INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–1261/2014/16–5–981–985
http://www.fspublishers.org

Full Length Article

Effects of Different Cropping Patterns on the Physiology and Quality of *Pseudostellariae heterophylla*

Sheng Lin[1,3], Jingjing Huangpu[3], Ting Chen[1,3], Linkun Wu[1,3], Zhongyi Zhang[2,3] and Wenxiong Lin[1,3*]
[1]*School of Life Science, Fujian Agriculture and Forest University, Fuzhou, Fujian province, China*
[2]*School of Crop Science, Fujian Agriculture and Forest University, Fuzhou, Fujian province, China*
[3]*Provincial Key Laboratory of Agroecological Processing and Safety Monitoring, Fuzhou, Fujian province, China*
*For correspondence: wenxiong181@163.com

Abstract

Pseudostellariae heterophylla has the problem of continuous cropping obstacle resulting in significant reductions in yield and quality. In this study, different cropping patterns were designed to investigate the effects on the physiology, yield and medicinal quality of *P. heterophylla*. Continuous cropping resulted in significant decline in the production and quality, the output and pharmacological components of two-year monoculture than of one-year monoculture of *P. heterophylla*. Compared with two-year monoculture, the rice -*P. heterophylla* and bean -*P. heterophylla* rotation increased the production and pharmacological components of *P. heterophylla*. These rotation patterns improved the photosynthetic capacity of *P. heterophylla*, and reduced the activity of protective enzymes and malondialdehyde content of *P. heterophylla*. The results indicated that rotation cropping can alleviate continuous cropping obstacles and improve the yield and quality of *P. heterophylla*. The rice- *P. heterophylla* rotation is more effective than bean- *P. heterophylla* to abate the continuous cropping obstacles of *P. heterophylla.* © 2014 Friends Science Publishers

Keywords: *Pseudostellaria heterophylla*; Continuous cropping obstacles; Rice -*P. heterophylla* rotation; Bean -*P. heterophylla* rotation; Physiology

Introduction

The *Pseudstellariae heterophylla* belongs to family *Caryophyllaceae* and is used as common and highly demanded traditional Chinese medicine. It is mainly planted in Fujian, Guizhou, Shandong, Jiangsu and Anhui provinces and source of major economic income to local farmers. However, like other medicinal plants such as *Rehmannia glutinosa*, *Panax ginseng*, *Radix notoginseng* and *Coptis chinensis*, *Angelica sinensis*, *P. heterophylla* suffers from continuous cropping obstacles (Lin *et al.*, 2005; 2012; Zeng *et al.*, 2012).

Most of medicinal plants with their roots used as medicine have more serious problem of continuous cropping obstacle than plants with their tissues used as medicines (Zhao, 2000). For example, the continuous cropping of *Salvia miltiorrhiza* resulted in exterior deformation of root, decreased tuberous products and low content of active ingredient (Zhang *et al.*, 2005). And the field was used to plant some medicinal plants where same medicinal plants cannot be grown in next couple of years or for a long time. This phenomenon exists in the cultivation of *R. glutinosa*, *P. ginseng* and *Codonopsis pilosula*. Two-year monoculture *R. glutinosa* had small plants, imbalanced root-shoot ratio and shortened growth period. The tuberous roots of *R. glutinosa* were unable to develop into enlarged and commercial product form (Zhang *et al.*, 2010; Wu *et al.*, 2011; Li *et al.*, 2012a). The emergence rate of ginseng fell below 30% in the field with two-year monoculture, and the periderm of most roots were lousy red and full of disease scar (Jian *et al.*, 2008). The problem is also very serious in the *P. heterophylla* continuous cropping which showed weak plant growth, aggravating diseases, decreased yield and poor quality (Zeng *et al.*, 2012).

Due to the limited farmland, planting conditions and economic incentive factors, continuous cropping area of medicinal plants is growing in recent years (Zhang *et al.*, 2011). And the problem of continuous cropping obstacle is becoming more serious. Therefore, to solve continuous cropping obstacle of medicinal plant is a critical issue. To curb the continuous cropping obstacle, some methods are used such as soil disinfection, application of chemical and organic fertilizer, adding biological agents or activated carbon, breeding disease-resistant varieties and improving cultivation system (Wei *et al.*, 2009). However, there is need of effective method to decrease continuous cropping obstacle.

