Bangladesh J. Bot. **46**(3): 1057-1062, 2017 (September) Supplementary

EFFECTS OF ENVIRONMENT AND GERMPLASM ON BERGENIN ACCUMULATION OF *BERGENIA PURPURASCENS* (J.D. HOOKER & THOMSON) ENGLER

SHI-YU WANG[1], FENG-GEN GUO*, LI-MEI ZHANG, YAN-PING CHEN, PING ZHOU AND WEN-HONG LONG[1]

College of Agronomy and Biotechnology, Yunnan Agricultural University, Kunming 650201, Yunnan Province, China

Keywords: *Bergenia purpurascens*, Bergenin content, Bergenin yield per plant, Environmental factors, Germplasm

Abstract

Bergenin, an important active compound with multiple pharmaceutical effects, is mainly extracted from *Bergenia purpurascens* (J.D. Hooker & Thomson) Engler. Long-term over-exploitation has resulted in an endangered status of wild *B. purpurascens* and its industrial cultivation is the only way to preserve wild resources and meet the market demand. In order to screen out best cultivation site and germplasm in relation to high level bergenin accumulation, 4 germplasms of *B. purpurascens* were simultaneously cultivated in 5 different sites of Yunnan Province. The results showed that Taian town was the best site for industrial scale cultivation of the germplasm of *B. purpurascens* collected from Yulong snow mountain accumulating high level of bergenin. The results also suggested that sufficient rainfall in June was beneficial for enhanced level of bergenin content.

Introduction

Bergenia purpurascens (J.D. Hooker & Thomson) Engler, an important medicinal plant belonging to *Saxifragaceae*, is widely used in China for the treatment of various diseases such as coughs, pneumonia, bronchitis, tuberculosis, diarrhoea, vomiting, fever, menorrhagia, excessive uterine hemorrhage, kidney stones and ulcer of large intestines (Li *et al.* 2006, Pan 1988, Pan and Soltis 2001, Wang *et al*. 2012, Yeo 1966, Yuan *et al.* 2011). It is distributed in South-west of China, North of Bhutan, North-east of India, North of Myanmar and Nepal and commonly cultivated as ground cover in Occident (Yeo 1966). Bergenin, the most important active compound in *B. purpurascens*, is reported to have anti-arthritic, anti-arrhythmic, anti-bacterial, anti-diabetic, anti-HIV, anti-hyperuricemic, anti-inflammatory, anti-oxidant, anti-tussive, antiulcerogenic, anti-urolithic, gastroprotective, hepatoprotective, hypolipidaemic, immuno-modulatory, neuroprotective effects and inhibitory effects on Citrus pathogens and bovine adrenal tyrosine hydroxylase (Bajracharya *et al.* 2011, Bashir *et al.* 2009, Dong *et al.* 2012, Li *et al.* 2006, Li *et al.* 2013, Lim *et al.* 2000, Nazir *et al.* 2007, Nazir *et al.* 2011, Patel *et al.* 2012, Rajkumar *et al.* 2010, Sajad *et al.* 2010, Zhang *et al.* 2003, Zhou *et al.* 2014, Zuo *et al.* 2012). It was first used for medicine production in 1972 and the average medical demand from 1995 to 2005 was about 4000 tons (fresh weight) every year. Due to the long-term over-exploitation *B. purpurascens* became an endangered species in China ten years ago and the industrial cultivation of *B. purpurascens* is the only way to preserve its wild resources and meet the medical demand. Since best germplasm containing high level of good quality bergenin is the important base for cultivation of this plant several projects about bergenin content determination and genetic diversity of *B. purpurascens*

*Author for correspondence: <yngfg@sina.cn>. [1]College of Horticulture and Landscape, Yunnan Agricultural University, Kunming 650201, Yunnan Province, China.

have been conducted since 2003 (Ji 2005, Jiang *et al.* 2010, Sun *et al.* 2010, Wang *et al.* 2012) and 4 promising germplasms were screened out for further research from 18 populations of *B. purpurascens* collected from different regions of Yunnan Province (Jiang *et al.* 2010). The environmental factors also play important role in bergenin accumulation, so screening of the best cultuvar as well as suitable cultivation site reflecting the effects of environmental factors on bergenin accumulation are other important tasks need to be addressed for industrial cultivation of *B. purpurascens*. The simultaneous cultivation of 4 *B. purpurascens* germplasms in five different sites of Yunnan Province and comparison of bergenin accumulation in the same germplasm cultivated in different sites possessing varied environmental conditions are described. The effects of environmental factors that affected bergenin accumulation for different germplasms are also explored by step-wise regression.

