



Article Seedling Survival Strategies of Zanthoxylum planispinum 'Dintanensis' and Zanthoxylum amatum 'Novemfolius', Based on Functional Traits in Karst Desertification Control

Yanghua Yu *^D, Yanping Song and Yitong Li

School of Karst Science, State Engineering Technology Institute for Karst Decertification Control, Guizhou Normal University, Guiyang 550001, China

* Correspondence: yuyanghua2003@163.com; Tel./Fax: +86-851-86690199

Abstract: The exploration of the functional traits of Zanthoxylum planispinum 'dintanensis' and Zanthoxylum amatum 'novemfolius' at the seedling stage may provide a scientific basis for the zoning of germplasm resources and the introduction of plant varieties. The seedlings of the above mentioned two species with an age of about 8 months were selected as the study material, and the structure, nutrients, and physiological traits of leaves and roots were determined, respectively, to reveal their survival strategies. The results demonstrated the following four key elements. (1) The leaf thickness, specific leaf area, and leaf δ^{15} N value of Z. planispinum 'dintanensis' were significantly higher than those of Z. amatum 'novemfolius' (p < 0.05). However, the root diameter and Ca content showed the opposite results, and the other traits were not significantly different. (2) Leaf functional traits except Ca, as well as specific root length, and root P, Ca, and physiological traits had strong dominant effects on the functional trait system. (3) Among the main functional traits, leaf K and root P preferred environmental selection, leaf C, N, and P favored stable inheritance, and specific leaf area, leaf δ^{15} N, and root Ca were affected by varieties. (4) There were significant synergies (promotion) and trade-offs (inhibition) between the functional traits, and the leaves and the root system were closely correlated. It is speculated that Z. planispinum 'dintanensis' may have a stronger defense system. Its leaves are mainly related to growth and defense functions, and its roots are mainly related to the regulation of functions.

Keywords: structural trait; element trait; physiological trait; leaf; root; karst

1. Introduction

Plant functional traits are characteristic attributes regarding their morphology, structure, physiology, and biochemistry. They characterize the strategies of plants to adapt to habitats [1], determine the level of productivity [2], and affect the elasticity of ecosystems [3]. So, the functional traits of plants are the core means for researchers to learn about the internal relationship between plants and the environment. The leaf is the specific site of photosynthesis and the valve of hydraulic safety, and its functional traits may reveal resource utilization and allocation strategies during plant growth and regulation [4], and the maintenance of photosynthetic capacity [5], and there are strong hydraulic, anatomical and economical traits [6]. The root system is an important organ for plants to absorb water and nutrients, and the fine roots are the main bearers of ecological metabolic functions. The functional traits of the root system were found to be closely related to plant phylogeny and could indicate resource utilization strategies [7], and they also had an impact on the content and stability of rhizosphere carbon [8]. Therefore, the study of the two main organs of the plant, i.e., the leaf and root system, will help to deepen an understanding of the changes in the patterns of plant nutrient and water balance [9], and to clarify the ecological strategies of plants to adapt to the environment.



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The seedling stage is a vulnerable stage in the life cycle of a plant, and it is very sensitive to environmental changes [10]. Seedlings showed phenotypic plasticity in the process of adapting to changes in habitat factors [11]. Thus, the seedling stage is a key parameter during the growth period to inform about plant adaptation strategy in the habitat. Thus, elucidating the survival strategy of seedlings may lay a foundation for the planning and utilization of germplasm resources. Researchers have carried out various studies on the functional traits of seedlings, and they have achieved various results. The first one is the ecological implication of indicators. Studies have shown that functional traits could reveal the growth strategies of seedlings [12], indicating their great potential for drought resistance [13], and these traits may be different from those of adult plants [14]. The second one is the environmental factors that affect the variation of functional traits. It has been reported that the functional traits of the roots of *Cunninghamia lanceolata* (Lamb.) Hook. across various geographical provinces are more stable than those of the leaves [15]. It has also been revealed that the growth, biomass distribution, and morphologies of seedlings of different plants respond differently to their light environment, and thus, plant groups were categorized according to the finding [16]. Studies have also shown that the seedling traits of the same plant group are affected by environmental factors such as altitude [17], and these variations of traits have their own biological scales of effects [18], with clearly differentiated scaling patterns. The third is the regulation and application of traits. For example, brassinosteroids and gibberellins have control effects on the traits of maize seedlings [19], and they are closely related to the induction function of endogenous hormones. By regulating the functional traits of seedling roots, a foundation can be laid for germplasm improvement and quality optimization [20]. Different root traits also affect phosphorus utilization [21], establishing a theoretical basis for the screening of target traits. However, there is no research on the functional traits of the seedlings of Z. planispinum 'dintanensis' and Zanthoxylum amatum 'novemfolius' adapting to a drought environment. This knowledge gap has been limiting theoretical research on scientific introduction and germplasm resource zoning. Additionally, it is not conducive to the promotion and application of improved varieties, nor to the formulation of nursery stock standards.

