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EFFECTS OF CROP ROTATION ON GROWTH AND PHYSIOLOGICAL INDEXES OF *PANAX NOTOGINGSENG*

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Abstract

Panax notoginseng is widely used as a kind of medicinal plant because of its anti-tumor, antioxidative, anti-inflammatory, and neuro-protective activities. Continuous cropping obstacle is currently a major problem restricting the development of the *P. notoginseng*. Crop rotation might reduce the replant problems, but little information is available on the effect of other crops rotation on soil cultivated with ginseng. In this study, we analyzed growth and physiological indexes, saponins and the expression of saponins synthesis genes from the *P. notoginseng* field after planting coix seed, dry rice, ginger, pepper, and tobacco. Six crop rotation modes were set up to explore the changes in the indicators of *P. notoginseng*. Physiological indicators were measured by a microplate reader. The content of the five saponins was determined by HPLC, and the expression of saponins synthesis genes was detected by real-time quantitative PCR. The results showed that the incidence rate of M_5 (*P. notoginseng*- ginger- coix seed- *P. notoginseng*) was the lowest (23.08%). The growth index in M_5 is the highest. The content of defense enzymes decreased with increasing crop rotation interval. A parallel comparison with the same interval period showed that the saponins content in the rotation fields of coix seed was slightly higher. The test results of soluble sugar, soluble protein and chlorophyll showed that the rotation sites of *P. notoginseng* and coix seed could improve the photosynthetic parameters and physiological indicators of *P. notoginseng* leaves. The above results indicated that M_5 was a suitable crop rotation mode for *P. notoginseng*. Coix seed rise proved to be more suitable as a previous crop of *P. notoginseng*. Choosing a reasonable crop rotation is more effective than increasing the rotation time.

Key words: *Panax notoginseng*; Growth characters; Protective enzyme

Abbreviations: PN, *Panax notoginseng*; **PE**, Protective enzyme; **GC**, Growth characters; **SSG**, Saponin synthesis gene.

Introduction

Panax notoginseng (Burk.) F.H. Chen has been cultivated for more than 400 years (Qiao *et al*., 2020). The main production area of Yunnan is in low-latitude and high-altitude climatic conditions, which is conducive to the accumulation of medicinal ingredients and biomass of *P. notoginseng*. Among them, Wenshan has the best quality and is the real estate of *P. notoginseng* (Qiao *et al*., 2020). Due to the large-scale planting of *P. notoginseng* in recent years, it is increasingly urgent to solve the problem of continuous cropping obstacles. Generally, the soil that was planted with *P. notoginseng* can only be replanted after five years (Wang, 2018a). Continuous cropping obstacles have become a major problem restricting *P. notoginseng* and even the development of the entire medicinal plant industry (Berg & Smalla, 2009).

After continuous cropping of medicinal plants, soil-borne diseases are aggravated and susceptible to disease, photosynthetic rate was reduced, which in turn affects the physiological activities of plants and ultimately lead to a decrease in yield. For example, after the continuous cropping of *P. notoginseng*, the seed germination rate, germination index, and seedling rate significantly reduced, and yield decreased (Sun *et al*., 2015). The continuous cropping of *Pinellia ternata* will inhibit the growth and development of the plant, reduce the content of chlorophyll, decrease the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), increase the content of MDA, proline, soluble sugar, and soluble protein, and decrease the yield and quality (An, 2018). Severe continuous cropping obstacles will not only reduce the output of medicine but also affect their medicinal quality. Medicinal qualities are various secondary metabolites of medicinal plants. Studies have shown that the levels of *andrographolide* and *dehydroandrographolide* in continuous cropping for 1 to 2 years are lower than those in continuous cropping land, by measuring the degree of lipid peroxidation and antioxidant enzyme activity of *Andrographis paniculata* leaf membrane. Studies have found that the content of MDA, SOD, POD, and CAT in the leaves of *A. paniculata* increased with the continuous cropping years and the main obstacle effect of continuous cropping on *A. paniculata* is the reduction of active ingredient content (Li *et al*., 2017).

Rotation and inter cropping are the main methods of bioremediation. After rotation with other crops, some soil indicators of *P. notoginseng* were restored. Some indicators have irregular changes, which may be caused by crop rotation and field fertilisation management measures (Zeng *et al*., 2020). Therefore, choosing a suitable crop rotation method will promote the alleviation of the continuous cropping obstaclesof *notoginseng* (Zhang *et al*., 2019). Based on common local crops, we designed six crop rotation modes to discuss the impact of phytoremediation. We calculated the growth indicators of *P. notoginseng* for repeated cropping and tested the changes in protective enzyme activity during the vegetative growth period, flowering period, and fruiting period. Count the changes in the content of the five saponins of *P. notoginseng* and the expression of synthetic genes under the five rotation modes, and finally determine the most suitable rotation mode for *P. notoginseng*, and screen out the most suitable rotation crops. By improving the farming system based on *P. notoginseng*, the continuous cropping obstacles of *P. notoginseng* can be reduced. In this study, we explored suitable crop rotations to improve the *P. notoginseng* planting system. The related mechanism of continuous cropping obstacles and physiological growth indicators has been clarified, which helps to solve the problem of continuous cropping obstacles.

Materials and Methods

Experimental design and sample preparation: This experimental site is located in Wenshan, Yunnan (104°13´N, 23°21´E, 1,750 m altitude). The climate is a typical subtropical monsoon. After harvesting *P. notoginseng* in 2015, 2016, and 2017, replant *P. notoginseng* in 2019. The six crop rotation modes are M_0(CK): *P. notoginseng*- *P. notoginseng*; M_1: *P. notoginseng*- Coix seed- *P. notoginseng*; M_2: *P. notoginseng*- Dry rice- *P. notoginseng*; M_3: *P. notoginseng*- Tobacco- pepper- Ginger- *P. notoginseng*; M_4: *P. notoginseng*- Coix seed- ginger– *P. notoginseng*; M_5: *P. notoginseng*- Ginger- Coix seed- *P. notoginseng* (Fig. 1). The leaves were collected, vacuum-sealed, and stored at -80^{o}C for enzyme activity testing. The whole *P. notoginseng* was collected during the harvest period (November 2020). Wet and dry weight, root length, the number of fibrous roots, the content of five saponins of notoginseng R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd, and the expression of four key genes(SS, DS, SE, FPPS) for the synthesis of saponins was measured.

