



Effects of Planting Density on the Growth, Taproots Yield and Quality of *Glycyrrhiza uralensis*

T.T. Jia¹, B. Chen¹, M. Ma¹

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ABSTRACT

Background: Suitable planting density has an important influence on the high yield and quality of medicinal plants. How to minimize the development of its rhizome while improving the yield and quality of its taproots is one of the scientific issues that are urgent to be solved in the development of the cultivated licorice at present.

Methods: In the present study, *G. uralensis* was used as the experimental material, in an effort to find a reasonable cultivation management measure that may limit the development of rhizomes of licorice but increase the yield and quality of taproots, three planting density treatments were set to explore the effects of density on the growth of the licorice and the taproots yield and quality of its taproot and rhizomes.

Result: The results showed that with the increase in cultivation density, rhizome development and growth of *G. uralensis* were significantly suppressed and their clonal spreading potential was significantly reduced. The development of the rhizome was significantly inhibited with the increasing of cultivation density. It was observed that using the medium-density treatment gave the maximum mean value of the total yield and the total amount of medicinally active components (glycyrrhizic acid, glycyrrhizin and flavonoids) of licorice taproots every square kilometre among the three treatments. In summary, the best planting treatment for *G. uralensis* was medium-density.

Key words: Flavonoids, *G. uralensis*, Glycyrrhizic acid, Liquiritin, Planting density, Rhizome, Yield.

INTRODUCTION

Glycyrrhiza uralensis Fisch. ex DC. is a perennial clonal plant of Leguminosae and its dried taproots and rhizomes have been used medicinally. *G. uralensis* is popular with consumers because the slices of its taproots can be infused for drinking and its extracts have a variety of pharmacological actions such as anti-inflammatory, antitussive, anti-bacterial, anti-viral and inhibit the proliferation of tumor cells (Qin *et al.*, 2015; Yu *et al.*, 2015; Deng *et al.*, 2017; Kwon *et al.*, 2020; Huan *et al.*, 2021). Over the past few decades, with demands for its roots and rhizomes have been increasing significantly, *G. uralensis* was suffering predatory exploitation making many of its wild populations at the edge of extinction (Hayashi *et al.*, 2009; Huang *et al.*, 2010; Li *et al.*, 2013). In order to ensure an adequate supply of the licorice, China has encouraged the cultivation of the medicinal plant, which has indeed alleviated the contradiction between its supply and demand to a certain extent (Alamgir, 2017). As cultivated licorice is usually harvested within two or three years and has a short growth period, its taproots and rhizomes appear to have a lower content of medicinally active components than wild licorice. Therefore, almost all of the taproots and rhizomes are processed into medicinal slices after harvesting (Zheng *et al.*, 2020). Although the chemical compositions of the taproots and rhizomes are similar, consumers prefer the larger diameter of the taproots slices, which has a red, smooth and intact epidermis (Huang *et al.*, 2019) and the larger the diameter of the slices, the higher the price (Long, 2017). Since there is a trade-off in resource allocation between the taproots and

¹Ministry of Education Key Laboratory of Xinjiang Phytomedicine Resource Utilization, College of Life Sciences, Shihezi University, Shihezi, Xinjiang-832003, The People's Republic of China.

Corresponding Author: M. Ma, Ministry of Education Key Laboratory of Xinjiang Phytomedicine Resource Utilization, College of Life Sciences, Shihezi University, Shihezi, Xinjiang-832003, The People's Republic of China. Email: mamiaogg@126.com

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rhizomes, over-developed rhizomes will inevitably lead to a reduction in the yield and quality of the taproots (Wani *et al.*, 2021).

Cultivation density is often considered a key factor affecting morphological characteristics, biomass allocation and secondary metabolite accumulation of plants. It changes the occupation of space and utilization of water and mineral nutrients of crop's below-ground organs by intraspecific competition (Wang *et al.*, 2016; Desouza *et al.*, 2021), which in turn leads to variation in yield (Xu *et al.*, 2021; Liu *et al.*, 2021; Musavi *et al.*, 2020) and quality of crop's economic organs (Gunri *et al.*, 2015; Omerand Ufuk, 2019; Zahra *et al.*, 2021). So density plays a critical role in regulating the biomass allocation among the organs of plants and intraspecific competition is also an important factor in shaping the rhizomes' development of clonal plants (Lima *et al.*, 2021). Therefore, we are devoted to find the effect of

cultivation density on growth, rhizomes development, yield and quality of taproots and rhizomes of *G. uralensis*, intending to provide a scientific basis for establishing a cultivation system that may effectively inhibit rhizomes' development but significantly increase the yield of taproots and rhizomes of *G. uralensis*.