Z. planispinum 'dintanensis' is a unique native plant in the karst dry-hot valley area of Guizhou, China. It has excellent properties, i.e., it can sustain a rocky habitat, it is calciumpreferred, and it is drought-resistant. The plant is famous for its 'pure fragrance, thick hemp flavor, excellent quality'. Additionally, it has been considered as a product-yielding species listed in the protected geographical areas, and as a preferred species for ecological industrial development. It has a planting area of more than 10,000 ha in China and has become a pillar industry for regional ecological construction and economic development. Z. amatum 'novemfolius' is the main variety of Z. amatum in the Sichuan and Chongqing regions. It has a large cultivation area in Jiangjin, Chongqing, which has won the title of "Hometown of Zanthoxylum in China". The species has also been introduced to the dry-hot valleys of Guizhou. As of now, the ages of the plants are mostly 3-5 years, and their growth and adaptability still need to be investigated. So, studying the functional traits of seedlings of these two varieties of Zanthoxylum can help us to explore the growth strategies of Z. amatum 'novemfolius' at the seedling stage in dry-hot valley areas. This provides a theoretical basis for introducing a fine variety, ecological restoration, and the utilization of germplasm resources. However, the research in this field is currently very limited, and there are no published literature reports. Therefore, this study selected the native plant *Z*. planispinum 'dintanensis', taking the introduced species Z. amatum 'novemfolius' as the control, to determine the functional traits of the leaf and root systems, mainly including the structural, nutrient, and physiological traits, which mainly reflect the resource acquisition and adaptive abilities of plants. These traits are the basis for selecting better traits in the future, and they can serve for trait regulation and germplasm creation. The purpose of elucidating their functional traits and growth strategies at the seedling stage is to answer the following two scientific questions: (1) to elucidate the differences in leaf and root

functional traits between *Z. planispinum* 'dintanensis' and *Z. amatum* 'novemfolius', and to reveal survival strategies at the seedling stage; (2) to screen the dominant functional traits, explore their sensitivities and selection tendencies, and to find out their internal correlation. In view of the above two scientific questions, this study made the following scientific hypotheses: (1) The resource acquisition and survival strategies of the local species of *Z. planispinum* 'dintanensis' and the exotic species of *Z. amatum* 'novemfolius' are different; (2) The dominance, selection tendency, and interaction of different plant functional traits were different. We hope to provide theoretical support for the introduction of *Zanthoxylum*, and to realize the scientific zoning and rational utilization of germplasm resources.

2. Materials and Methods

2.1. Study Site

The study area was located in Huajiang, Guanling, Anshun. The uniqueness of the habitat is indicated as a dry-hot climate, a deep valley, and with rocky desertification. The area has a dry and hot climate, mainly a subtropical humid monsoon climate, an annual average rainfall of approximately 1100 mm, with uneven seasonal distribution, severe drought in winter with spring and summer droughts, abundant heat resources, an annual average temperature of 18.4 °C with maximum and minimum temperatures of 32.4 and 6.6 °C, respectively, an annual total accumulated temperature of 6542.9 °C, a warm and dry winter and spring, and a hot and humid summer and autumn. The annual frost-free period is about 337 days, and the monthly mean meteorological data are shown in Table 1. The valley is 530–1473 m above sea level, with a 943 m elevation. The groundwater is deeply buried in the deep valley. Rocky desertification has developed in the Beipan River Basin of the upper reaches of the Pearl River. The exposed bedrock rate is between 50% and 80%, and carbonate rock accounts for 78.45%. The soil is mainly a calcareous soil of discontinuous soil mass. The vegetation is mainly natural secondary forest and Z. *planispinum* 'dintanensis' plantations. Although there is a lot of rainfall in this area, but the combination of geological and seasonal drought, as well as the tendency of plantation xerification, make the habitat tend to be arid.

