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Article

Effects of Weeds Interference Frequency on the Yield and Quality of *Glycyrrhiza uralensis* Fisch in an Arid and Semi-Arid Area of Northwest CHINA

Dongqing Wang [1,2], Bin Ma [1,2,4,*], Hua Liu [1,2], Yangmei Bao [3], Ying Niu [1,2] and Ming Li [1,2]

[1] Institute of Forestry and Grassland Ecology, Ningxia Academy of Agriculture and Forestry Sciences, Yinchuan 750002, China; wangdonqing2046@163.com (D.W.); nxhmsliu@163.com (H.L.); nyingxtbg@163.com (N.L.); lm.nxpl@163.com (M.L.)
[2] Key Laboratory of Desertification Control and Soil and Water Conservation of Ningxia, Yinchuan 750002, China
[3] School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 102488, China; bouzi1224@163.com
[4] State Key Laboratory for Quality Ensurance and Sustainable Use of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, 100700, China
* Correspondence: mbin89@163.com

Abstract: Globally, weeds interfere with agricultural production activities and have a very serious impact on agriculture and animal husbandry. Identifying a safe and reliable weed control strategy might increase yield, production net income, and improve crop quality. A field experiment was carried out to explore the effects of weeds interference frequency on the yield and quality of *Glycyrrhiza uralensis* Fisch in an arid and semi-arid area of northwest China. The experiment consisted of seven treatments and were (1) no weeding, marked as WF0; (2)-(7) artificial weeding by hoe once every 1, 2, 4, 6, 8, 10 weeks after emergence, marked as WF1, WF2, WF4, WF6, WF8 and WF10, respectively. We found that the higher weeding frequency had a higher the plant height, photosynthesis, yield and quality. The highest yield was obtained in the WF1 treatment, while the cost of weeding was the highest among all treatments. The concentration of liquiritin and glycyrrhizic acid were increased by 53.24% and 36.57%, with the highest nitrogen metabolism enzymatic activities and quality in WF4 treatment. WF4 treatment exhibited the largest increases in the net income among all treatments in both growing seasons, with respective increases up to 71.39 % and 78.81%. These findings suggested that weeding once every 4 weeks would be an effective and sustainable measure to control weeds in an arid and semi-arid area.

Keywords: weeds interference frequency; yield and quality; sustainable agriculture; the optimal ratio of medicinal materials and weeds; *G.uralensis*

1. Introduction

Globally, weeds interfere with agricultural production activities and have a very serious impact on agriculture and animal husbandry [1, 2]. Weeds and crops form a complex system in the ecosystem, competing directly for resources, including water, nutrients, light and space, which can affect the growth, yield and quality of crops, ultimately leading to substantial economic losses[3]. Weeds caused 45% of yield loss; however, this percentage may rise to 94-96% in rice, 50% in pulses, 72% in sugarcane and nearly 90% in almost all vegetables with an increase in weed interference[4]. In traditional agriculture, farmers had a proactive approach to weed removal, clearing them as soon as they were noticed[5]. Nonetheless, hand weeding is time-consuming, labor-intensive and tedious, leading to higher production costs [6]. Although chemical weed control using herbicides has been the most popular and effective method [7, 8], it affects the quality and safety of crops and pollutes the environment. In addition, a single herbicide application cannot control the entire weeds over the growing season[9]. Moreover, the evolution of herbicide resistance has also become a major problem, further complicating the process of weed control [10]. All these factors can significantly impact the sustainable development of farmland ecosystems. Therefore, identifying a safe and reliable weed control strategy that can not only increase yield and production net income but also improve crop quality is of great significance.

Previous studies have shown that some weed control strategies can improve the yield and quality of cultivated crops. Weed diversity mitigates crop yield loss. For instance, yield loss decreased from 60% to 30% in wheat paddocks with an increase in the weed species richness from 7 to 20 [11, 12]. As one of the common weeds of grain fields, corn cockle (*Agrostemma githago* L.) promotes crop growth and improves the yield and quality in the wheat field by secreting gibberellin, agrosfemin and allantoin into the soil [13, 14].

Proactive management of weed diversity may increase crop productivity[15]. Most studies have shown that weeds impact crop yield, the yield is sub-correlated with the intensity of weed disturbance and constantly clearing all weeds during the entire growth period is the best approach to increase output. A previous study found that corn fields without weed interference had the highest grain yield[16]. Continuous weeding significantly reduced weed density and weed dry matter and increased weed control efficacy, ultimately improving plant growth, yield attributes and the overall yield of groundnut [17]. Similar results were reported in wheat[7], rice [18] and other crops[19]. Some studies have also suggested that weeds not only interfere with crop yield but also affect quality. The yield of the paddy field without weed interference was about 50% higher than that of the weed field and rice processing, and appearance quality and seed nitrogen accumulation were also the best[20]. Other studies have shown that although weed interference affects crop growth and yield, they have no significant effect on quality. Notably, an increasing duration of weed interference negatively affected crop height, head diameter and 1000-kernel weight but not seed oil content[21]. Three times of artificial weeding in the early stage of the *Radix bupleuri* field significantly increased the root weight but had little effect on the total amount of saponin A and saponin D[22]. It can be seen that weed control has different effects on the yield and quality of cultivated crops. Photosynthesis [23, 24], anti-stress enzyme activity [25, 26] and nitrogen metabolism during growing season significantly affect crop yield and quality. However, the impact of weed interference frequency on these plant indicators has not been fully tested.

Licorice (*Glycyrrhiza uralensis* Fisch) is a perennial herb of the genus *Glycyrrhiza* in the family Leguminosae. It is one of the most popular traditional Chinese herbal medicines and has been used for over 2000 years in China. Its dried roots and rhizomes are widely used in Eastern and Western countries as medicine. This species contains numerous active ingredients [27, 28], including liquiritin (LQ) and glycyrrhizic acid (GA), which are the main medicinal components specified in the Chinese Pharmacopoeia. The artificial cultivation of wild *Licorice* began in the 1980s in China and has become one of the leading industries in recent years. Weed control is a main technical obstacle restricting the large-scale cultivation of *Licorice*.