Growth indicator test: Emergence rate, seedling retention rate, and morbidity rate were counted and the plant height, stem circumference, leaf length, were measured at the vegetative growth period (April to June), flowering period (July to August), and fruit setting period (September to November). The stem thickness of P. notoginseng was measured at a distance of 20 cm from the ground by using vernier calipers. In October 2020, three P. notoginseng were selected, and the root length, root diameter and fibrous root number of the root system were determined. It is dried to constant weight at 55^{o}C in an oven, and its dry matter above and below ground was measured.

Physiological index test: Healthy and comparable P. notoginseng leaves have been selected in June, August, and October 2020. After cold extraction, store in the refrigerator at -80^{o}C. The SOD activity was analyzed by the nitro blue tetrazolium (NBT) colour method (Giannopolitis & Ries, 1977). The guaiacol method was analyzed by the determination of POD activity (Li *et al*., 2020). CAT was analyzed by ultraviolet spectrophotometry (Aebi, 1984). The content of malondialdehyde was analyzed by the thiobarbituric acid (TBA) method (Wang *et al*., 2018b). The content of glutathione reductase (GR), ascorbate peroxidase (APX), and soluble sugars were analyzed by ultraviolet spectrophotometry (Nakano & Asada, 1981; Ma & Cheng, 2003). The soluble protein was analyzed by Coomassie Brilliant Blue G-250 (Moran *et al*., 2000). The determination of chlorophyll and carotenoids adopts the direct immersion extraction method (Nayyar *et al*., 2009).

HPLC analysis: HPLC was quantitated by reverse phase HPLC (LC-20A High Performance Liquid Chromatography System (SPD-20A detector; Shimadzu Company, Kyoto, Japan)) to determine the components of tPNS (ginsenosides Rg_1, Re, Rb_1, Rd, and notoginsenoside R_1). 0.200 g of each sample (grind into powder and pass through a 60-mesh sieve) was put into 10 mL centrifuge tube. Add 8 mL 70% methanol to the sample tube, mix well, and soak overnight. After overnight, the saponins were extracted by Ultrasonic cleaner (KQ-250S, Kunshan Ultrasonic Instrument Company) at 40 kHz frequency for 60 min. After cooling to room temperature, add 70% methanol to make up the weight. Centrifuge the resulting solution at 12,000 rpm (13,200 × g) for 10 min, and then the supernatant was taken and filtered with filter membrane (0.45 μm, Millipore Company, USA). Chromatographic separation using ultrapure water system (UPW, Shanghai Ultrapure Company), Waters HPLC system equipped with Waters 1525 Binary pump, Waters 1500 column thermostat, Waters 2487 Dual λ Absorbance detector (DAD) and Waters 2996 Photodiode Array detector (PAD). The chromatographic columns are Waters Symmetry C18 reverse column (5 μm, 4.6 mm × 250 mm) and Waters Sunfire C18 reverse column (5 μm, 4.6 mm × 250 mm), both with Waters C18 guard column (5 μm, 20 mm) × 4 mm), UV spectra: Shimadzu UV-2401A spectrophotometer (Shimadzu Instruments Co., Ltd, Tokyo, Japan). The chromatographic conditions are: the temperature of the column oven is 30°C, the sample volume is 20 μL, and the flow rate of the mobile phase is 2 $mL\cdot min^{-1}$ and the wavelength is set to 203 nm. Acetonitrile and water were used as mobile phases, using a linear gradient elution procedure. The gradient elution procedure 0~10 min, 10~20% acetonitrile; 10~20 min, 20% acetonitrile; 20~40 min, 20~30% acetonitrile; 40~60 min, 30~44% acetonitrile; 60 ~70 min, 44~100% acetonitrile; 70~80 min, 100% acetonitrile. The resulting solution was directly loaded. Ginsenosides Rg1, Re, Rb1, Rd, and notoginsenoside R1 were selected as the target compounds as the five components with the highest content in P. notoginseng.