Month	Rainfall/mm	Land Surface Available Temperature/°C Evaporation/mm Rainfall/mm		Temperature/°C	Radiation /(W·m ^{−2})	Humidity /%
January	17.00	16.49	0.51	12.17	227.26	80.02
February	10.40	10.32	0.08	14.79	320.71	70.44
March	12.60	12.51	0.09	19.15	373.30	67.19
April	33.50	32.84	0.66	26.40	572.70	64.11
May	31.20	30.65	0.55	26.20	465.80	67.24
June	253.00	125.07	127.93	27.41	582.78	78.13
July	156.60	112.47	44.13	27.40	585.91	78.62
August	75.60	70.09	5.51	28.70	761.22	73.05
September	296.40	105.78	190.62	25.30	587.69	73.80
Öctober	101.00	80.77	20.23	23.80	332.78	70.10
November	57.60	45.43	12.17	14.30	282.56	68.47
December	43.50	36.05	7.45	12.00	247.72	72.34

Table 1. Meteorological data statistics (2019 data) [22].

2.2. Plot Setting and Sample Collection

During the higher seedling growth period of 11–12 June 2022, three standard quadrats of 10×10 m were set up for each of *Z. planispinum* 'dintanensis' and *Z. amatum* 'novemfolius' in their bases, with similar environments (Table 2). Then, a number of 20 seedlings with heights of 30–40 cm, with no visible signs of diseases or pests to the naked eye, were randomly selected. All seedlings were sown in the nursery site during October 2021. At the seedling stage, thinning and tending measures were adopted, and the seedling growth environment was consistent without transplanting. The seeds

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of *Z. planispinum* 'dintanensis' were locally harvested, while the seeds of *Z. amatum* 'novemfolius' were from Jiangjin, Chongqing; all were collected from their respective adult mother trees. Select pure varieties, healthy growth, full fruits, stable yield, no pests and diseases as the seed trees. The picked seeds are dried in the sun, shelled, and sown in time to prevent damage due to heat.

Table 2. Basic situation of the seedling plots.

Variety	Altitude /m	Longitude	Latitude	Seedling Age/Month	Seedling Height/m	Insect Feed Rate
ZP	620	105°41′30.17″ E	35°39′49.35″ N	8	0.3–0.4	Less
AP	620	105°41′30.17″ E	35°39′49.35″ N	8	0.3–0.4	More

ZP: Zanthoxylum planispinum 'dintanensis'; AP: Zanthoxylum armatum 'novemfolius'. The same below.

All of the leaves and roots of 20 seedlings in each quadrat were collected. Only the primary roots were sampled, since the secondary roots had not fully grown by that time, and they were retained mostly on the primary roots. After collection, the samples were washed with tap water, and then rinsed 3 times with deionized water. In each quadrat, with the quadrate as the scale, 10 leaves and 10 roots were selected from all of the plants. After recording the number, the thickness, area, fresh biomass, saturated (in water for 12 h) fresh biomass of the leaf, as well as the diameter, length, and fresh biomass of the root were measured immediately, to prevent the impact of water loss on the test results. Among the measurements was each first-order branch root on the taproot; since plant root development in the karst area is greatly affected by underground cracks, the total root system could not be measured in this study. Then, all samples were dried in an oven at 65 °C to constant mass, and the biomass as dry wight was determined. The leaf thickness and root diameter were measured using a vernier caliper, the leaf area was measured using Delta-T Devices (Cambridge, UK), the root length was measured using a tape measure, and the mass was weighed using an analytical balance.

2.3. Sample Processing and Testing

All of the remaining fresh samples of leaves and roots were dried following the same procedure as that described before, being thoroughly mixed with the previously dried samples, and then ground and filtered through a 0.25 mm sieve for the analyses of chemical elements and stable isotopes. Plant carbon (C), nitrogen (N), and their stable isotopes were determined using elemental analyzer-stable isotope ratio mass spectrometry (Vario ISOTOPE Cube—IsoPrime IRMS, Elemental); a stable carbon isotope is denoted as δ^{13} C, and a stable nitrogen isotope is denoted as δ^{15} N. The contents of phosphorus (P), potassium (K), and calcium (Ca) were determined via perchloric acid-sulfuric acid digestion–molybdenum antimony anti-colorimetric–UV spectrophotometry, a flame spectrophotometer method, and inductively coupled plasma optical emission spectroscopy (ICP-OES) methods, respectively. Then, via calculation, the following parameters were obtained: the specific leaf area was the ratio of the leaf area to the dry mass, the leaf dry matter content was the ratio of the dry weight to saturated fresh mass, the specific root length was the ratio of the root length to dry weight, and the stoichiometric ratio was the element quality ratio.

