

## QUALITY ZONING OF *EPIMEDIUM BREVICORNU* MAXIM. BASED ON THE CORRELATIONS AMONG ENVIRONMENTAL FACTORS AND INFLUENCE OF PATH ANALYSIS

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### Abstract

*Epimedium brevicornu* Maxim. rich in flavonoids is used as a medicinal plant. The purpose of this study was to predict the adaptive zones, and quality zones, provide experimental data support for the development and utilization of *E. brevicornu*. ArcGIS was used to extract environmental factors associated with *E. brevicornu*, SPSS was used to analyze the correlation between the icariin content and these environmental factors. In the end, the quality zoning was performed based on the icariin contents of collected samples. A significant negative correlation between the July vapor pressure and the icariin content was observed. Quality *E. brevicornu* was mainly distributed in parts of Shanxi, Shaanxi, Gansu. The icariin contents of plants in the southern region of Gansu Province were higher than others. In the present study, the quality zones were identified and the influence paths of environmental factors were analyzed.

### Introduction

*Epimedium brevicornu* Maxim. (Fig. 1), belonging to Berberidaceae is mainly distributed in the provinces of Gansu, Shaanxi, Shanxi, Henan. It has been used as a traditional Chinese medicine (TCM) for a long time (Ma *et al.* 2011). It was first recorded in Shen Nong Ben Cao Jing (Han Dynasty of China). Traditionally, *E. brevicornu* has the function of invigorating the kidney and reinforcing yang, strengthening bones and muscles. Ma *et al.* (2011) have reported that more than 260 compounds were isolated from *Epimedium*, including flavonoids, lignins, ionones and other compounds. As the major constituents, flavonoid biosynthesis are easily affected by light, temperature and water content (Cominelli *et al.* 2005, Azuma *et al.* 2012). It was also reported that phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) play important roles in the synthesis and metabolism of flavonoids and are vulnerable to the influence of the natural environment, which can lead to changes in flavonoid contents (Dick *et al.* 2011). Icariin, one of the main components of flavonoids, has been demonstrated that it has extensive pharmacological effects by numerous pharmacology studies and clinical practice. It has preventive and therapeutic effects on liver cancer, osteoporosis, cardiovascular diseases and Alzheimer's disease (Li *et al.* 2014, Fang and Zhang 2017, Qian *et al.* 2018, Angeloni *et al.* 2019).

With the arrival and development of natural medicinal products, the market demand for *E. brevicornu* is increasing significantly. At present, *E. brevicornu* mainly depends on the extraction of wild resources, while causing ecological destruction, the yield and quality are also affected by the local climate and environment. Hence, establishing nature reserve and quality regions is one of great significance for *E. brevicornu*.

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Fig. 1. The pictures of *E. brevicornu* and its growing environment, photographed in Diebu County, Gansu Province.

Using species distribution models (SDMs) is a main solution in ecology, conservation, evolution and wildlife management. SDMs are statistical models that relate species distribution data with information on the environmental and/or spatial characteristics of those locations, such models are used to estimate species distributions under the impacts of environmental factors. Although a wide variety of SDMs have been introduced, the maximum entropy model (MaxEnt) is better than other models, for its better discrimination of suitable versus unsuitable areas (Phillips *et al.* 2006). MaxEnt has been widely used to predict distributions of many species (Fitzpatrick *et al.* 2013, Cao *et al.* 2016) in conjunction with geographic information systems (GIS).

In the present study, MaxEnt was used to predict the adaptive zones and the quality zones of *E. brevicornu* in China, using occurrence data. The key environmental factors influencing the icariin content were found by Statistical Product and Service Solutions (SPSS) (Yan *et al.* 2020). It was aimed to identify the key environmental factors affecting the icariin content in order to construct maps of their potential quality distributions and facilitate the formulation of appropriate protection and development measures.

### Materials and Methods

Samples were collected from *E. brevicornu* distribution areas in Henan, Shanxi, Shaanxi, Ningxia, Gansu and Chongqing Provinces in July. Plants were collected from each site, and information for each sample site was obtained using global positioning system (GPS) data. Thirty nine distribution points of *E. brevicornu* were obtained from the Flora of China (<http://www.iplant.cn/>) and the Botanical Image database of China (<http://ppbc.iplant.cn/>). In the end, 68 GPS points indicating the locations and 29 samples were collected. Microsoft Excel was used to save geographical distribution data in CSV format. Combined with the map data, ArcGIS

10.3 (ESRI, Redlands, CA, USA) was used to draw a map of the geographical locations of the distribution points of *E. brevicornu* (Fig. 2). The map data were downloaded from the National Geomatics Center of China (<http://ngcc.sbsm.gov.cn/>).

