.0033
B-6	22.01±0.37	0.8964±0.0078	77.50±4.02	238.49±20.7	12.18±0.42	433.77±3.02	4.68±0.0057
C-1	21.23±0.16	0.8986±0.0044	108.01±3.02	414.80±13.9	35.83±3.13	254.54±2.89	5.35±0.0088
C-2	22.31±0.25	0.8769±0.0017	104.18±3.53	384.49±7.2	46.83±4.66	211.21±2.79	5.33±0.0120
C-3	21.98±0.23	0.9012±0.0036	133.17±25.5	369.70±18.3	60.21±2.40	254.54±2.89	5.31±0.0145
C-4	18.62±0.19	0.8276±0.0053	120.70±6.24	479.25±24.6	68.94±1.97	340.90±2.10	5.67±0.0115
D-1	28.58±0.11	1.0232±0.0031	50.88±0.99	240.76±11.2	33.09±1.81	132.67±3.34	5.47±0.0348
D-2	24.37±0.23	0.9557±0.0029	129.63±1.81	254.45±26.3	64.33±1.54	207.94±6.05	5.68±0.0233

D-3	23.84±0.26	0.9814±0.0039	108.69±1.90	235.17±11.8	59.86±1.91	191.75±3.67	5.61±0.0321
D-4	25.01±0.31	0.9631±0.0025	123.78±1.16	258.50±7.4	32.47±0.79	145.45±3.10	5.75±0.0120
E-1	24.03±0.37	1.0429±0.0024	37.97±14.22	646.40±48.4	10.78±0.21	142.13±2.85	6.52±0.0252
E-2	24.97±0.17	0.9875±0.0036	35.08±8.22	507.27±21.2	20.74±1.07	215.22±2.68	6.14±0.1645
E-3	25.31±0.09	1.0332±0.0074	36.67±2.05	512.51±25.5	39.54±0.29	439.68±4.04	6.50±0.0440
E-4	25.19±0.11	1.0194±0.0215	37.17±1.99	479.30±42.2	58.40±1.62	567.65±2.06	5.59±0.0305
E-5	19.71±0.23	0.9635±0.0946	24.94±0.90	683.70±16.8	34.18±1.35	260.30±3.53	6.56±0.0240

Values are means±SE (n = 3)

Table 3. The metal element content of soil samples from five fields.

Soil sample	rhizosphere soil						
	Total Ca (g/kg)	Total Mg (g/kg)	Total Al (g/kg)	Total Fe (g/kg)	Total Mn (g/kg)	Total Cu (mg/kg)	Total Ni (mg/kg)
A-1	1.855±0.011	1.572±0.006	12.971±0.282	7.454±0.056	0.429±0.003	13.599±0.140	15.358±0.238
A-2	1.772±0.031	1.546±0.018	12.821±0.507	7.511±0.191	0.450±0.018	14.058±0.429	15.938±0.718
A-3	1.553±0.010	1.472±0.011	12.349±0.279	7.195±0.132	0.456±0.008	12.421±0.348	14.513±0.384
A-4	1.600±0.017	1.411±0.004	12.483±0.608	6.931±0.184	0.433±0.031	13.153±0.376	13.955±0.384
A-5	1.535±0.009	1.369±0.001	12.464±0.416	6.739±0.080	0.418±0.015	12.578±0.273	13.718±0.155
A-6	1.764±0.010	1.286±0.010	11.983±0.218	6.484±0.046	0.429±0.010	14.078±0.222	13.598±0.314
B-1	1.232±0.028	1.248±0.014	11.294±0.359	6.494±0.099	0.306±0.007	10.209±0.174	15.035±0.353
B-2	1.169±0.013	1.197±0.004	10.729±0.094	6.228±0.027	0.306±0.001	9.756±0.104	14.009±0.276
B-3	1.182±0.004	1.199±0.003	10.989±0.067	6.270±0.026	0.312±0.015	10.366±0.106	14.507±0.195
B-4	1.084±0.013	1.188±0.004	10.474±0.262	5.201±0.073	0.264±0.013	8.201±0.128	14.431±0.232
B-5	1.271±0.009	1.195±0.008	11.758±0.129	5.420±0.064	0.326±0.007	10.729±0.308	14.740±0.303
B-6	1.242±0.015	1.212±0.007	12.140±0.178	5.479±0.060	0.306±0.007	9.545±0.256	15.118±0.248
C-1	1.779±0.011	1.147±0.008	13.738±0.383	6.998±0.102	0.722±0.034	18.058±0.403	13.730±0.210
C-2	1.456±0.001	0.749±0.01	18.172±0.42	5.711±0.088	0.459±0.01	15.298±0.76	11.309±0.30