In the past 40 years, *Licorice* has been comprehensively studied in terms of plant systematic classification[29], chemical composition[30], pharmacological efficacy [31, 32], separation and extraction methods [33-35], metabonomics [36, 37], clinical application [38-40], etc. Most studies on artificial *Licorice* cultivation have largely focused on seed treatment [41, 42], the best sowing and harvesting date [43, 44], water stress [37, 45], fertilization [46] and pest control[47], with only a handful of studies on weed control and herbicides. Herbicides pose significant challenges to the safe medical use of licorice.

We hypothesized that (*i*) different weed interference frequencies might affect the growth, photosynthetic physiology and enzyme activity of *Licorice*, and then change its yield and quality and (*ii*) the effects of different weed interference frequencies on the yield and quality might have a critical value, which is conducive to improving the economic benefits of its ecological planting. The present study aimed to (*i*) identify the differential efficacy of weed interference frequencies on yield and quality and (*ii*) determine the optimal interference frequency and the cost of ecological planting.

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2. Materials and Methods

2.1. Study Site

The field experiment was conducted in 2021 and 2022 at Tianjizhang village of *Licorice* cultivation base, Yanchi County, Ningxia, in northwest China (Long. 107º16′48"E, Lat. 37°48′0"N, ca. 1427 m above sea level). The region is characterized by a typical continental monsoon climate with a mean annual temperature of 7.7°C, total annual precipitation of 290 mm and annual evaporation of 2132 mm. The soil type is mainly aeolian sandy soil, sierozem, which is barren and vulnerable to wind erosion. The region is a typical desert steppe area, dominated by xerophytes and mesoxerophytes.

2.2. Experimental Set-Up

One-year-old transplanted seedlings of *Licorice* were planted in early May at a depth of approximately 15 cm with a 30 × 12 cm spacing row and harvested in mid-October in each year (2021-2022). The planting density was about 27 plants m^{-1}. Each plot had an area of 3 × 5 m.

The weed control test was started after the emergence of *Licorice*. The experiment consisted of seven treatments and was carried out as a randomized complete block (RCB) design with three replications. The treatments were (1) no weeding, marked as WF0; (2)-(7) artificial weeding by hoe once every 1, 2, 4, 6, 8, 10 weeks after emergence, marked as WF1, WF2, WF4, WF6, WF8 and WF10, respectively. All plots, whether weeding or not weeding, received the same agronomic management practice.

2.3. Weeds Investigation

Three quadrats (1 × 1 m) were randomly selected in the three plots without weeding. Weeds were removed from the ground and kept in ovens at 80°C to a constant weight. Then, aboveground biomass accumulation (g/m^2) was calculated. Weed species and density were also recorded.

2.4. Sampling and Measurements

2.4.1. Growth index

In mid-September of each year, 30 healthy plants with uniform growth were chosen for plant height (vertical height through the center of the crown) and crown size (length and width through the center of the crown) measurement using steel tape (accuracy of 0.1 cm) in different treatment plots. The ground diameter was measured using a vernier caliper (accuracy of 0.01 mm).

2.4.2. Photosynthetic parameters

Healthy leaves were selected at 2/3 of the plant from the ground on a sunny day at the end of August of each year for the measurement of the net photosynthetic rate (*Pn*, $\mu mol/m^2 \cdot s$), transpiration rate (*Tr*, $mmol/m^2 \cdot s$), stomatal conductance (*G*, $mmol/m^2 \cdot s$) and intercellular carbon dioxide (CO_2) concentration (*Ci*, μmol/mol) using a LI-COR 6800 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA). The reference (CO_2) was set to 400 μmol/mol, light intensity was 1000 $\mu mol/m^2 \cdot s$, temperature was 24°C and water vapor pressure difference (VPD) was 1.0 kPa. Three leaves were selected for each treatment as three replicates. The measurement time was between 9:00 and 11:00 A.M., and leaves were tiled to cover the whole leaf chamber (6 cm^2). The chlorophyll soil plant analysis development (SPAD) value was measured using a portable chlorophyll SPAD meter (SPAD-502 Plus, Konica Minolta, Japan).

2.4.3. Antioxidant and nitrogen metabolism enzymes

Healthy leaves were collected at the end of August every year and stored in a dry ice sampling box. The leaves were taken back to the laboratory and placed in a refrigerator at -80°C for determination. Antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and nitrogen metabolism enzymes, including glutamine synthetase (Gs), nitrate reductase (NR), nitrite reductase (NiR) and glutamate synthase (NG), were determined by spectrophotometry (BioPhotometer, Eppendorf, Germany) according to the instructions of the corresponding kits (provided by Suzhou Michy Biomedical Technology Co., Ltd., China).

2.4.4. Yield and effective components

All *Licorice* roots in each plot (3 × 5 m) were dug out in mid-October each year and dried naturally. Its dry weight was measured and the yield per hectare was calculated. The content of LQ and GA were determined by high-performance liquid chromatography (HPLC) according to the 2020 edition of Chinese Pharmacopoeia.

2.5. Data Analysis

Analysis of variance (ANOVA) was estimated using Statistical Analysis System software (SAS 8.1) and means were compared following a protected least significant difference (LSD) procedure at a 5% level of probability using the Duncan Multiple Range Test (DMRT). Data from the two seasons were examined independently. The box line chart, correlation analysis graph and principal component analysis (PCA) graph were performed using Metware Cloud — a free online platform for data analysis (https://cloud.metware.cn).

3. Results

3.1. Weeds Investigation

There were 9 species of weeds in the non-weeding plot, belonging to 4 families and 7 genera (Table 1). Weeds with a higher density and aboveground biomass were *Setaria viridis* and *Chenopodium album*, with a density of 27 m^{-2} and 7 m^{-2} and biomass of 62.12 $g \cdot m^{-2}$ and 236.71 $g \cdot m^{-2}$, respectively. The weed densities in the plot without weeding was 46 m^{-2}, about 465000 ha^{-1}.

