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The photosynthetic stress responses of five pepper species are consistent with their genetic variability

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Abstract

The aim of the study was to investigate the genetic distances and their relationships among pepper species using photosynthetic features under different stresses and genetic variability. The photosynthetic features under drought, waterlogging and low-temperature stresses, rDNA internal transcribed spacer (ITS) sequences of nuclear genome and *trn*H-*psb*A sequence of chloroplast genome of 25 varieties from 5 pepper species *Capsicum annuum* L. (CA), *Capsicum baccatum* L. (CB), *Capsicum chinense* Jacquin. (CC), *Capsicum frutescens* L. (CF) and *Capsicum pubescens* Ruiz & Pavon (CP) were analyzed and used to construct the dendrograms. The results showed the photosynthetic rate of different pepper species could be greatly but differentially decreased by stresses. For example, CB and CF had the smallest and the highest decrease to drought, CC had the highest decrease to waterlogging, and CP had the smallest decrease to low temperature. The ITS sequences of 25 pepper varieties are 591-619 bp in length and have GC% between 51.1% and 64.5%. Their *trn*H-*psb*A sequences are 537-558 bp in length and have GC% between 27.2% and 28.5%. The cluster analysis of the five pepper species based on the changes in P_N under stresses is similar to that based on genetic variability, that is, CP clusters with CB, and CC clusters with CA after first clusters with CF. In addition, the clustering methods based on the photosynthetic stress responses and genetic variability are unable to completely distinguish pepper varieties within the same species. The results indicate that similarly to genetic variability, changes in P_N under stresses (specifically the stress corresponding to the climate of plant's original habitat) could be used to identify genetic distance of pepper species.

Additional key words: gene sequence, pepper species, photosynthetic rate, stresses.

Introduction

Pepper is an important vegetable and condiment. It is widely planted in the world. International Plant Genetic Resources Board (IBPGR) has classified pepper species into five species: *C. annuum* L., *C. baccatum* L., *C. chinense* Jacquin, *C. frutescens* L., and *C. pubescens* Ruiz & Pavon. *C. pubescens* Ruiz & Pavon and *C. baccatum* L. are quite distinct morphologically, while the near continuous overlap in morphological characteristics among *C. annuum* L., *C. chinense* Jacquin, and *C. frutescens* L. led various authors to recognize them as a species complex (Pickersgill 1971, McLeod *et al*. 1982). *C. annuum* L. is the most differentiated, widely cultivated worldwide (Pickersgill 1997); *C. chinense* Jacquin and *C. frutescens* L. mainly cultivated in USA; *C. pubescens* Ruiz & Pavon is a plateau-type species with strong cold resistance and mainly distributed in the Andes. The chromosome pairing of interspecific pepper hybrids during meiosis showed there is a very small number of univalents during meiosis of the interspecific hybrids of *C. chinense* Jacquin and *C. frutescens* L., indicating that their chromosomes are highly homologous. *C. frutescens* L. is closest to *C. chinense* Jacquin, followed next by *C. annuum* L. and then by *C. baccatum* L. (Egawa 1985, Egawa and Tanaka 1986). The pollen fertile rate of hybrid of *C. frutescens* L. and *C. chinense* Jacquin is only 28% and that of intraspecific hybrid is 75%, suggesting that *C. frutescens* L. and *C. chinense* Jacquin are distant relatives (Bahadur 2003). Genomic DNA polymorphism

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Abbreviations: CA – *Capsicum annuum* L.; CB – *Capsicum baccatum* L.; CC – *Capsicum chinense* Jacquin.; CF – *Capsicum frutescens* L.; CP – *Capsicum pubescens* Ruiz &Pavon; *E* –transpiration rate ; g_s – stomatal conductance; ITS – rDNA internal transcribed spacer; P_N – net photosynthetic rate; WUE – water-use efficiency.
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analysis of 13 species of *C. baccatum* L. and one species of *C. annuum* L. using RFLP technology found that interspecific genetic variation of *C. annuum* L. and *C. baccatum* L. is great (Lefebvre *et al*. 1993). Southern hybridization bands of total 21 varieties of the five cultivated pepper species by RFLP technology found that these 21 varieties have 4 distinct clusters, three of which are closely connected clusters presented in *C. annuum* L., *C. frutescens* L. and *C. baccatum* L., and the other one only presented in *C. chinense* Jacquin and *Capsicum chacoens*, indicating a closer relationship among the former three species and between the latter two species (Prince *et al*. 1995). Although *C. annuum* L. is the widely cultivated and most focused species in pepper breeding worldwide (Pickersgill 1997), its genetic basis is relatively narrow (Prince *et al*. 1995) and barely contains outstanding characteristics of other cultivated speices, which significantly constrains the progress of pepper breeding. The heterosis in pepper is very obvious because the yield of elite hybrids normally is 50% higher than that of traditional varieties. Therefore, study on the genetic relationships of different pepper species is important for finding new parental resources, extending gene pool and effective utilizing pepper germplasm, and an effective way to resolve the bottleneck of pepper breeding.