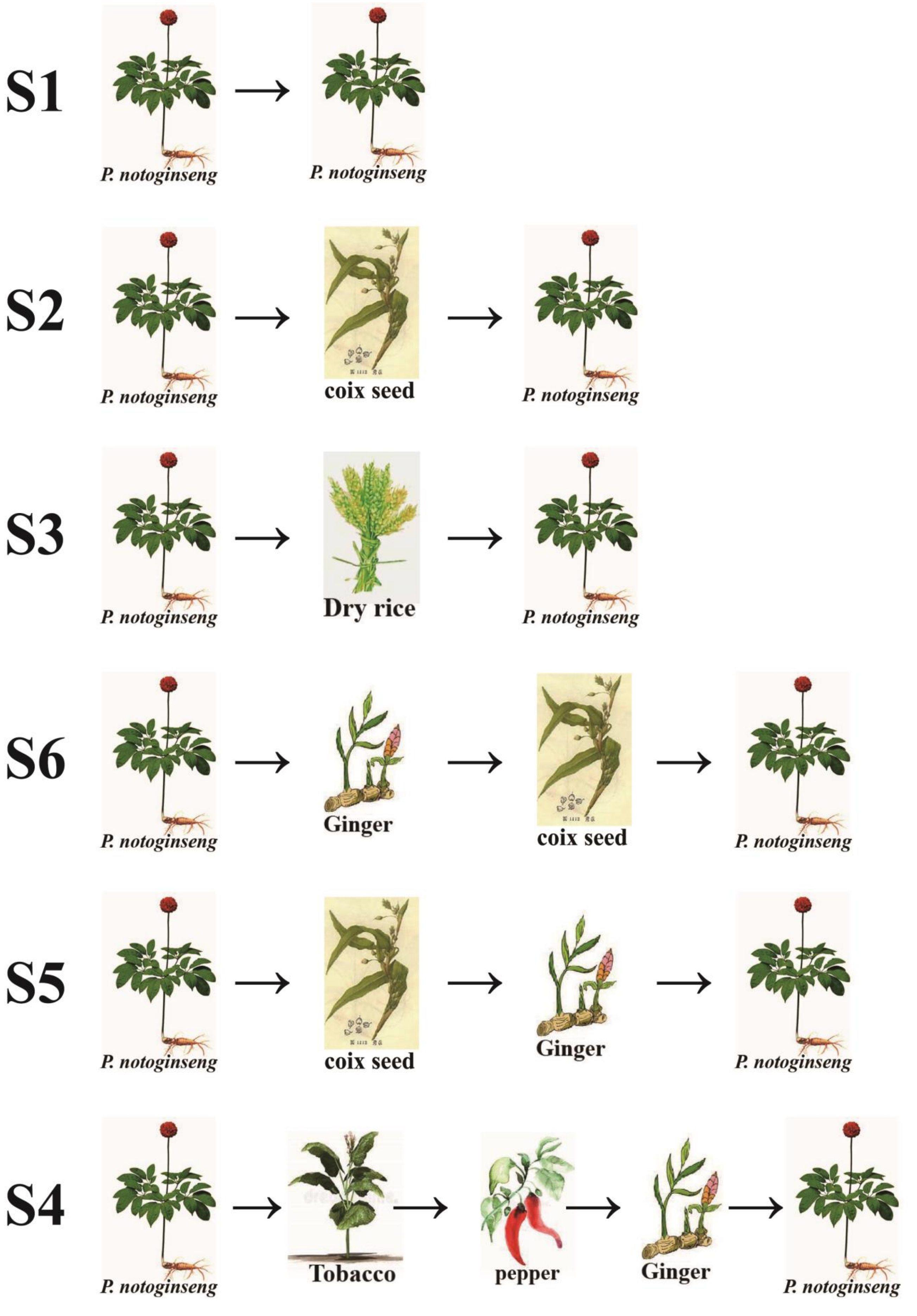

Fig. 1. Illustration of different rotation modes.

Table 1. Primers for gene expression of *Panax notoginseng*.

Gene	The upstream primer (5'-3')	Downstream primers (3'-5')
PnACT2	TCCAAGGGTGAATATGATGAATCG	AACCTCTCCAAAGAGAATTTCTGAGT
FPPS	CAAGAAGCATTTCCGACAA	CTCTCCTACAAGGGTGGTGA
SE	AAACACCAGGCTTTCTTACCC	GAAGTGATGCAGCTTTCTCCC
SS	GGACTTGTTGGATTAGGGTTG	ACTGCCTTGGCTGAGTTTTC
DS	ACCGCCGTTGAGATTAGATG	ATAGGGCAATGATAAGGGGAG

Table 2. The germination rate of four varieties under different rotation modes.

Model	Seed lot number	Base	Emergence rate	Area
M0	ZMML	112	72%	34
M1	ZMPYJ	114	86%	26
M2	ZMYSH	115	90%	8
M3	ZMML	113	72%	6
M4	ZMYSH	116	90%	5
M5	ZMYSH	116	91%	15

Note: Seedlingemergence rate = (Number of emergent seeds ÷ Number of testedseeds)*100%
M_0 (CK): *P. notoginseng- P. notoginseng*; M_1: *P. notoginseng-* Coix seed- *P. notoginseng*; M_2: *P. notoginseng-* Dry rice- *P. notoginseng*; M_3: *P. notoginseng-* Tobacco- Pepper- Ginger- *P. notoginseng*; M_4: *P. notoginseng-* Coix seed- Ginger– *P. notoginseng*; M_5: *P. notoginseng-* Ginger- Coix seed- *P. notoginseng*

Fig. 2. Growth status of *Panax notoginseng* under different cropping rotations.

qPCR analysis: The total RNA was isolated from the leaves of P. notoginseng using the polysaccharide polyphenol plant kit (TIANGEN, Beijing, China). Reverse transcription according to the PrimeScriptTM RT kit (Takara, Shiga, Japan). The cDNA after reverse transcription was diluted and used TaKaRa's TB Green Premix Ex Taq Ⅱ(Tli RNaseH Plus) kit for fluorescent quantitative PCR. The primers are listed in Table 1. The amplification curves of five key genes and reference genes for saponin synthesis were shown in Fig. 1.

Results

Changes in biomass and yield: The biomass of P. notoginseng under different cropping rotation patterns in the field was monitored and counted (Fig. 2). There was no significant difference in seedling survival rate at the same interval ($p<0.05$). The emergence rate of M_4 and M_5 was significantly higher than CK ($p>0.05$, Table 2), while other treatments fell between these values.The seedling retention rate showed a gradient change, in the order of $M_5 > M_4 > M_1 > M_2 > M_3 > M_0$. It is basically consistent with the germination rate (Table 3). No significant differences were observed for the root rot among the treatments except M0. We disassembled and analyzed different symptoms and found that root rot is the most serious and often representative. When root rot occurs severely, other diseases also occur more severely (Table 4).