		2	8		4	2	9
C-3	1.480±0.021	0.748±0.01	18.857±0.53	5.680±0.105	0.446±0.02	18.151±0.34	11.960±0.50
		3	9		1	7	7
C-4	1.481±0.007	0.721±0.00	17.280±0.29	4.332±0.043	0.412±0.01	18.274±0.84	11.672±0.32
		6	8		3	9	6
D-1	1.394±0.038	0.672±0.01	15.367±0.82	4.705±0.136	0.251±0.00	19.151±0.31	12.945±0.72
		9	3		8	9	5
D-2	1.398±0.018	0.661±0.01	14.560±0.34	3.518±0.094	0.192±0.00	19.948±0.45	12.192±0.51
		3	6		6	1	1
D-3	1.358±0.005	0.655±0.00	14.746±0.23	3.483±0.028	0.197±0.00	19.107±0.11	12.423±0.21
		6	0		5	2	1
D-4	1.439±0.017	0.618±0.01	14.678±0.18	3.669±0.099	0.300±0.02	22.901±0.24	12.820±0.36
		3	2		9	9	3
E-1	1.237±0.002	0.589±0.00	14.722±0.15	10.701±0.07	0.898±0.03	15.141±0.58	11.976±0.15
		3	0	9	8	5	1
E-2	1.210±0.023	0.642±0.01	11.989±1.16	10.027±0.03	0.915±0.03	14.964±0.92	13.105±0.17
		2	4	1	1	1	1
E-3	1.892±0.003	1.663±0.01	18.187±0.35	11.095±0.03	1.174±0.04	17.111±0.44	24.451±0.36
		6	1	8	6	8	4
E-4	1.855±0.009	1.590±0.00	18.084±0.20	10.188±0.01	1.086±0.00	17.176±0.44	24.217±0.42
		7	9	0	6	8	1
E-5	1.453±0.008	1.629±0.02	20.624±0.27		0.655±0.02	17.891±0.10	22.031±0.23
		3	3	9.221±0.112	4	4	0

Values are means±SE (n = 3)

Table 4. Polyphenol oxidase, catalase, urease and acid phosphatase activity of soil samples from five fields.

Soil sample	rhizosphere soil			
	Polyphenol oxidase m(purpurogallin)/ (mg.g ⁻¹ .soil.3h ⁻¹)	Catalase (mL of 0.1 mol/L KMnO ₄ g ⁻¹ .soil)	Acid phosphatase m(phenol)/ (mg.g ⁻¹ .soil.24h ⁻¹)	Urease m(NH ₃ -N)/ (mg.g ⁻¹ .soil.24h ⁻¹)
A-1	1.24±0.022	0.209±0.0042	28.15±7.15	0.683±0.0729
A-2	1.22±0.018	0.242±0.0058	27.71±1.09	0.448±0.1702
A-3	1.35±0.036	0.232±0.0142	31.74±3.64	0.549±0.0151
A-4	1.08±0.035	0.231±0.0053	31.19±1.55	0.530±0.0296
A-5	1.15±0.019	0.266±0.0156	33.24±2.64	0.504±0.1105
A-6	1.10±0.014	0.219±0.0046	31.33±3.84	0.436±0.0245
B-1	2.45±0.075	0.152±0.0054	32.16±4.63	0.077±0.0521
B-2	2.50±0.028	0.153±0.0032	26.98±0.48	0.069±0.0260