2.4. Data Analysis

All data were organized and preprocessed using Microsoft Office Excel. In detail, firstly, Shapiro–Wilk function was used to test the normal distribution of the data, and lg transformation was performed if the data did not conform to the normal distribution. Then, a one-way analysis of variance (ANOVA) and least significant difference (LSD) methods were adopted to conduct multiple comparisons to test for the significant differences between the functional traits of the two *Zanthoxylum* varieties. Secondly, principal component analysis (PCA) was performed to screen out the main traits from all

of the functional trait indicators. Then, intraspecific and interspecific sensitivity indexes were calculated, respectively. Intraspecific refers to the internal species of *Zanthoxylum planispinum* 'dintanensis' or *Zanthoxylum armatum* 'novemfolius', and interspecific refers to between the two *Zanthoxylum* species. Intraspecific is to judge the changes within a single species, while interspecific is to judge the overall changes of two *Zanthoxylum* species. The calculation method was referred to the reference [23]. The intra-species and inter-species sensitivity indicators were calculated, respectively, by dividing the difference between the respective maximum and minimum values by the minimum value of a trait. The inter-species sensitivity was calculated using the maximum and minimum values of all values of a trait. The formula is $M_i = (I_{imax} - I_{imin})/I_{imin}$, where *M* is the sensitivity index and *I* is the functional trait. Finally, Pearson correlation analysis was used to reveal the correlation characteristics between the main characters, at a very significant and a significant level of $\alpha = 0.01$ and $\alpha = 0.05$, respectively. The data in the figures and the tables are the mean \pm standard deviation. Origin 2021 (version 2021, Originlab Corporation, Hampton, NY, USA) was used to make the figures.

3. Results and Analysis

3.1. Leaf Functional Traits

3.1.1. Leaf Structural Traits

The leaf thickness and specific leaf area of *Z. planispinum* 'dintanensis' were significantly higher than those of *Z. amatum* 'novemfolius' (p < 0.05, same as following), suggesting that *Z. planispinum* 'dintanensis' had a stronger ability to utilize and to preserve resources and to resist diseases. The leaf dry matter content of *Z. amatum* 'novemfolius' was higher, but the difference was not significant (Figure 1a).



Figure 1. Cont.



Figure 1. Leaf functional traits of *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'. Different letters represent significant differences at the level of p < 0.05. (a) The comparison of the leaf structural traits between *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'. (b) The comparison of the leaf element traits between *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'. (c) The comparison of the leaf element stoichiometry between *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'. (d) The comparison of the leaf physiological traits between *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'. (d) The comparison of the leaf physiological traits between *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'.

3.1.2. Leaf Nutrient Traits and Stoichiometry

In general, the contents of C, N, P, K, and Ca in the leaves of *Z. amatum* 'novemfolius' were higher, but the differences were not significant. It thus indicates a similar ability of these two varieties to adjust and to store nutrients (Figure 1b). Neither were the ratios of C:N, C:P, or N:P significantly different (Figure 1c), supporting their consistency in regulating leaf nutrient balance.

3.1.3. Leaf Physiological Traits

The δ^{13} C values of *Z. planispinum* 'dintanensis' and *Z. amatum* 'novemfolius' were (-31.53 ± 0.22) % and (-31.33 ± 0.20) %, respectively, with no significant differences. Additionally, both varieties had small variations, indicating no significantly different long-term water use efficiency between the two varieties. The δ^{15} N value was higher in *Z. planispinum* 'dintanensis', and 2.43 times that of *Z. amatum* 'novemfolius', suggesting that some volatile substances in *Z. planispinum* 'dintanensis' leaves were released, and that this resulted a strong fractionation effect (Figure 1d).