Table 1. Weeds investigation of non-weeding plots.

	Family	Genus	Species	Density（no.·m-2）	Biomass（g·m-2）
1	Poaceae	Setaria	*S. viridis*	27	62.12
2	Poaceae_	Echinochloa	*E. crusgalli*	2	3.84
3	Chenopodiaceae	Corispermum	*C. declinatum*	2	16.52
4	Chenopodiaceae	Chenopodium	*C. album*	7	236.71
5	Chenopodiaceae	Chenopodium	*C. foetidum*	2	4.80
6	Chenopodiaceae	Chenopodium	*C. aristatum*	1	1.09
7	Compositae	Artemisia	*A. scoparia*	3	0.38
8	Chenopodiaceae	Salsola	*S. collina*	1	1.72
9	Geraniaceae	Geranium	*G.sibiricum*	1	2.64
total				46	329.82

3.2. Growth of Licorice

Different weed interference frequencies had different effects on growth in both growing seasons (Table 2). Generally, the growth index value showed a downward trend with a decrease in the weeding frequency. The plant height, ground diameter and crown width of *Licorice* treated with WF1 were the highest, and the values in 2021 were 51.17 cm, 5.51 mm and 963.23 cm^2 respectively, which were 31.54, 31.19% and 94.55% higher than those of WF0 treatment (lowest values), and the difference was significant ($P < 0.05$). The values in 2022 were 42.67 cm, 5.13 mm and 747.52 cm^2 respectively, which were also significantly increased by 81.96, 95.05 and 137.39% compared with WF0 treatment (lowest values).

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Table 2. Effect of weeds interference frequencies on the growth.

Year	Weeds interference frequency	Plant height （cm）	Ground diameter （mm）	Crown size （cm²）
2021	WF1	51.17±6.59a	5.51±0.23a	963.23±38.11a
	WF2	49.43±5.51ab	5.01±0.19bc	823.63±55.35b
	WF4	43.30±1.55bc	5.07±0.15b	809.19±62.07b
	WF6	40.50±1.23c	4.93±0.25bcd	672.96±90.32c
	WF8	42.37±5.95bc	4.64±0.24cd	730.74±46.48bc
	WF10	39.33±2.52c	4.54±0.21de	504.04±30.34d
	WF0	38.90±1.73 c	4.20±0.26e	495.11±17.91d
2022	WF1	42.67±1.45a	5.13±0.40a	747.52±20.26a
	WF2	34.89±5.05ab	3.93±0.49b	586.41±46.01b
	WF4	36.89±7.04ab	3.83±0.25b	574.07±51.27bc
	WF6	31.44±2.72bcd	3.73±0.47b	470.52±91.19d
	WF8	33.00±6.69bc	3.77±0.85b	491.41±60.78cd
	WF10	25.00±5.03cd	2.67±0.25c	322.15±24.30e
	WF0	23.45±5.18d	2.63±0.23c	314.89±13.45e

Note: The crown width of this study was defined as the multiplication of two diameters passing through the center of the crown[48].

Meanwhile, no significant difference in plant height index was found between WF1 and WF2 treatments in 2021 ($P > 0.05$). Similarly, no significant difference was detected between WF1 and WF2 or WF4 treatments in 2022 ($P > 0.05$); however, the difference between WF1 and other treatments was significant in both growing seasons ($P < 0.05$). Overall, no significant differences in plant height, ground diameter and crown width were observed between WF2 and WF4, WF6 and WF8, WF10 and WF0 treatments ($P > 0.05$).

3.3. Photosynthetic Parameters

The effects of different weed interference frequencies on photosynthetic parameters were also different in both growing seasons (Table 3). The effects of different treatments on the *Pn* were WF1 > WF4 > WF2 > WF6 > WF8 > WF10 > WF0 in 2021 and WF1 > WF4 > WF2 > WF8 > WF6 > WF10 > WF0 in 2022. The effects on the *Tr* were WF1 > WF4 > WF2 > WF8 > WF6 > WF10 > WF0 in 2021 and WF1 > WF4 > WF2 > WF6 > WF8 > WF10 > WF0 in 2022.

Table 3. Effect of weeds interference frequencies on photosynthetic parameters.

Year	Weeds interference frequency	*Pn* (μmol/m²·s)	*Tr*(mmol/m²·s)	*G* (mmol/m²·s)	*Ci*(μmol/mol)	SPAD
2021	WF1	34.88±1.35a	16.19±1.07a	890.64±137.21a	309.03±13.07a	44.90±4.97a
	WF2	25.49±2.56bc	13.97±2.03bc	557.30±147.16bc	299.63±19.21a	41.53±4.71ab
	WF4	28.57±1.48b	15.80±0.36 ab	629.25±104.12b	300.38±11.03a	40.80±4.00ab
	WF6	24.62±2.41c	13.43±1.28c	491.88±115.33bc	322.00±30.00a	41.07±1.40ab
	WF8	24.06±1.54c	13.56±0.83c	550.73±59.40bc	310.73±19.40a	40.00±5.01ab
	WF10	22.15±3.67cd	12.97±1.46cd	431.91±43.81cd	293.71±10.77a	38.33±2.72ab
	WF0	18.87±1.09d	11.02±1.03d	300.71±10.54d	297.62±18.33a	33.77±7.35b
2022	WF1	22.43±3.23a	12.15±1.71a	603.21±102.03a	325.30±12.50a	40.17±5.29a
	WF2	20.21±1.66ab	10.39±0.81ab	446.60±83.45bc	295.74±5.03a	36.53±1.18a
	WF4	20.24±4.27ab	10.68±1.30ab	581.78±81.98ab	299.37±40.59a	36.60±3.38a
	WF6	16.15±0.75c	10.10±1.43ab	447.70±118.98bc	317.73±14.08a	34.87±4.95ab
	WF8	17.57±1.21bc	9.36±1.28bc	430.27±116.35bc	315.40±21.86a	35.77±1.40ab

	WF10	14.87±0.63cd	9.04±2.92bc	308.39±16.75cd	283.54±49.72a	33.80±1.30ab
	WF0	11.46±1.34d	7.02±0.56c	241.63±20.23d	301.78±10.44a	28.80±6.94b

Pn, *Tr*, *G* and SPAD values in the WF1 treatment within 2 years were the highest. Compared with the WF0 treatment (lowest values), these parameters increased by 0.85, 0.47, 1.96 and 0.33 times and 0.96, 0.73, 1.50 and 0.39 times in 2021 and 2022 respectively, and the differences were significant (P < 0.05). However, no significant differences in these parameters were found between WF2 and WF4 treatments and among WF6, WF8 and WF10 treatments (P > 0.05).