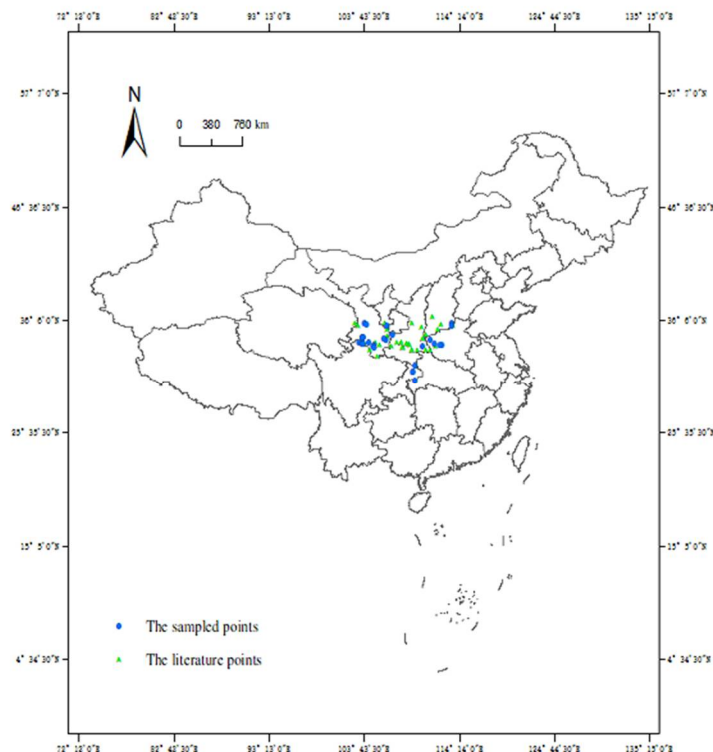


Fig. 2. The distribution map of *E. brevicornu* based on the 68 GPS points.

The plant samples were grouped according to the collection site, and the icariin content of each sample was determined by high-performance liquid chromatography (HPLC). A Purospher Star LP C18 column (250×4.6 mm, 5 μm) was used because mobile phase A was acetonitrile and mobile phase B was water. The gradient elution process was as follows: 24-25% (A) for 0-30 min; 26-45% (A) for 30-31 min; and 45-47% (A) for 31-45 min. The detection wavelength was 270 nm (Table 1).

Climatic data covering the climatic period (1979-2000) was obtained from the World Climatic Database (<http://worldclim.org/>) at a spatial resolution of 1 km<sup>2</sup>. This database provides information on 19 climatic factors (Bio1-Bio19), as well as precipitation (Prec1-Prec12), solar radiation (SRad1-SRad12), water vapor pressure (Vapr1-Vapr12) and wind speed (Wind1-Wind12) data. Topographic factors were extracted from elevation data, including the slope (Slo) and aspect (Asp). The data of 61 soil types (Soil) was obtained from the Institute of Soil Science, Chinese Academy of Sciences (<http://www.issas.ac.cn/>) at a spatial resolution of 1 km<sup>2</sup>. These environmental factors were extracted by masks in ArcGIS 10.3 and then imported into SPSS 18.0 (IBM corp., Armonk, NY, USA) for Pearson correlation analysis. If the correlation coefficient between two environmental factors  $|r| \geq 0.8$ , only one factor was selected to establish a niche

model (Zhang *et al.* 2019). Finally, 18 environmental factors were selected: Asp, Bio1, Bio3, Bio4, Bio12, Bio15, Prec1, Prec6, Prec8, Prec9, Slo, Soil, SRad8, SRad12, Vapr1, Vapr7, Wind1, and Wind7 (Table 2).