3.2. Root Functional Traits

3.2.1. Root Structural Traits

The root diameter of *Z. planispinum* 'dintanensis' was significantly smaller than that of *Z. amatum* 'novemfolius', and its variation was narrower as well. Additionally, the root length of *Z. planispinum* 'dintanensis' was also shorter than that of *Z. amatum* 'novemfolius', although the difference was not significant. The results suggested that *Z. planispinum* 'dintanensis' might absorb the available nutrients in niche spaces through a fine root strategy. There was no significant difference in the specific root length between the two varieties, but *Z. planispinum* 'dintanensis' exhibited less variation, indicating that the native species *Z. planispinum* 'dintanensis' may possess a more stable ability to withstand adversity (Figure 2a).



Figure 2. Cont.



Figure 2. Root functional traits of *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'. Different letters represent significant differences at the level of p < 0.05. (a) The comparison of the root structural traits between *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'. (b) The comparison of the root element traits between *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'. (c) The comparison of the root element stoichiometry between *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'. (d) The comparison of the root physiological traits between *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'. (d) The comparison of the root physiological traits between *Zanthoxylum planispinum* 'dintanensis' and *Zanthoxylum armatum* 'novemfolius'.

3.2.2. Root Nutrient and Chemical Traits

There were no significant differences in root C, N, P, and their stoichiometric ratios, as well as the K content between the two varieties, indicating that *Z. planispinum* 'dintanensis' and *Z. amatum* 'novemfolius' had similar abilities to maintain a large number of elements and to regulate nutrient balance (Figure 2b,c). However, the Ca content was higher in *Z. planispinum* 'dintanensis' (Figure 2b), suggesting a possible regulatory mechanism of *Z. planispinum* 'dintanensis' for Ca uptake in a high Ca environment.

3.2.3. Root Physiological Traits

There was no significant difference in root δ^{13} C between the two varieties (Figure 2d). Combined with the results of root diameter and length, it indicated that *Z. amatum* 'novemfolius' might counteract the disadvantage of thick roots by increasing the root length to

obtain a larger specific surface area and physiological activity, thereby increasing the respiration rate. Similarly, there was no significant difference in $\delta^{15}N$, suggesting consistent volatilization patterns of the root secondary metabolites of the two varieties.

3.3. Screening for Major Functional Traits

According to the principal of an eigenvalue of >1 and a cumulative contribution rate of >90%, the functional traits were extracted into five principal components. Then, according to the loading cutoff of >0.80, 14 factors with a big influence were extracted. In detail, there were nine leaf functional traits, including specific leaf area. Those were leaf thickness, dry matter content, C, N, P, K, δ^{13} C, and δ^{15} N. Similarly, there were five root functional traits, including specific root length, P, Ca, δ^{13} C, and δ^{15} N. The results demonstrated that leaf functional traits play a dominant role, especially the leaf structural traits and physiological traits (Table 3).

Factor	Principal Component (PC)						
	PC 1	PC 2	PC 3	PC 4	PC 5		
Specific leaf area	-0.963	-0.018	-0.214	0.058	0.154		
Leaf δ^{15} N	-0.923	0.320	-0.077	-0.057	0.189		
Leaf dry matter content	0.911	0.054	-0.016	0.302	0.274		
Leaf thickness	-0.852	0.483	-0.200	0.007	0.021		
Root diameter	0.766	-0.472	0.034	-0.430	0.057		
Root length	0.694	0.042	0.414	0.376	0.452		
Specific root length	0.250	0.965	0.039	0.025	-0.061		
Root $\delta^{15}N$	0.151	-0.891	-0.388	-0.176	-0.048		
Leaf K content	-0.288	0.867	-0.311	0.086	-0.248		
Leaf N content	-0.463	0.860	-0.108	0.159	0.095		
Leaf P content	-0.430	0.838	0.221	-0.181	-0.174		
Root P content	0.243	0.182	0.941	-0.148	0.026		
Leaf δ ¹³ C	0.364	-0.191	0.896	0.207	-0.118		
Root Ca	0.398	-0.211	0.874	0.029	-0.178		
Root K	-0.539	0.221	0.797	0.111	0.114		
Root K	0.008	-0.304	-0.777	-0.342	0.432		
Root C	0.431	0.391	-0.571	-0.507	0.278		
Root $\delta^{13}C$	0.200	0.118	0.166	0.955	-0.085		
Leaf C	0.141	0.307	0.188	0.194	-0.902		
Leaf Ca	0.221	0.340	-0.506	0.477	0.594		
Eigenvalue	5.909	5.086	4.910	2.149	1.946		
Cumulative contribution rate/%	29.546	54.974	79.525	90.272	100		

Table 3. Principal component analysis of plant functional traits.