Materials and Methods

Four wild germplasms of *Bergenia purpurascens* were collected from Baimang Snow Mountain (BM), Yulong Snow Mountain (YL), Xiaoxueshan Mountain (XXS) and Xiaozhongdian town (XZD) in Yunnan Province, P.R. China, respectively. They were identified following standard method (Pan and Soltis 2001) and used for breeding seedlings by cutting propagation. All seedlings of these four germplasms were respectively cultivated in 5 sites of Yunnan Province (Table 1) in late April and early May of 2011. In total 12 blocks of 4 germplasms and 3 replications in each cultivation site were arranged in a canopy of 30% relative light intensity by randomized block design. Fifteen seedlings were transplanted in each block and the spacing in the rows and spacing between rows were 30×30 sq.cm. The same field management measures were applied to all the five cultivation sites. Five individuals in each block were randomly harvested in December of 2012 and the average rhizome dry weight per plant was calculated after being dried in an oven at 60°C for 6 days. Then all the dry rhizomes in each block were ground to powders and used for the determination of bergenin content.

Table 1. Information about five cultivation sites in Yunnan Province.

Site code	Position of cultivation sites	North latitude (NL)	East longitude (EL)	Altitude /meter
YS	Yaoshan Town, Qiaojia county	27°08′33″	103°00′12″	2520
YAU	Yunnan Agricultural University, Kunming	25°07′48.6"	102°44′44.6″	1929
TA	Taian Town, Yulong County	26°51′50.3″	100°02′16.7″	2559
JD	Jingding Town, Lanping County	26°28′35″	99°25′40″	2470
JT	Jiantang Town, Xianggelila County	27°50′6.4″	99°44′11.6″	3280

The bergenin contents of rhizomes in all blocks were determined by the Agilent Technologies 1200 series (Agilent Technologies Inc., USA) according to the HPLC method as reported by Jiang *et al.* (2010). Three replications were conducted for each block and the average bergenin content of each block was calculated. Because more than 90% of bergenin were accumulated in rhizomes (Jiang *et al.* 2010), the bergenin yield per plant for each block ≈ the average bergenin content in rhizomes × the average rhizome dry weight per plant.

The climatic data of 5 cultivation sites during 2011 - 2012 were obtained from the Yunnan Provincial Climate Center. Their month average amounts of 9 categories, namely soil temperature, air temperature, highest temperature, lowest temperature, humidity, rainfall, rain day, sunshine hours and sunshine per cent were calculated and in total 108 climatic factors were obtained. Eight soil factors of 5 sites in April of 2012, namely total nitrogen, total phosphorus, total potassium, effective nitrogen, fast-effective phosphorus, fast-effective potassium, organic matter and pH value were examined according to the methods of Bao (2000).

The original data were input to Microsoft Excel 2003 first. Analysis of variance of bergenin content and bergenin yield per plant among 4 germplasms and 5 cultivation sites, step-wise regression analysis of 4 germplasms between bergenin accumulation and 119 environmental factors and path analysis of 4 important factors for each germplasm were conducted by the SPSS 17.0 software (Du 2010, Zhang 2008).

Results and Discussion

Although *Bergenia purpurascens* has been commonly cultivated as ground cover in Occident for long time, no report on its high-effective cultivation as medicinal plant has been found so far. There are some reports on bergenin contents of *B. purpurascens* (Ji 2005, Jiang *et al*. 2010, Sun *et al*. 2010), but all these contents were interaction results of genotypes and environmental factors because wild samples were collected to examine bergenin contents in all these reports. Screening out best cultivation site and germplasm and revealing main environmental factors affecting bergenin accumulation are important tasks for the industrial cultivation of *B. purpurascens.*

In order to screen out the best site for industrial cultivation of *B. purpurascens*, five sites with different latitude, longitude and altitude were carefully selected in our research work (Table 1). Because bergenin content was negatively correlated to the biomass, two indices, namely bergenin content and bergenin yield per plant were used in screening out best cultivation site and best germplasm. The bergenin contents and bergenin yields per plant of 4 germplasms among 5 cultivation sites were listed separately and synthetically in Tables 2 and 3.