The plant height of *P. notoginseng* was significantly different under different crop rotations ($p>0.05$, Table 5). The number of plant height was highest in M_3, followed by M_5. The change range of plant height was $M_3 > M_5 > M_1 > M_4 > M_0 > M_2$ concentration, and all treatments increased the fastest from the vegetative growth period to the flowering period, and the plant height changed slowly after the flowering period. The petiole length of M_5 and M_3 was significantly higher ($p>0.05$, Table 5). The variation range of petiole length is $M_5 > M_3 > M_0 > M_2 > M_4 > M_1$, all treatments had little change in the three growth periods, and the increase in petiole length was stable. The number of branches of M_2 was significantly more than other treatments (Table 5). After the completion of the vegetative growth period, the number of branches remained unchanged. With the increase of the interval, the number of branches began to increase. No significant differences were observed in the leaf length and width parameters of *P. notoginseng* under different rotation modes ($p>0.05$, Table 5). The increase in leaf length and leaf width in the same period is the same. The leaf length and leaf width gradually increase as the interval increases. It is interesting to note that the final amount of leaf length and leaf width is just the opposite of the increasing order. Indicating that the greater the development base of leaf length and leaf width in the early stage as the longer the interval time. The increase in stem circumference in M_5 was significantly higher and M_3 was significantly lower than that of CK ($p<0.05$), while other treatments fell between these values. The number of stem circumference was highest in M_5, followed by M_3, while the treatments of M_2 and CK had the lowest number of stem circumference ($p>0.05$, Table 5).

Table 3. Germination potential of *Panax notoginseng* under different crop rotation modes.

Model	Seed lot number	Base	Seedling retention rate
M0	ZMML	112	56%
M1	ZMPYJ	114	72%
M2	ZMYSH	115	71%
M3	ZMML	113	64%
M4	ZMYSH	116	76%
M5	ZMYSH	116	85%

Seedlingstorage potential = (Number of regularsurviving seedlings ÷ Total number of plantedseeds)*100%

M_0 (CK): *P. notoginseng*- *P. notoginseng*; M_1: *P. notoginseng*- Coix seed- *P. notoginseng*; M_2: *P. notoginseng*- Dry rice- *P. notoginseng*; M_3: *P. notoginseng*- Tobacco- Pepper- Ginger- *P. notoginseng*; M_4: *P. notoginseng*- Coix seed- Ginger– *P. notoginseng*; M_5: *P. notoginseng*- Ginger- Coix seed- *P. notoginseng*

In the statistics of production, the taproot length and tuber volume in M_3 was significantly bigger than that of CK ($p<0.05$, Table 6). The difference is not significant under the same interval, but significant under different intervals. The overall pattern is three years apart > two years> one year > continuous cropping. A parallel comparison in the same interval shows that M_1 and M_5 are better than M_2 and M_4 in root size and yield ($p>0.05$, Table 6). Once again, the indexes of the rotation land where coix seed rotation are better. In summary, the growth indicators of *P. notoginseng* all increase with the extension of the interval, and the order of increase is 2-3 years < 1-2 years < 0-1 year. The rotation pattern with Coix seed as the previous crop in the same interval is always better than others ($M_1 > M_2$; $M_5 > M_4$).

Physiological indicators: No significant differences were observed for the SOD activity among the treatments, but they are all significantly lower than CK ($P>0.05$). The SOD value showed a positive correlation trend with the increase of time (Fig. 3a). The increment of each treatment was the same, indicating that different crop treatments had little effect on the SOD value.

The peroxidase of *P. notoginseng* increased greatly in continuous cropping and showed a downward trend as the interval increased. The POD value of the five treatments all stable increased. The measured values of M_2 and M_4, M_1 and M_5 were the same (Fig. 3b), Indicated that choosing the right crop rotation can reduce the rotation time.

The catalase has a particularly violent response to the interval period ($p>0.05$, Fig. 3c). There are three gradients as a whole the gradients correspond to the interval years. In the same interval, The CAT value would be reduced by planting coix seed ($M_2 > M_1$, $M_4 > M_5$, Fig. 3c). This shows that coix seed planting has a defense and decomposition effect on *hydrogen peroxide*. The CAT value of the five crop rotation modes increased first and then decreased over time, indicated that the catalase content would gradually decrease in the later stage of plant growth, which may be related to the change of the substrate hydrogen peroxide content.

The MDA content in the *P. notoginseng* land with coix seed rotation for 1 year was higher than that of the *P. notoginseng* land with 3 years of rotation (Fig. 3d). This result shows that planting coix seed improves the antioxidant capacity of plants. The MDA value of each treatment increased first and then decreased over time.

The APX activity of M_2 treatment was significantly higher than that of other treatment groups ($p>0.05$, Fig. 3e), indicating that APX would have a considerable level effect on the antioxidant capacity of plants. Each treatment showed a trend of decreasing first and then increase with time, indicating that the H_2O_2 content of *P. notoginseng* would rise briefly in the late growth period.

There were no significant differences in the GR among the treatments ($p<0.05$, Fig. 3f). The GR value becomes more and more stable with the later stage of plant growth. The GR value fluctuates slightly when root rot is common. From the flowering stage to the fruiting stage, the GR value gradually stabilized.

Table 4. Germination potential of *Panax notoginseng* under different crop rotation modes.

Model	Root rot	anthrax	Plague	Hemp leaf disease	Black spot	Round spot
M0	6.69%	0.15%	0.22%.	0.13%	0.71%	0.66%
M1	2.43%	0.11%	0.10%	0.07%	0.33%	0.29%
M2	2.44%	0.07%	0.12%	0.08%	0.67%	0.53%
M3	2.51%	0.12%	0.18%	0.10%	0.18%	0.23%
M4	2.10%	0.10%	0.16%	0.03%	0.61%	0.59%
M5	1.87%	0.05%	0.13%	0.02%	0.78%	0.82%

Plant incidence = (Number of intected plants/Total number of surveyed plants)*100%

M_0 (CK): *P. notoginseng*- *P. notoginseng*; M_1: *P. notoginseng*- Coix seed- *P. notoginseng*; M_2: *P. notoginseng*- Dry rice- *P. notoginseng*; M_3: *P. notoginseng*- Tobacco- Pepper- Ginger- *P. notoginseng*; M_4: *P. notoginseng*- Coix seed- Ginger– *P. notoginseng*; M_5: *P. notoginseng*- Ginger- Coix seed- *P. notoginseng*

Table 5. Effects of different crop rotation modes on the growth indicators of *Panax notoginseng*.