**Table 1. The geographic details and icariin contents of the collected *E. brevicornu*.**

No.	Location	Longitude	Latitude	Icariin (%)
1	Agan forest farm, Qilihe district, Gansu Province	103°47'29.3"	35°53'33.3"	1.47
2	Badu, Long County, Shaanxi Province	106°52'40"	34°45'14"	0.56
3	Baihe, Li County, Gansu Province	104°51'24.4"	33°51'57.3"	1.17
4	Caoping, Li County, Gansu Province	104°48'51.2"	33°40'51.34"	1.23
5	Changcao, Fengjie County, Chongqing Province	109°21'49.1"	30°32'3"	1.13
6	Checun, Song County, Henan Province	112°8'52.4"	33°47'43.8"	0.87
7	Dangchang County, Gansu Province	104°15'36.8"	34°5'42.9"	0.82
8	Dangchuan forest farm, Maiji District, Gansu Province	106°8'26"	34°20'37.1"	0.97
9	Dayugou, Zhuoni County, Gansu Province	103°34'44.2"	34°31'4.1"	1.81
10	Dianga, Diebu County, Gansu Province	103°16'6.9"	34°2'10"	1.43
11	Duguan, Lushi County, Henan Province	111°0'50"	34°14'56.5"	0.51
12	Liupanshan, Jinyuan County, Ningxia Province	106°18'22.5"	35°39'30.6"	0.54
13	Liuquan, Lingchuan County, Shanxi Province	113°29'38.1"	35°48'36.7"	0.98
14	Magedang, Lingchuan County, Shanxi Province	113°26'6.1"	35°34'55.1"	0.87
15	Maiji District, Gansu Province	105°58'52.7"	34°24'32.1"	0.88
16	Miaogou, Danfeng County, Shaanxi Province	110°13'3"	33°46'16"	0.44
17	Nalang, Zhuoni County, Gansu Province	103°42'44.7"	34°30'40.4"	1.10
18	Nalang, Zhuoni County, Gansu Province	103°34'41.6"	34°30'58.5"	1.45
19	Panjiaba, Wudu District, Gansu Province	104°52'10.16"	33°34'43.3"	1.81
20	Pingan, Fengjie County, Chongqing Province	109°10'49.8"	31°20'48.8"	0.53
21	Qiugou, Zhuoni County, Gansu Province	103°34'53"	34°30'59"	1.13
22	Sandaomen, Zhenping County, Shaanxi Province	109°24'30.1"	31°56'35.9"	0.86
23	Shizimiao, Luanchuan County, Henan Province	111°33'36.3"	34°0'34.3"	0.82
24	Taizi, Fengjie County, Chongqing Province	109°10'3.8"	31°20'32.1"	0.81
25	Wangzang, Diebu County, Gansu Province	103°36'32.54"	33°57'2.45"	1.36
26	Wangzang, Diebu County, Gansu Province	103°33'44"	33°59'36.1"	1.15
27	Wangzang, Diebu County, Gansu Province	103°41'41.1"	33°54'28.3"	0.82
28	Xinlongshan, Yuzhong County, Gansu Province	104°3'25.12"	35°47'4"	2.09
29	Yaoshan, Lushan County, Henan Province	112°25'13.30"	33°46'34.4"	0.53

These 29 samples were collected from Henan, Shanxi, Shaanxi, Ningxia, Gansu and Chongqing Provinces.

MaxEnt V3.4.1 ([http://biodiversityinformatics.amnh.org/open\\_source/maxent/](http://biodiversityinformatics.amnh.org/open_source/maxent/)) was used for model building. The 25% of the distribution data were randomly selected as the test set, and 75% of the data were selected as the training set. The maximum number of background points was 10,000, and 10 replicates were used. Environmental layers were set for continuous variables

except for soil types. The receiver operating characteristic curve (ROC) was chosen to evaluate the prediction accuracy of the model. A jackknife test was performed to measure the importance of variables, and response curves were plotted. The results were obtained in logistic format and with ASC file outputs, and other parameters were held constant at default values (Merow *et al.* 2013).

**Table 2. The selected environmental variables.**

Environmental Variable	Abbreviation	Unit
Aspect	Asp	°
Annual mean temperature	Bio1	°C
Isothermality	Bio3	1
Temperature seasonality	Bio4	°C
Annual precipitation	Bio12	mm
Standard deviation of precipitation seasonality	Bio15	1
Precipitation	Prec1	
	Prec6	mm
	Prec8	
Slope	Prec9	
	Slo	%
Soil type	Soil	1
Water vapor pressure	Vapr1	kPa
	Vapr7	
Solar radiation	SRad8	kJ m <sup>-2</sup> day <sup>-1</sup>
	SRad12	
Wind speed	Wind1	m s <sup>-1</sup>
	Wind7	

These environmental variables were downloaded from the World Climatic Database, then selected by SPSS.

