小麦—簇毛麦染色体代换系、易位系特异蛋白组分的双向电泳比较分析

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摘 要 利用双向电泳技术,对栽培小麦(AABBDD)、染色体代换系(6V/6A)、易位系(6VS/6AL)、(6VS/6DL)和簇毛麦(VV)的叶片全蛋白进行了比较研究。在栽培小麦、代换系和两个易位系中检测到超过 350 个蛋白组分,它们的分子量范围是 10 ~ 110 KD,等电点在 4.5 ~ 8.6 之间。栽培小麦、6V/6A、6VS/6AL、与 6VS/6DL 之间的双向电泳谱型极为相似,但与簇毛麦不同。在代换系、两个易位系和簇毛麦中检测到了特异蛋白组分 16 KD/pI5.0,而在栽培小麦中未检测到该组分,这些结果表明 16 KD/pI5.0 蛋白可能定位于簇毛麦 V 染色体短臂上。

关键词 染色体代换系;双向电泳;簇毛麦;白粉病;特异蛋白;易位系

Comparison of the Protein Composition and Analysis of the Specific Protein from Wheat-*Haynaldid villosa* Chromosome Substitution Line and Translocation Line by 2D-PAGE

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Abstract With 2D-PAGE techniques, comparison of leaf protein in cultivar wheat (AABBDD), Wheat-*Haynaldia villosa* chromosome substitution line (6V/6A), translocation lines (6VS/6AL), (6VS/6DL) and *H. villosa* (VV) have been made in this study. Over 350 protein species were detected in the leaf of cultivar wheat, chromosome substitution line and two translocation lines. Their molecular weight ranged from 10 ~ 110 KD and pI from 4.5 ~ 8.6. 2D-PAGE patterns of leaf protein are almost the same among cultivar wheat, 6V/6A, 6VS/6AL, 6VS/6DL; but different from *H. villosa*. A specific protein (16 KD/pI5.0) was found in substitution line, two translocation lines and *H. villosa*, but not found in the cultivar wheat. These results suggested that the protein (16 KD/pI5.0) may be located in the short arm of V chromosome of *H. villosa*.

Key words chromosome substitution line; 2D-PAGE; *Haynaldia villosa*; powdery mildew; specific protein; translocation lines

1 Introduction

Wheat-*H. villosa* chromosome substitution line (6V/6A) was bred through hybridization of *H. villosa* (VV) with *T. durum* (AABB), then hybrid F_1 was pollinated freely by cultivar wheat (AABBDD).

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Chromosome translocation lines 6VS/6AL, 6VS/6DL were obtained by selection from hybrid progenies which were bred through hybridization of substitution line 6V/6A with agronomic parent combined with radiation treatment[1,2]. Substitution line 6V/6A and translocation lines 6VS/6AL, 6VS/6DL, which were thought to contain the powdery mildew resistance-related gene *pm*21, were used by many researchers in China, as experimental materials for isolating and cloning powdery mildew resistance-related genes by using molecular marker[3~5], transformation competent artificial chromosome (TAC) library construction[6], micro-dissection cloning[7] and cDNA library screening techniques. Up to now, about 40 powdery mildew resistance gene loci (*pm*1 – *pm*28) have been identified[8] and the complete powdery mildew resistance gene or fragments being cloned were reported[9~18]. But it is necessary for further investigation of resistance mechanisms in wheat, and full utilization of resistant resources. In this study, with the two-dimensional PAGE techniques, the protein compositions were compared and the specific protein from wheat-*Haynaldia villosa* substitution line and translocation lines was analyzed. The results may be helpful for the understanding of powdery mildew resistance mechanisms at biochemical level.

2 Materials and methods

2.1 Materials and culture

Wheat-*Haynalia villosa* 6V/6A chromosome substitution line, 6VS/6AL, 6VS/6DL translocation lines were kindly provided by prof. Z. M Yang, Department of plant Genetics and Breeding, China Agricultural University. Seeds of wheat (*Triticum aestivum* AABBDD), *Haynalia villosa* (VV), chromosome substitution line, translocation lines were sterilized for 5 min with a solution of 0.1% $HgCl_2$ and then washed twice with tap water and germinated in petri dishes on filter paper moistened with distilled water for 3 days at 23 ~25℃.