3.4. Sensitivity to Changes in Major Functional Traits

The sensitivity index can represent the sensitivity or dullness of different functional traits to a variable response. A high sensitivity means a low anti-interference ability. Among the 14 main functional traits, high intraspecific and interspecific sensitivity were leaf K and root P, indicating that they were more prone to environmental selection. The intraspecific and interspecific sensitivities of leaf C, N, and P all had small variations, suggesting their stable heritabilities and lagged responses to habitat changes. The intraspecific and interspecific sensitivities of leaf δ^{15} N, specific leaf area, and root Ca had big variations, indicating that they were heavily affected by varieties (Table 4).

No.	Functional Traits	ZP	AP	Interspecific
1	Leaf thickness	0.11	0.14	0.50
2	Specific leaf area	0.15	0.05	0.56
3	Leaf dry matter content	0.11	0.21	0.33
4	Leaf C content	0.03	0.04	0.04
5	Leaf N content	0.03	0.02	0.04
6	Leaf P content	0.12	0.05	0.13
7	Leaf K content	0.44	0.53	0.70
8	Leaf $\delta^{13}C$	0.01	0.01	0.01
9	Leaf $\delta^{15}N$	0.13	0.12	1.78
10	Specific root length	0.32	0.11	0.32
11	Root P	0.19	0.21	0.25
12	Root Ca	0.08	0.11	0.82
13	Root δ^{13} C	0.02	0.01	0.02
14	Root $\delta^{15}N$	0.50	0.02	0.50

Table 4. Sensitivity analysis of changes in main functional traits.

3.5. Correlations between Major Functional Traits

According to Table 5, there were highly significant positive correlations between leaf $\delta^{15}N$ with leaf thickness and specific leaf area, and between leaf $\delta^{13}C$ with root Ca. There were significant positive correlations of leaf N with leaf thickness, leaf P, and leaf K; of specific leaf area with leaf thickness; and of root P with leaf $\delta^{13}C$ and root Ca. In contrast, root $\delta^{15}N$ was significantly and negatively correlated with leaf N, and with P and specific root length.

Table 5. Correlation analysis of main functional traits.

Index	Leaf Thickness	Specific Leaf Area	Leaf N	Leaf P	Leaf $\delta^{13}C$	Specific Root Length	Root P
Specific leaf area	0.857 *	1					
Leaf dry matter content	-0.739	-0.850 *					
Leaf N	0.835 *	-0.332	1				
Leaf P	0.722	0.459	0.851 *	1			
Leaf K	0.721	0.335	0.903 *	0.809			
leaf δ ¹³ C	-0.534	0.285	-0.322	-0.052	1		
Leaf $\delta^{15}N$	0.961 **	0.932 **	0.720	0.626	-0.469		
Specific root length	0.244	-0.278	0.708	0.716	0.051	1	
Root P	-0.307	-0.426	-0.079	0.278	0.881 *	0.268	1
Root Ca	-0.619	-0.596	-0.472	-0.129	0.974 **	-0.058	0.872 *
Root $\delta^{15}N$	-0.484	-0.044	-0.827 *	-0.857 *	-0.242	-0.838 *	-0.466

There is no significant (*) or extremely significant (**) correlation between some indicators, and they are not written out.

4. Discussion

4.1. Characteristics of the Seedling Leaf Functional Traits of the Two Zanthoxylum Varieties

Traits such as leaf thickness and dry matter content affected the cellulose content and palatability of plants [24]; they also had an impact on the digestion of herbivores, and thus they are important defensive traits. According to an on-site investigation during sampling, the insect herbivory rate of *Z. amatum* 'novemfolius' leaves was ~60%, much higher than the ~30% insect herbivory rate of *Z. planispinum* 'dintanensis' leaves [25]. It is speculated that *Z. planispinum* 'dintanensis' may adopt thicker leaves and volatilize some metabolites that are harmful to herbivores, to defend against insect pests. The reason for this is that *Z. planispinum* 'dintanensis' is a native species and it has a dry-hot ecotype. It has already developed a relatively stable adaptive mechanism and a stronger defense against common diseases in the area. As a contrast, *Z. amatum* 'novemfolius' is an exotic species and has a wet-hot ecotype. Its defense mechanism has not yet been fully developed, and the smaller leaves and higher nutritional contents make it preferred by animals. Studies have confirmed that the plant functional traits were closely correlated to the ability to resist pests and diseases [26]. In the future, the studies, including leaf texture, nutrient elements, and metabolites, to explore the plant's ability to resist pests, may provide a strong practical value.