	Growth index	Processing mode	Vegetative growth period	Flowering period	Fruit set
Different rotation mode processing	Plant height (cm)	M0	43.81 ± 7.71	45.52 ± 4.12	47.21 ± 7.31
		M1	44.41 ± 6.62	51.13 ± 3.43	53.41 ± 4.42
		M2	50.41 ± 9.62	51.44 ± 5.44	53.88 ± 7.62
		M3	48.70 ± 7.75	59.40 ± 11.44	61.31 ± 9.32
		M4	51.10 ± 7.37	57.42 ± 5.44	59.60 ± 10.4
		M5	52.6 ± 4.64	58.12 ± 3.11	64.10 ± 3.12
	Petiole length (cm)	M0	9.56 ± 2.31	10.58 ± 1.42	10.64 ± 2.22
		M1	9.71 ± 0.81	10.32 ± 2.32	10.50 ± 0.52
		M2	9.61 ± 1.80	10.44 ± 1.01	10.58 ± 1.62
		M3	10.12 ± 1.88	11.21 ± 0.81	11.34 ± 2.71
		M4	9.98 ± 1.02	11.51 ± 0.50	10.9 ± 0.32
		M5	11.02 ± 0.98	13.10 ± 2.12	13.3 ± 2.36
	Number of branches	M0	3+1	4	4
		M1	4	4	4
		M2	4+1	4+2	4+2
		M3	4+1	4+1	4+1
		M4	4+1	4+1	4+1
		M5	5	5	5
	Leaf length (cm)	M0	11.51 ± 1.51	12.51 ± 2.10	13.42 ± 2.12
		M1	11.72 ± 1.20	13.43 ± 1.61	13.66 ± 0.31
		M2	11.50 ± 2.51	13.54 ± 1.11	13.54 ± 0.61
		M3	12.21 ± 1.51	13.84 ± 0.51	13.92 ± 1.01
		M4	10.51 ± 2.52	12.71 ± 3.11	13.42 ± 0.40
		M5	13.22 ± 2.41	13.52 ± 2.51	13.82 ± 1.31
	Leaf width (cm)	M0	4.12 ± 0.63	5.23 ± 0.81	6.26 ± 0.93
		M1	4.71 ± 0.94	5.31 ± 0.31	5.52 ± 0.43
		M2	4.41 ± 0.82	5.51 ± 0.40	5.64 ± 0.44
		M3	4.53 ± 0.35	5.25 ± 0.64	5.22 ± 0.75
		M4	4.94 ± 0.31	5.82 ± 0.45	6.10 ± 0.51
		M5	5.02 ±0.96	5.35 ± 0.65	5.72 ± 1.21
	Stem circumference (cm)	M0	0.482 ± 0.190	0.489 ± 0.008	0.546 ± 0.038
		M1	0.514 ± 0.008	0.548 ± 0.018	0.544 ± 0.028
		M2	0.468 ± 0.048	0.514 ± 0.142	0.528 ± 0.008
		M3	0.522 ± 0.122	0.546 ± 0.162	0.546 ± 0.112
		M4	0.484 ± 0.020	0.522 ± 0.002	0.594 ± 0.028
		M5	0.518 ± 0.462	0.555 ± 0.142	0.604 ± 0.092
	Total incidence	M0	71.54%	89.23%	90.39%
		M1	23.44%	44.62%	47.69%
		M2	18.76%	30.77%	55.38%
		M3	28.32%	32.31%	36.92%
		M4	20.52%	27.69%	45.38%
		M5	9.61%	24.73%	23.08%

Note: 4+1 means 4-5, 4+2 means 4-6

M_0(CK): *P. notoginseng*- *P. notoginseng*; M_1: *P. notoginseng*- Coix seed- *P. notoginseng*; M_2: *P. notoginseng*- Dry rice- *P. notoginseng*; M_3: *P. notoginseng*- Tobacco- Pepper- Ginger- *P. notoginseng*; M_4: *P. notoginseng*- Coix seed- Ginger– *P. notoginseng*; M_5: *P. notoginseng*- Ginger- Coix seed- *P. notoginseng*

Table 6. Effects of different cropping patterns on root tubers of *Panax notoginseng*

Model	Main root length	Number of roots	Root fresh weight	Root dry weight	Root length	Tuber diameter
M0	18.11 ± 0.20ab	16b	7.71 ± 3.72c	2.91 ± 0.93c	1.72 ± 0.31b	0.77 ± 0.11c
M1	22.31 ± 0.31a	24b	16.82 ± 0.97a	4.95 ± 0.81ab	2.43 ± 0.31b	1.33 ± 0.08b
M2	11.45 ± 0.14b	46a	17.27 ± 1.49a	5.33 ± 0.52a	2.05 ± 0.31b	1.54 ± 0.52b
M3	13.67 ± 0.76b	18c	16.69 ± 7.53a	6.01 ± 1.34a	4.33 ± 0.31a	2.67 ± 0.61a
M4	18.24 ± 0.75ab	54a	17.11 ± 2.39a	5.79 ±0.97a	3.51 ± 0.31ab	1.35 ± 0.99b
M5	23.63 ± 0.76a	56a	14.33 ± 5.84b	5.77 ± 1.21a	4.21 ± 0.31a	2.23 ± 0.84a

M_0 (CK): *P. notoginseng*- *P. notoginseng*; M_1: *P. notoginseng*- Coix seed- *P. notoginseng*; M_2: *P. notoginseng*- Dry rice- *P. notoginseng*; M_3: *P. notoginseng*- Tobacco- Pepper- Ginger- *P. notoginseng*; M_4: *P. notoginseng*- Coix seed- Ginger– *P. notoginseng*; M_5: *P. notoginseng*- Ginger- Coix seed- *P. notoginseng*