Table 2. Bergenin contents (%) among five cultivation sites.

Sites	Germplasm BM	Germplasm YL	Germplasm XZD	Germplasm XXS	Integration of four germplasms
YS	12.34 cC	13.40 bB	9.04 bB	8.25 bB	10.76 cB
YAU	8.58 bB	8.58 aA	9.05 bB	7.56 bAB	8.44 bA
TA	8.21 bB	9.17 aA	10.23 cB	6.51 aA	8.53 bA
JD	6.91 aAB	7.64 aA	5.88 aA	7.96 bB	7.10 aA
JT	6.10 aA	7.68 aA	6.36 aA	7.48 bAB	6.91 aA

Different small and capital letters in the same column represent significant differences (p < 0.05%) and extremely significant differences (p < 0.01%) among five sites, respectively.

The results in Tables 2 and 3 showed that there were extremely significant differences (p < 0.01%) in bergenin contents and bergenin yields per plant among 5 cultivation sites in all four germplasms. This phenomenon indicated that cultivation site played an important role in bergenin accumulation and screening of best site was an essential work. In bergenin content, germplasms BM and YL produced most bergenin in site YS while germplasm XZD accumulated most bergenin in site TA. From an integrative view of 4 germplasms, site YS was extremely significantly higher than other 4 sites in bergenin content. In bergenin yield per plant, germplasms YL, XZD and XXS obtained highest yields in site TA while germplasm BM ranked second position in site TA and was not significantly different from the first position in site YAU. From an integrative view of 4 germplasms, site TA was extremely significantly higher than other 4 sites in bergenin yield per plant. Based on the cultivation efficiency TA was the best site for industrial cultivation of *B. purpurascens* among the five researched sites.

Table 3. Bergenin yields per plant (g) among five cultivation sites.

Sites	Germplasm BM	Germplasm YL	Germplasm XZD	Germplasm XXS	Integration of 4 germplasms
YS	0.44 aA	0.85 aAB	0.51 aA	0.42 aA	0.56 aA
YAU	1.04 cC	0.75 aA	0.52 aA	0.42 aA	0.68 abAB
TA	1.00 cC	1.52 cD	1.23 cC	1.34 cC	1.27 dC
JD	0.75 bB	1.10 bBC	0.57 aAB	1.30 cBC	0.93 cB
JT	0.66 bB	1.17 bC	0.68 bB	0.93 bB	0.86 bcB

Different small and capital letters in the same column represent significant differences ($p < 0.05\%$) and extremely significant differences ($p < 0.01\%$) among five sites, respectively.

The bergenin contents and yields per plant among 4 germplasms in 5 cultivation sites were compared separately and synthetically in Tables 4 and 5.

Table 4. Bergenin contents (%) among four germplasms grown in five different sites.

Germplasms	Site YS	Site YAU	Site TA	Site JD	Site JT	Integration of 5 sites
BM	12.34 bB	8.58 aA	8.21 bAB	6.91 bAB	6.10 aA	8.43 abA
YL	13.40 bB	8.57 aA	9.17 bcB	7.64 bcB	7.68 bB	9.29 bA
XZD	9.04 aA	9.05 aA	10.23 cB	5.88 aA	6.36 aAB	8.11 abA
XXS	8.25 aA	7.56 aA	6.51 aA	7.96 cB	7.48 bAB	7.55 aA

Different small and capital letters in the same column represent significant differences ($p < 0.05\%$) and extremely significant differences ($p < 0.01\%$) among four germplasms, respectively.

Table 5. Bergenin yields per plant (g) among four germplasms grown in five different sites.

Germplasms	Site YS	Site YAU	Site TA	Site JD	Site JT	Integration of 5 sites
BM	0.44 abA	1.04 dC	1.00 aA	0.75 aA	0.66 aA	0.78 aAB
YL	0.85 cB	0.75 cB	1.52 cB	1.10 bB	1.17 cB	1.08 bB
XZD	0.52 bA	0.52 bA	1.23 bAB	0.57 aA	0.68 aA	0.70 aA
XXS	0.42 bA	0.42 aA	1.34 bcB	1.30bB	0.93 bAB	0.88 abAB

Different small and capital letters in the same column represent significant differences ($p < 0.05\%$) and extremely significant differences ($p < 0.01\%$) among 4 germplasms, respectively.