3.4. Antioxidant Enzyme Activities

Generally, the values of antioxidant enzyme activity in different treatments showed an increasing and then a decreasing trend in both growing seasons (Table 4). Notably, the WF1 treatment exhibited the lowest SOD, CAT and POD values.

Table 4. Effect of weeds interference frequencies on antioxidant enzyme activities.

Year	Weeds interference frequency	SOD (U/g FW)	CAT (μmol/min/g FW)	POD (U/g FW)
2021	WF1	415.23±60.15d	128.36±17.58e	89.16±3.57e
	WF2	802.89±66.61c	300.04±10.20c	92.73±21.40de
	WF4	947.39±50.11c	399.19±55.07b	160.49±32.10c
	WF6	1122.11±58.57b	411.90±45.74b	181.89±32.10bcd
	WF8	1203.29±129.87b	511.35±30.34a	210.42±17.84bc
	WF10	2080.94±119.27a	234.78±5.96d	385.04±7.69a
	WF0	1967.78±93.04a	145.63±13.16e	256.79±92.73b
2022	WF1	1648.03±246.22c	27.87±11.15c	21.93±8.00c
	WF2	1817.54±42.030bc	54.25±13.54c	30.67±11.72bc
	WF4	1891.68±155.685bc	164.43±22.15b	34.00±9.17bc
	WF6	1990.55±154.53ab	206.98±17.16a	40.67±1.15c
	WF8	2255.41±204.68a	217.55±19.32a	50.67±15.28b
	WF10	1811.35±164.48bc	164.41±27.28b	124.33±18.50a
	WF0	1784.57±249.06bc	161.76±29.38b	102.33±27.32a

In 2021, the SOD activity in WF10 and WF0 treatments were significantly higher than that in other treatments (P < 0.05), which was 4.01 and 3.74 times higher than that in WF1 treatment, respectively. The CAT activity in the WF8 treatment and POD activity in the WF10 treatment were significantly higher than those in other treatments (P < 0.05). In 2022, the SOD activity in WF8 treatment was the highest, and the change in CAT and POD activities was consistent with that in 2021.

3.5. Nitrogen Metabolism Enzyme Activities

Similarly, the activities of nitrogen metabolism enzymes in different treatments also exhibited an increasing and then a decreasing trend in both growing seasons (Table 5). The value of higher weeding frequencies was significantly higher than that of lower weeding frequencies. NR, NiR, NG and Gs activities in WF1, WF2, WF4 and WF6 treatments were higher, while those in WF8, WF10 and WF0 treatments were lower. NR, NiR and NG activities in the WF4 treatment were the highest. Compared with the WF0 treatment, these parameters increased by 1.76, 0.67, and 2.19 times and 6.93, 0.56, and 2.33 times in 2021 and 2022 respectively, and the differences were significant (P < 0.05). Gs activities were the highest in WF6 and WF4 treatment in 2021 and 2022 respectively.

Table 5. Effect of weeds interference frequencies on nitrogen metabolism enzyme activities.

Year	Weeds interference frequency	NR (nmol/min/gFW)	NiR (μmol/h/gFW)	NG (nmol/min/gFW)	Gs (μmol/h/gFW)
2021	WF1	197.89±25.41b	4.70± 0.50d	196.16±18.75b	12.47±0.94ab
	WF2	215.28±12.58ab	4.90±0.37cd	205.40±10.52b	12.56±0.61ab
	WF4	239.58±22.24a	7.05±0.29a	384.95±24.30a	13.15±0.84ab
	WF6	210.89±14.29ab	6.26±0.35b	390.32±8.83a	13.31±0.59a
	WF8	128.59±19.64c	5.65±0.68bc	158.70±13.49c	12.04±0.69bc
	WF10	91.20±11.31d	4.90±0.48d	153.46±9.01c	10.90±0.47cd
	WF0	86.74±7.44d	4.22±0.13d	122.45±12.51d	9.76±0.80 d
2022	WF1	98.59±19.64d	3.55±0.68c	205.40±8.44b	9.47±0.94ab
	WF2	147.89±25.41c	5.13±1.01ab	208.70±13.49b	10.56±1.42a
	WF4	252.91±39.14a	6.05±0.29a	359.68±11.99a	10.80±1.41a
	WF6	188.61±23.69b	5.32±0.89a	344.54±9.28a	10.36±0.52a
	WF8	90.02±11.14d	3.68±0.74c	190.21±11.60b	10.15±0.84ab
	WF10	61.20±11.31de	3.65±1.11c	120.62±24.08c	9.26±0.71ab
	WF0	31.91±4.54e	3.89±0.27bc	108.07±9.81c	8.51±1.09b

3.6. Yield and Effective Components

The yield showed a downward trend with a decrease in the weeding frequency in both growing seasons (Figure 1). The highest yield was obtained in the WF1 treatment, and the dry weight reached 2536.46 and 2122.20 kg/ha in 2021 and 2022 respectively, which was 71.41% and 55.65% higher than that without weeding respectively, and the difference was significant ($P < 0.05$). However, no significant differences were observed among WF1, WF2 and WF3 treatments and between WF8 and WF10 treatments ($P > 0.05$).