MATERIALS AND METHODS

Test material

The seeds of *G. uralensis* used for the test were collected in October 2018 from the town of Burqin (86°28'73"E, 41°38'83"N), in China and were dried at room temperature. Glycyrrhizic acid (Batch No.: C11654946) and liquiritin (Batch No.: C11602211), methanol, formic acid, anhydrous ethanol, acetate, aluminum trichloride and sulfuric acid (98%) were bought from Macklin Biochemical (Shanghai Macklin Biochemical Co, Ltd) and acetonitrile was purchased from Thermo Fisher Scientific, Inc. (LCMS grade, Thermo Fisher Scientific, USA).

Experimental design

On 10 April 2019, uniformly sized seeds of *G. uralensis* were placed in a beaker and were soaked in sulfuric acid solution with concentration of 98% for 0.5 h, then were rinsed with running water until no sulfuric acid residue remained on the seed coat and then were soaked in distilled water for 8 hours at room temperature. The seeds with full imbibition were selected and planted in plastic buckets (75 cm in diameter and 80 cm in height) with sandy soil (sand: loam mixture ratio of 3:7) as the cultivation substrate, total nitrogen content $1.21 \text{ g} \cdot \text{kg}^{-1}$, total phosphorus content $0.82 \text{ g} \cdot \text{kg}^{-1}$, total potassium content $2.57 \text{ g} \cdot \text{kg}^{-1}$, organic matter content $21.25 \text{ g} \cdot \text{kg}^{-1}$, available phosphorus content $39.33 \text{ mg} \cdot \text{kg}^{-1}$, available potassium $53.54 \text{ mg} \cdot \text{kg}^{-1}$ and 45 seeds of *G. uralensis* were evenly sown in each bucket at a depth of 1 cm. As emerging of the fourth true leaf, excess seedlings were removed according to the experimental design as following on 10 May 2019.

Three density treatments were designed in this study: low-density ($10 \text{ plants} \cdot \text{bucket}^{-1}$, corresponding to $2.25 \times 10^5 \text{ plants} \cdot \text{hm}^{-2}$), medium-density ($20 \text{ plants} \cdot \text{bucket}^{-1}$, amounting to $4.5 \times 10^5 \text{ plants} \cdot \text{hm}^{-2}$), high-density ($30 \text{ plants} \cdot \text{bucket}^{-1}$, being equal to $6.75 \times 10^5 \text{ plants} \cdot \text{hm}^{-2}$). Ten days after the density setting of the seeding, fertilizers were added to each bucket according to the common level of the *G. uralensis* field fertilization with $14.99 \text{ g} \cdot \text{m}^{-2}$ of urea ($\text{N} \geq 46\%$), $23.99 \text{ g} \cdot \text{m}^{-2}$ of potassium calcium super phosphate ($\text{P}_2\text{O}_5 \geq 46\%$) and $10.49 \text{ g} \cdot \text{m}^{-2}$ of potassium sulphate ($\text{K}_2\text{O} \geq 50\%$), which were added in 5 times at 3-week intervals. Ten repetitions were set for each density treatment.

During the experiment, each bucket was rehydrated by weighing method at 10:00 am (Beijing time) every day, maintaining the relative soil moisture at about 70%. All buckets were randomly placed in an open area on the Ministry of Education Key Laboratory of Xinjiang Phytomedicine Resource Utilization at Shihezi University

campus with a distance of 100 cm between two adjacent buckets to avoid shading among the treatments and the position of each bucket was randomly changed every week to eliminate the influence of light and marginal effect.

Measurement of biomass and morphological parameters

On October 1, 2020, all the materials were harvested after the height and crown width of each plant were measured, the complete below-ground part of each plant was carefully rinsed with running water until the surface was free of floating soil. The length and the maximum diameter of the taproots were measured for each plant; the number of rhizomes (length $\geq 1 \text{ cm}$), rhizome shoot (length $< 1 \text{ cm}$) and ramet were counted.