Leaf δ^{13} C correlated positively with long-term water use efficiency [27,28]. No significant difference was seen in the seedling leaves of the two varieties, suggesting that their long-term water use efficiency was not significantly different. However, this was not consistent with the ecotype habits of these two Zanthoxylum varieties. The reasons might be that firstly, the precipitation in the first half of 2022 in the study area was relatively abundant, being higher than the historical period. There was less soil water deficit, and even drainage measures needed to be taken to prevent flooding in the nursery. Therefore, water was not a dominant or a limiting factor affecting the growth of seedlings. Secondly, both of the two varieties belong to the Zanthoxylum genus and share a common origin. During the later environmental selection process, although they evolved different traits, their water use capacity was controlled by their individual genetic characteristics and was highly stable, and this was also related to their climate-controlled ecotype. In the future, it is necessary to study the interpretation rates of genetics and habitat (soil moisture, light, etc.) on the water use capacities of different varieties of Zanthoxylum. In addition, there were no differences in leaf nutrient element traits between the two varieties of *Zanthoxylum*, indicating a similar nutrient retention capacity shared by them.

Plant secondary metabolites can represent environmental adaptability, including metabolic substances such as osmoregulation and antioxidants [29], have many functions such as allelopathic effects, drug components, signal transduction, and ecological adaptation [30,31], making their roles complicated in plant growth and physiology. The results of the present study suggested that more volatile substances were released from the leaves of *Z. amatum* 'novemfolius' seedlings [32], resulting in an effect in N isotope fractionation. The reason for this may be that chlorophyll was degraded, triggered a series of changes in physiological processes, and became a source of energy [33]. It may also be that *Z. planispinum* 'dintanensis' has evolved a special defense system and adaptive mechanism during its long-term adaptation to the environment [34]. In production practice, the main body of planting also employed the leaf flavor as a simple indicator for the screening of provenance. However, how leaf traits and quality traits are correlated and what is the underlying mechanism have not been elucidated yet, and these await further exploration.

4.2. Characteristics of Root Functional Traits of the Two Zanthoxylum Varieties

Plant root traits characterized the individual plant's strategy to cope with heterogeneous environments [35]. The thinner the roots, the better the ability to absorb nutrients. Previous studies have shown that root tips have evolved to be narrower and narrower from a nutrient-rich environment to a nutrient-deficient environment, in seeking to utilize nutrients efficiently [36]. It is speculated from our results that *Z. planispinum* 'dintanensis' prefers to obtain available nutrients in niche spaces through the fine root strategy and has evolved it into a stable trait during its long-term adaptation to the habitat. Compared with *Z. planispinum* 'dintanensis', *Z. amatum* 'novemfolius' might compensate for the deficiency of low surface area by increasing the root length to obtain nutrients and water. In the future, it is necessary to determine the morphology and diameter of root tip micro-domains to clarify the relationship between these root traits and nutrient utilization. Among the root structure traits, the specific root length of the two varieties did not vary significantly. Presumably, it is because the similar natural environment in the nursery caused the identical availability of soil resources, resulting in specific environmental selection pressure. Moreover, the nutrient-holding capacities of the root systems of these two varieties were also not significantly different, and this was presumed to be related to the lower heterogeneities of nursery habitats.

Karst soil is rich in Ca due to its high inheritance of Ca in the parent rock, and accordingly, plants have developed a specific mechanism to adapt to the high Ca environment [37]. As an important biogenic element, Ca functions for both nutrient and signal transduction, and it is a key nutrient element and messenger substance [38]. As a Ca-loving plant, *Z. planispinum* 'dintanensis' possesses a specific Ca-dependent mechanism. The results of the present study demonstrated that the root Ca content of *Z. amatum* 'novemfolius' was significantly higher than that of *Z. planispinum* 'dintanensis'. The reason for this might be that *Z. planispinum* 'dintanensis' has a stronger buffer capacity to the fluctuation of Ca content, so as to maintain Ca^{2+} in a suitable range. In addition, the Ca content in the root system of *Z. planispinum* 'dintanensis' may also be limited by the type and quantity of soil Ca components, and it may also have a synergistic absorption relationship with elements such as C and N. It is speculated that *Z. planispinum* 'dintanensis' has a mechanism of affecting the distribution of other elements by regulating Ca. However, the related mechanism remains elusive. As an exotic species, the suitability of the introduction of *Z. amatum* 'novemfolius' needs to be further investigated.