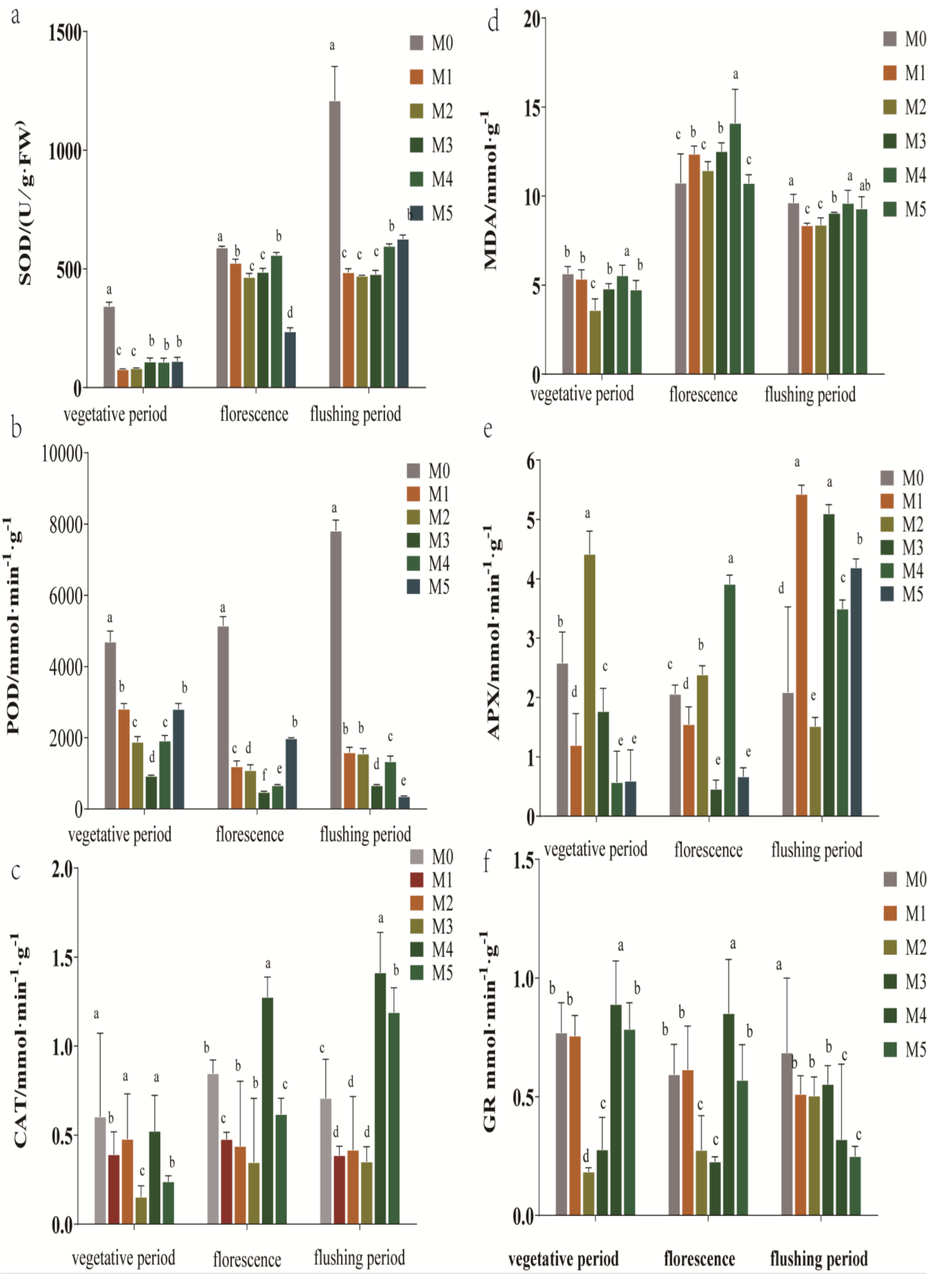

Fig. 3. The effect of different crop rotation modes on physiological indicators of *Panax notoginseng*: a. Superoxide dismutase (SOD) b. Peroxidase (POD) c. Catalase (CAT) d. Malondialdehyde (MDA) e. Ascorbate Peroxidase (APX) f. Glutathione Reductase (GR).
(The data in the figure uses SPSS 22 for significance analysis, and a, b, and c indicate significance. The same is true for the following table).