This study designed rice -*P. heterophylla* and bean -*P. heterophylla* rotation cropping modes in the GAP (Good Agriculture Practice) experimental field, and analyzed

To cite this paper: Lin, S., J. Huangpu, T. Chen, L. Wu, Z. Zhang and W. Lin, 2014. Effects of Different Cropping Patterns on the Physiology and Quality of *Pseudostellariae heterophylla. Int. J. Agric. Biol.*, 16: 981–985

physiological indexes, yield and quality of *P. heterophylla* under different cropping patterns. The results aimed to explore the effect of different rotation cropping modes on abatement of *P. heterophylla* continuous cropping obstacle, and might provide a theoretical reference for resolving the problems associated with *P. heterophylla* continuous monoculture.

Materials and Methods

Experimental Conditions and Crop Husbandry

The experiment was conducted in the GAP (Good Agriculture Practice) experimental field of *P. heterophylla*, Zherong County, Ninde Municipality, Fujian Province, P.R. China in 2009-2011. The *P. heterophylla* cultivar, 'Zheseng-2', was used for this study, planted in December and harvested in July every year. The field trial with three replications consisted of five treatments including one-year monoculture (NP), two-year consecutive monoculture (CM), rice -*P. heterophylla* rotation (RP), bean -*P. heterophylla* rotation (BP) and fallow treatment (CK, kept uncultivated). The experimental plot was 5 × 5 m (25 m^2) in each treatment. Individual *P. heterophylla* root was planted in plots at 5 × 10 cm among plants. One-year monoculture was made on December 23, 2010. The planting of two-year consecutive monoculture, rice -*P. heterophylla* and bean -*P. heterophylla* rotation was made on December 23, 2009 and 2010. The rice and bean were respectively planted in July, 2010 and harvested in November, 2010. The *P. heterophylla* completely germinated in March, 2011, and were harvested in July, 2011. The soil properties are shown in Table 1.

Determination of Different Component in *P. heterophylla* Root

Root moisture contents, total ash, acid insoluble ash content, water soluble extract and alcohol soluble extract content of *P. heterophylla* were determined.

Determination of Leaf Photosynthesis Indexes and Chlorophyll Content of *P. heterophylla*

In May, 2011 of *P. heterophylla* growth peak period, three plants of *P. heterophylla* were selected for photosynthesis indexes measurement. The chlorophyll content was measured by SPAD-502 chlorophyll meter. The LI-6400 photosynthetic apparatus was used to measure net photosynthetic rate (P_n), intercellular CO_2 concentration (Ci) and stoma conductance (g_s). Three plants of *P. heterophylla* were used to determine the biomass and physiological indicators.

Antioxidants Enzyme Activity and Malondialdehyde Content Assays

The 0.5 g of *P. heterophylla* leaf was weighed and grinded into homogenate using 2 mL phosphate buffer (50 mM phosphate, pH 7.0, 1% w/v PVP). The homogenate was moved into 10 mL centrifuge tube and centrifuged at 1000 g for 10 min. The supernatant was used to detect superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activity, malondialdehyde (MAD) and protein content. The methods were referred to the book of plant physiology experiment (Wang, 2006).

Polysaccharide and Saponin Content in *P. heterophylla* Root

The polysaccharide and saponin content were determined. The 100 mg glucose was dissolved by distilled water and diluted to 100 mL volume. Glucose solution was used to make standard curve. The 10, 20, 40, 60 and 80 µL glucose solution was removed into 15-mL tube and respectively added distilled water to 2 mL volume. Each concentration of glucose solution had three replications. The 2.0 mL of distilled water was used as blank control. In every tube, 1.0 mL of phenol and 5 mL of concentrated sulfuric acid were added and kept for 5 min. After incubation in boiling water for 15 min, the tubes were removed and cooled to room temperature. The optical absorption of the mixture was obtained at 490 nm. The standard curve was made by using glucose concentration levels as abscissa and absorbance as ordinate, and linear regression equation was $y = 0.0081 x - 0.0039$, $R^2 = 0.9999$.

