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Original Research

How do Environmental Variables Affect the Suitable Habitat of Medicinal Plants? A Case Study of *Citrus medica* L. var. *sarcodactylis* Swingle in China

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Abstract

Citrus medica L. var. *sarcodactylis* Swingle (Rutaceae: *Citrus*) is one of the traditional bulk Chinese medicinal materials in China with the effects of bacteriostasis, anti-inflammatory, anti-oxidation, anti-cancer cells, regulating the immun. In our paper, MaxEnt and ArcGIS were applied to simulate the suitable areas of *C. medica* L. var. *sarcodactylis* in China from the perspectives of bioclimate, soil, topographic factors and human activities Results showed that 1) temperature annual range (Bio7), annual precipitation (Bio12), human footprint (Hf), elevation (El) and precipitation seasonality (Bio15) were identified as the dominant environmental variables related to the distribution of *C. medica* L. var. *sarcodactylis*. The area of the total, most, moderately and poorly suitable habitat of *C. medica* L. var. *sarcodactylis* in China were 177.36×10^4 km^2, 22.27×10^4 km^2, 51.96×10^4 km^2 and 103.13×10^4 km^2 respectively. The range of the most suitable habitat was the narrowest, which was located in the middle east of Sichuan, western Chongqing in the upstream of the Yangtze River Basin, southern Guizhou and western Guangxi in the upstream of the Pearl River Basin, central and southern Yunnan and Southeast Tibet in the Middle-Lower reaches of the Southwest River Basin and western Taiwan.

Keywords: *Citrus medica* L. var. *sarcodactylis* Swingle, MaxEnt, key environmental variables, suitable habitat

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Introduction

Citrus medica L. var. *sarcodactylis* Swingle (Rutaceae: *Citrus*) is one of the traditional bulk Chinese medicinal materials in China, after a long period of biological evolution, natural and artificial selection, it has formed some distinctive cultivated varieties [1]. *C. medica* L. var. *sarcodactylis* contains contains flavonoids, coumarin, sugar, volatile oil, citric acid, amino acids and other chemical components, and its roots, stems, leaves, flowers and fruits can be used as medicine, with a unique role in disease prevention, health care and longevity [1, 2] (Fig. 1). Traditional Chinese medicine believes that *C. medica* L. var. *sarcodactylis* has the effects of rational gasification of phlegm, anti emesis and anti distension, soothing the liver and strengthening the spleen, while modern scientific medicine have shown that it has the effects of bacteriostasis, anti-inflammatory, anti-oxidation, anti-cancer cells, regulating the immune system [3]. The essential oil extracted from *C. medica* L. var. *sarcodactylis* has the effect of relieving pressure and inspiring people, and has been used in the food, drug and cosmetics industries [4]. *C. medica* L. var. *sarcodactylis* is native to India and widely cultivated in Europe (France, Italy and Germany), the United States and Southeast Asia [1]. Its main producing areas in China include Guangdong, Fujian, Chongqing, Sichuan and Zhejiang, especially Jiangjin in Chongqing and Zhaoqing in Guangdong have the largest planting area and the highest yield [5].

In recent years, due to the government's emphasis on the Chinese medicine industry and increased investment, the planting area of *C. medica* L. var. *sarcodactylis* has continued to expand [1]. The biological characteristics show that *C. medica* L. var. *sarcodactylis* is not resistant to cold, drought and frost. Therefore, it is suitable for planting in areas with sufficient rainfall and no freezing in winter, and its requirements for the original environment are very strict. In some regions, due to the lack of scientific planting zoning and development planning guidance, farmers have carried out large-scale bergamot planting in non optimal growth areas, resulting in low fruit survival rate, serious diseases and pests, poor product quality and fruit deformity. On the other hand, the current domestic research on artificial cultivation of *C. medica* L. var. *sarcodactylis* is not deep and wide enough, which results in the lack of comprehensive, systematic and scientific planting technical specifications to guide farmers in production areas to introduce or expand scientifically. Based on these factors, it is necessary to analyze the suitability of *C. medica* L. var. *sarcodactylis* in China.