Photosynthesis is the basic physiological process and a source of biological energy for plants. It is affected not only by internal factors, but also by external factors such as temperature, moisture, and carbon dioxide. Abnormal climate changes such as drought, waterlogging, and cold can affect photosynthesis and are expected to lower transpiration along with reduced leaf area. Optimization of N economy under limited water supply may be accomplished by increased leaf N content per area (Weih *et al*. 2011, Farquhar *et al*. 2002). Some genes in plant have specific roles under abiotic stress conditions (Huang *et al*. 2011, Sun *et al*. 2009, Frankel *et al*. 2003). Although the effects of stresses on photosynthesis have been extensively studied (Ou *et al*. 2011, Fu *et al*. 2009), few are on their application in their classification. In this paper, we studied the photosynthetic characteristics of five pepper species under different stresses, applied these characteristics to identify the genetic distance in combination with genetic sequence analysis, and further investigated the linkage between photosynthetic characteristics and genetic diversity, hoping to provide a theoretical rationale for expanding the application of studies on photosynthesis.

Materials and methods

Plants: Five pepper species, *C. annuum* L. (CA), *C. baccatum* L. (CB), *C. chinense* Jacquin. (CC), *C. frutescens* L. (CF) and *C. pubescens* Ruiz & Pavon (CP), were provided by the United States Department of Agriculture and the Vegetable Institution of Hunan Academy of Agricultural Science. Each species has 5 varieties from different provinces (Table 1). Each pepper variety was planted in 3 plots with size of 2 m × 5 m with 50 plants per plot and grown at normal fertilization and watering condition. They were planted for three consecutive times. DNA was extracted from leaves according to the method of Rogers and Bendic (1998).

Experimental design: During July to August of 2010, three healthy seedlings with similar growth conditions were selected from each of variety and transplanted into a tub with diameter of 1 m. The tubs were placed outdoor and plants were grown under normal fertilization and watering conditions at 35 ± 0.5°C and irradiance of 750 ± 610 μmol m^{-2} s^{-1} during the day. During flowering period, peppers were assigned into three groups with 15 plants of each species in each group. One group was treated with drought by stopping water supply, one group was over-irrigated to submerge the aerial parts of the seedlings, the other group was placed in an artificial climate box with temperature of 15°C, light intensity of 800~1,000 μmol m^{-2} s^{-1} for 12 h a day and relative humidity of 60%. The treatments were conducted in 5 replicates for several consecutive days till one species shows severe wilt symptom.

Table 1. Source and accession of pepper species.

Accession	Country	Category
PI 171565	Turkey	*Capsicum annuum* L.
PI 645487	Panama	
PI 451762	Israel	
PI 193472	Ethiopia	
PI 640503	Australia	
PI 267729	Guatemala	*Capsicum baccatum* L.
PI 215699	Peru	
PI 215700	Peru	
PI 281310	Brazil	
PI 260595	Brazil	
PI 645555	Niger	*Capsicum chinense* Jacquin.
PI 209028	Puerto Rico	
PI 406987	Panama	
PI 281435	United States	*Capsicum chinense* Jacquin.
PI 215736	Peru	
PI 355395	Ecuador	*Capsicum frutescens L.*
PI 640907	Nigeria	
PI 263109	Former Soviet Union	
Grif 9286	Costa Rica	
PI 159261	United States, Georgia	
PI 593620	Guatemala	*Capsicum pubescens* Ruiz & Pavon
PI 593639	Guatemala	
Co 0709	Unknown	
Co 4895	Unknown	
Co 2415	Unknown	