B-3	2.45±0.113	0.128±0.0002	36.09±3.65	0.078±0.0048
B-4	1.34±0.031	0.153±0.0004	32.88±1.62	0.019±0.0332
B-5	1.17±0.043	0.147±0.0057	35.98±3.29	0.087±0.0297
B-6	1.47±0.031	0.140±0.0031	34.22±2.95	0.057±0.0211
C-1	2.11±0.004	0.133±0.0184	29.25±1.41	0.478±0.0324
C-2	2.45±0.014	0.142±0.0108	36.56±1.56	0.518±0.0355
C-3	2.43±0.011	0.153±0.0008	39.60±2.97	0.485±0.0093
C-4	1.71±0.038	0.135±0.0195	40.03±2.81	0.384±0.0355
D-1	2.44±0.070	0.133±0.0092	28.16±2.83	0.285±0.0173
D-2	1.22±0.014	0.094±0.0022	28.25±1.52	0.217±0.0097
D-3	1.28±0.037	0.084±0.0153	43.39±6.51	0.113±0.0513
D-4	1.46±0.088	0.122±0.0030	36.73±6.14	0.278±0.0042
E-1	2.46±0.024	0.123±0.0105	31.53±1.58	0.232±0.0941
E-2	2.08±0.052	0.131±0.0059	33.28±1.23	0.228±0.0081
E-3	2.62±0.025	0.109±0.0052	47.97±3.53	0.173±0.0462
E-4	2.41±0.032	0.080±0.0061	40.09±2.06	0.213±0.0190
E-5	1.49±0.061	0.120±0.0006	20.74±1.29	0.125±0.0125

Values are means±SE (n = 3)

Table 5. Rusty root index of Fe (II) inducing rusty root experiment.

No.	The concentrations of Fe(II)	Rusty-root grade	Number of ginsengs	Rusty root index
Fe0	0	0	8	0.27
		1(<10%)	3	
Fe1	0.67g/kg	0	5	0.28
		1(<10%)	3	
		2(10%-25%)	1	
Fe2	1.34g/kg	0	3	0.38
		1(<10%)	3	
		2(10%-25%)	1	
		3(25%-50%)	2	
		4(>50%)	1	
Fe3	2.68g/kg	0	1	0.44
		1(<10%)	4	
		2(10%-25%)	1	
		3(25%-50%)	2	
		4(>50%)	1	
Fe4	5.36g/kg	0	1	0.44
		1(<10%)	5	
		3(25%-50%)	1	
		4(>50%)	2	

Fe5	10.72g/kg	1(<10%)	1	0.67
		2(10%-25%)	1	
		3(25%-50%)	3	
		4(>50%)	1	

4. Discussion

Non-rhizosphere and rhizosphere soils of ginseng roots sampled from five ginseng cultivation fields in Changbai Mountains were used to identify the relationship between rusty root and soil characteristics in our study. Soil bulk density, soil moisture, total iron (Fe), total manganese (Mn) concentrations and polyphenol oxidase (PPO) activity were significantly higher in rusty root groups than healthy root groups by two-sample test, respectively. High soil bulk density and moisture of non-rhizosphere soils in rusty root groups may lead to poor soil permeability, ventilation and low oxygen content, which could promote the reduction of insoluble metal ions into soluble ions, especially Fe (III) into Fe (II) which also provides the basis for Fe (II) oxidation [26]. Rahman and Punja [11] suggested that excessive Fe (II) in rhizosphere soil can be toxic to plant roots, and ginseng could induce host defense responses, producing phenolic compounds. An increase in PPO activity of the rhizosphere soil is closely related to its efficiency in promoting oxidation of polyphenols to quinones in the soil [27]. Quinones can bind with amino acids, metal ions and peptides, etc., to form initially humic acid molecules and pigment under suitable conditions [28,29]. Phenolic compounds secreted by ginseng roots are oxidized by PPO in rhizosphere soils and sequestered metal ions consequently form pigments molecules. These adhere to the ginseng root surface, making it red-brown. This was also suggested by Rahman and Punja [11], based on quantification of phenolic and metals in tissues from rusty and healthy root.