The results in Table 4 showed that YL and BM produced more bergenin than XZD and XXS in site YS while XZD accumulated more bergenin than XXS and BM in site TA. The data in Table 4 also showed that XZD presented less bergenin than other 3 germplasms in site JD while YL and XXS gave more bergenin than BM and XZD in site JT. There were no significant differences among 4 germplasms in site YAU. From an integrative view of five sites YL was better than XXS and not significantly different from BM and XZD. Table 5 showed that YL produced more bergenin yields per plant than other three germplasms in site YS, TA and JT. In sites JD, YL occupied second rank and was not significantly different from the first rank XXS. In sites YAU, YL also occupied second rank but was extremely significantly lower than first rank BM in bergenin yields per plant. From an integrative view of five sites, germplasm YL produced higher bergenin yield per plant than BM, XZD and XXS but YL was not significantly different from XXS in bergenin yield per plant. Based on 2 indices YL was the best germplasm for industrial cultivation of *B. purpurascens* for bergenin production among the four germplasms studied.

Table 2 showed that germplasm BM produced 12.34 and 6.10% bergenin in site YS and JT respectively while germplasms YL obtained 13.40 and 7.68% bergenin in site YS and JT respectively. This phenomenon indicated that environmental factors played important role in bergenin accumulation. In order to discover the main environmental factors that affected bergenin contents, step-wise regression analysis and path analysis were conducted by SPSS 17.0 software for germplasms BM and YL. For germplasm BM, rainfall of June, sunshine percent of December, fast-effective phosphorus and east longitude were four main factors to affect bergenin content and their path coefficients were 1.1386, 0.2288, –0.0387 and –0.0162, respectively. As to germplasm YL, rainfall of June, north latitude, air temperature of June and soil temperature of October were 4 main factors that affected bergenin content and their path coefficients were 0.8627, 0.5537, 0.3364 and –0.0374, respectively. Although the main environmental factors that affected the bergenin contents of germplasms BM and YL were different, rainfall of June was the most important factor shared by them. This result suggested that sufficient rainfall in June (about 195 mm) was beneficial for the increase of bergenin content. The step-wise regression analysis of bergenin yields per plant of 4 germplasms showed that the most important factors for germplasms BM, YL, XZD and XXS were sunshine hours of January, sunshine hours of December, humidity of January and rainfall of October, respectively and their path coefficients were 0.788, 1.0019, –1.0153 and 1.0707, respectively.

Three conclusions could be drawn from this research. Firstly Taian town was the best site for industrial cultivation of *B. purpurascens*. Secondly the germplasm collected from Yulong snow mountain was the best source for industrial cultivation of *B. purpurascens*. Finally, sufficient rainfall in June was beneficial for the increase of bergenin content. It is suggested that looking for the genes encoding key enzymes for the bergenin biosynthesis pathway in *B. purpurascens* should be a valuable work in the future.

Acknowledgements

The authors are grateful to National Natural Science Foundation of China (81060341) for financial support.

References

Bajracharya GB, Maharjan R and Maharjan BL 2011. Potential antibacterial activity of *Bergenia purpurascens*. Nepal J. Sci. Tech. **12**:157-162.

Bao SD 2000. Soil agrochemistry analysis. Agricultural Press of China, Beijing.

Bashir S and Gilani AH 2009. Antiurolithic effect of *Bergenia ligulata* rhizome: an explanation of the underlying mechanisms. J. Ethnopharmacol. **122**: 106-116.

Dong CM, Yang LC, Zou C, Zhao PJ, Pu H and Zhang Y 2012. The research progress of bergenin. J. Kunming Med. Univ. **33**(1): 150-154.

Du JJ and Chen ZW 2010. Realization of path analysis by using linear regression of SPSS. Chin Biol. Bull. **45**(2): 4-6.

Jiang HJ, Guo FG, Zhang LM, Chen YP and Yang SC 2010. Comparison of bergenin contents of *Bergenia purpurascens* among different regions in Yunnan Province. J. of Yunnan Agr. Univ. **25**(6): 895-898.