Each plant was separated into above-ground organ (stem and leaf), taproot and rhizome and the rhizomes of each treatment group were scanned by Expression 1100XL scanner (Epson company, Japan) and the scanned images were performed uniformly by using the image analysis software (WinRHIZO Pro 2013) to calculate the total length, total surface area and total volume of rhizomes of each plant. Then the above-ground organ, taproot and rhizomes were dried in an oven at 70°C to a constant weight and their biomass was measured by an analytical balance (Beijing Sartorius Instrument Systems Co. Ltd.). The biomass of below-ground organs (taproot and rhizome), the percentage of taproot biomass (taproot's biomass/below-ground organ's biomass) and the percentage of rhizome's biomass (rhizome biomass/below-ground organ's biomass) were calculated.

Measurement of the content of major active components of the taproots of *G. uralensis*

The contents of glycyrrhizic acid and glycyrrhizin were detected by the ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method (Agilent Technologies Inc., California, USA), the method of the standard curve, UHPLC and MS condition reference to detail indicated in a previous study (Fu *et al.*, 2013). The ion pairs, fragmentation voltages and collision energies used for quantitative analysis were shown in Table 1. The content of total flavonoids was determined by a UV-visible spectrophotometer (UV-1900, Shimadzu Corporation, Shanghai) at 334 nm with the LI standard (CAS#551-15-5) as the control.

Data analysis

Statistical analysis of all experimental data was performed by IBM SPSS 19.0 (IBM Corp, Armonk, NY, USA) software. One-way ANOVA was used to compare the differences in plant morphological parameters, rhizome development index, taproots yield and secondary metabolite content of the licorice under the different density treatment conditions. Duncan multiple range test ($\alpha=0.05$) was used to test for significant difference and the same letter means no significant difference among the two treatments, while the different means there is a significant difference between them, Origin 2019 software was used for plotting.

Table 1: Mass spectrometry conditions, regression equations and correlation coefficients of 3 compounds.

Compounds	Parent ion ^y (m/z)	Daughter ion (m/z)	Ionization mode	Voltage (V)	Collisional energy (V)	Retention time (min)	Regression equation	Correlation coefficients R^2	Linear overn (g/mL)
Glycyrrhizic acid	821.2	350.9*	-	62	42	2.48	$Y=87.2 X -3.76$	0.9992	1.0-978.0
		113.0		62	56				
		134.9		2	18				
Glycyrrhizin	417.0	254.9*	-	52	20	1.00	$Y=750.9 X+356.9$	0.9999	1.0-992.3
		134.9		52	30				

RESULTS AND DISCUSSION

Effect of cultivation of density on morphological parameters of *G. uralensis*

Influenced by environmental factors, plant inevitably develops certain morphological, structural and physiological metabolic characteristics that are suitable for the environment. Morphological changes are the most intuitive indicators that may reflect the adaptive status of a plant to cultivation density and may reflect the energy allocation strategies of different organs (Luo and Dong, 2002). The results of the present study showed that the most vigorous growth potential of *G. uralensis* was observed under low-density treatment, which showed the highest values of plant height, crown width, taproots length and taproots diameter among the three treatments (Fig 1). Compared with the low-density treatment, the plant height of *G. uralensis* was reduced by 14.32% and 8.78%; its crown width was reduced by 15.89% and 27.18%; its taproots length was shortened by 14.19% and 16.07%; and its taproots diameter was decreased by 13.84% and 24.23% under the medium-density and high-density planting conditions, respectively. In summary, the growth and development of rhizomes of *G. uralensis* were significantly inhibited by the increase in planting density and their clonal spreading potential was significantly reduced. This may be due to the fact that under low-density condition, individuals have more space to grow with less intraspecific competition and the space, nutrients, water and light resources required for growth are relatively adequate. On the contrary, plant growth is poor under high-density planting condition because the available space and resources of each single plant is very limited and the intraspecific competition intensity is relatively high (Gao, 2017).