2.2 2D polyacrylamide gel electrophoresis

Two-dimensional polyacrylamide gel electrophoresis was performed according to the method of O'Farrell et al[19], with the use of a modified procedure: the one dimensional 3% polyacrylamide gel, containing 9 M urea 0.98 g; 2% nonionic detergent p40 40 μL, 30% polyacrylamide 340 μL, ampholytes 30 μL(pH 3 ~10) and 60 μL(pH 5 ~7); ddH_2O 1.09 mL, TEMED 2 μL, 10% APS 10 μL, were each cast in glass tubes (120 mm × 3 mm) and were respectively pre-electrophoresed for 15 min at 200 V, 30 min at 300 V and 60 min at 400 V. The protein samples were dissolved in the sample buffer which contained ddH_2O 4.0 mL, 500 mM Tris-HCl (pH6.8) 1.0 mL, glycerol 0.8 mL, 10% SDS 1.6 mL, β-mercaptothanol 0.4 mL, 10% (w/v) bromophenol blue 0.2 mL. The first dimension was isoelectrofocusing, 60 μL protein samples (4 ~6 mg · mL^{-1}) were located in each glass tube; electrophoresis was run for 14 ~16 h at 400 V. After eletrofocusing, the gels were removed from the tubes by shattering the glass and placing in equilibration buffer which contained 6 mM Tris-HCl (pH6.8), β-mercaptoethanol, 10% glycerol, 2% (w/v) SDS for 20 min. The second dimension was a 12.5% SDS-PAGE that was performed according to the method of Laemmli[20].

The tube gels were placed on top of second dimension gels, 1% agarose was overlayered and allowed to polymerize. Cylindrical gels were run at a constant voltage of 80 V for 5.5 h in a Bio-Rad unit. Gels were stained with 0.4% $AgNO_3$ solution[21].

3 Results

3.1 Comparison of 2D-PAGE pattern of leaf protein among cultivar wheat (AABBDD), chromosome substitution line (6V/6A) and *H. villosa* (VV)

Over 350 protein species were identified in leaf of cultivar wheat and substitution line. Same 2D-PAGE patterns of leaf protein were observed between the cultivar wheat and translocation line (6V/6A). Their molecular weight ranged from 10 ~40 KD and pI from 4.5 ~8.6, most of the leaf protein molecular weight ranged from 20 ~110 KD (Fig. 1 A, B). But there are quite differences of protein patterns among the cultivar wheat, substitution line and *H. villosa* (Fig. 2). The differences of 2D-PAGE protein patterns including the protein number, protein molecular

