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. And every populations had several subpopulations (except KROMI). The locations of sampled populations were reported in Table1.

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

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

DNA extraction and ISSR amplification Within each subpopulation we selected 15 individuals to extract and mixed DNA, then PCR amplifications were 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 conducted by following program: 94℃ for7 min; Followed by 35cycles of for30s, 50℃for45s, 72℃for1min; and ended with 72℃for7min.

Results and discussions

Genetic diversity Nine primer pairs produced 66 polymorphic bands, the average percentage of polymorphic bands was 81.48. At the population level, the percentage of polymorphic bands ranged from 43.21 to 59.26, with the average of 50.21. The Nei's gene diversity index was 0.2414 and Shannon diversity index was 0.3719. These results suggested that there was 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, can be clustered into three groups. Moreover, the findings implied that a correlation among the populations. The significantly high correlation between geographical and genetic distance were observed($r=0.494$, $0.05>P>0.01$). Further analysis on genetic relationship suggested relatively high genetic similarity among populations, ranged from 0.7003 to 0.9409.

Conclusions 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.