Pearson correlation showed that there was a significant positive correlation between rusty root index and pH, N, Fe, Mn, Al, Zn, Ca and Ni. Plant root can influence their environment by secreting oxidizing substances into the rhizosphere [30]. Ginseng root under iron toxicity stress can produce oxidizing substances, resulting in oxidation and deposition of metal ions (especially iron), which can be largely responsible for the symptoms in epidermal and underlying cortical cells of ginseng root. Thus rusty root index becomes higher with increase of metal ion content (especially Fe) in the rhizosphere. pH and nitrate also are involved in controlling Fe-cycling [26]. The soils were acidic with pH_{H2O} ranging from 4.29 to 6.56. The neutral pH conditions and nitrogen content are conducive to Fe (II) oxidation [26]. Higher pH and N content in rhizosphere may drive the oxidation of metal ions. Ginseng rusty root may be a physiological disorder that is closely related to soil environmental factors. Fe (III) reduction and Fe (II) oxidation could be an important influencing factor giving rise to rusty root; soil moisture and bulk density of non-rhizosphere soil can also promote the reduction of Fe (III) to Fe (II), and higher pH, N and PPO content of rhizosphere soils are important during oxidation and sequestration of metal ions. Increasing rusty root index with increasing addition of Fe (II) indicated that Fe (II) is a main factor inducing rusty root of *P. ginseng*.

5. Conclusions

Fe (III) reduction and Fe (II) oxidation could be very important in increasing the incidence of rusty root. Soil moisture and bulk density of non-rhizosphere soil, and pH, N and PPO content of rhizosphere soils were heavily involved in the reduction, oxidation and sequestration of metal ions (especially Fe). Physiological factors may be very important roles giving rise to ginseng rusty root.

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References

- [1] Kang K S, Yokozawa T, Kim H Y and Park J H 2006 Study on the nitric oxide scavenging effects of ginseng and its compounds *Journal of Agricultural and Food Chemistry* **54** 2558-62
- [2] Sagar S M, Yance D and Wong R K 2006 Natural health products that inhibit angiogenesis: a potential source for investigational new agents to treat cancer *Current Oncology* **13** 1426
- [3] Koo H N, Jeong H J, Choi I Y, An H J, Moon P D, Kim S J, Jee S Y, Um JY, Hong S H and Shin S S 2007 Mountain grown ginseng induces apoptosis in HL-60 cells and its mechanism have little relation with TNF- α production *The American Journal of Chinese Medicine* **35** 169-82
- [4] Shin B K, Kwon S W, Park J H 2015 Chemical diversity of ginseng saponins from *Panax ginseng* *Journal of Ginseng Research* **16** 287-98
- [5] Shi W, Wang Y, Li J, Zhang H and Ding L 2007 Investigation of ginsenosides in different parts and ages of *Panax ginseng* *Food Chemistry* **102** 664-8
- [6] Reeleder R D, Roy R and Capell B 2002 Seed and root rots of ginseng (*panax quinquefolius* L) caused by *cylindrocarpon destructans* and *fusarium* spp *Journal of Ginseng Research* **26** 151-8
- [7] Seifert K A, McMullen C R, Yee D, Reeleder R D and Dobinson K F 2003 Molecular differentiation and detection of ginseng-adapted isolates of the root rot fungus *Cylindrocarpon destructans* *Phytopathology* **93** 1533-42
- [8] Yang D C, Kim Y H, Yun K Y, Kwon JN, Kang H M and Lee S S 1997 Red-colored phenomena of ginseng (*Panax ginseng* CA Meyer) root and soil environment *Korean Journal of Ginseng Science* **21** 91-7
- [9] Campeau C, Proctor J T A, Jackson C J C and Rupasinghe H P V 2003 Rust-spotted north american ginseng roots: phenolic, antioxidant, ginsenoside, and mineral nutrient content *Hortscience* **38** 179-82
- [10] Punja Z K, Wan A, Goswami R S, Verma N, Rahman M, Barasubiye T, Seifert K A and Lévesque C A 2007 Diversity of *fusarium* species associated with discolored ginseng roots in british columbia *Canadian Journal of Plant Pathology* **29** 340-53
- [11] Rahman M and Punja Z K 2005 Biochemistry of ginseng root tissues affected by rusty root symptoms *Plant Physiology and Biochemistry* **43** 1103-14