Due to the characteristics of fixed growth and immobility, plants have evolved a variety of adaptability to environmental changes [6]. The developmental process of plants of different species or genotypes has different responses to different environmental conditions [7]. Even different tissues and organs of the same plant at different development stages have different responses to different environmental stresses at the molecular, cellular and morphological levels [8, 9]. These changes in the development process are crucial for plant adaptation to the environment and crop production. Climate change characterized by global warming has become one of the important environmental problems [10]. The increase of CO_2 concentration accelerates global warming and causes glacier melting and sea level rise, which in turn causes a chain reaction of frequent extreme weather such as floods, typhoons and heat waves, seriously affecting the sustainability of ecosystem structure and function [6, 11]. Under the premise of environmental change caused by global climate change, the growth process, suitable planting areas and disastrous factors of crops have also changed. Scientific simulation and prediction of the impact of environmental factors and their changes on the growth and production of plants and crops, and discussion of agricultural development strategies to cope with global changes, have become major issues that need to be addressed in implementing the global sustainable agricultural development strategy [12].

Medicinal plant ecological regionalization is a systematic analysis and regional division of the distribution sites of traditional medicine growing in a specific environment, and is a common method for the suitability division of traditional medicine that is not clear about the potential planting areas [13-15]. In recent years, the maximum entropy model (MaxEnt) has been applied to the study of habitat zoning of various medicinal plants under climate change scenarios since its good prediction ability. Zhang et al. [17] compared

Fig. 1. Field overview of *C. medica* L. var. *sarcodactylis* planting. a plant; b) fresh fruit; c) dried fruit slices.

the changes of the suitable habitat of wild *Anemarrhena asphodeloides* in China under the future climate change scenarios by MaxEnt, and their results provided a theoretical basis for the protection of this medicinal plant. She et al. [16] selected MaxEnt to predicting the suitable habitat of the important medicinal resource plant *Notopterygium incisum* in the Three Rivers Headwater Region of China in the present and 2050s, and believed that the results would be conducive to the scientific protection and rational utilization of its resources. Dad and Rashid [19] mapped the current and future geographical distributions of *Aconitum heterophyllum*, *Fritillaria cirrhosa*, *Meconopsis aculeata* and *Rheum webbianum* in Kashmir-Himalaya using MaxEnt. Li et al. [18] simulated the potential distributions of the genus *Nardostachys* under current and future (2050s and 2070s) climatic conditions. The above proves that MaxEnt model is an effective tool for the study of medicinal plant habitats and can be used for the suitability analysis of *C. medica* L. var. *sarcodactylis.*

The habitat environment is an important external factor for the formation of genuine medicinal materials, which directly affects their growth and development and the formation of effective ingredients [20]. With the deepening understanding of genuine medicinal materials, researchers try to use different mathematical methods to explain the impact of ecological environment on their quality, such as light, temperature, water, air, soil, etc., and explore the best ecological suitable area for the growth of genuine medicinal materials, so as to scientifically guide their introduction and expansion [14, 21-23]. Researches of *C. medica* L. var. *sarcodactylis* has focused on, physiologically active compounds [2, 24], and the quality of medicinal materials [4, 5], while no reports have been found on its suitability analysis. In this paper, by analyzing the importance of modeling, the domain variables affecting the distribution of *C. medica* L. var. *sarcodactylis* were screened, MaxEnt and ArcGIS were used to simulate the suitable areas of *C. medica* L. var. *sarcodactylis* in China from the perspectives of bioclimate, soil, topographic factors and human activities. The main purposes of this study are: 1) to explore the key environmental variables that affect the distribution of *C. medica* L. var. *sarcodactyli* and analyze their relationship 2) to provide scientific guidance for reasonable planning and high-quality cultivation of *C. medica* L. var. *Sarcodactyli.*

Experimental

Materials

Data of Species Occurrence

We conducted field investigation, searched Global Biodiversity Information Facility (GBIF, https://www.gbif.org/), the Chinese Virtual Herbarium (CVH, https://www.cvh.ac.cn/), and consulted literature to obtain the occurrence data of *C. medica* L. var. *Sarcodactylis* [25, 26]. We used ENMTOOLs to process the data to ensure the uniqueness of distribution points in each grid.