Effects of planting density on the development of rhizomes of *G. uralensis*

Intraspecific competition is considered to be an important factor in shaping rhizome traits (Li *et al.*, 2020). In the present study, with the increase in cultivation density, rhizome development and growth of *G. uralensis* were significantly suppressed and their clonal spreading potential was significantly reduced, the resource allocation pattern between taproots and rhizomes was changed and more resources were invested in the growth of the taproots, thus showing that the number of rhizomes, shoots, clonal ramets, total length, total surface area, total volume, biomass and biomass percentage of the rhizome decreased significantly with increasing of the plant density (Fig 2). Compared with the low-density treatment, these above parameters decreased by 18.44%, 9.38%, 34.29%, 24.49%, 44.89%, 51.04%, 53.36% and 19.52%, respectively under the medium-density condition and those of the high-density treatment reduced by 39.27%, 40.01%, 46.67%, 50.99%, 70.09%, 78.17%, 82.55% and 33.45%, respectively. This is similar with the results of Baoqing Dai and Yan Wang's study on knotweed (*Zoysia japonica* Steud.) (Dai and Wang, 2013),

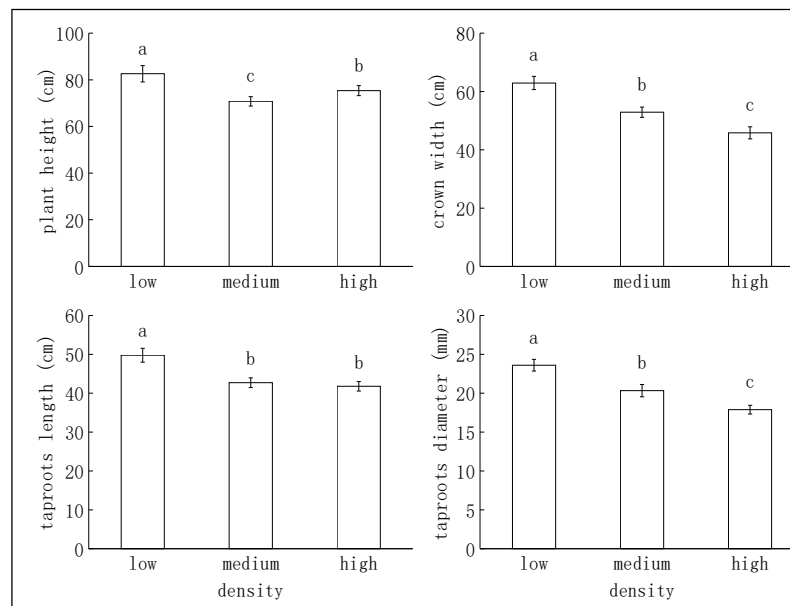


Fig 1: Effects of planting density on themorphological parameter of *G. uralensis* (Mean±SE). Different lowercase letters indicate significant differences among different treatments ($P<0.05$).