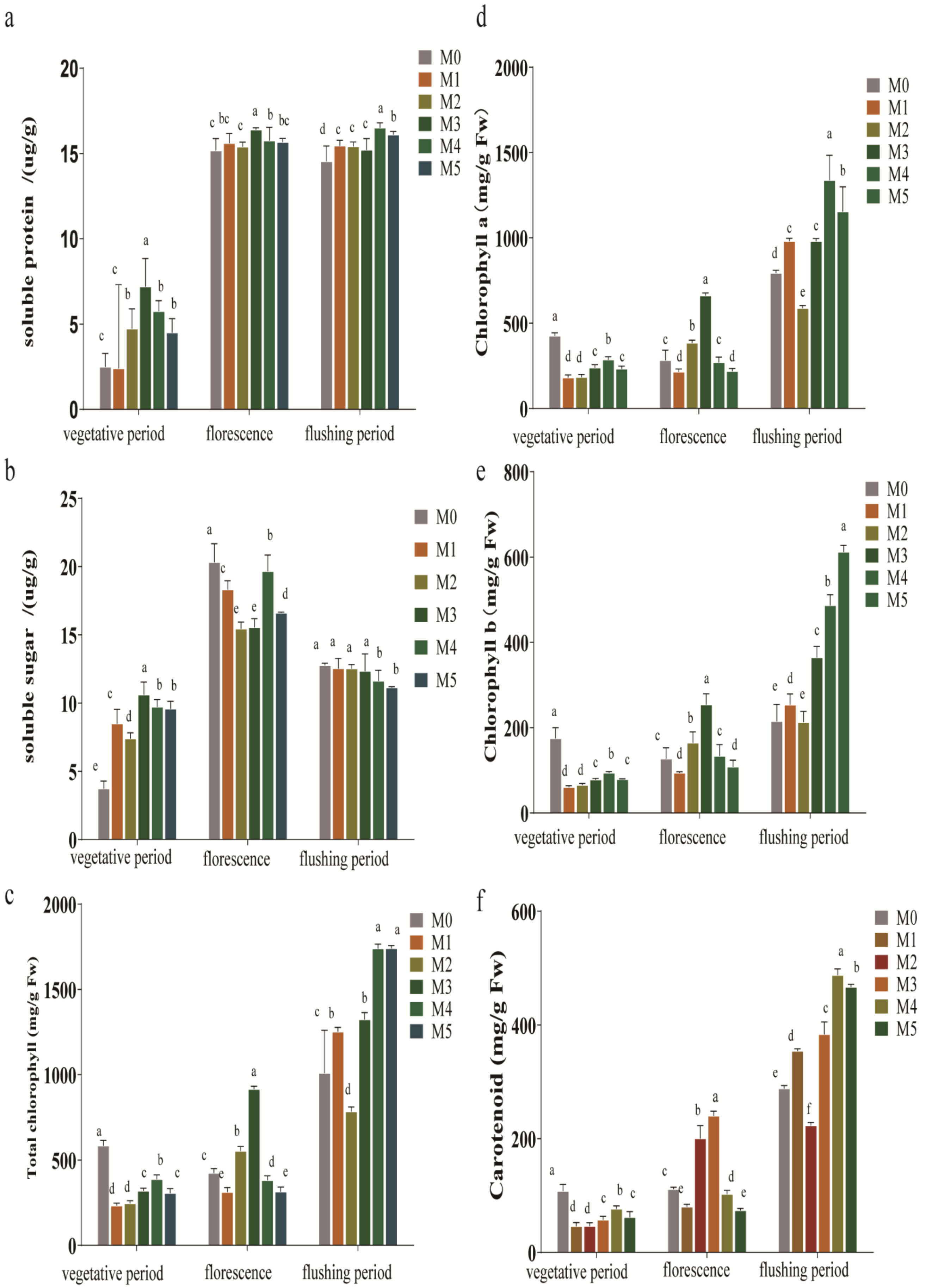

Fig. 4. The effect of different crop rotation modes on other physiological indicators of *Panax notoginseng*: a. soluble protein. b. soluble sugar. c. total chlorophyll. d. chlorophyll a. e. chlorophyll b. f. carotenoids.

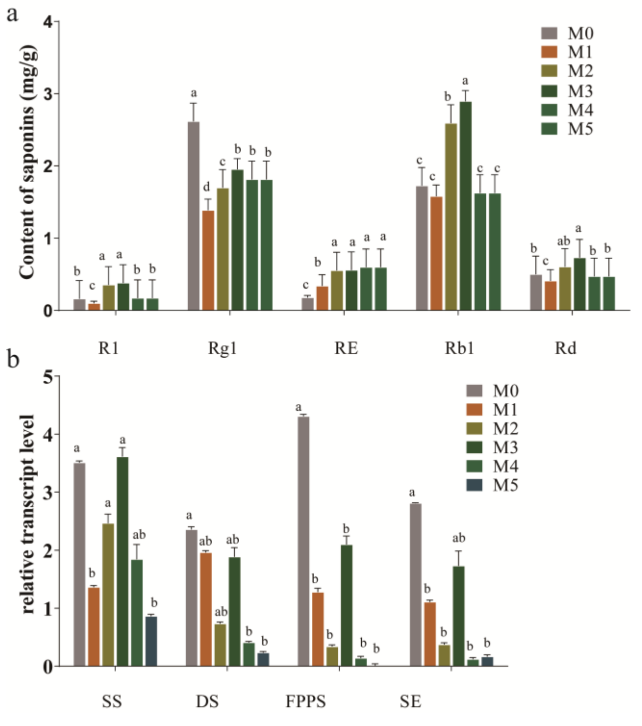

Fig. 5. Effects of different cropping rotations on contents of main saponins (a) and expression levels of saponin synthesis genes (b) in *P. notoginseng*. The five saponins include: notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1 and ginsenoside Rd. The relative expression levels of FPPS.SS, SE and DS in saponin synthesis pathway were detected using PnAct2 as reference gene.

Other indicators: The soluble protein content of M_2 treatment was significantly higher than that of other treatment groups ($p>0.05$, Fig 4a). Meanwhile, the soluble protein content increased with the increase of interval years. This is because plant biomass accumulates sufficient after the interval increases, leading to a strength of the metabolic level. The content of soluble protein in continuous cropping is relatively low, indicating that the continuous cropping of notoginseng reduces the content of osmotic adjustment substances, which ultimately leads to the weakening of plant resistance.

The soluble sugar content of the control group was significantly higher than other treatments ($p>0.05$, Fig. 4b). The soluble sugar shows a trend of increasing with the increase of the interval. The decrease of soluble sugar in the continuous cropping of *P. notoginseng* is the result of the decline in the metabolism under adversity.

The chlorophyll and carotenoid content of coix seed rotation land was higher than that of the same interval ($M_1>M_2$, Fig. 4c- f). The chlorophyll of each treatment group still showed a trend of increasing with the growth of years. At the same time, the growth levels of chlorophyll a, b, and carotenoids were synchronized. The differences were stable under the treatment of the five rotation modes. The content of chlorophyll and carotenoids in continuous cropping fields (CK) was higher than others ($p>0.05$). The chlorophyll content has exceeded four times that of carotenoids, indicating that plants are accelerating growth. The reason is that continuous cropping fields have a higher mortality rate and the remaining *P. notoginseng* has enough nutrition and growth space.