Environmental Variables

According to the daily observation data of 824 meteorological stations in China from 1991 to 2020 provided by data set of China's surface climate data from the China Meteorological Data Sharing Service System (http://data.cma.cn/), after excluding the daily average temperature and the missing measurement value of precipitation at 20-20 h, and converting snow, ice and fog into precipitation, 19 bioclimatic variables are calculated. For more accurate prediction, we downloaded DEM, soil, radiation and human activity data (Table 1).

Modelling Process

MaxEnt software operation procedure was as follows. 1) The occurrence data of *C. medica* L. var. *sarcodactylis* in "CSV" format and the environmental variable in "ASC" format (including bioclimatic variables, elevation and human footprint) were imported into the "sample" and "environmental layers" data boxes of MaxEnt software (V3.4.4) respectively. 2) "Create response curves" and "Do jackknife to measure variable importance" were selected respectively to analyze the relationship between variables and presence probability of *C. medica* L. var. *sarcodactylis* and measure the importance of variables. 3) In the initial model, "Random test percentage" was set to 25%, while in the reconstructed model, "random seed" was selected, and the "replicates" was set to 10 [26-28].

Results and Discussion

Selection of Environmental Variables

In order to reduce the influence of multi-collinearity among 19 bioclimatic variables, Pearson correlation analysis method was used [26, 28]. Firstly, MaxEnt was used to calculate the percent contribution of 19 environmental variables, and the variables whose percent contribution rate was greater than 0 were retained. Thereafter, the Pearson's coefficients between two variables with percent contribution greater than 0 corresponding to 123 occurrence data of *C. medica* L. var. *sarcodactylis* were analyzed using SPSS (Fig. 2). Thirdly, by comparing the percentage contribution of the variables with the absolute value of the coefficient greater than 0.85, the higher one was retained. Finally, in addition to elevation, a total of 17 variables were selected to establish the prediction model of *C. medica* L. var. *sarcodactylis* (Table 1).

Table 1. Environmental variables used in this study.

Type	Variables	Description	Unit	Percent contribution of initial model	Selected /Eliminated
Bioclimate	Bio1	Annual Mean Temperature	ºC	3.7	Selected
	Bio2	Mean Diurnal Range(Mean of monthly (max temp - min temp)	ºC	2.9	Selected
	Bio3	Isothermality (BIO2/BIO7) (* 100)	/	1.1	Eliminated
	Bio4	Temperature Seasonality (standard deviation *100)	ºC	1.5	Eliminated
	Bio5	Max Temperature of Warmest Month	ºC	3.4	Selected
	Bio6	Min Temperature of Coldest Month	ºC	0.4	Eliminated
	Bio7	Temperature Annual Range (BIO5-BIO6)	ºC	19.9	Selected
	Bio8	Mean Temperature of Wettest Quarter	ºC	1.1	Eliminated
	Bio9	Mean Temperature of Driest Quarter	ºC	0.4	Eliminated
	Bio10	Mean Temperature of Warmest Quarter	ºC	0.7	Eliminated
	Bio11	Mean Temperature of Coldest Quarter	ºC	3.1	Eliminated
	Bio12	Annual Precipitation	mm	30.1	Selected
	Bio13	Precipitation of Wettest Month	mm	0.2	Eliminated
	Bio14	Precipitation of Driest Month	mm	3.5	Eliminated
	Bio15	Precipitation Seasonality (Coefficient of Variation)	/	5.0	Selected
	Bio16	Precipitation of Wettest Quarter	mm	2.4	Eliminated
	Bio17	Precipitation of Driest Quarter	mm	13.9	Selected
	Bio18	Precipitation of Warmest Quarter	mm	1.2	Eliminated
	Bio19	Precipitation of Coldest Quarter	mm	5.5	Eliminated
DEM	El	Elevation	m	/	Selected
Soil	PH	Potential of hydrogen	/	/	Selected
	T-C	Topsoil organic carbon	%	/	Selected
	T-sand	Topsoil sand fraction		/	Selected
	USDA	Topsoil USDA texture classification	name	/	Selected
	Depth	Reference soil depth	m	/	Selected
Radiation	UV-B3	Mean UV-B of Highest Month	kJ/m^2	/	Selected
Human activities	Hf	Human footprint index	/	/	Selected

