ISSR analysis on genetic diversity of wild *Agrostis stolonifera* L . Germplasm resources

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Introduction *Agrostis stolonifera* L . is a major cool season turf grass in China .ISSR(Zietkiewicz E ,1994) molecular markers were used to detect the genetic diversity among three populations of wild and one of cultivated *Agrostis stolonifera* L . collected from Guizhou .

Materials and methods

Plant material The plants used in this study were sampled from three natural populations and one cultivated population of *Agrostis stolonifera* L . located in Guizhou . Each populations had several subpopulations with the exception of the cultivated population KROMI . The locations of sampled populations shown in Table 1 .

Table 1 *Natural and cultivated populations of* Agrostis stolonifera *L . used in this study .*

Name of populations		origins	No . of subpopulations
Natural populations	BSE	Southeastern of Biji	6
	BNW	Northwestern of Biji	7
	LPS	Liu Panshui	5
Cultivated population	KROMI	Dushan	1

DNA extraction and ISSR amplification Fifteen individuals were selected from each subpopulation for extraction of mixed DNA . PCR amplifications were then performed in a final volume of 25μL containing 50ng DNA templates , 1μL(2mmol/L) dNTPs , 2μL10×PCR buffer ,2μL (25mmol/L) Mg^{2+} ,0 .4U(2 .5U/L) Taq polymerase ,1μL Primer(10pmol) .Reactions were carried out under the following regime : 94℃ for 7 min ; Followed by 35 cycles of for 30s ,50℃ for 45s ,72℃for1min ;and ended with 72℃for7min .

Results and discussion

Genetic diversity Nine primer pairs produced 66 polymorphic bands , the average percentage of polymorphic bands was 81 .48 . At population level , the percentage of polymorphic bands ranged from 43 .21 to 59 .26 , with an average of 50 .21 .The Nei's gene diversity index was 0 .2414 and Shannon diversity index was 0 .3719 . These results suggested a rich genetic diversity among the natural populations of Agrostis stolonifera L .

Amova The genetic differentiation coefficient was 0 .492 and the gene flow was 0 .5164 among three wild populations , It revealed a significant genetic differentiation .

Genetic relatives The Nei's genetic similarity coefficient of the sub-populations ranged from 0 .4074 to 0 .9123 , with three cluster groups . Moreover , the findings implied a correlation among the populations . A significantly high correlation between geographical and genetic distance was observed($r=0.494$, $0.05>P>0.01$) .Further analysis on genetic relationship suggested relatively high genetic similarity among populations ,ranging from 0 .7003 to 0 .9409 .

Conclusion There was a rich genetic diversity and significant genetic differentiation among the natural populations of Agrostis stolonifera L . and the cultivated populations showed a significant genetic difference from natural populations .

Reference

Zietkiewicz E ,Rafalaki A ,Labuda D , 1994 .Genome fingerprinting by simple sequence repeat (SSR) —anchored polymerase chain reaction amplification . *Genomics Journal* , 20 ,176～183 .