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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 *Puccinia triticina*), *Yr9* for resistance to stripe rust (*P.striiformis* f. sp. *tritici*) and *Sr31* for resistance to stem rust (*P. graminis* f. sp. *tritici*). Since *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 *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 (*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 *Pm8* for resistance to powdery mildew (Zeller, 1973). However, in many genetic backgrounds, especially wheat lines of CIMMYT origin, the expression of *Pm8* is suppressed by a gene(s) located in chromosome 1A (Ren *et al.*, 1997) or 7D (Zeller *et al.*, 1993). In addition, the translocation may contribute positively to agronomic traits such as yield and drought tolerance (Rajaram *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 *et al.*, 1987).

Singh *et al*. (1990) used SDS-PAGE to examine genetic linkage between the genes controlling secalins (*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 *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, *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

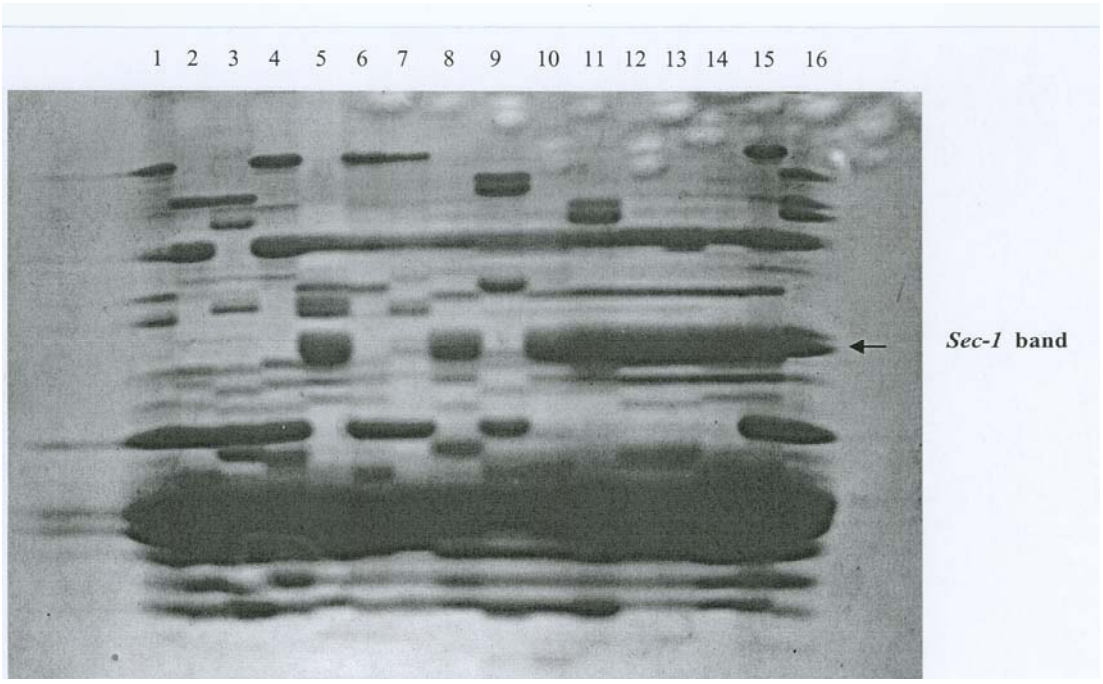

Figure 1. Banding patterns of seed protein extracts from eight Iranian wheat cultivars and various controls subjected to SDS-PAGE electrophoresis. The *Sec-1* band is indicated. For the cultivars listed below the presence and absence of *Sec-1*, and therefore 1BL.1RS, is indicated by (+) and (-), respectively. From left to right: 1. Alvand (-), 2. Darab 2 (-),3. Tajan (-),4. Nicknejad (-), 5. Mahdavi (+), 6. Zarrin (-), 7. Alamoot (-), 8. Atrak (+), 9. Federation (-), 10. Fed*4/Kavkaz (+), 11. Disponent (+), 12. Skorospelka 35 (+), 13. Kavkaz (+), 14. Gabo 1DL.1RS (+), 15. Clement (+) and 16. Avocet S*3/*Yr9* (+).

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 multi-pathotype 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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شناسایی ژن های مقاومت *Yr9, Lr26* و *Sr31* به بیماری زنگهای گندم در گروهی از ارقام ایرانی با استفاده از مارکر پروتئینی

ف. افشاری

چکیده

قطعه کروموزومی 1RS گرفته شده از رقم پتکوس چاودار حامل سه ژن مقاومت به سه زنگ مهم گندم به ترتیب *Lr26* برای مقاومت به زنگ قهوه‌ای (*Puccinia triticina*)، *Yr9* برای مقاومت به بیماری زنگ زرد (*P.striiformis* f. sp. *tritici*) و *Sr31* برای مقاومت به زنگ سیاه (*P.graminis* f.sp. *tritici*) میباشد. از آنجایکه *Sec-1* پروتئین کاملا" پیوسته با سه ژن مقاومت به زنگهای ذکر شده میباشد میتواند به عنوان ابزاری در جهت شناسایی و تایید حضور و یا عدم حضور ژن های فوق در مواد ژنتیکی گندم باشد. در این آزمایش تعداد هشت رقم تجاری و مهم گندم به نامهای الوند، داراب۲، تجن، نیک نژاد، مهدوی ، زرین، الموت و اترک که در سطح وسیع در ایران کشت میشوند همراه با هفت لاین استاندارد که دارای این ژنهای مقاومت میباشند به عنوان شاهد مثبت و یک لاین که فاقد این ژن ها بود به عنوان شاهد منفی در این تحقیق مورد استفاده قرار گرفتند. نتایج SDS-PAGE بر روی ژل نشان داد که دو رقم مهدوی و اترک حامل *Sec-1* میباشند در نتیجه قطعه کروموزومی 1BL:1RS با حضور سه ژن *Yr9, Lr26* و *Sr31* در این دو رقم تایید شد و شش رقم باقیمانده فاقد ژن‌های فوق میباشند. با توجه به اینکه از هشت رقم فوق به جز رقم الموت مابقی دارای مقاومت قابل قبولی در سطح کشور میباشند میتوان نتیجه گرفت که تنوع ژنتیکی مقاومت در این ارقام با منابع مختلف مقاومت وجود دارد و عامل مقاومت در آنها فاکتور(های) بجز قطعه کروموزومی 1RS میباشد.