Key Environmental Variables

In order to screen the key environmental variables that affect the distribution of *C. medica* L. var. *Sarcodactylis*, we comprehensively compared the regularized training gains, percent contribution rates and permutation importance of each variable. Fig. 3 showed that the regularized training gain of temperature annual range (Bio7) was the highest (1.47), and its percent contribution rate (18.25 %) and permutation importance (39.68 %) were at a higher level, indicating that it was one of the most important variables affecting the distribution of *C. medica* L. var. *Sarcodactylis* (Fig. 3). The training gain and percent contribution of annual precipitation (Bio12) was1.23 and 44.47 %, indicating the importance of this variable. During the modeling of "without variables", the human footprint (Hf) had the largest decrease in the training gain value, indicating that it contains unique information related to the distribution of *C. medica* L. var. *sarcodactylis* and has a great impact on its distribution (Fig. 3). Through the above comprehensive comparison method, temperature annual range (Bio7), annual precipitation (Bio12), human footprint (Hf), elevation (El) and precipitation seasonality (Bio15) were identified as the dominant environmental variables related to the distribution of *C. medica* L. var. *sarcodactylis* (Fig. 3).

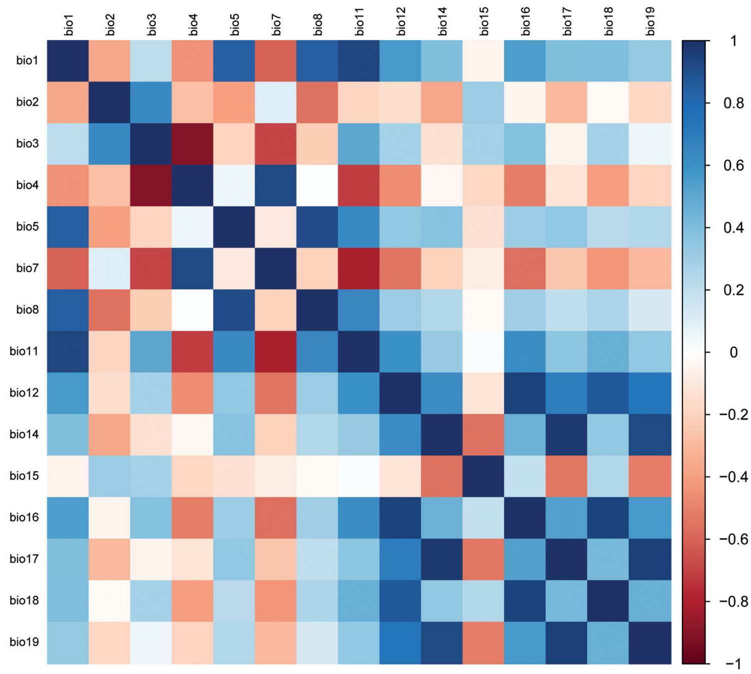

Fig. 2. Pearson correlation matrix of environmental variables.

Since the 20th century, with the rapid growth of population and economy, human beings have had a great impact on their own living environment [29, 30]. Climate change and human activities are considered to be the leading factors affecting the structure and function of terrestrial ecosystems [31, 32]. Our analysis showed that human footprint was the key variables affecting the distribution of *C. medica* L. var. *sarcodactylis*, and the appropriate range was ≥6.99. *C. medica* L. var. *sarcodactylis* is mostly cultivated in China, and the impact of agricultural activities on its distribution cannot be ignored. Cutting, grafting and high-pressure propagation are the main propagation methods of *C. medica* L. var. *sarcodactylis* [3]. In recent years, with the continuous optimization of agricultural industrialization, the continuous adjustment of agricultural planting structure, and the influence of market regulation, the planting area of *C. medica* L. var.