Gas exchange was determined during 10:00–11:00 h (Beijing time) in mid-August (heading stage) using a portable photosynthesis system (*LI-6400, LI-COR, Inc*., Lincoln, NE, USA) with a 3 cm × 2 cm (length × width) leaf chamber with air humidity of 47.50 ± 1.20%. The net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) were measured at the irradiance of 1,000 μmol m^{-2} s^{-1}, temperature of 35 ± 0.5°C, and native CO_2 concentration of approximately 358.70 ± 5.43 μmol(CO_2) mol^{-1}. Water-use efficiency (WUE) was calculated according to WUE = P_N/E. Each measurement was performed 5 times a day for 3 d.

ITS and *trn*H-*psb*A sequences analysis ITS primers were: F: 5'-TCCTCCGCTT ATTGA TAT GC -3' and R: 5'-GGAAGGTAAAAGTC AAGG-3' as reported by White *et al*. (1990). *trn*H-*psb*A primers were F: 5'- GTTATGCATGAACGTA ATGCTC-3' and R: 5'- CGCGCATGGTGGATTCACAATCC -3' as reported by Peterson *et al*. (2004). The reactions were performed in a 50 mm^3 system containing 5.0 mm^3 10 × PCR buffer, 1 mm^3 50 mM forward and reverse primers, 1 mm^3 10 mM dNTPs, 1.5 U Taq, and 40 ng template under the following conditions: predenaturation at 94°C for 4 min followed by 32 cycles of 94°C for 45 s, 55°C or 50°C for 45 s, and 72°C for 45 s and 72°C for 10 min. The PCR products were fractionated on 1% agarose gel, and imaged with the *GelLogic 100* image system (*Bio-Rad*, USA). The target fragments were isolated from the agarose gel under UV radiation, reclaimed and purified with a reagent kit (*Tiangel* mini purification kit, *TianGen Biotech Co*., LED, China), and directly evaluated by bi-directional sequencing. Sequences were analyzed and clustered by *DNAMAN* (*LynnonBiosoft*, USA) and *MEGA 4.1* software (*Center for Evolutionary Medicine and Informatics*, USA) using unweighted pair group method with arithmetic mean (UPGMA).

Statistical analysis: Parametric data were analyzed using analysis of variance (*ANOVA*) in *Excel* (2003). *P* values less than 0.05 and 0.01 were considered as statistically significant and extremely significant, respectively.

Results

The effects of stresses on photosynthetic characteristics of five pepper species: The changes in P_N, E, g_s, and WUE under the same stress conditions did not differ significantly among the five varieties of each species, but differed significantly among different species.

After five days of drought treatment, P_N of species CA, CC, and CF decreased by 90%, whereas those of species CB and CP decreased by 80%. The decreases in P_N were significantly different between the former three and the latter two. P_N also decreased in response to waterlogging stress: among the five species, CC had the greatest decrease of 98.95%, CP had the smallest of 86.37%, and the other three decreased by 90%. P_N decreased relatively smaller in response to low-temperature stress: that in CP was relatively smaller than those in the other four species (Table 2).

Although the E of each species decreased significantly in response to drought and waterlogging stresses, the decreases in E among pepper species were not significant (Table 3). The decreases of E to low-temperature stress can be divided into 2 levels: CP had relatively smaller decrease than the others.

g_s of the five species decreased significantly under drought stress. Among them, g_s of CC and CP dropped about 64%, and g_s of species CA, CF and CB dropped about 60%. g_s changed significantly in response to waterlogging: CC had the greatest decrease of 96.67% and CP had the smallest decrease of 79.41% and these changes in g_s differed significantly among the five pepper species. The decreases in g_s to low-temperature stress were divided 2 levels: CP decreased by 26.47%, while the others decreased by about 60% (Table 4).

WUE of all the five species increased in response to drought stress: those of CB and CP increased by up to 69%, while those of CA, CB, and CF only increased by 30%. Under waterlogging stress, WUE of CC significantly decreased by 79.25%; WUE of CA and CF slightly increased; and WUE of CB and CP significantly increased. In addition, at low-temperature stress, WUE of all the five species except CP only changed slightly (Table 5).

Table 2. Percentage of descendant of net photosynthetic rate (P_N) in different pepper species. *Capital* and *lowercase letters* mean significance at 0.01 and 0.05 levels, respectively. Means of 15 replications ± SE.