- [12] Campeau C, Proctor J T A, Murr D P and Schooley J 2003 Characterization of north american ginseng rust-spot and the effects of ethephon *Journal of Ginseng Research* **27** 188-94
- [13] Punja Z K, Wan A M and Rahman M 2006 Role of fusarium species in rusty root development on ginseng roots *Phytopathology* **96** S170-1
- [14] Liu X, Yang Z, Gao L, Xiang W, Zhang B, Xie Z and You J 2014 Comparison of the characteristics of artificial ginseng bed soils in relation to the incidence of ginseng red skin disease *Experimental Agriculture* **50** 59-71
- [15] Lee S S, Lee M G and Choi K T 1999 Rusty-root tolerance and chemical components in 4-year old ginseng superior lines *Journal of Ginseng Research* **23** 61-6
- [16] Choi J E, Lee J S, Yoon S M and Cha S K 2002 Comparison of inorganic elements and epidermis structures in healthy and rusty ginseng *Korean Journal of Crop Science* **47** 161-6
- [17] Zhang X, Cao Y, Tian Y and Li J 2014 Short-term compost application increases rhizosphere soil carbon mineralization and stimulates root growth in long-term continuously cropped cucumber *Scientia Horticulturae* **175** 269-77
- [18] Li Z, Guo S, Tian S, Liu Z and Long B 1997 Study on the causes for ginseng red skin sickness occurred in albic bed soil *Acta Pedologica Sinica* **34** 328-35
- [19] Wang Y, Li Z, Sun Y, Guo S, Tian S and Liu Z 1997 Studies on the genesis of ginseng rust spots *Journal of Ginseng Research* **21** 69-77
- [20] Rumberger A, Yao S, Merwin I A, Nelson E B and Thies J E 2004 Rootstock genotype and orchard replant position rather than soil fumigation or compost amendment determine tree growth and rhizosphere bacterial community composition in an apple replant soil *Plant Soil* **264** 247-60
- [21] Moodie C D, Smith H W and McCreery R A 1951 Laboratory manual for soil fertility *Soil Science* **71** 400
- [22] Walkley A and Black I A 1934 An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method *Soil Science* **37** 29-38
- [23] Adamczyk B, Adamczyk S, Kukkola M, Tamminen P and Smolander A 2015 Logging residue harvest may decrease enzymatic activity of boreal forest soils *Soil Biology and Biochemistry* **82** 74-80
- [24] Rattan R K, Datta S P, Chhonkar P K, Suribabu K and Singh A K 2005 Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater-a case study *Agriculture, Ecosystems and Environment* **109** 310-22
- [25] Guan S Y 1986 *Soil Enzymes and Research Methods* (China: Agricultural Science Press) pp 274-97
- [26] Hu M and Li F 2014 Soil microbe mediated iron cycling and its environmental implication *Acta Pedologica Sinica* **51** 683-98
- [27] Liu R, Dai Y and Sun L 2015 Effect of rhizosphere enzymes on phytoremediation in PAH-contaminated soil using five plant species *Plos One* **10** e0120369

- [28] Duran N and Esposito E 2000 Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review *Applied Catalysis B: Environmental* **28** 83-99
- [29] Trasar-Cepeda C, Leiros M C, Seoane S and Gil-Sotres F 2000 Limitations of soil enzymes as indicators of soil pollution *Soil Biology and Biochemistry* **32** 1867-75
- [30] Fu Y, Yu Z, Cai K and Shen H 2010 Mechanisms of iron plaque formation on root surface of rice plants and their ecological and environmental effects :A review *Plant Nutrition and Fertilizer Science* **16** 1527-34