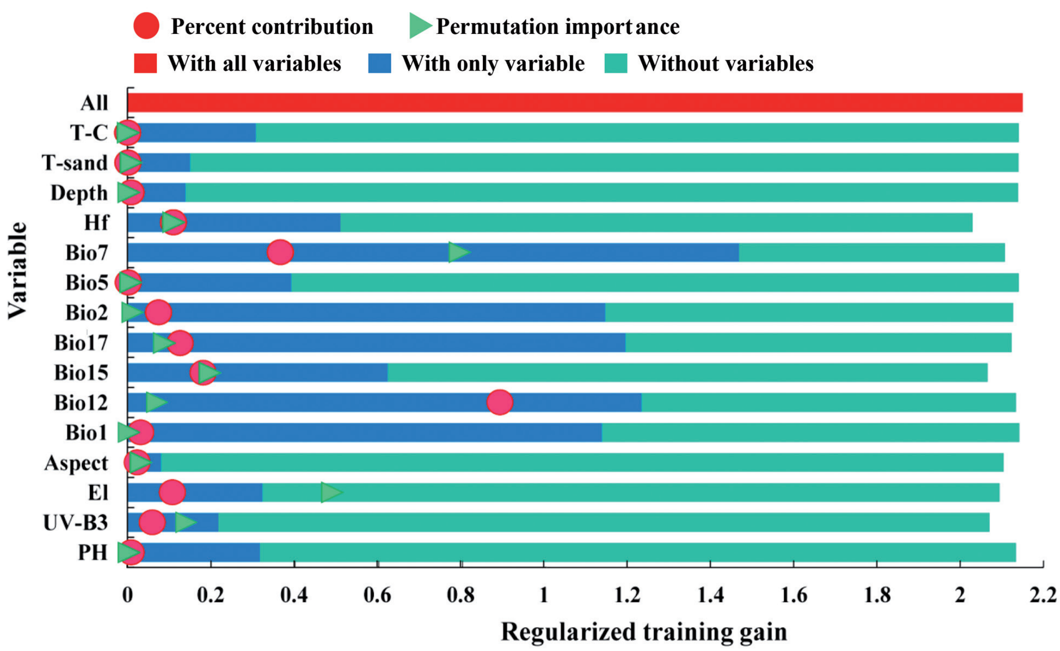

Fig. 3. Regularized training gain, percent contribution and permutation importance of environmental variables.

sarcodactylis has expanded [33, 34]. However, Yue et al. [35] investigated the resources of *C. medica* L. var. *sarcodactylis* in Guangdong, and the results showed that due to the influence of production technology, market conditions, economic benefits and other factors, the planting area of *C. medica* L. var. *sarcodactylis* in Guangdong was shrinking year by year, and even sporadic cultivation was found in only 13 of the 40 sample plots visited. The above results all prove the importance of human activities to the cultivation and distribution of *C. medica* L. var. *sarcodactylis*.

Relationship between Key Environmental Variables and Presence Probability of *C. medica* L. var. *sarcodactylis*

Fig. 4 showed that there was a strong response relationship between the distribution of *C. medica* L. var. *sarcodactylis* and the key environmental variables. Specifically, the presence probability of *C. medica* L. var. *sarcodactylis* reached the peak when the annual precipitation (Bio12) was 1311.72 mm, and then gradually decreased with the increase of precipitation (Fig. 4a). With the increase of the temperature annual range (Bio7), the presence probability of *C. medica* L. var. *sarcodactylis* showed a significant downward trend, which indicated that this variable has a negative impact on it's presence (Fig. 4b). With the increase of human footprint, the presence probability of *C. medica* L. var. *sarcodactylis* showed an upward trend, and they were basically positively correlated (Fig. 4c). When the elevation (El)<347.12 m, it had a positive impact on the presence probability of *C. medica* L. var. *sarcodactylis*, while when the elevation>347.12 m, it had a negative impact on its presence probability (Fig. 4d).

According to literature, there are more than 20 species of citrus plants of Rutaceae in the world, which are mainly planted in the southeast and south of Asia [36]. In China, there are about 14 species of citrus plants, which are cultivated in the south of the Yangtze River basin. *C. medica* L. var. *sarcodactylis* is mainly distributed in the tropical and subtropical regions of Guangdong, Guangxi, Zhejiang, Sichuan, Fujian, etc. and likes a warm, humid and sunny environment [1, 2, 4]. By analyzing response curves, the relationship between the key variables and the presence probability of *C. medica* L. var. *Sarcodactylis* was obtained. Results showed that the suitable ranges of temperature annual range (Bio7) and annual precipitation (Bio12) were <28.07ºC and >964.67 mm respectively, which were similar to its growth habits. Importance analysis showed that temperature annual range (Bio7) was a domain temperature variable affecting the geographical distribution pattern of *C. medica* L. var. *sarcodactylis*. When temperature annual range (Bio7) was higher than 28.07ºC, the presence probability of *C. medica* L. var. *sarcodactylis* decreased rapidly, indicating that too large temperature difference was not conducive for its survival. Comparing our simulation with the Plant Hardiness Zone Map (PHZM) drew by Widlechne [37], *C. medica* L. var. *sarcodactylis*t was mainly distributed in the range of 9-11 grades (- 6.6~4.5ºC). rom the perspective of adaptability to temperature variables, this medical plant was suitable for planting in most areas south of 31°N in China.

