RESEARCH NOTES

Protein Marker Assisted Identification of \( Yr9, Lr26 \) and \( Sr31 \) Genes in a Group of Iranian Wheat Cultivars

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ABSTRACT

The 1RS chromosome segment derived from Petkus rye carries genes for resistance to three wheat rust diseases, namely \( Lr26 \) for resistance to leaf rust (caused by \textit{Puccinia triticina}) \( Yr9 \) for resistance to stripe rust (\textit{P. striiformis} f. sp. \textit{tritici}) and \( Sr31 \) for resistance to stem rust (\textit{P. graminis} f. sp. \textit{tritici}). Since \textit{Sec-1} is tightly linked with the three rust resistance genes electrophoresis it is a useful method to identify and confirm the presence of three rust resistance genes in current wheat populations. SDS-PAGE was used to examine eight Iranian wheat cultivars for resistance to three rusts. The eight Iranian wheat cultivars examined were Alvand, Darab 2, Tajan, Nicknejad, Mahdavi, Zarrin, Alamoot and Atrak. The SDS-PAGE results showed that cultivars Mahdavi and Atrak have \textit{Sec-1} bands and are therefore likely to carry the 1BL.1RS translocation and the linked genes \( Yr9, Lr26 \) and \( Sr31 \).

Keywords: Rust Resistance, Secalins (\textit{Sec-1}), SDS-PAGE.

INTRODUCTION

Much of the widely adapted wheat germplasm generated and distributed by CIMMYT throughout spring wheat production areas in low latitude countries carry a 1BL.1RS translocation first identified in European wheat germplasm by Mettin et al. (1973) and Zeller (1973). The 1BL.1RS segment carries genes for resistance to three rusts, namely \( Lr26, Yr9, Lr26 \), and gene \textit{Pm8} for resistance to powdery mildew (Zeller, 1973). However, in many genetic backgrounds, especially wheat lines of CIMMYT origin, the expression of \textit{Pm8} is suppressed by a gene(s) located in chromosome 1A (Ren \textit{et al.}, 1997) or 7D (Zeller \textit{et al.}, 1993). In addition, the translocation may contribute positively to agronomic traits such as yield and drought tolerance (Rajaram \textit{et al.}, 1983).

On the negative side, wheat lines with the translocation generally produce flours of a lower quality than their non-1BL.1RS counterparts (Dhaliwal \textit{et al.}, 1987).

Singh \textit{et al.} (1990) used SDS-PAGE to examine genetic linkage between the genes controlling secalins (\textit{Sec-1}) and genes for resistance to the three rust diseases of wheat. They found no recombination among the three rust resistant genes. The rust resistance genes were located 5.4±1.7 cM from the \textit{Sec-1} locus, thus suggesting a close linkage. Due to the lack of pairing between the wheat and rye chromatin (1B and 1BL.1RS) in the wheat background, \textit{Sec-1} acts as a marker for \( Lr26, Yr9 \) and \( Sr31 \). The eight wheat cultivars used in this study, except for Alamoot cultivar, have shown good levels of stripe rust resistance in Iran (Afshari, 2004a).

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MATERIALS AND METHODS

This experiment was carried out in Sydney University to identify the presence or absence of the 1BL.1RS translocation in a set of Iranian wheat cultivars. 1BL.1RS wheat-rye translocation lines including Fed*4/Kavkaz, Disponent, Skorospelka 35, Kavkaz and Gabo 1DL.1RS (positive control lines) and Federation wheat were provided by Dr. H. S. Bariana, Dr. R. A. McIntosh and line Avocet S*3/Yr9 was supplied by Dr. C. R. Wellings from Sydney University-Australia. The eight Iranian wheat cultivars were Alvand, Darab 2, Tajan, Nicknejad, Mahdavi, Zarrin, Alamoot and Atrak.