The area under the receiving operator curve (AUC) was used to evaluate the accuracy of the prediction results, and AUC values ranged from 0 to 1. When the value was less than 0.60, the model prediction failed; values from 0.60-0.70 indicates that the model performed poorly; values from 0.70-0.80 reflects average performance; values from 0.80 - 0.90 suggests the model outputs are relatively accurate; and values from 0.90 - 1.00 meant that the model predictions are accurate and highly reliable (Wisz *et al.* 2008).

All the selected environmental factors were input into the MaxEnt model to delimit the potential adaptive zones of *E. brevicornu* and the results ranged from 0 to 1.00, the adaptive zones were divided into the following three types: non-adaptive zones (0 - 0.25), low-adaptive zones (0.25 - 0.50) and high-adaptive zones (0.50 - 1.00).

SPSS 18.0 was used to analyse the correlation between the icariin content and all environmental factors, and multiple linear regression (MLR) analysis was conducted. The results obtained with the linear regression equation of icariin contents and the adaptive zoning results for *E. brevicornu* were superimposed to obtain the quality zones with the raster calculation function in ArcGIS. An icariin content of 5 mg/g was specified in the Chinese Pharmacopoeia as the threshold

value for regional division. Thus, the *E. brevicornu* quality zones were divided into the following three types: low-quality zones (0-5 mg/g), middle-quality zones (5-12 mg/g) and high-quality zones ( $\geq 12$  mg/g). Finally, the area of each region was calculated.

## Results and Discussion

Results showed that the average test AUC of the replicate runs was 0.980, and the standard deviation was 0.003 (Fig. 3), indicating that the prediction results were highly accurate and reliable. The relative contributions of the environmental factors and the jackknife test results (Fig. 4) showed that the key factors that influenced the adaptive zones of *E. brevicornu* were Prec9 (25.3%), Soil (17.3%), Vapr1 (17.2), Vapr7 (16.4%), Bio4 (12.8%), and SRad8 (3.9%), and the cumulative per cent contribution exceeded 90%.

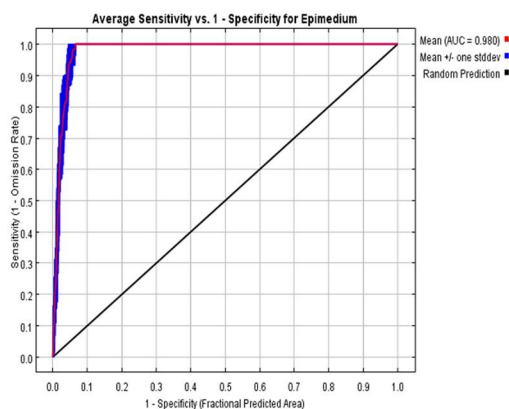


Fig. 3. The area under the receiving operator curve which was obtained by MaxEnt.

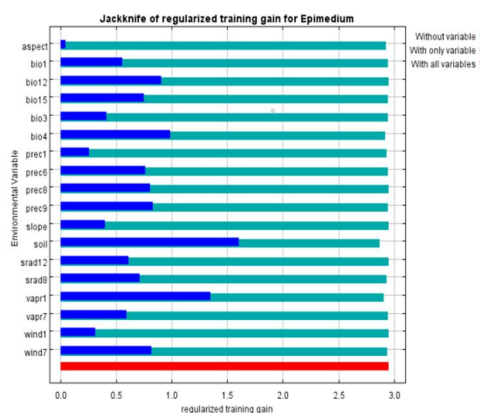


Fig. 4. The jackknife test results which were obtained by MaxEnt.