The saponin content in *P. heterophylla* root was determined by using Agilent HPLC system (Agilent HPLC system, USA). The chromatographic system consisting of Agilent 1260 HPLC system with a reversed-phase column Zorbax Extend-C18 (150 mm×4.6 mm, 5 µm column) was used at a flow rate of 1.0 mL min^{-1}. The solvent system was solvent A (30% acetonitrile) and solvent B (70% water). The injected volume was 10 µL. The UV detector was performed at 203 nm. The chromatographic data were recorded and processed with Agilent empower workstation. The standard of ginseng saponin Rb1 was dissolved in methanol and diluted into 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 mg mL^{-1}. The standard curve was made by using saponin concentration levels as abscissa and area as ordinate, and linear regression equation was $y=0.0026x+0.0318$, $R^2=0.9999$.

The polysaccharide and saponin content were extracted from *P. heterophylla* root and determined as described by Zeng *et al.* (2012).

Results

Photosynthetic Indexes and Biomass of *P. heterophylla*

The discretion of photosynthetic rate showed the ability of plant to produce assimilates. Different photosynthetic indexes can directly reflect the strength of photosynthesis. The increase of chlorophyll contents in leaves of newly planted *P. heterophylla* was 137%, and photosynthetic rate was 200% of one year monoculture (Table 2).

Table 1: Soil physicochemical properties of experimental area

Treatments	Organic matter (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	Available N (mg 100 g^{-1})	Available K (mg kg^{-1})	Available P (mg kg^{-1})	pH
Fallow	38.31	5.01	0.47	19.77	19.78	21.07	185.89	5.7
One-year monoculture	38.88	2.02	0.47	20	18.2	30.09	174.52	5.18
Two-year consecutive monoculture	30.53	4.48	0.60	24.31	20.3	61.23	197.27	5.49
Rice -*P. heterophylla* rotation	26.74	1.90	0.54	19.55	23.8	38.42	242.79	5.24
Bean -*P. heterophylla* rotation	33.38	1.68	0.76	23.85	15.4	54.94	211.18	5.59

Table 2: Effects of different cropping patterns on the photosynthetic parameters, biomass and yield of *P. heterophylla*

Treatment	Chlorophyll (mg g^{-1} FW^{-1})	Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (μmol H_2O_2 m^{-2} s^{-1})	Ci (μmol m^{-2} s^{-1})	Transpiration rate (μmol CO_2 m^{-2} s^{-1})	Biomass (g $plant^{-1}$)	Yield (kg ha^{-1})
One-year monoculture	44.83±5.8a	8.698±2.79a	0.24±0.016a	283±0.58a	3.91±0.21a	4.01±0.6298a	7103.55±241.50a
Two-year consecutive monoculture	32.74±6.3b	4.2413±1.79d	0.11±0.005c	257±0.93b	2.12±0.06c	3.03±1.1489c	4082.10±193.50bc
Rice -*P. heterophylla* rotation	47.11±4.9a	8.022±1.82b	0.19±0.016b	281±0.73a	3.69±0.15b	3.71±1.2984b	6555.90±271.20ab
Bean -*P. heterophylla* rotation	44.91±4a	6.4687±1.85c	0.12±0.019c	259±0.48b	2.34±0.14c	3.12±1.1212c	4602.15±198.00b

The lowercase letters behind data represented significant differences ($P<0.05$)

Stoma conductance, intercellular CO_2 concentration and transpiration rate of newly planted *P. heterophylla* were higher than of one year monoculture. This indicates that monoculture reduces photosynthesis and restrains growth of *P. heterophylla* (Table 2).

The chlorophyll content and photosynthetic rate of RP rotation was respectively 140% and 200% of one year monoculture *P. heterophylla*, and other photosynthesis indexes of RP rotation were also higher than of one year monoculture. The effect of rice -*P. heterophylla* rotation to improve photosynthesis was more significant than of bean -*P. heterophylla* rotation. The increase of chlorophyll content and enhance of photosynthesis indexes were beneficial to plant growth and biomass accumulation. Compared with the biomass of one year monoculture *P. heterophylla*, the increase of biomass in rice -*P. heterophylla* reached significant level (Table 2).

Antioxidants Enzymes Activity and Malondialdehyde Content

The consecutive monoculture increased the activity of protective enzyme and content of MAD. The activity of SOD in leaves of two-year consecutive monoculture was significantly higher than of one-year monoculture, and reached 272% of one-year monoculture (Table 3). Activity of POD and CAT of two-year consecutive monoculture was also significantly higher than of one-year monoculture. The MAD content of two-year consecutive monoculture was 130% of one-year monoculture (Table 3).