Species	CK	Drought stress		Waterlogging stress		Low-temperature stress	
	P_N [μmol m^{-2} s^{-1}]	P_N [μmol m^{-2} s^{-1}]	–%	P_N [μmol m^{-2} s^{-1}]	– %	P_N [μmol m^{-2} s^{-1}]	– %
CA	17.73 ± 0.86^{Bb}	2.11 ± 0.37^{Db}	88.09^{Aa}	1.09 ± 0.07^{Cb}	93.85^{Bb}	2.31 ± 0.21^{Cb}	86.97^{Aa}
CB	16.49 ± 1.28^{Bb}	3.10 ± 0.40^{Aa}	81.20^{Cb}	1.64 ± 0.04^{Bb}	90.05^{Cc}	2.53 ± 0.49^{Cb}	84.65^{Ab}
CC	17.17 ± 1.32^{Bb}	2.31 ± 0.95^{Cb}	86.54^{Ba}	0.18 ± 0.01^{Dc}	98.95^{Aa}	2.37 ± 0.27^{Cb}	86.19^{Aa}
CF	21.77 ± 0.82^{Aa}	2.42 ± 0.39^{Cb}	88.88^{Aa}	1.41 ± 0.11^{Bb}	93.52^{Bb}	2.91 ± 0.14^{Bb}	86.63^{Aa}
CP	15.78 ± 1.89^{Cb}	2.88 ± 0.03^{Ba}	81.74^{Cb}	2.15 ± 0.23^{Aa}	86.37^{Dd}	4.71 ± 0.15^{Aa}	70.15^{Bc}

Table 3. Percentage of descendant of transpiration rate (E) in different pepper species. *Capital* and *lowercase letters* mean significance at 0.01 and 0.05 levels, respectively. Means of 15 replications ± SE.

Species	CK	Drought stress		Waterlogging stress		Low-temperature stress	
	E [mmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	–%	E [mmol m^{-2} s^{-1}]	–%	E [mmol m^{-2} s^{-1}]	–%
CA	3.26 ± 0.45^{Cb}	0.28 ± 0.09^{Cc}	91.41^{Aa}	0.18 ± 0.09^{Cb}	94.47^{Aa}	0.45 ± 0.14^{Cb}	86.19^{Aa}
CB	3.79 ± 0.26^{Bb}	0.42 ± 0.11^{bA}	88.91^{Ba}	0.30 ± 0.11^{Ba}	92.08^{Bb}	0.57 ± 0.17^{Bb}	84.96^{Aa}
CC	3.36 ± 0.49^{Cb}	0.37 ± 0.08^{Bb}	88.98^{Ba}	0.17 ± 0.08^{Cb}	94.94^{Aa}	0.50 ± 0.14^{Bb}	85.11^{Aa}
CF	3.21 ± 0.47^{Cb}	0.29 ± 0.08^{Cc}	90.96^{Aa}	0.19 ± 0.08^{Cb}	94.08^{Aa}	0.46 ± 0.11^{Cb}	85.66^{Aa}
CP	4.05 ± 0.58^{Aa}	0.44 ± 0.10^{Aa}	89.13^{Aa}	0.39 ± 0.10^{Aa}	90.37^{Bc}	0.86 ± 0.12^{Aa}	78.76^{Bb}

Table 4. Percentage of descendant of stomatal conductance (g_s) in different pepper species. *Capital* and *lowercase letters* mean significance at 0.01 and 0.05 levels, respectively. Means of 15 replications ± SE.