The response curve of the environmental variable showed the logistic probability of the species' presence in the MaxEnt model. According to the response curve (Fig. 5), the suitable range of Prec9, Vapr1, Vapr7, Bio4, and SRad8 are 84.61 - 146.15 mm, 0.25 - 0.39 kPa, 1.36 - 2.33 kPa, 757.14 - 885.71,  $1.73 \times 10^4$ - $1.89 \times 10^4$   $\text{kJm}^{-2}\text{day}^{-1}$  the soil types are brown soil, cinnamon soil, gray-cinnamon soil, loessioisol, alluvial soil and mountain meadow soil.

The results showed that *E. brevicornu* mainly distributed in Henan, Shanxi, Shaanxi, Ningxia, and Gansu with the range of  $31.6 - 36.8^\circ \text{N}$ ,  $102.1 - 113.7^\circ \text{E}$  (Fig. 6), the marginally suitable areas and highly suitable areas of it were  $1.56 \times 10^5 \text{ km}^2$  and  $0.99 \times 10^5 \text{ km}^2$ , respectively, the total area was approximately  $2.6 \times 10^5 \text{ km}^2$ .

As a popular tool to predict the pattern of specie distribution, MaxEnt is commonly used, theoretically, it can provide acceptable results from limited information (Hernandez *et al.* 2006). The adaptive regions of plants can be estimated by using MaxEnt. For medicinal plants, the active ingredients content is also important, only estimating the adaptive regions cannot meet the pharmaceutical market demand. Studies have shown that most of the active ingredients are secondary metabolites, which are affected by environmental stresses, a certain correlation between environmental factors and chemical compounds exists (Cao *et al.* 2016).

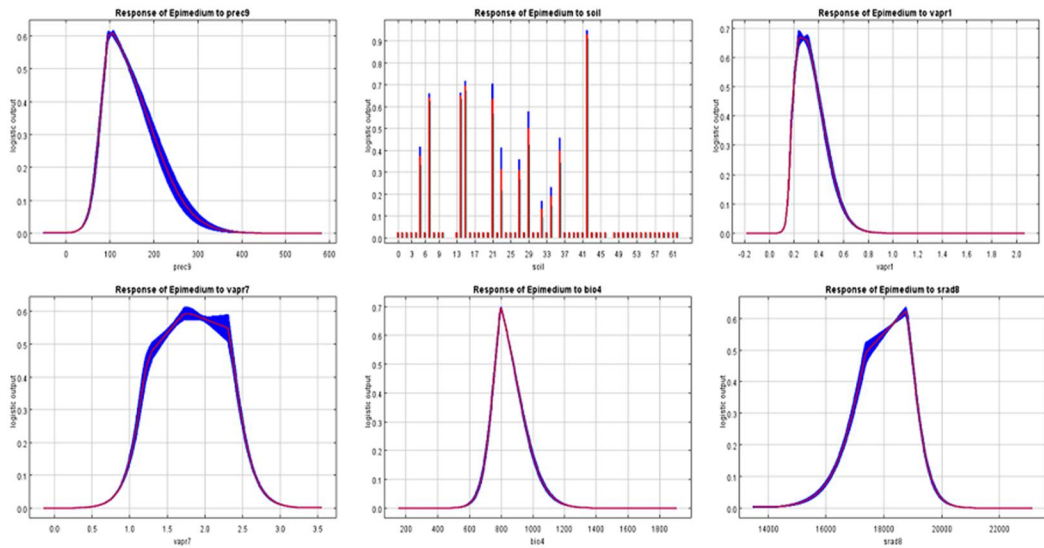


Fig. 5. The response curves of the environmental variables which were obtained by MaxEnt.