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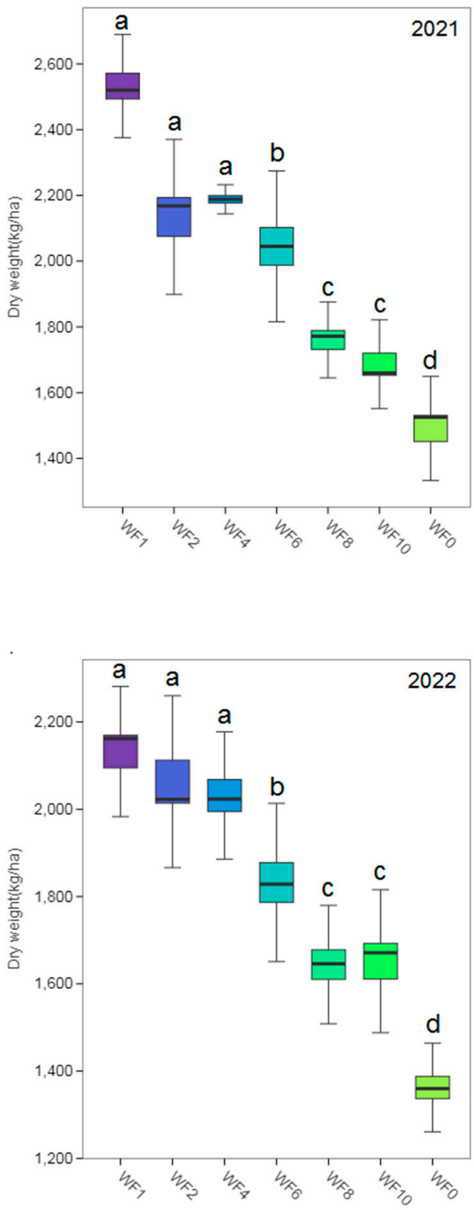

Figure 1. Effect of weeds interference frequencies on yield in 2021and 2022.

The contents of LQ and GA in different treatments were much higher than those specified in the 2020 edition of Chinese Pharmacopoeia (LQ: 0.50%, GA: 2.00%) (Figures 2 and 3). The highest LQ and GA were found in the WF4 treatment in both growing seasons, and the lowest value was found in the WF0 treatment. In 2021, LQ content increased by 35.67% and 53.24% in the WF4 treatment compared with the WF1 and WF0 treatments. The GA increased by 11.74% and 36.57% in the WF4 treatment compared with the WF1 and WF0 treatments. In 2022, LQ and GA increased by 19.23% and 21.30% in the WF4 treatment compared with the WF1 treatment respectively and by 24.71% and 35.48% compared with the WF0 treatment respectively.

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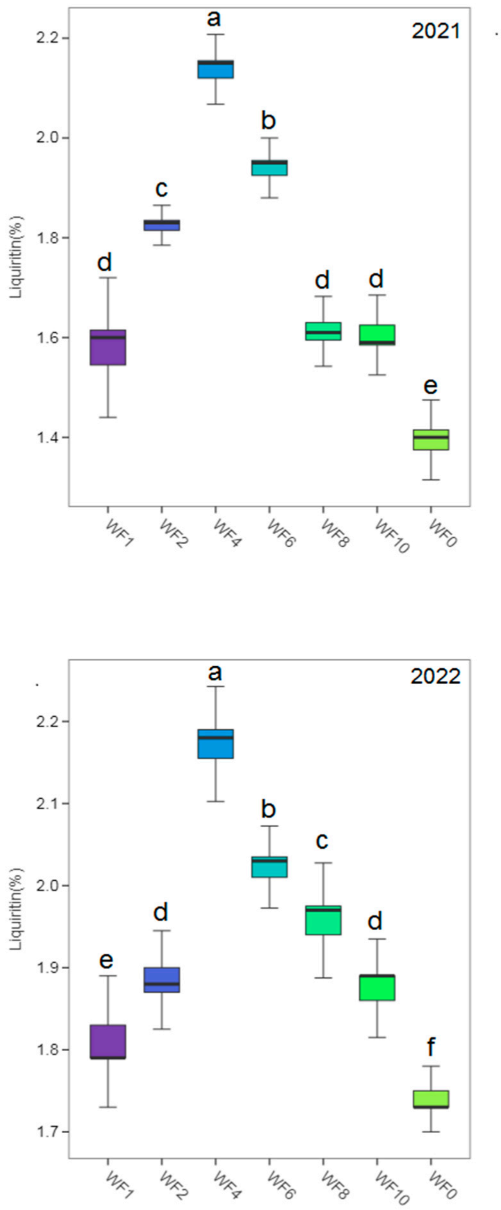

Figure 2. Effect of weeds interference frequencies on liquiritin in 2021and 2022.

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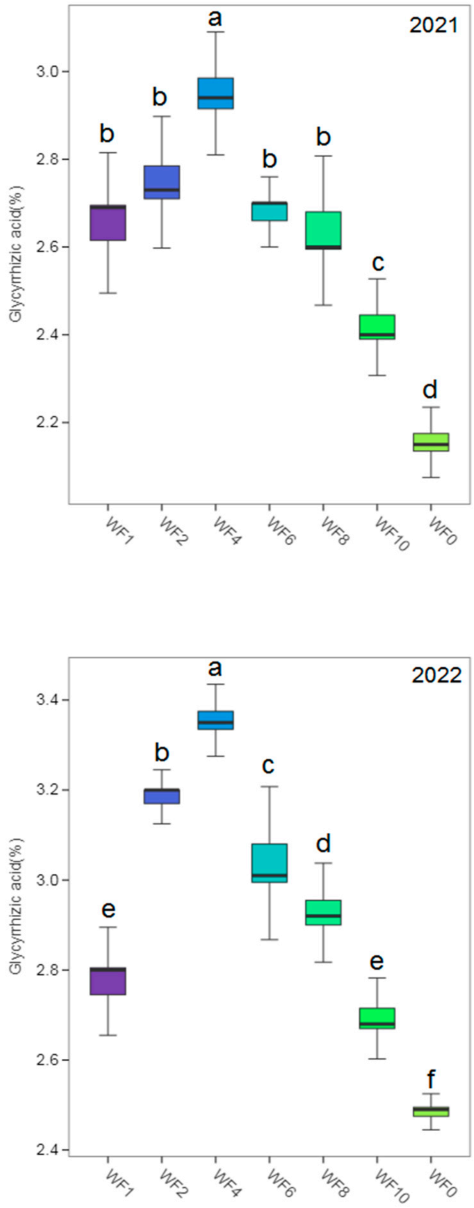

Figure 3. Effect of weeds interference frequencies on glycyrrhizic acid in 2021and 2022.