Species	CK	Drought stress		Waterlogging stress		Low-temperature stress	
	g_s [mol(H_2O) m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	–%	g_s [mol(H_2O) m^{-2} s^{-1}]	–%	g_s [mol(H_2O) m^{-2} s^{-1}]	–%
CA	0.47 ± 0.08^{Bb}	0.18 ± 0.03^{Bb}	61.70^{Bc}	0.06 ± 0.01^{Ba}	87.23^{Cb}	0.17 ± 0.02^{Cb}	63.82^{Aa}
CB	0.43 ± 0.08^{Bb}	0.17 ± 0.02^{Bb}	60.46^{Bc}	0.04 ± 0.01^{Cb}	90.69^{Bb}	0.21 ± 0.04^{Bb}	51.16^{Ba}
CC	0.43 ± 0.08^{Bb}	0.15 ± 0.03^{Cb}	65.11^{Aa}	0.01 ± 0.00^{Dc}	97.67^{Aa}	0.18 ± 0.03^{Cb}	58.13^{Ba}
CF	0.55 ± 0.02^{Aa}	0.21 ± 0.05^{Aa}	61.81^{Bc}	0.08 ± 0.01^{Aa}	85.45^{Cb}	0.19 ± 0.03^{Bb}	65.45^{Aa}
CP	0.34 ± 0.02^{Cb}	0.12 ± 0.03^{Cb}	64.70^{Ab}	0.07 ± 0.02^{Aa}	79.41^{Dc}	0.25 ± 0.07^{Aa}	26.47^{Cb}

Table 5. Percentage of descendant of water-use efficiency (WUE) [μmol(CO_2) mmol(H_2O)$^{-1}$] in different pepper species. *Capital* and *lowercase letters* mean significance at 0.01 and 0.05 levels, respectively. Means of 15 replications ± SE.

Species	CK	Drought stress		Waterlogging stress		Low-temperature stress	
	WUE	WUE	± %	WUE	± %	WUE	± %
CA	5.44 ± 0.75^{Bb}	7.54 ± 0.14^{Bb}	38.60^{Bb}	6.06 ± 1.14^{Bb}	11.39^{Cb}	5.13 ± 0.14^{Bb}	-5.69^{Cc}
CB	4.35 ± 0.68^{Cb}	7.38 ± 0.36^{Bb}	69.65^{Aa}	5.47 ± 1.36^{Cb}	25.74^{Bb}	4.44 ± 0.36^{Cb}	2.06^{Bb}
CC	5.11 ± 0.71^{Bb}	6.24 ± 0.33^{Dc}	22.11^{Cb}	1.06 ± 0.33^{Dc}	-79.25^{Dc}	4.74 ± 0.33^{Cb}	-7.24^{Cc}
CF	6.78 ± 1.21^{Aa}	8.34 ± 0.18^{Aa}	23.00^{Cb}	7.42 ± 1.18^{Aa}	9.43^{Cb}	6.33 ± 0.18^{Aa}	-6.63^{Cc}
CP	3.89 ± 0.55^{Dc}	6.55 ± 0.13^{Cc}	68.38^{Aa}	5.51 ± 1.13^{Cb}	41.64^{Aa}	5.48 ± 0.13^{Ba}	40.87^{Aa}

Based on the photosynthetic characteristics of the five species under drought stress, we constructed their UPGMA tree using *MEGA 4.1* software and found that the cluster trees of the five species based on P_N, E, g_s, and WUE can be divided into two branches with some differences. For example, based on decline extents of P_N, CB and CP were classified as one branch, while CC first clustered with CF and then with CA (Fig. 1*A*). Based on the decline of E, CA and CF were in one cluster, while CC first clustered with CB, then with CP (Fig. 1*B*). Based on the decline in g_s, CC and CP were in one cluster, while CA first with CB and then with CF (Fig. 1*C*). Based on the increase in WUE, CB and CP were in one cluster, while CC first clustered with CF and then with CA (Fig. 1*D*). By comparison, the UPGMA trees established based on the changes in P_N and WUE were similar, while those first clustered with CC and CF and then with CA were different in cluster distances.

The dendrogram under P_N to waterlogging stress showed that CC was located in the outermost (Fig. 1*E*). The dendrogram under P_N to low-temperature stress showed that the five species also could be divided into two large branches, cluster CA and CF and cluster CC merged into one branch and CB and CP constituted the other branch (Fig. 1*F*).

ITS and *trn*H-*psb*A sequences analysis: The length of ITS sequences of the 25 pepper varieties were 591–619 bp with variation up to 28 bp. GC contents were between 51.1% and 64.5%. The lengths of ITS1 and ITS2 were around 230 bp and 222 bp, respectively. ITS2 has higher GC content and larger varations in length. Statistic analysis of the 25 varieties of the five species found that species CA had the largest variations in both ITS length and GC content, while CP had the smallest. UPMGA tree established based on ITS sequences found the CC normally fisrt cluster with CF, then with CA, while CB and CP were in another cluster; the varieties of each species mostly in one cluster with some exceptions. For example, PI209028 of species CC first clustered with PI 263109 of species CF and then with other varieties of CF, and PI 406987 of species CC first clustered with PI 645487 of CA and then with other CA (Fig. 2*A*).