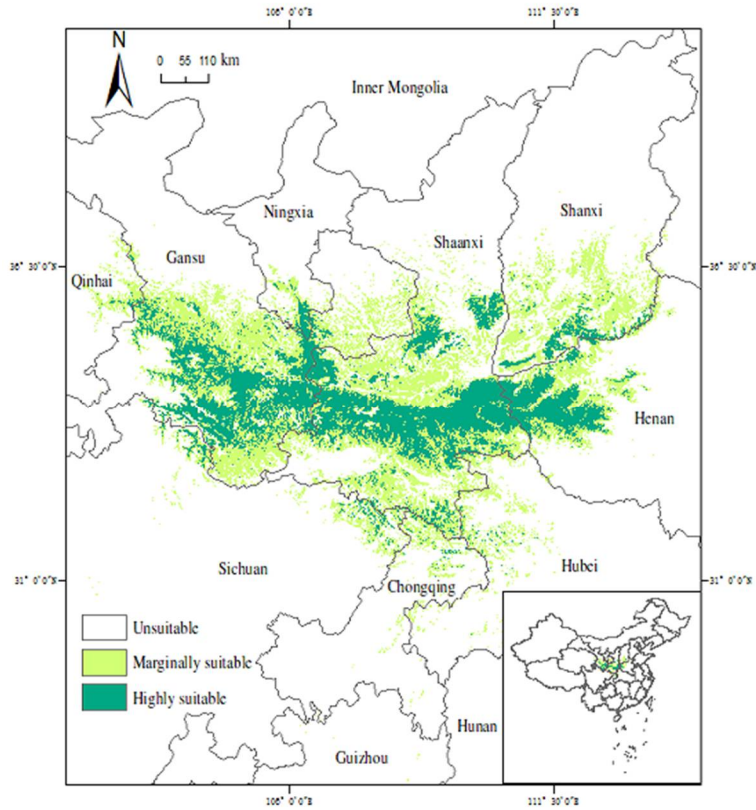


Fig. 6. The adaptive zones of *E. brevicornu* which were based on the result of MaxEnt by ArcGIS.

According to the results of MaxEnt, the adaptive zones of *E. brevicornu* are located primarily in a narrow strip in central China, which are consistent with the actual distributions. Five climatic factors (Prec9, Vapr1, Vapr7, Bio4, and SRad8) and soil types were the key environmental factors, which were determined by the jackknife test. The relationship between the environment and the existence probability of *E. brevicornu* was analyzed. As a precipitation-variable, Prec9 is a major factor affecting the distribution. It indicated that the distributions of species are influenced by environmental factors, often through species-specific physiological thresholds of temperature and precipitation tolerance. Most studies demonstrate soil effects on plant growth (Ehrenfeld *et al.* 2005), changes in soil types occur over a geological time frame, so any changes due to climate change are likely to be small (Ray *et al.* 2015). Soil types had the second highest contribution to the distribution. It showed that mountain meadow soil is the most suitable soil type for its growth.

Ecological and environmental data were extracted with ArcGIS according to the location information for the 29 collected samples, and the correlations between the icariin contents and environmental factors were analysed. Bio15, Prec9, SRad8, Vapr1 and Vapr7 were significantly correlated with the icariin content. A stepwise regression analysis of the icariin content and environmental factors was conducted using MLR, and the regression equation was as follows:  $Y=2.178-0.678X$ , where Y is the icariin content and X is Vapr7. A significance test of the model resulted in the following statistical terms:  $F = 26.331$ ,  $P = 0.000$ ,  $r = 0.703$ , and  $R^2 = 0.494$ , indicating that the equation had significant effects and that the linear relationship between Vapr7 and the icariin content was significant and could thus be used in subsequent analyses.

According to the results of the regression analysis, Vapr7 is the crucial environmental factor. The icariin content was negatively correlated with Vapr7. The icariin content displayed a gradually increasing trend when Vapr7 values decreased; icariin, as a secondary metabolite of plants, is closely related to the growth environment.

Based on the mathematical correlations between the icariin contents and environmental factors, the spatial calculation function in ArcGIS was used to obtain the quality zones (Fig. 7).

The results showed that the middle-quality zones and high-quality zones for *E. brevicornu* were mainly distributed in a narrow strip located between the latitudes of 32° N and 36° N and the longitudes of 102° E and 113° E; this region includes parts of Shanxi, Shaanxi and Gansu Provinces, with a total area of approximately  $1.2 \times 10^5$  km<sup>2</sup>.

As the quality map showed, the middle-quality zones and high-quality zones were consistent with the high-adaptability area, according to the icariin content ( $\geq 5$  mg/g), which indicated that the high-adaptability environment of *E. brevicornu* had a promoting effect on the accumulation of icariin. Thus, the plants in this region could meet the requirements of the Chinese Pharmacopoeia and the market demands for medicinal materials. Furthermore, the area of high-quality zones was approximately  $1.4 \times 10^4$  km<sup>2</sup>, which is located in the southern Gansu Province. The region has long been recognized as a high-quality producing region of *E. brevicornu*. This result is consistent with the present field investigation results.