Compared with protective enzymes activity and MAD content of two-year consecutive monoculture, SOD, POD and CAT activity in leaves of *P. heterophylla* significantly decreased under different rotation patterns. The SOD, POD and CAT activity of rice -*P. heterophylla* rotation were respectively 54.81, 90 and 65.48% of two-year consecutive monoculture (Table 3). And SOD, POD and CAT activity of bean -*P. heterophylla* rotation were respectively 83.65, 96.33 and 86.28% of two-year consecutive monoculture (Table 3). It indicates that rotation can effectively alleviate the effects of superoxide anion free radical caused by *P. heterophylla* consecutive monoculture.

Yield of *P. heterophylla* under Different Cropping Patterns

There was significant difference of *P. heterophylla* yield between different cropping patterns (Table 2). The *P. heterophylla* yield of two-year consecutive monoculture was the lowest, and 57.46% of one-year monoculture indicates that consecutive monoculture resulted in sharp decline of *P. heterophylla* yield. The *P. heterophylla* yield of rice -*P. heterophylla* rotation was 92.29% slightly lower than of one-year monoculture. The yield of *P. heterophylla* roots in bean -*P. heterophylla* rotation was 64.79% of one-year monoculture and lower than of rice -*P. heterophylla* rotation. It showed that compared with bean -*P. heterophylla* rotation, rice -*P. heterophylla* rotation was more effective to increase the yield of *P. heterophylla* after consecutive monoculture.

Quality of *P. heterophylla* under Different Cropping Patterns

For the quality of *P. heterophylla*, the national pharmacopoeia stipulated that water content is not more than 14%, aqueous extract not less than 25%, ethanol-soluble extract not less than 6% and ash content not more than 4% of dry weight (Table 4). The quality of different samples is according to the stipulation of national pharmacopoeia. The main medicinal components of *P. heterophylla* were polysaccharides and saponin (Fig. 1). The polysaccharides and saponin of two-year consecutive monoculture were significantly lower than of one-year monoculture, respectively 81.5% and 73.3% of one-year monoculture. In rice -*P. heterophylla* rotation, polysaccharides and saponin were respectively 116% and

Table 3: Antioxidants activities and MDA content in leaves of *P. heterophylla*

Treatments	SOD (U min^{-1} g^{-1})	POD (U min^{-1} g^{-1})	CAT (H_2O_2, mg g^{-1})	MAD (nmol g^{-1})
One-year monoculture	0.008±0.0007c	0.24±0.01d	41.60±0.82c	1.38±0.09b
Two-year consecutive monoculture	0.021±0.0009a	0.31±0.03a	58.12±1.12a	1.64±0.18a
Rice -*P. heterophylla* rotation	0.011±0.0011c	0.27±0.02c	37.78±0.65c	1.39±0.09b
Bean -*P. heterophylla* rotation	0.017±0.0006b	0.29±0.04b	50.01±0.73b	1.40±0.26b

Table 4: The quality of *P. heterophylla* under different cropping patterns

Treatment	Moisture (%)	Water-soluble extract (%)	Alcohol-soluble extract (%)	Total ash (%)	Polysaccharide (%)	Total saponin (%)
One-year monoculture	8.94±0.0007a	43.5±0.0012a	8.46±0.0051c	3 ±0.0024a	9.94±1.6b	0.3813±0.0042b
Two-year consecutive monoculture	8.37±0.0007b	43.3±0.0044a	9.08 ±0.0031b	3.22±0.0022a	8.10±0.14d	0.2777±0.0075d
Rice -*P. heterophylla* rotation	7.96±0.0006c	39.4±0.0007b	8.79±0.0019c	3.29±0.0019a	11.51±1.9a	0.3317±0.0076c
Bean -*P. heterophylla* rotation	8.3 ±0.0004b	43.7±0.0083a	10.88±0.0012a	2.98±0.004a	8.99±1.6c	0.513±0.0105a

The lowercase letters behind data represented significant differences (P<0.05)