Ji LJ 2005. Bergenin HPLC determination of two species of bergenia growing in Tibet. Acta Bot. Boreali-Occidentalia Sin. **25**(2): 397-399.

Li BH, Wu JD and Li XL 2013. LC-MS/MS determination and pharmacokinetic study of bergenin, the main bioactive component of Bergenia purpurascens after oral administration in rats. J. Pharm. Anal. **3**(4): 229-234.

Li WC, Guo FG, Zhang LM, Yu HM, Li X and Lin C 2006. The situation and prospect of research on *Bergenia purpurascens*. J. Yunnan Agr. Univ. **21**(6): 845-850.

Lim HK, Kim HS, Choi HS, Oh S and Choi J 2000. Hepatoprotective effects of bergenin, a major constituent of *Mallotus japonicus*, on carbon tetrachloride-intoxicated rats. J. Ethnopharmacol. **72**: 469-474

Nazir N, Koul S, Qurishi MA, Taneja SC, Ahmad SF, Bani S and Qazi GN 2007. Immunomodulatory effect of bergenin and norbergenin against adjuvant-induced arthritis - A flow cytometric study. J. Ethnopharmacol. **112**(2): 401-405

Nazir N, Koul S, Qurishi MA, Najar MH and Zargar MI 2011. Evaluation of antioxidant activity and antimicrobial activites of bergenin and its derivatives obtain by chemoenzymatic synthesis. Eur. J. Med. Chem. **46**: 2415-2420

Pan JT 1988. A conspectus of the genus *Bergenia* Moench. Acta Phytotaxon. Sin. **26**(2): 120-129.

Pan JT and Soltis DE 2001. *Bergenia* Moench. In: Wu ZY & Raven PH Flora of China. Vol.8 (Brassicaceae through Saxifragaceae). Science Press, Beijing and Missouri Botanical Garden Press, St. Louis.

Patel DK, Patel K, Kumar R, Gadewar M and Tahilyani V 2012. Pharmacological and analytical aspects of bergenin: a concise report. Asian Pac. J. Trop. Dis. **2**(2): 163-167.

Rajkumar V, Guha G, Kumar RA and Mathew L 2010. Evaluation of antioxidant activities of Bergenia ciliata rhizome. Rec. Nat. Prod. **4**(1): 38-48.

Sajad T, Zargar A, Ahmad T, Bader GN, Naime M and Ali S 2010. Antibacterial and anti-inflammatory potential Bergenia ligulata. Am. J. Biomed. Sci. **2**(4): 313-321.

Sun XG, Huang WH, Ma M, Guo BL and Wang GY 2010. Comparative studies on content of arbutin, bergenin and catechin in different part of Bergenia purpurascens and B. crassifolia. China J. Chin. Mat. Med. **35**: 2079-2082.

Wang BX, Zhao H and Li YX 2012. The research progress of the genus Bergenia. Chin. J. Spectrosc. Lab. **29**: 367-370.

Wang SY, Guo FG, Zhang YH and Yang ZA 2012. ISSR analysis of 18 Bergenia purpurascens resources in Yunnan. Chin. Agr. Sci. Bull. **28**(7): 114-118.

Yeo P F 1966. A revision of the genus Bergenia Moench. Kew Bull. **20**(1): 113-148.

Yuan JL and Suo JL 2011. Research progress in medicinal plant genus Bergenia Moench. J. Baoji Univ. Arts and Sci. **31**(1): 46-50.

Zhang YH, Fang LH, Lee MK and Ku BS 2003. In vitro inhibitory effects of Bergenin and norbergenin on bovine adrenal tyrosine hydroxylase. Phytother. Res. **17**: 967-969.

Zhang L 2008. The application of SPSS in Biostastitics (Second edition). Press of Xiamen University, Xiamen.

Zhou HX and Chen YS 2014. Anti-hyperuricemic activity of bergenin and its mechanism. Acta Univ. Med. Anhui. **49**(1): 63-67.

Zuo PP, Li MM, Hu JH, Yao TS, Li HJ, Liu HQ and Ran C 2012. Inhibitory effect of bergenin against citrus pathogens. South China Fruits **41**(4): 1-4.

(Manuscript received on 8 April, 2017; revised on 28 August, 2017)