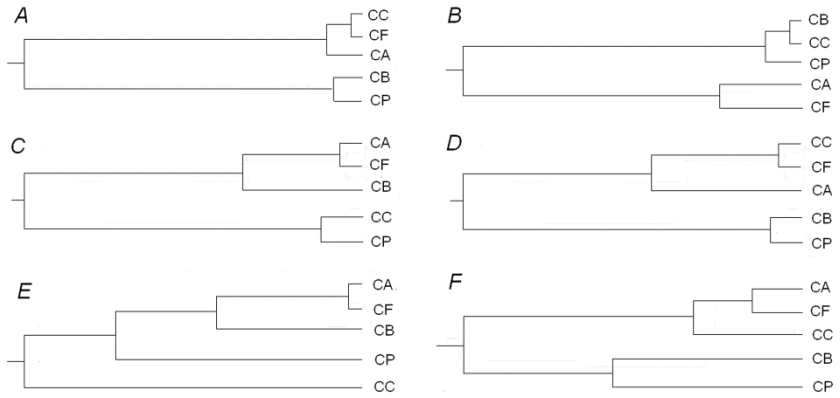

Fig. 1. UPGMA dendrograms based the decreases or ascension of photosynthetic rate (P_N) (*A*), transpiration rate (*E*) (*B*), stomatal conductance (g_s) (*C*), and water-use efficiency (WUE) (*D*) to drought, UPGMA dendrograms based the decreases of P_N to waterlogging (*E*) and to low temperature (*F*). Means of 15 replications ± SE.

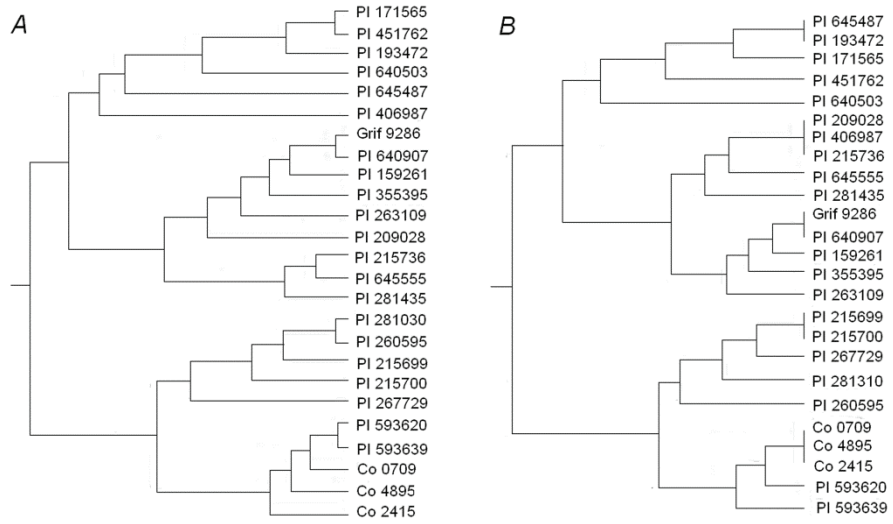

Fig. 2. UPGMA dendrograms based ITS sequences (*A*) and *trn*H-*psb*A sequences (*B*).

The lengths of *trn*H-*psb*A sequences of the 25 pepper varieties were in the range of 537–558 bp with variation of 21 bp and their GC contents were relatively low in the range of 27.2% – 28.5% with high similarity of 89.59%. The lengths of *trn*H-*psb*A sequences of the five varieties of species CA varied from 537 to 558 bp, which is greater than others such as 2 bp among different varieties of species CF and CP. UPGMA tree based on *trn*H-*psb*A sequences showed that the five species were divided into two branches: CB and CP as one branch and CC, CA and CF as the other branch, where CC first clustered with CF and then with CA. Some varieties within the same species could not be distinguished by their *trn*H-psbA sequences, such as PI 645487 and PI 193472 of species CA, PI 215699 and PI 215700 of species CB, PI 209028, PI 406987 and PI 215736 of species CC, PI 355395, PI 640907 and Grif 9286 of species CF, and PI 593620 and PI 593639 of species CP (Fig. 2*B*).