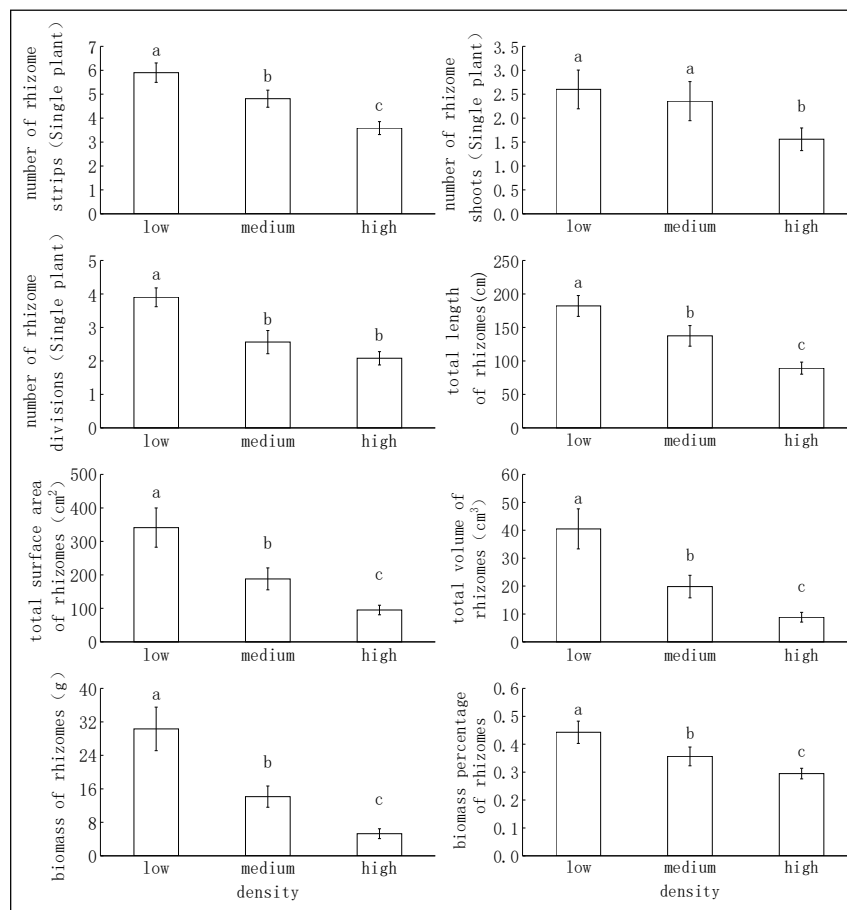


Fig 2: Effects of planting density on clonal propagation potential (rhizome development) of *G. uralensis* (Mean±SE). Different lowercase letters indicate significant differences among different treatments ($P<0.05$).

which showed that the rhizome's development and spread were significantly inhibited under high-density planting condition. This may be because the increased planting density intensified the intraspecific competition for space, light, water and mineral elements, leading to the blockage of horizontal extension of the rhizomes.

Effects of planting density on yield of the licorice's economic organs

Cultivation density also affects the final yield of a crop (Gunaeni *et al.*, 2021). The results of this study showed that the biomass of above-ground organs, below-ground organs and taproots of a single individual under low-density cultivation treatment were the highest among the three treatments and all the three indexes decreased with the increasing of cultivation density (Fig 3). Compared with the low-density treatment, the above-ground organs biomass decreased by 35.96% and 56.37% in the medium- and high-density treatment groups, respectively; the below-ground organs biomass declined by 42.04% and 73.77%, respectively and the taproots biomass reduced by 33.06% and 66.81%, respectively.

Although the taproots biomass of an individual was the lowest under the high-density condition, the percentage of taproots' biomass of it was the highest, the percentage of taproots biomass in the high-density treatment group increased by 25.56% and 9.58% compared with the low and medium-density treatments, respectively, which indicated that increasing cultivation density within a certain range could effectively promote the resources allocation to taproots.

However, as planting density increases, there is often a trend towards inconsistencies in crop yield between an

individual's and a population's. In the present study, the total yield (mean value of taproots biomass per plant \times number of plants in a certain area) of taproots was the highest in the medium-density treatment in a certain area (Table 2), which increased by 34.44% and 33.87% compared with those of the low and high-density treatments, respectively. This indicated that the increase in planting density resulted in a significant decrease of taproots' biomass of an individual, but the variation in total taproots' yield in a certain area of farmland showed a parabolic curve pattern. This may be due to the fact that the increase in number of the taproots to some extent compensate for the decrease in single taproots' biomass. However, when the seedling density continuously increased and reached a high level, the increase of the taproots' number could not effectively make up the decrease in the yield of single taproot, which might be the reason why the total yield of the taproots of *G. uralensis* under high-density planting conditions was significantly lower than that of the medium-density treatment group.

Effects of planting density on quality of the licorice's economic organs

The two most important factors determining the market price of medicinal material are the medicinal quality and the morphological character of the taproots and the medicinal quality plays a dominant role, which is mainly depended on the content of triterpenoids (Luo *et al.*, 2021) and flavonoids (Hu *et al.*, 2019) in the taproots. Content of glycyrrhetic acid, glycyrrhizin and total flavonoids is traditionally used to characterize the quality of the licorice in the Pharmacopoeia of the People's Republic of China (2020 edition)(Chinese Pharmacopoeia Commission *et al.*, 2019) and in this study, planting density had significant effects on the content of