Temperature and precipitation are important factors that restrict the distribution of plant species in latitude and longitude respectively, and they jointly restrict the geographical distribution pattern of plants [38-40]. Response curve of the annual precipitation (Bio12)

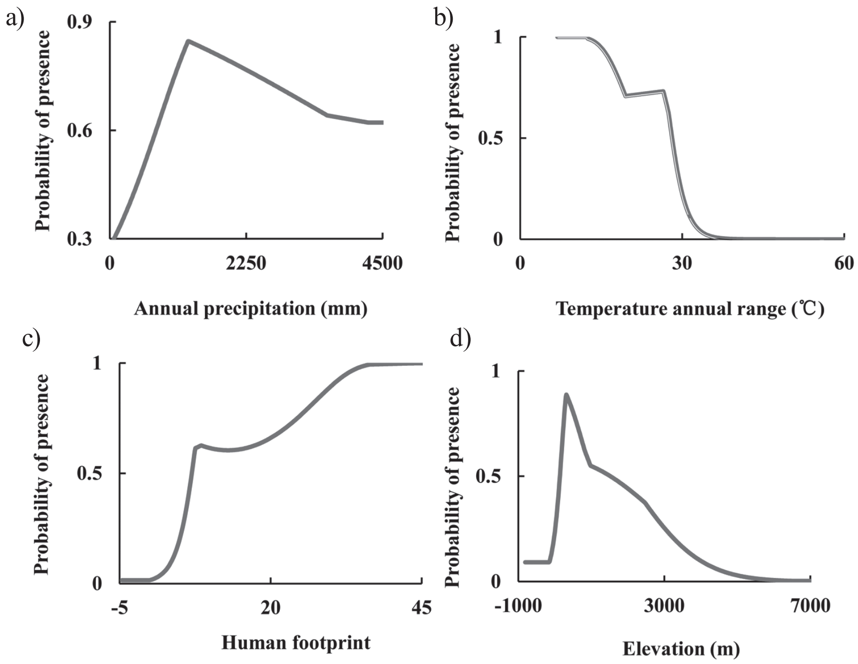

Fig. 4. Response curves of dominant variables.

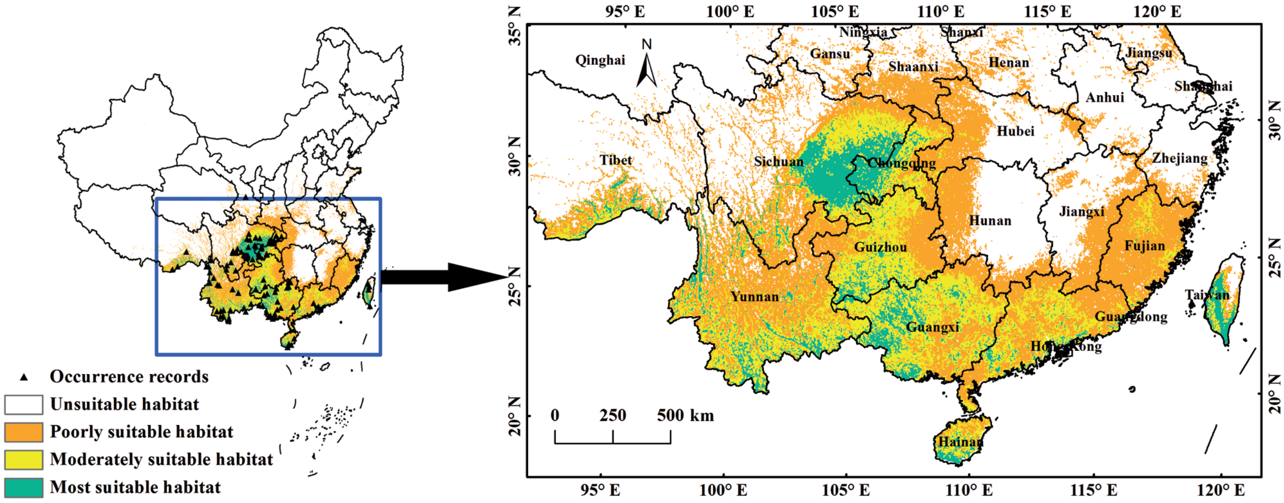

Fig. 5. Suitable habitat of *C. medica* L. var. *sarcodactylis* simulated by MaxEnt.