3.7. Economic Benefit

The change trend in the gross income and dry weight of *Licorice* under different treatments was similar, while the net income changed differently in both growing seasons. WF4 treatment exhibited the largest increases in the net income among all treatments in both growing seasons, with respective increases up to 71.39 % and 78.81% compared with the WF0 treatment. The net income in the WF6 treatment was the second and that in the WF0 treatment was the lowest. The WF4 treatment did not differ significantly from the WF6 treatment ($P > 0.05$) but significantly differed from other treatments ($P < 0.05$).

Table 6. Effect of weeds interference frequencies on Economic Benefit .

Year	Weeds interference frequency	Weeding costs (CNY/ha)	Gross income (CNY/ha)	Net income (CNY/ha)
2021	WF1	12000	40583.31±1276.38a	16583.31±1276.38bc
	WF2	6000	33973.09±1986.02b	15973.09±1986.03cd
	WF4	3000	35011.40±353.84 b	20011.40±353.84a
	WF6	1950	32720.22±1834.66b	18770.22±1834.66ab
	WF8	1500	28103.03±946.79c	14603.03±946.79cd
	WF10	1200	27122.79±1186.75c	13922.79±1186.75de
	WF0	0	23675.86±1413.25d	11675.85±1413.25e
2022	WF1	12000	33955.20±1312.96 a	9955.20±1312.96d
	WF2	6000	33225.60±1743.35a	15225.60±1743.35b
	WF4	3000	32548.80±1177.42 a	17548.80±1177.42a
	WF6	1950	29336.00±1452.55 b	15386.00±1452.55ab
	WF8	1500	26294.40±1086.57c	12794.40±1086.57c
	WF10	1200	26325.60±1358.29c	13125.60±1358.29bc
	WF0	0	21814.40±815.04d	9814.40±815.04d

Note: In 2021 and 2022, the total input of each treatment was 12000 CNY / ha (seedling, land rent and other inputs, excluding weeding costs), and the price of dry goods was 16 CNY / kg in 2021 and 2022.

3.8. Combined Correlation Analysis of Growth and Physiological Indexes with Yield and Effective Components

The correlation between growth and physiological indexes (photosynthetic indicators, antioxidant enzyme activity, nitrogen metabolism enzyme activity) and yield and effective components of *Licorice* was analyzed, and the Mantel test results were as follows.

In 2021, the dry weight of *G.uralensis* was significantly positively correlated with GD, *Pn*, SPAD, CS, *Tr* and G (Sorted by the size of the correlation coefficient) and negatively correlated with SOD, POD and CAT. LQ was positively correlated with NG, NiR, Gs and NR. GA was significantly positively correlated with Gs and NR (Figures 4 and 5).

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Figure 4. Correlation analysis of growth and physiological indexes with yield and effective components in 2021.

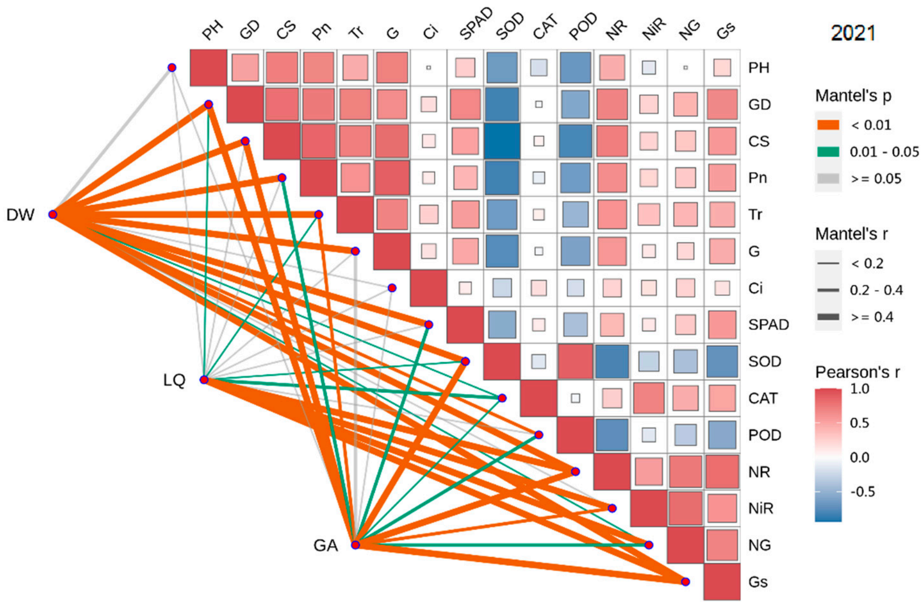

Figure 5. Combined correlation analysis of growth and physiological indexes with yield and effective components in 2021.

In 2022, the dry weight of *G.uralensis* was significantly positively correlated with Pn, Tr, SPAD, CS, PH and GD and negatively correlated with POD, CAT and SOD. Meanwhile, LI was significantly positively correlated with NR, NG and Gs. Additionally, GA was significantly positively correlated with Gs, NR, NiR and NG (Figure 6 and Figure 7).

