ISSR Markers for Alfalfa Conferring Resistance to Common Leafspot

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ISSR markers for alfalfa conferring resistance to common leafspot

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Introduction Common leafspot caused by *Pseudopeziza medicaginis* (Lib .) Sacc . is a worldwide severe disease of alfalfa . The employment of resistant materials is the most effective method to prevent the outbreak of disease , and its selection is of great importance . As an aid to selection , molecular markers is well-established , with which genotypes can be selected . The objective of this study is to select several ISSR molecular markers related to common leafspot resistance gene of alfalfa , and expect to establish molecular marker-assisted selection system in the breeding of alfalfa in future .

Materials and methods Resistant and susceptible lines of five alfalfa cultivars—Iroquois , Saranac , Shahe , Jingyang and Shawan were developed by Yuan Qing-hua (2003) . For every cultivar , 10 highly susceptible and 10 highly resistant plants were selected through fungal inoculation and resistance tests . With DNA extraction , resistant and susceptible DNA pools of every cultivar were established , and then equally mixed five resistant DNA pools to construct a mixture resistant DNA pool . Using the same method , a mixture susceptible DNA pool was established . 65 ISSR primers were applied to selecting molecular markers related to common leafspot resistance gene in this study , accompanied with BSA (Michelmore RW et al . 1991) . ISSR primers produced characterized band between the mixture susceptible DNA pool and mixture resistant DNA were selected and then were tested between susceptible and resistant DNA pools of the five cultivars . Characterized bands appeared in DNA pools of five cultivars were cut from 2 .0% agarose gel , purified with Agarose Gel DNA Purification Kit Ver .2 .0 (TaKaRa) , cloned and sequenced .

Results Eleven ISSR primers produced characterized bands between mixture susceptible and resistant DNA pools , four of which still appeared in susceptible or resistant DNA pools of at least three cultivars . The four markers were selected to be related to common leafspot resistance gene of alfalfa . The sequence of the primers and the length of its PCR products were shown in Table 1 . Primer 22 produced two characterized bands in susceptible DNA pools of five alfalfa cultivars (Figure 1) . Primer 6 produced one characterized band in five susceptible DNA pools and primer 834 produced one characterized band in three resistant DNA pools .

Table 1 *The sequence of ISSR primers and its products .*

Primer	Sequence (5 -3)	Susceptible band	Resistant band
6	$(AC)_8TC$	636 bp	—
		450 bp	—
22	$(TC)_8CC$	673 bp	—
834	$(AG)_8YT$	—	446 bp

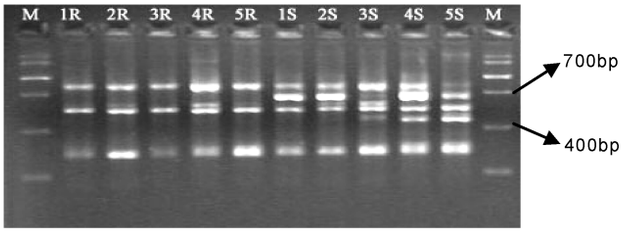

Figure 1 *Amplified results of Primer 22 in susceptible and resistant DNA pools of five alfalfa cultivars .*

Conclusions Because of the complex genetic nature of alfalfa , to construct the mixture susceptible DNA pool and mixture resistant DNA pool of five cultivars is necessary . The four ISSR markers may be closely linked to common leafspot resistance gene of alfalfa . To test the linkage distance between the four molecular markers and disease resistance genes , a BC1 population should be established . For the closely linked ISSR markers , SCAR (sequence characterized amplified region) primers should be designed according to the sequenced fragment to ensure repeatability and stability of the experiments .

References

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