showed that the value suitable for the presence of *C. medica* L. var. *sarcodactylis* needs to be higher than 964.67 mm. In the process of photosynthesis, each part of dry matter formed by citrus plants needs 300-500 parts of water, and the annual precipitation suitable for planting citrus plants is 1000-1500mm. Therefore, in order to achieve high and stable yield of citrus, irrigation measures should be taken to supplement water equivalent to 800~900 mm rainfall in areas with annual rainfall of 200~600 mm [41, 42]. Under the background of global climate change, the annual average temperature in China has increased by 0.4~0.5ºC in the past 100 years, and the precipitation has shown obvious regional characteristics [43]. In south China, it shows a warm and humid trend, and the whole South shows a tendency of being drier in the northwest and wetter in the southeast [43]. From above-mentioned, the division of *C. medica* L. var. *sarcodactylis* planting areas should also determine whether the local precipitation and irrigation conditions can meet these requirements in addition to temperature factors.

Simulation of Suitable Habitat

The result simulated by MaxEnt and calculated by ArcGIS showed that the distribution range of the most suitable habitat was the narrowest, mainly located in the middle east of Sichuan, western Chongqing, southern Guizhou, western Guangxi, central and southern Yunnan and southeast Tibet, with an area of 22.27×10^4 km^2 (Fig. 5). The moderately suitable habitat extended to the periphery along the most suitable habitat, with an area of 51.96×10^4 km^2, mainly distributed in northeast Sichuan, central Chongqing, northern Guizhou, most of Guangxi, central Guangdong, southern Yunnan and most of Hainan (Fig. 5). The poorly suitable habitat of *C. medica* L. var. *sarcodactylis* were mainly distributed in the northeast and south of Sichuan, the north of Chongqing, the south of Shaanxi, the west of Hubei, the west of Hunan, the northeast and northwest of Guizhou, the east of Jiangxi, most of Fujian, most of Guangxi, Guangdong, southeast of Tibet and north central Yunnan,accounting for 58.15% of the total suitable habitat (Fig. 5).

C. medica L. var. *sarcodactylis* is mainly cultivated in China with few wild species. With the cultivation and introduction, *C. medica* L. var. *sarcodactylis* has been widely planted in Sihui, Chaoshan, Yunfu and Yunan of Guangdong, Tianlin, Longlin, Lingle, Guanyang, Daxin and Yongfu of Guangxi, Kunming, Yuxi, Chuxiong, Xinping, Yimen, Eshan and Pu'er of Yunnan, Jiangjin, Yongchuan, Yunyang and Kai counties of Chongqing, Anxian, Hejiang, Yibin, Muchuan, Ya'an, Hongya, Jiajiang, Qianwei and Xingjing of Sichuan [5]. The overlap between our simulation results and these areas is very high, indicating the accuracy of MaxEnt. In this study, MaxEnt simulated the presence probability of *C. medica* L. var. *sarcodactylis* under the combination of dominant natural environmental variables, but it could not involve production management and the impact on fruit yield and quality. In the actual production, not only the dominant natural resources, but also the social and economic factors such as labor, field management, production cost and market demand should be considered.

Conclusions

As a commonly used medicinal material in China, the introduction and cultivation of *C. medica* L. var. *sarcodactylis* in various suitable habitats will alleviate the imbalance between the shrinking supply of wild resources and the increase of market demand. In addition to the traditional production areas, the site selection range can be reasonably planned according to the dominant environmental factors affecting the distribution of *C. medica* L. var. *sarcodactylis* and the change trend of future suitable areas, so as to improve the quality and output and promote industrial development.

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Conflict of Interest

The authors declare no competing interests.

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