Detection of germplasm accessions and SSR markers for rust resistance in Zoysiagrass (*Zoysia japonica* Steud.)

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Introduction

Zoysiagrass (*Zoysia japonica* Steud.) which characterized by wide adaptation, stress tolerance, requires less management input, is extensively used as a promised warm-season turfgrass, grazing forage and soil conservation species. Zoysia rust (caused by *Puccinia zoysiae* Diet.), is a common disease occurring on zoysia turf. The objective of this research was to evaluate the rust resistance of 162 accessions, and to develop SSR markers linked to this resistance trait by transferability methods.

Materials and Methods

A total of 162 accessions collected from six sites of Liaoning, Shandong and Anhui Provinces in China in 2008. Rust disease infection of each accession was evaluated in 2008 and 2009 two consecutive years in a field of the China Agricultural University Experimental Station in Beijing. In addition to the field evaluation, an greenhouse inoculation test was conducted. Urediospore suspension solution of *Puccinia zoysiae* pathogen was sprayed on leave surface, with approximately inoculum concentration of 1×10^5 urediospore ml^{-1}, The inoculated plants were kept at 20 ℃, 100% humidity for 24h in dark. After 2 weeks, the disease infection rate of each accession was scored according to Roelfs (1984).

Genomic DNA was extracted from frozen leaves of tested materials using a modified CTAB method described by Jin et al. (2004). A set of 67 SSR primer pairs derived from wheat, barley and perennial ryegrass that previously identified as linked with rust resistant genes were screened on 4 individuals randomly selected from Shandong province. Strong amplified primer pairs were assessed among16 individuals, including 8 resistant types and 4 susceptible types. PCR reaction was conducted according to Gui et al. (2008), PCR products were electrophoresed on 8.0% non-denatured polyacrylamide gel, and stained by silver staining method.

Results

Out of the 162 accessions, 9 accessions (5.6%) were high resistant and 17 accessions (10.5%) were moderate resistant, whereas, 100 accessions (61.7%) were moderate susceptible and 33 accessions (20.4%) were high susceptible.

Figure 1. Uredinium (left) and urediniospores (middle, 10×; right, 100×) of *Puccinia zoysiae* Diet

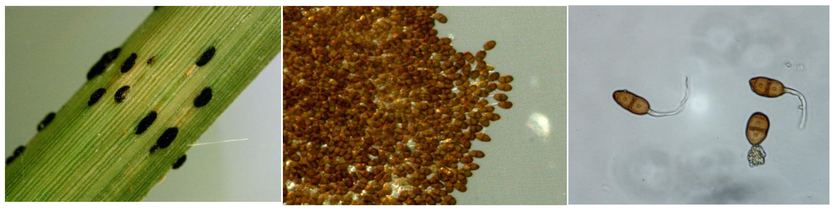

Figure 2. Telium (left) and teliospores (middle, 10×; right, 100×) of Puccinia zoysiae Diet

Table 1. Efficacy of SSR primer pairs (PPs) derived from wheat, barley and perennial ryegrass in zoysiagrass

Library	Number of tested PPs	Strong amplified PPs	Polymorphic PPs
Wheat	35	12 (34.3%)	11 (91.2%)
Barley	12	6 (50.0%)	3 (50.0%)
Perennial ryegrass	20	17 (85.0%)	14 (82.4%)
Total	67	35 (52.2%)	28 (80.0%)

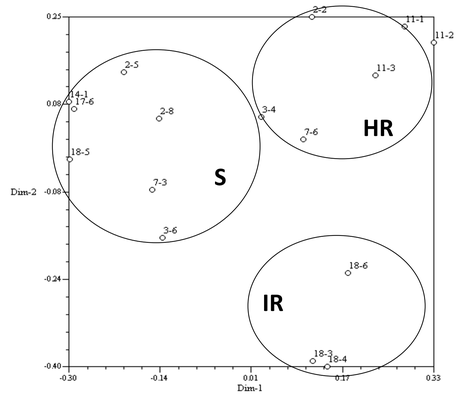

Figure 3. Principal component analysis of tested zoysiagrass accessions based on effective SSR markers.

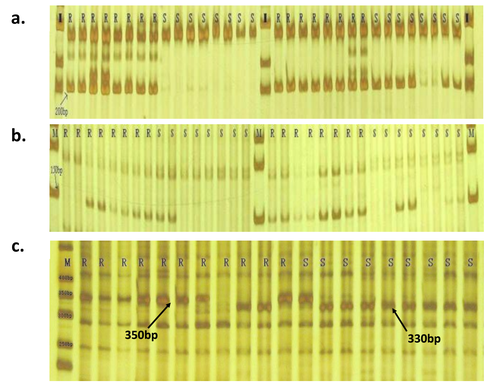

Figure 4. Specific bands between rust disease resistant and susceptible accessions amplified by lpssr059 (a.), LpSSRH01A07 (b.) and Xgwm533 (c.) markers.

Figure 5. Divergent selection effects for susceptible (top) versus resistant (bottom) to rust disease by one cycle phenotype selection in zoysiagrass.

A total of 35 markers amplified clear SSR type bands, the transfer rates of wheat, barley and perennial ryegrass rust resistance linked SSR markers to zoysiagrass were 34.3%, 50% and 85%, respectively. The SSRs of perennial ryegrass are higher conservative in zoysiagrass than that of wheat and barley. Twenty eight markers of 35 working primer pairs amplified polymorphic bands, and three markers including LpSSRH01A07, LpSSR059 and Xgwm533 consistently produced contrasting bands with fragment size ranging from 200 to 350 bp between rust resistant and susceptible accessions.

Cited Reference

Roelfs A.P., 1984. Race specificity and methods of study. In: Bushnell, Roelfs, The cereal rust. Vol .1 Origins, Specificity, Structure and Physiology. Academic Press, Orlando.2,131-164.