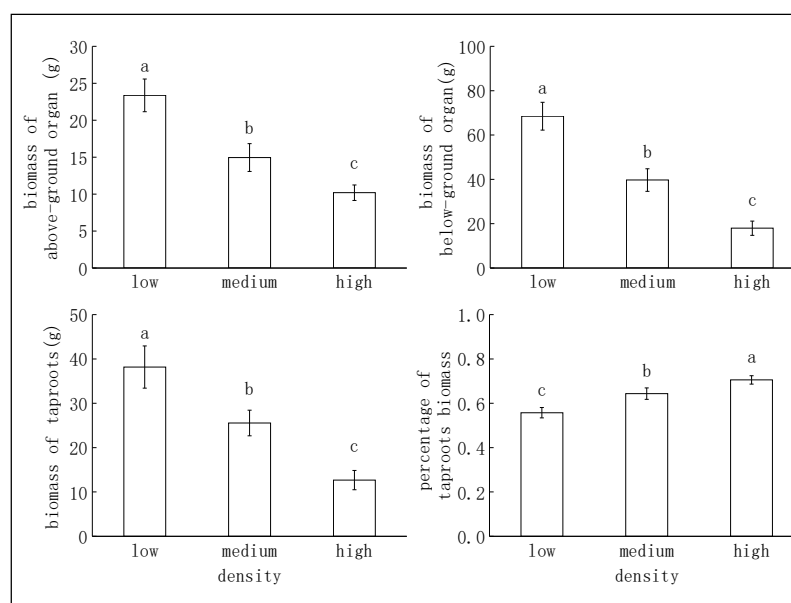


Fig 3: Effects of planting density on the taproots yield of *G. uralensis* (Mean \pm SE). Different lowercase letters indicate significant differences among different treatments ($P<0.05$).

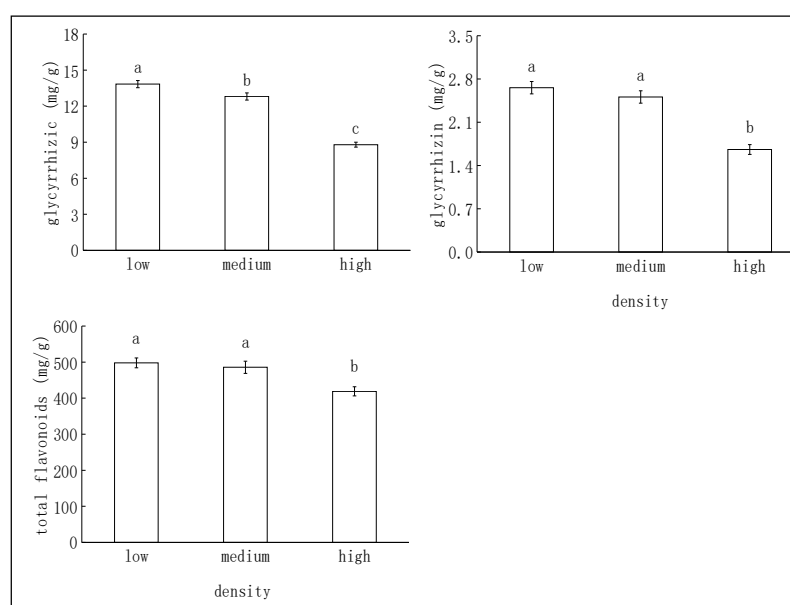


Fig 4: Effects of planting density on the quality of *G. uralensis* (Mean±SE). Different lowercase letters indicate significant differences among different treatments ($P<0.05$).

Table 2: The total yield of *G. uralensis* taproots per hectare among different treatments (Mean±SE).

Treatments	The total yield of taproots (Kg hm ⁻²)
Low-density	8588.25±86.475
Medium-density	11497.5±103.38
High-density	8552.25±77.625

these three secondary metabolites in the taproots of *G. uralensis* and the content of these active components significantly decreased with the increase of cultivation density (Fig 4). Compared to the low-density treatment, the content of glycyrrhetic acid, glycyrrhizin and total flavonoids in the medium-density treatment group decreased by 7.45%, 5.64% and 2.46%, respectively, while the content of these components in the high-density treatment group decreased by 36.43%, 37.61% and 15.86%, respectively.

The contents of glycyrrhetic acid, glycyrrhizin and total flavonoids in the high-density treatment were reduced by about 16% to 38%, respectively, than those in the low-density treatment, while there was no difference in the contents of these three active components among low and medium-density treatments.

CONCLUSION

Exploring a reasonable planting density of *G. uralensis* is one of the essential ways to increase its' taproots yield and optimize its quality. Planting with low density, *G. uralensis* can't fully utilize the sunlight and the nutrients in the soil, its' yield will be reduced; planting with high density, the competition for nutrients between individuals will increase and photosynthesis will be hindered, its' quality will be inferior. In conclusion, *G. uralensis* planting with medium density not only significantly inhibits the growth and

development of its rhizomes, but also ensures the maximum yield and optimal quality of the licorice population.

Conflict of interest: None.

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