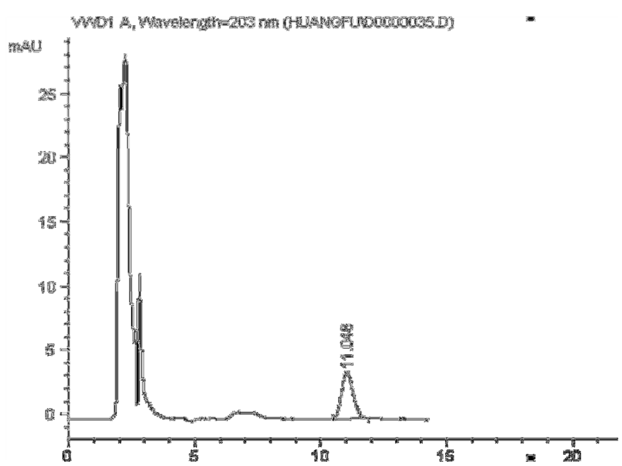

Fig. 1: HPLC map of ginsenoside Rb1 in control

86.8% of one-year monoculture, and higher than of two-year consecutive monoculture. The polysaccharides and saponin of bean -*P. heterophylla* rotation were respectively 90.4% and 134% of one-year monoculture. Two rotation patterns could increase medicinal ingredients and alleviate the effect of continuous cropping obstacle on *P. heterophylla* quality (Table 4).

Discussion

Continuous cropping destroyed the protective mechanism of photosynthetic system and reduced non-photochemical quenching coefficient (NPQ), causing light energy absorbed by PSII system not to be used for photosynthesis or dissipated (Zeng *et al.*, 2012). In addition, the cell density of leaves and roots declined, and leaves emerged abnormal morphology and internal structure, which resulted in decline of photosynthetic rate (Zeng *et al.*, 2012). It was found that the chlorophyll content, photosynthetic rate, stoma conductance, intercellular CO_2 concentration and transpiration of one-year monoculture were higher than of two-year consecutive monoculture. It concludes that continuous monoculture significantly reduced leaf photosynthetic capacity of *P. heterophylla*.

The SOD, POD, CAT activity and MAD content increased, indicating that protective enzyme system was disturbed, membrane lipid was seriously destroyed and growth of *P. heterophylla* was inhibited in two-year consecutive monoculture. Yu *et al.* (2003) studied cucumber continuous cropping, and found that chlorophyll content, transpiration and photosynthetic rate decreased in cucumber leaves of continuous cropping, the activity of POD and SOD increased and the growth of leaves was hindered. Other studies on medicinal plants also concludes that continuous cropping had direct negative effect on photosynthetic efficiency, root and protective enzymes activity which were major causes of continuous cropping obstacles (Zhang *et al.*, 2010).

After crop rotation, the chlorophyll content, photosynthetic rate and other parameters related to photosynthesis of *P. heterophylla* were higher than of two-year consecutive monoculture. The total biomass of one-year monoculture increased, and activity of protective enzymes, MDA content and damage of membrane lipid all decreased, which alleviate continuous cropping obstacles of *P. heterophylla* to a certain extent (Table 3).

Crop rotation promoted the recovery of photosynthetic capacity and stability of physiological state in *P. heterophylla* plant which had positive effect on biomass, yield and quality. The yield, polysaccharide and saponin contents of rice -*P. heterophylla* were respectively 92.29%, 116% and 86.8% of one-year monoculture. Under bean -*P. heterophylla* rotation, the production, polysaccharide content and total saponin content of *P. heterophylla* amounted to 64.79%, 90.4% and 134.6% of one-year monoculture. It indicate that rice -*P. heterophylla* rotation had more significant abatement effect on continuous cropping obstacles than bean -*P. heterophylla* rotation. And several studies showed the crops rotating with rice have the advantages for soil quality and crop yield, such as soybean-rice (Popp *et al.*, 2005), rape-rice (Li *et al.*, 2012b) and wheat-rice rotations (Ma *et al.*, 2013).

The results of this study showed that crop rotation could reduce the continuous cropping obstacle to a certain extent and improve the yield and quality of *P. heterophylla*. However, it is very complicated to study mechanisms of continuous cropping obstacles and related abatement, which is comprehensive to external performance of multiple factors between *P. heterophylla* and rhizosphere soil. More research through the coordination of different crops rotation are needed, which can reduce the accumulation of autotoxic substances and improve the structure and functional diversity of microbial community, to restore ecosystem function of rhizosphere soil and overcome the obstacles of continuous cropping. Therefore, on the basis of results of field observation and different index measurement, further studies for the abatement effect of different cropping patterns on *P. heterophylla* auto-toxicity and improvement of soil rhizosphere micro-ecological system are needed.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant no. 2012CB126309, U1205021 and 81303170).