Saponins content and gene expression: Five saponins of *P. notoginseng*, i.e. *notoginsenoside R1*, *ginsenoside Rg1*, *ginsenoside Re*, *ginsenoside Rb1* and *ginsenoside Rd*, were studied as main medicinal ingredients. The content of five saponins in the continuous cropping *P. notoginseng* was exceptionally high ($p>0.05$, Fig. 5a). Because severe root rot in continuous cropping caused damage to the cell wall structure, which made the saponins of the secondary metabolites more likely to be extracted by external solvents, resulting in false high levels. There were significant differences in the content of notoginsenoside processed by different rotation methods. The saponin content contained in the *P. notoginseng* increases with the increase of the interval. In the same interval, the Dry rice on the content of notoginseng saponins is always better than others. The order of performance is dry rice greater > coix seed > ginger (M2>M1; M5>M4). The use of Dry rice as the previous crop is beneficial to increase the content of notoginsenoside.

The results of relative fluorescence quantitative PCR showed that the expression of each gene in M_0 and M_3 treatments was always higher than other treatments ($p>0.05$, Fig. 5b), which was consistent with the HPLC detection results. In different crop rotations at the same interval, the dry rice on the expression of *notoginsenoside* synthesis genes was always better than that of Coix seed ($M_2 > M_1$). Simultaneously, using Dry rice as the previous crop would increase the expression of key enzymes for saponin synthesis.

Discussion

The results of this study show that with the increase of the interval, the growth indicators and saponins content of *notoginseng* will increase significantly, which is consistent with the results of previous studies (Tang *et al*., 2020). The difference is that with the optimization of the field management system and the improvement of technical means, the overall growth and incidence of *P. notoginseng* after three years have been normal. However, the protective enzyme content of *P. notoginseng* with an interval of two years and less than two years still showed continuous cropping obstacles (Yao *et al*., 2019). Soluble protein and soluble sugar are the basis of plant physiological and biochemical metabolism, and their content directly reflects the adaptability of plants to adversity (Xuan *et al*., 2012). To resist the harm caused by continuous cropping, plants will actively accumulate osmotic substances such as soluble sugars and soluble proteins to regulate cell osmotic potential and maintain water balance, thereby protecting cells and their membrane structure functions (Jorge *et al*., 2015). In this study, the content of soluble sugar and soluble protein increased sharply during the flowering period, related to the root rot disease in the *P. notoginseng* field due to the increased rainfall in Wenshan from July to August. Research on secondary metabolites showed that the control group's saponins synthesis gene expression and five saponins content detection results were significantly better than other crop rotation models, indicating that the secondary metabolites of medicinal plants will increase under adversity (Huang *et al*., 2004; Narollhi *et al*., 2014). Chlorophyll and carotenoids showed a steady growth trend with the growth period, and the content of both decreased with the decrease of the interval, indicating that continuous cropping would lead to a decrease in the chlorophyll content and photosynthetic rate of *P. notoginseng* (GlleU & Fller, 2007; Herzog *et al*., 2015). When setting several crop rotation modes, we found that the yield of Ginger after planting *P. notoginseng* is high. Ginger proved to be more suitable as an aftercrop for *P. notoginseng*.

One of the mechanisms of the continuous cropping disorder of *P. notoginseng* is that continuous cropping interferes with the internal material metabolism process of *P. notoginseng*, reduces the protective enzyme activity of *P. notoginseng* leaves, and hinders the regular growth of *P. notoginseng* (Wei *et al*., 2012). Transcriptome analysis confirmed that Rg1 stress up-regulated genes related to the cell membrane, cell wall breakdown and reactive *oxygen species* (ROS) metabolism. Further cell analysis showed that Rg1 induced the accumulation of ROS (O_2^- and H_2O_2) in root cells by inhibiting the activity of *ascorbate peroxidas*e (APX) and the enzymes involved in the *ascorbate-glutathione* (ASC-GSH) cycle. Exogenous antioxidants (ASC and gentiobiose) helped cells remove excessively accumulated ROS by promoting SOD activity and ASC-GSH cycle. In general, the autotoxin Rg1 induces root cell death by inducing excessive ROS accumulation (Yang *et al*., 2018). Protective enzymes such as SOD, POD, and CAT play an important role in balancing the production and elimination. Under the synergistic action of the three, the ROS in the organism can be maintained at a low level. To prevent plants from being damaged by external environmental stress. ASA-GSH cycle is another ROS removal system in plants, and it also plays an indispensable role in removing ROS.

Crop rotation is a momentous agricultural treatment method that inhibits pests and diseases and increases yield (Abawi & Widmer, 2000; Guong *et al*., 2012; Peters *et al*., 2003). In the research on plants of the same genus as *P. notoginseng*, it takes at least three years for American ginseng to rotate, indicating that it takes time to alleviate obstacles to continuous cropping by rotation (Jiao *et al*., 2019). At the same time, Li *et al*. also found that the abundance of potentially beneficial bacteria of *P. notoginseng* also increased significantly with the increase of crop rotation (Li *et al*., 2020). Severe root rot prevalence is also one of the main reasons for continuous cropping obstacles in *P. notoginseng*. Rotation can improve the soil microbial community of *P. notoginseng* and reduce the occurrence of soil-borne diseases (Larkin & Lynch, 2018; Jin *et al*., 2019). Crop rotation is a long-term effective management measure. The implementation of reasonable crop rotation makes the layout of crops relatively stable, conducive to the rational use of agricultural machinery, fertilizers, pesticides, and other production resources. Finally, form a set of rotation technology system. So that various technical measures can be efficiently matched. To achieve better results, this will be the key to alleviating or even eradicating continuous cropping obstacles.

Conclusions

To explore the most suitable crop rotation model for *P. notoginseng*, improve the planting system based on *P. notoginseng*, and finally alleviate the obstacles to continuous cropping. It is suggested that to use Coix seed as the previous crop of *P. notoginseng*, promoting the research progress of exploring the scientific model of crop rotation. The shortcoming is the lack of soil microbial analysis results. We will continue to study the soil microbial diversity and abundance of *P. notoginseng* with five rotation modes.

In general, Growth physiological indicators, saponin synthesis gene expression, and product content indicate that Coix seed is the most suitable predecessor for *P. notoginseng.* Dry rice is a sub-suitable crop. Based on current technology, it is recommended to plant notoginseng at an interval of three years or more.

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