



University of Kentucky
UKnowledge

International Grassland Congress Proceedings

XXI International Grassland Congress / VIII
International Rangeland Congress

Analysis of ISSR Markers Linked to Disease Resistance Gene of F1 in Alfalfa

Fang Meng

Chinese Academy of Agricultural Sciences, China

Qinghua Yuan

Chinese Academy of Agricultural Sciences, China

Follow this and additional works at: <https://uknowledge.uky.edu/igc>



Part of the [Plant Sciences Commons](#), and the [Soil Science Commons](#)

This document is available at <https://uknowledge.uky.edu/igc/21/12-2/4>

The XXI International Grassland Congress / VIII International Rangeland Congress took place in Hohhot, China from June 29 through July 5, 2008.

Proceedings edited by Organizing Committee of 2008 IGC/IRC Conference

Published by Guangdong People's Publishing House

This Event is brought to you for free and open access by the Plant and Soil Sciences at UKnowledge. It has been accepted for inclusion in International Grassland Congress Proceedings by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

Analysis of ISSR markers linked to disease resistance gene of F₁ in alfalfa

Meng Fang, Yuan Qing-hua*

Institute of Animal Sciences, CAAS, Beijing 100094, China

* Corresponding author. E-mail: yuanqinghud@hotmail.com

Key words alfalfa, F₁, common leaf spot, ISSR markers, disease resistance gene

Introduction Common leaf spot of Alfalfa (*Medicago sativa* L.) which is caused by *Pseudopeziza medicaginis* (Lib.) Sacc. is the most serious disease in this crop. Breeding and using resistant varieties is the best method for preventing and controlling the leaf spot disease. This research attempted to find some markers linked to disease resistance gene of F₁ of alfalfa.

Materials and methods The materials included 6 highly resistant plants, 6 highly susceptible plants and 4 moderately resistant plants. We established highly resistant × highly resistant cross, highly susceptible × highly susceptible cross, moderately resistant × moderately resistant cross. Firstly, the materials in these hybrid combinations were selected according to the results of disease investigation after inoculation. Secondly, ISSR (Zietkiewicz E et al, 1994) was employed to detect molecular markers linked to the resistant gene of Common leaf spot in resistant and susceptible DNA pools composed of 12 highly resistant plants and 12 highly susceptible plants which were selected from moderately resistant × moderately resistant cross. Finally, the results were verified by 24 single plants above and 75 single plants from highly resistant × highly resistant cross and 50 single plants from highly susceptible × highly susceptible cross.

Results 32 of 93 ISSR primers amplified clear and steady bands. There are 6 ISSR primers that amplified polymorphic bands between resistant and susceptible DNA pools (Figure 1). The length of bands was 430-900bp (Table 1). After that, the 6 ISSR markers were verified by 24 plants above and 75 highly resistant single plants and 50 highly susceptible single plants. Markers 9-R920, 20-R750, 21-R430, 818-R680 were found to be significantly associated with resistance in F₁ progeny, and 866-S800 was found to be significantly associated with susceptibility in F₁ progeny (Table 2).

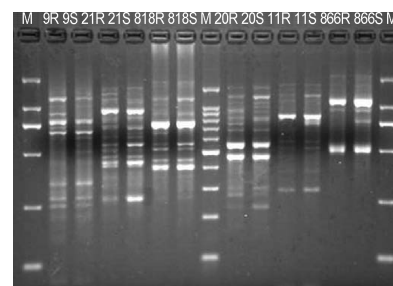


Figure 1 Amplified results of 6 ISSR primers between resistant and susceptible DNA pools.

Table 1 Sequence and Annealing temperature of ISSR primers.

Primer	Resistant	Sensitive
9	920bp	-
11	-	750bp
20	750bp	-
21	430bp	-
818	680bp	-
866	-	800bp

Table 2 Chi square test results of validation of 6 ISSR markers in resistant and susceptible plants.

markers	χ^2 test
9-R920	$\chi^2 = 4.18$ P < 0.05
11-S750	$\chi^2 = 1.44$ P ≥ 0.05
20-R750	$\chi^2 = 39.38$ P < 0.05
21-R430	$\chi^2 = 38.95$ P < 0.05
818-R680	$\chi^2 = 4.10$ P < 0.05
866-S800	$\chi^2 = 5.68$ P < 0.05

Conclusions When the 6ISSR primers were tested, we found that the resistant markers appeared in some susceptible single plants. That's because the inheritance of resistance to *P. trifolium* in these progeny was varied (D.E.Obert et al, 2000). I think the 5 ISSR markers (9-R920, 20-R750, 21-R430, 818-R680, 866-S800) would be valuable in breeding program.

References

- Zietkiewicz E, Rafalski A, Labuda D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification [J]. *Genomics* 20:176-183.
- D.E.Obert, D.Z.Skinner, D.L.Stuteville. 2000. Association of AFLP markers with downy mildew resistance in autotetraploid alfalfa. *Molecular Breeding* 6:287-294.