References

Jian, Z.Y., W.Q. Wang, L. Men and Z.L. Zhang, 2008. Research progress on continuous cropping obstacles of *Panax* plants. *Mod. Chin. Med.*, 10: 3–5

Li, C.F., L.X. Yue, Z.K. Kou, Z.S. Zhang, J.P. Wang and C.G. Cao, 2012a. Short-term effects of conservation management practices on soil labile organic carbon fractions under a rape–rice rotation in central China. *Soil Till. Res.*, 119: 31–37

Li, Z.F., Y.Q. Yang, D.F. Xie, L.F. Zhu, Z.G. Zhang and W.X. Lin, 2012b. Identification of autotoxic compounds in fibrous roots of Rehmannia (*Rehmannia glutinosa* Libosch.). *PLoS One*, 7: e28806

Lin, G.M., J.B. Zhang, C.H. Hou, S.G. Wang, J.D. Yuan and W.X. Lin, 2005. Studies on strain characteristics of *Pseudostellaria heterophylla* varieties. *J. Chin. Mater. Medica*, 30: 422–426

Lin, M.Z., H.B. Wang and H.F. Lin, 2012. Effects of *Pseudostellariae heterophylla* continuous cropping on rhizosphere soil microorganisms. *Chin. J. Ecol.*, 31: 106–111

Ma, Y.C., X.W. Kong, B. Yang, X.L. Zhang, X.Y. Yan, J.C. Yang and Z.Q. Xiong, 2013. Net global warming potential and greenhouse gas intensity of annual rice–wheat rotations with integrated soil–crop system management. *Agric. Ecosyst. Environ.*, 164: 209–219

Popp, M., P. Manning, P. Counce and T. Keisling, 2005. Rice–soybean rotations: opportunities for enhancing whole farm profits or water savings. *Agric. Syst.*, 86: 223–238

Wang, X.K., 2006. *Principles and Techniques of Plant Physiology and Biochemistry Experiment*, Higher Education Press, Beijing, China

Wei, X.C., J.M. Yang, K.Z. Liao, M.W. Shi, X.L. Ma and F. Jiang, 2009. The cause of continuous cropping obstacles and integrated control measures. *Shanghai Veget.*, 2: 61–62

Wu, L.K., H.B. Wang, Z.X. Zhang, R. Lin, Z.Y. Zhang and W.X. Lin, 2011. Comparative metaproteomic analysis on consecutively *Rehmannia glutinosa*-monocultured rhizosphere soil. *PLoS One*, 6: e20611

Yu, J.Q., S.F. Ye, M.F. Zhang and W.H. Hu, 2003. Effects of root exudaws and aqueous root extracts of cucumber(Cucumis sativus) and allelochemicals on photosynthesis and antioxidant enzymes in cucumber. *Biochem. Syst. Eco1.*, 3l: 129–139

Zeng, L.J., M.Z. Lin, Z.F. Li, L.Q. Dai, J. Li, J.J. Li, Z.Y. Zhang and W.X. Lin, 2012. Effects of continuous cropping on photosynthesis and medicinal quality of *Pseudostellariae heterophylla*. *Acta Agron. Sin.*, 38: 1522–1528

Zhang, C.L., Q. Sun and Q. Ye, 2005. Obstacle Effect of continuous cropping on *Salvia miltiorrhiza* growth. *Acta Bot. Boreali-Occident. Sin.*, 25: 1029–1034

Zhang, Z.Y., M.M. Niu, T. Chen, J. Li, L.Y. Lin and W.X. Lin, 2011. Research on medicinal plant autotoxicity and innovation of cultivation technology. *Mod. Chin. Med.*, 13: 4–7

Zhang, Z.Y., W.J. Yin, J. Li, J.F. Du, Y.H. Yang, X.J. Chen and W.X. Lin, 2010. Physio-ecological properties of continuous cropping Rehmannia glutinosa. *Chin. J. Plant Eco.*, 34: 547–554

Zhao, Y.J., 2000. The importance and application prospects of allelopathy in medicinal plant cultivation. *Chin. Trad. Herb. Drug*, 31: 1–4

(Received 25 October 2013; Accepted 26 February 2014)