Ten mg. of crushed endosperm from 10 seeds were shaken in a small tube with 100μl 75% (v/v) ethanol for five minutes. The samples were vortexed and centrifuged at 13,000 xg for 5 minutes to recover the supernatant. To 50μl supernatant, 50μl of protein extraction buffer was added to obtain a final sample. SDS-PAGE was carried out using the buffer of Laemmli (1970). The separating gel contained 12% (w/v) acrylamide cross-linked with 0.213% N’N’-Methylene-bis-acrylamide (75:1 weight ratio of acrylamide:bisacrylamide), 0.1% (w/v) SDS in 375mM Tris.Cl buffer (pH=8.8). The stacking gel contained 4% (w/v) acrylamide cross linked with 0.11% (w/v) N’N’-Methylene-bis-acrylamide, 0.1% (w/v) SDS, and 125mM Tris.Cl buffer (pH=6.8). The gel w run in a 21 x 16.5 x 0.7 cm glass and electrophoresed in a vertical dual gel unit (Sigma-Aldrich). Electrophoresis was carried out at a constant electric current of 15mA per gel for approximately 5-6 hours, until the bromophenol blue dye migrated to 1.5-2cm above the gel base. The gel was

fixed and stained in a solution containing 0.1% (w/v) coommassie blue R250 (Sigma), 50% (v/v) methanol, 7% (v/v) acetic acid and 3% (v/v) glycerol for 40 minutes. The gel was then rinsed with distilled water and destained in 10% (v/v) acetic acid and 30% (v/v) methanol for 20 minutes as described by Liu et al. (1989). The gel was washed in distilled water for 50 minutes with gentle shaking. The gel was dried between two cellophane sheets and kept as a permanent record.

RESULTS AND DISCUSSION

The SDS-PAGE results showed that among the 8 Iranian wheat cultivars Mahdavi and Atrak carried the 1BL.1RS translocation (Figure 1) and the linked genes Yr9, Lr26 and Sr31. This confirms the results of multipathotype tests reported by Afshari (2004b). Both cultivars showed the presence of a thick Sec-1 band in common with the positive control lines/cultivars Fed*4/Kavkaz, Disponent, Skorospelka 35, and Kavkaz with the 1BL.1RS translocation and Gabo 1DL.1RS. The Sec-1 band did not present in Federation or in the six Iranian cultivars, Alvand, Darab 2, Tajan, Nicknejad, Zarrin and Alamoot.

As Sec-1 is tightly linked with the three rust resistance genes; electrophoresis is a useful method to identify and confirm the presence of rye 1RS chromatin for identification of the three wheat rust resistant genes. Seven of the eight cultivars (except for Alamoot) were resistant to stripe rust in Iran (Afshari, 2004a), indicating the likelihood of a genetic diversity of resistance running through these cultivars, and other mechanisms are involved in their resistance. In addition, using this protein marker is a quick method for screening wheat germplasm for these resistance genes in different laboratories without any greenhouse facilities in a short period of time.

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REFERENCES

شناسایی زن های مقاومت 26، Yr9، Lr26 به بیماری زنگهای گندم در گروهی از
ارقام ایرانی با استفاده از مارک بروتینی

ف. افتخاری

چکیده
قلعته کروموزومی 1RS گرفته شده از رقم تکوس جاودان حامل سه زن مقاومت به سه زنگ مهم گندم به ترتیب ۲۶ برای مقاومت به زنگ فوهای P. striiformis f. sp. tritici و Yr9 برای مقاومت به زنگ سیاه P. graminis f. sp. tritici و این سه زنگ به شکل‌های مختلف و در ماده میشنوده می‌باشد.

در این آزمایش تعادل هشته‌ها و تعداد هشته‌های تجاری و مهم گندم به نامهای اندازه‌گیری دارا هستند. نتایج نشان‌داد که میزانJRQ نسبتی مرور به عنوان دلیل‌های اصلی در پیشگیری از حمل و نگهداری از مقاومت در برابر پاتوم، می‌باشد.

SDS-PAGE نتایج مورد امتحان در این بررسی ژن‌های زنگهای گندم، میزان میکروژن و مقاومت به تهیه می‌شود.

در این مطالعه میزان مقاومت به جریان Yr9، Lr26 و ۲۶ در حامل ۱RS و ۱BL:۱RS می‌باشد. میزان مقاومت به جریان Yr9، Lr26 و ۲۶ در حامل ۱RS می‌باشد. این میزان مقاومت به حمل و نگهداری در حامل ۱RS به عنوان میکروژن و مقاومت به تهیه می‌شود. در این مطالعه میزان مقاومت به حمل و نگهداری در حامل ۱RS به عنوان میکروژن و مقاومت به تهیه می‌شود.