Based on the results, it may be inferred that the path of the environmental factors that influences the icariin content were as follows: when the vapor pressure (Fick and Hijmans 2017) decreased, the vapor pressure deficiency (Massmann *et al.* 2019) increased, and the evapotranspiration (Maes and Steppe 2012) increased, leading to an increase in soil water evaporation and the intensification of plant transpiration. A reduction in vapor pressure can lead to the generation of environmental drought stress (Krasensky and Jonak 2012), thus influencing biological enzymes (Dick *et al.* 2011) and further affecting the accumulation of icariin. The experimental results are consistent with the results on the impacts of plant flavonoids reported by Zhang *et al.* (2020).



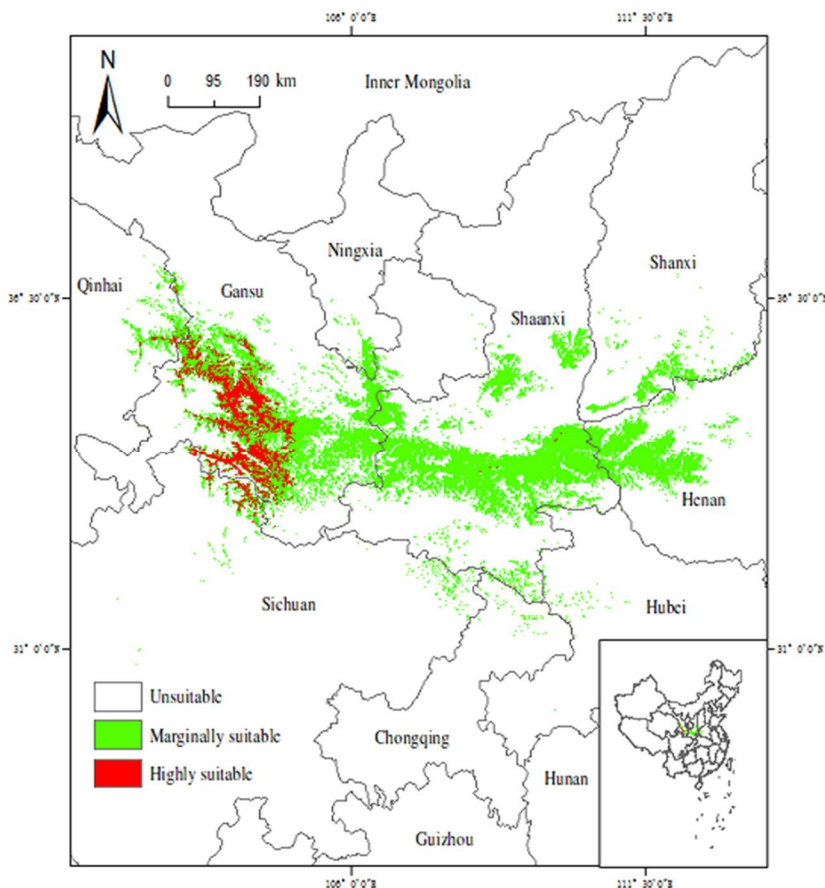


Fig. 7. The quality zones of *E. brevicornu* which were based on the adaptive zones by ArcGIS.

The fundamental niche of a species is generally described by SDMs, rather than its realized niche (Wang *et al.* 2010), many factors affecting the predictions of the SDMs are not taken into account. In this work, some factors were not considered, such as species interactions, self-diffusion ability, geographical barriers and socioeconomic factors. Furthermore, the climate data comes from the World Climate Database and covers the period from 1979 to 2000, ignoring the update in climatic information due to climate change (McMahon *et al.* 2011). All of the above factors may affect the inaccuracy of the predictions, leading to the generation of one-sided results. Therefore, adjusting the comprehensiveness and accuracy of the environmental variables used is one of the ways to improve this study.

In the present study, MaxEnt was used to predict the adaptive zones and the quality zones of *E. brevicornu*. These explorations revealed that the environment plays a major role in the distribution. Prec9, Vapr1, Vapr7, Bio4, and SRad8 and soil types are the key environmental factors to the adaptive zones. Meanwhile, Vapr7 is the crucial environmental factor to the quality zones for its negative correlation with the icariin content. Overall, this study provides a reference for the management of plant resources, which could avoid the exhaustion of wild *E. brevicornu* resources.

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