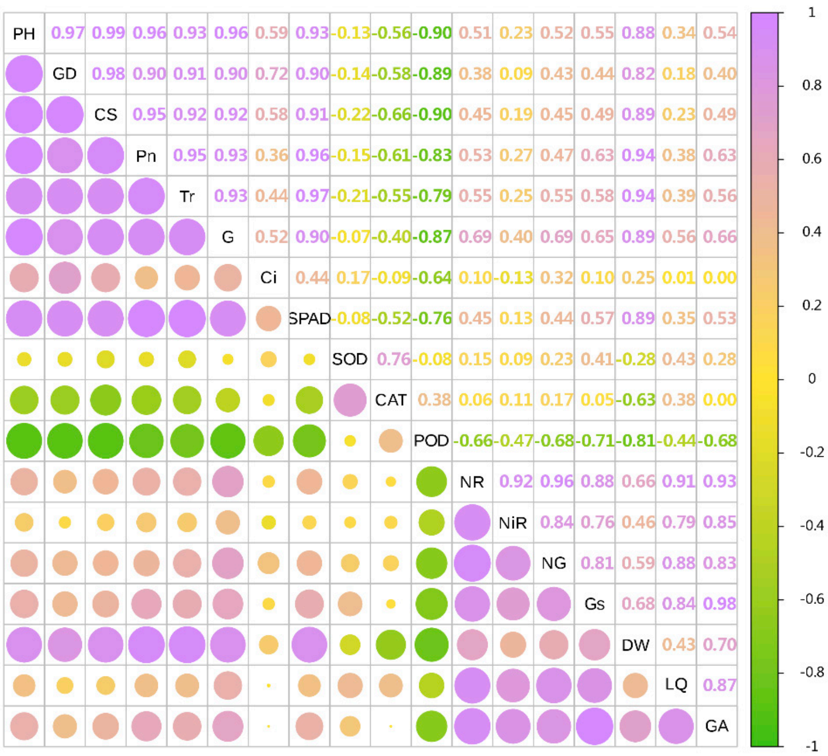

Figure 6. Correlation analysis of growth and physiological indexes with yield and effective components in 2022.

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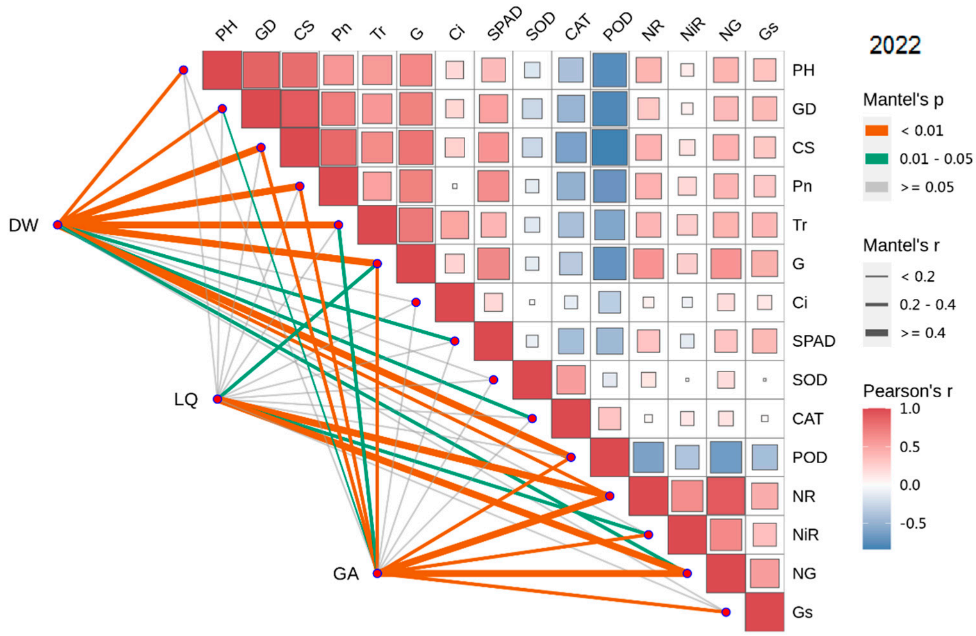

Figure 7. Combined correlation analysis of growth and physiological indexes with yield and effective components in 2022.

3.9. PCA of Weed Interference Frequencies

To assess the difference between the seven weed interference frequencies in the field, PCA was performed on 18 indicators, including growth, photosynthetic indicators, antioxidant enzyme activity, nitrogen metabolism enzyme activity, yield and effective components of *G.uralensis* (Figure 8).

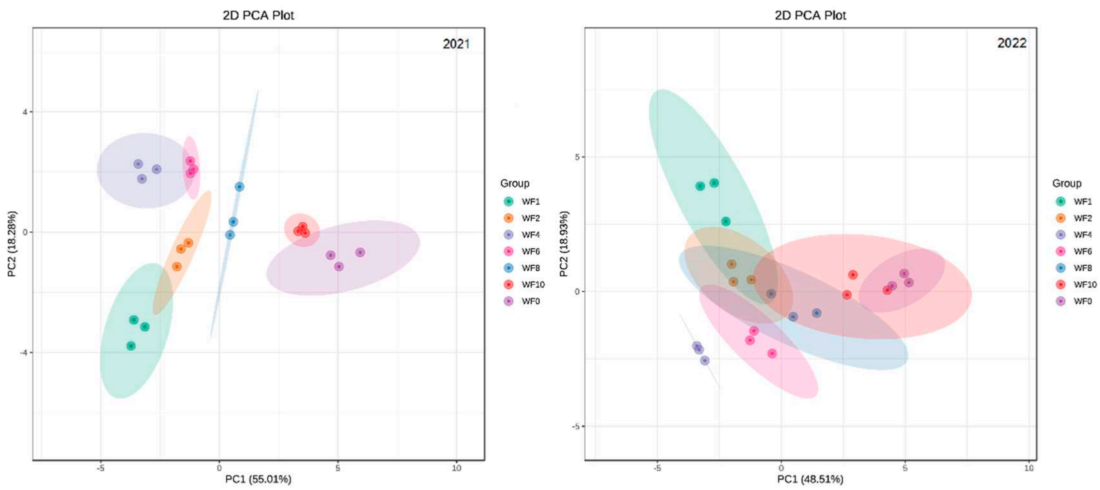

Figure 8. The PCA of Weed Interference Frequencies in 2021 and 2022.

PCA showed that the total variance of the model described by PC1 and PC2 axes was 73.29% in 2021 and 67.44% in 2022, which suggested that there were differences among the seven treatments.