Discussion

The evolution rate of ribosomal DNA ITS sequence was rapid and can provide a wealth of variable sites. Therefore, it is not only widely used for species identification, but also for genetic distance analysis within species (Christopher *et al*. 2009, Vijaykumar *et al*. 2010, Yan *et al*. 2010). Cytoplasmic DNA as maternal inheritable material is relative stable compared to nuclear DNA and has lower variation frequency. Therefore, it is more suitable as a molecular marker for the species origination and evolution. In plant chloroplast genes, some regions, particularly the noncoding regions, have relatively high nucleotide substitution rate, thus those regions can be used not only for the reconstruction of phylogenetic relationships between species, but also for the intra-

species genetic diversity analysis. Since the noncoding region of *trn*H-psbA gene has small external selection pressure and high evolutionary rate, it has been widely used in intraspecies phylogenetic analysis and phylogeography studies (John *et al*. 2009, Font *et al*. 2009). Pepper has strong ecological adaptation ability and distributes widely in the world with high degree of geographical heterogeneity and wide geographical terrain. Different ecoclimatic conditions are favorable for the formation of peppers with different genetic backgrounds. Moreover, geographic isolation has hindered gene flow between pepper species, resulting in higher level of differentiation. Therefore, classification of pepper solely based on morphology is not reliable. Jarret (2008) analyzed genetic diversity of pepper species CA, CC, and CF using multiple nuclear DNA fragments and chloroplast fragments and found that *trn*H-*psb*A and *trn*L-*trn*T fragment can effectively distinguish the pepper species, but not varieties of the same species. Similarly to their results, our study also found that *trn*H-*psb*A sequence could be used to distinguish the five pepper species. Meanwhile, the variation among ITS sequences was greater than that of *trn*H-*psb*A sequence, therefore is better than *trn*H-*psb*A sequence to distinct pepper species. However, it is not as accurate as *trn*H-*psb*A sequence. In summary, clustering based on ITS and *trn*H-*psb*A sequences indicate that these two sequences can be used to distinguish species, but not varieties within species. Combining these two sequences can effectively distinguish different varieties of pepper species.

According to the principles of plant physiology and ecology, plant ecologic types may not necessarily appear in its morphology. They are more reflected in their differences in physiological and biochemical characteristics (Liu and Mao 2008). When the plant growth conditions were unable to meet those requirements, some plant characteristics would alter significantly. For example, water deficit could cause partial stomatal closure and increase resistance to CO_2 flow, resulting in reduced substrate of photosynthesis and photosynthetic rate. This paper showed that P_N of pepper species differed only slightly under natural conditions, but significantly under stress conditions. Moreover, the size of impact of different stress to different species was different. P_N decreases of CB and CP were relatively small to drought stress, CC had the highest decrease to waterlogging and CP had the smallest one to low temperature. These results showed that different species had different resistance to different stresses, CB and CP had stronger resistance to drought, CC had the weakest resistance to waterlogging and CP had the strongest resistance to low temperature. WUE is determined by plant P_N and E, namely consumption per unit mass of water and the amount of CO_2 fixed by plants. The value of WUE can reflect adaptation ability of plant to stress (Fischer and Turner 1978). The WUE increases of CB and CP were higher than the others and it indicated that the two species had stronger resistance to stress. Plants partially close stomata and increase the resistance to CO_2 flow, which leads to a reduced substrate income and to a decrease in P_N. This happens when such growth conditions such as chilling, high temperature and mild water deficit (Fischer and Turner 1978) conflict with the normal requirements. Our results showed in all cases that g_s decreased under stress and especially under waterlogging. Taking together, our study shows that pepper has stronger resistance to drought and weak resistance to waterlogging which is consistent with the fact that pepper originated in the tropical regions of Latin America. Moreover, clustering based on the changes in P_N of the five species under drought stress is consistent with that based on genetic analysis, but different from that based on E and g_s, which may be related to the fact that P_N is affected by multiple factors. And clustering based on the changes in P_N under waterlogging and low-temperature stresses is similar to the cluster-based gene sequences. In conclusion, the study suggests that the P_N under stresses can be used as an important criterion for genetic diversity analysis and species identification of pepper.

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