Combined with the root δ^{13} C values of the two varieties, our results suggested small differences in root respiration rates and the activities between them [39]. It may be because of the weather that the root system was in water-saturated soil for a long time. Soil water content affected the root respiration intensity and rate. At the same time, the δ^{13} C values of roots were higher than those of leaves, consistent with the results reported by Diao [39]. This was related to the transport route of high δ^{13} C substances from leaves to roots [40], and it was also impacted by the fractionation of substances such as sucrose during their synthesis. In addition, from what was revealed by the δ^{15} N of the root system, the metabolites released by the root system and leaf behaved quite differently, indicating certain differences in the physiological functions of the underground and aerial parts of the two *Zanthoxylum* varieties. This may be due to the fact that the respective metabolites exert different effects, and it may also have practical value for a plant to construct its system of defense against external threats. In the future, isotopic methods may be adopted to study the functions of root respiration and vitality, and to explore the internal relationships between roots and leaves, branches, bark, soil, etc.

4.3. Trade-Offs and Synergies among Major Functional Traits

Leaf thickness and specific leaf area had highly significant positive correlations with leaf δ^{15} N. Both of these two structural traits affect the ability of plants to capture light and to utilize nutrients, and to respond to element content and climate changes [41]. They also regulate heat and water [42], further affecting physiological processes in leaves such as photosynthesis and respiration, and they act on the utilization and fractionation of nitrogen. Thus, there are strong synergistic relationships between them. This indicated to a certain extent that easily observable phenotypic traits such as leaf thickness could serve as simple trait indicators to direct production practice. The results of this study also revealed a very significant enhancement effect between root Ca and leaf δ^{15} N. The reason for this is that the abundant Ca element in the environmental background of the karst area mediates ecological function and resource utilization, and it restricts the stability and heterogeneity of the ecosystem. As the starting point of plant Ca transport, root Ca has nutrient and messenger functions for material synthesis, secondary metabolism, etc. [36], and further exerts a synergistic effect on the fractionation of nitrogen.

Leaf N had a significant enhancement effect on leaf P and K. N and P are the basic elements of biological proteins and genetic materials, and both of them are restrictive elements. They help each other and have a same-direction effect for growth and for physiological activities. K is an essential element in the regulation of photosynthesis and osmotic substances, and it has a synergistic gain with the nitrogen element of the synthetic organism. However, there is no significant correlation between P and K, and it may be

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related to the trade-offs and synergies between the nutritional and messenger functions of the elements. Root P and Ca were synergistic. It might be due to the fact that Ca and P were converted into insoluble phosphorus in closed storage through precipitation, adsorption, immobilization, and microbial absorption [43]. Furthermore, the Ca content in karst areas was higher than those of Fe and Al and participated in the main chemical reaction. However, there was no similar pattern in the leaves. Presumably, this is related to nutrient reabsorption in the leaves, and a shorter distance between plants and the environment [44]. Moreover, it is also affected by the difference in the element transfer rate. Root δ^{15} N exhibited a significant negative effect on leaf N and leaf P. It may be that physiological activities such as root respiration and metabolism affected the microbe and extracellular enzyme activities involved in N and P cycles. Nevertheless, there was no significant effect on the contents of N and P in the root system itself. The reasons for this need to be further explored.

5. Conclusions

The local species of *Z. planispinum* 'dintanensis' has stronger ability to utilize and to preserve resources and to resist diseases, and builds stronger defense system by releasing volatile substances and forming fine roots. By increasing root length, *Z. amatum* 'novemfolius' can obtain a larger specific surface area and stronger physiological activity. Leaf functional traits (except Ca), specific root length, P, Ca, and physiological traits of the root had greater dominance on the trait system. Leaf K and root P tended to be selected by environment; leaf C, N, and P tended to be inherited stably, which was more greatly affected by variety than by specific leaf area, leaf δ^{15} N, or root Ca. Leaf and root functional traits had strong interaction through synergistic and trade-off effects.

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