4. Discussion

4.1. Effect of Weed Interference Frequencies on Growth of Licorice

The present study found that the degree of different weed interference frequencies affecting normal growth was inconsistent. The plant height, ground diameter and crown in WF1, WF2 and WF4 treatments were higher (Table 2). The study was conducted in the arid and semi-arid areas of China, with low rainfall and high evaporation. Plant growth is often affected by water stress. In this study, there were about 465000 weeds per hectare in *Licorice* field, which consumed approximately 157.33 kg of $(NH_4)_2SO_4$, 41.95 kg of $Ca(H_2PO_4)_2$ and 62.93 kg of K_2SO_4[49]. Since nutrient absorption in weeds is often faster and higher than in crop plants[50], it might be that weeds consumed the N, P, K and water required for the normal growth of *Licorice*, ultimately affecting its morphological structure.

4.2. Effect of Weed Interference Frequencies on Photosynthetic Parameters of Licorice

This study found that an increase in weed interference affected water, nutrients and light available for growth, leading to the occurrence of a series of adaptive changes in photosynthetic physiology. The *G* of without weeding treatment was significantly lower than that of other treatments, and the SPAD value was also lower (Table 3). This might be due to weed competition, leading to leaf water deficit, stomatal limitation[51] and photosynthetic pigment degradation[52], reducing the *Pn* value and ultimately the energy and material provided for growth. Contrarily, treatment with a high weeding frequency was less affected by weed competition, and photosynthetic parameters were relatively high, leading to the production of dry matter. This was consistent with the effect of weeds on growth and yield.

4.3. Effect of Weed Interference Frequencies on Antioxidant Enzyme Activities of Licorice

Plants can spontaneously induce osmotic regulation and catalyze a series of antioxidant enzymes under a stressful environment, thus removing excess reactive oxygen species and alleviating stress damage [53]. The antioxidant enzyme activity increases when plants are under stress[54]. In this study, the activities of the three enzymes in different treatments showed an increasing and then decreasing trend (Table 4). The content of the three types of enzymes was lower in the treatment of weed removal once a week, which might be due to lower levels of weed stress, strong photosynthetic capacity, and relatively low active oxygen-free radicals that destroy the cell membrane. The decrease in photosynthetic capacity reduced the transmission rate of photosynthetic electrons with the aggravation of weed interference, which was conducive to the flow of electrons to molecular oxygen, resulting in the production of superoxide radicals in plants and the accumulation of reactive oxygen species in cells [55] and an increase in the content of three enzymes in *Licorice* leaves. When the degree of weed interference exceeded the adaptation range, weed stress damaged the cell membrane system and the physiological system in plants, and the activity of antioxidant enzymes showed a decreasing trend.

4.4. Effect of Weed Interference Frequencies on Nitrogen Metabolism Enzyme Activities of Licorice

Nitrogen is a key plant nutrient known to affect primary and secondary metabolism in plants. The lack of nitrogen nutrition leads to the accumulation of carbon-based secondary metabolites such as terpenoids and phenols [56, 57]. The current study found that low weed interference frequency treatments promoted NR, NiR, NG and Gs activities, while high weed interference frequency significantly inhibited its enzyme activities. The WF4 treatment had higher nitrogen metabolism enzyme activities (Table 5). This might be because the study area is arid and semi-arid, the land is barren and the weeds consume a certain amount of nitrogen under low weed interference frequency. Mild nitrogen deficiency might promote an increase in nitrogen metabolism enzyme activities. However, under high weed interference frequency, weeds consume a large amount of nitrogen, resulting in an extreme lack of nitrogen nutrition in *Licorice*, which might inhibit the nitrogen metabolism pathway during growth, lowering the activities of nitrogen metabolism enzymes.

4.5. Effect of Weed Interference Frequencies on Yield and Effective Components of Licorice

Our data showed that the effects of different weed interference frequencies on yield were consistent with those on growth and photosynthesis. The yields in the WF1, WF2 and WF4 treatments were higher, and the yield in the WF0 treatment was the lowest. Since *Licorice* with higher plant height, crown width and ground diameter and better growth had stronger photosynthetic capacity, more dry matter was accumulated, and the corresponding yield was higher. It is inferred that the growth status of the underground and aboveground parts of the plant has a direct positive correlation with its photosynthetic capacity.

The effects of different weed interference frequencies on the accumulation of active ingredients and the activities of nitrogen metabolism enzymes were also consistent. WF4-treated *Licorice* had higher LQ and GA contents. This might be because secondary metabolites of *Licorice* increase to varying degrees under the condition of mild weed interference to protect the carbon/nitrogen nutrient metabolism balance and adapt to the weed-stress environment. Therefore, it was inferred that a moderate weed interference frequency would help improve the accumulation of active ingredients.

5. Conclusions

In summary, different degrees of weed interference frequencies had different effects on the yield and quality of *Licorice*. WF1, WF2 and WF4 treatments exhibited higher yields, followed by WF6, WF8 and WF10 treatments, with the lowest yield observed in WF0 treatment. Moderate weed interference frequency was beneficial to increase the content of LQ and GA. In addition, it was proved that WF4-treated *Licorice* had higher LQ and GA contents, which was consistent with its high economic benefits. Therefore, considering the quality and economic benefits of the local ecological planting of *Licorice*, the WF4 treatment would not only significantly improve the quality and economic benefits and save labor but also promote the sustainability of the farmland ecosystem. It would be a green ecological and efficient measure to prevent and control weeds in farmland. The study findings have certain reference significance for regulating the quality of Chinese medicinal materials using weeds in the future. Further studies should explore the optimal ratio of medicinal materials and weeds.

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Abbreviations

PH	Plant height
GD	ground diameter

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CS	crown size
Pn	net photosynthetic rate
Tr	transpiration rate
G	stomatal conductance
Ci	intercellular CO2 concentration
SOD	superoxide dismutase
POD	peroxidase
CAT	catalase
GS	glutamine synthetase
NR	nitrate reductase
NiR	nitrite reductase
NG	glutamate synthase
DW	dry weight
LQ	liquiritin
GA	glycyrrhizic acid

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