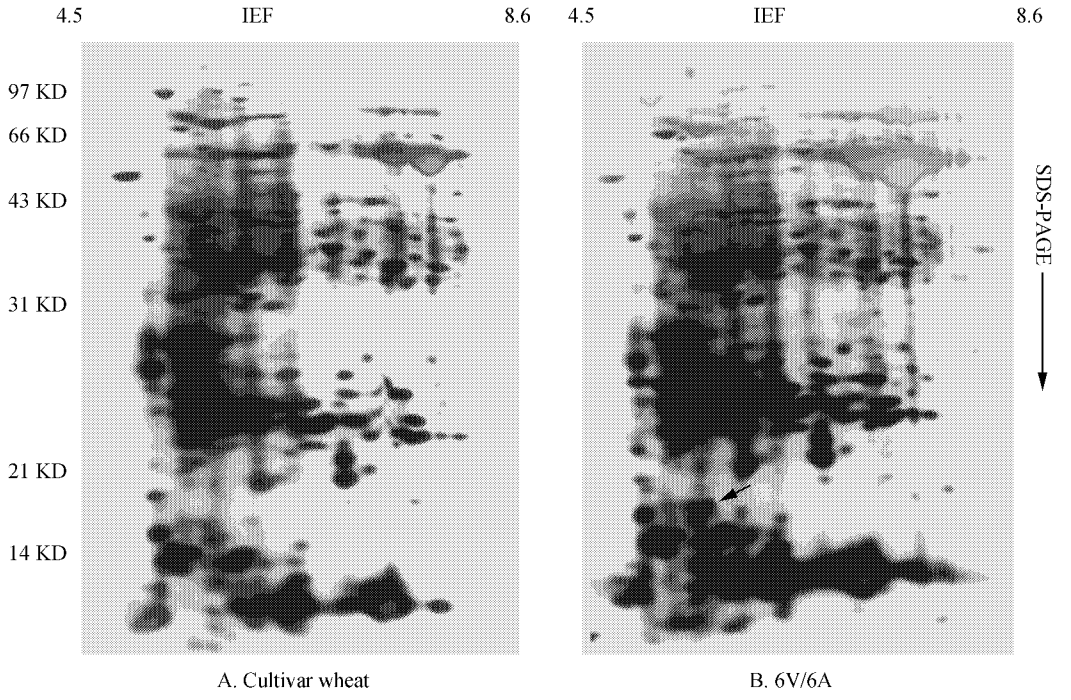

Fig. 1　2D-PAGE protein patterns of leaf protein of cultivar wheat and chromosome substitution line (6V/6A)

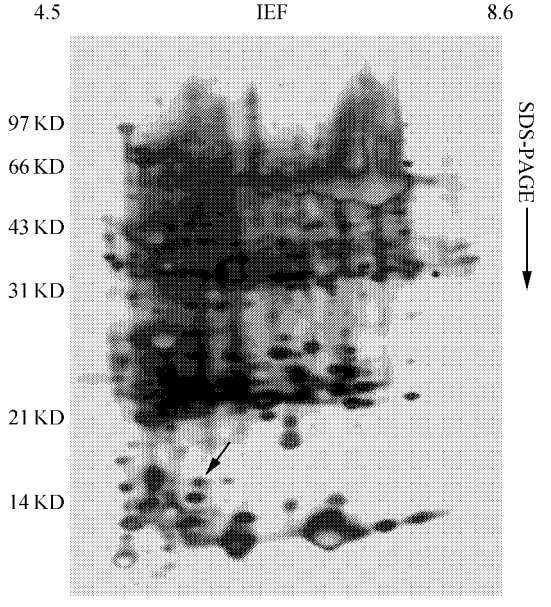

Fig. 2　2D-PAGE protein pattern of *H. villosa*.

weight and pI were caused due to the different genomics compositions of cultivar wheat and *H. villosa*.

3.2　Comparison of 2D-PAGE patterns of leaf protein between the translocation line 6VS/6AL and 6VS/6DL

To examine the difference of protein species existing between 6VS/6AL and 6VS/6DL, the protein compositions were analyzed by 2D-PAGE analysis, leaf protein patterns of 6VS/6AL, 6VS/6DL were shown in Fig. 3.

These results indicated no obvious difference in protein species, molecular weight and pI occurred between the 6VS/6AL and 6VS/6DL.

3.3　Specific protein existed in chromosome substitution line and translocation line

By the comparison of 2D-PAGE patterns of leaf protein, one specific protein was found in the chromosome substitution line (6V/6A), two translocation lines (6VS/6AL, 6VS/6DL) and *H. villosa* (VV). The specific protein was located in the region below the molecular weight 20 KD and acid region. The molecular weight and pI of this protein were 16 KD and 5.0, respectively. But it was not found in cultivar wheat (Fig. 4). We have not known whether this protein was related to the V-chromosome existing in 6V/6A, 6VS/6AL, 6VS/6DL and *H. villosa* (VV). In the translocation line 6VS/6DL, there was another specific protein (14 KD/pI4.6) (Fig. 4 D indicated by arrow) lost, but it was present in cultivar wheat, 6V/6A, 6VS/6AL and *H. villosa* (VV). But it needs further investigation, we did not discuss the protein in this paper.

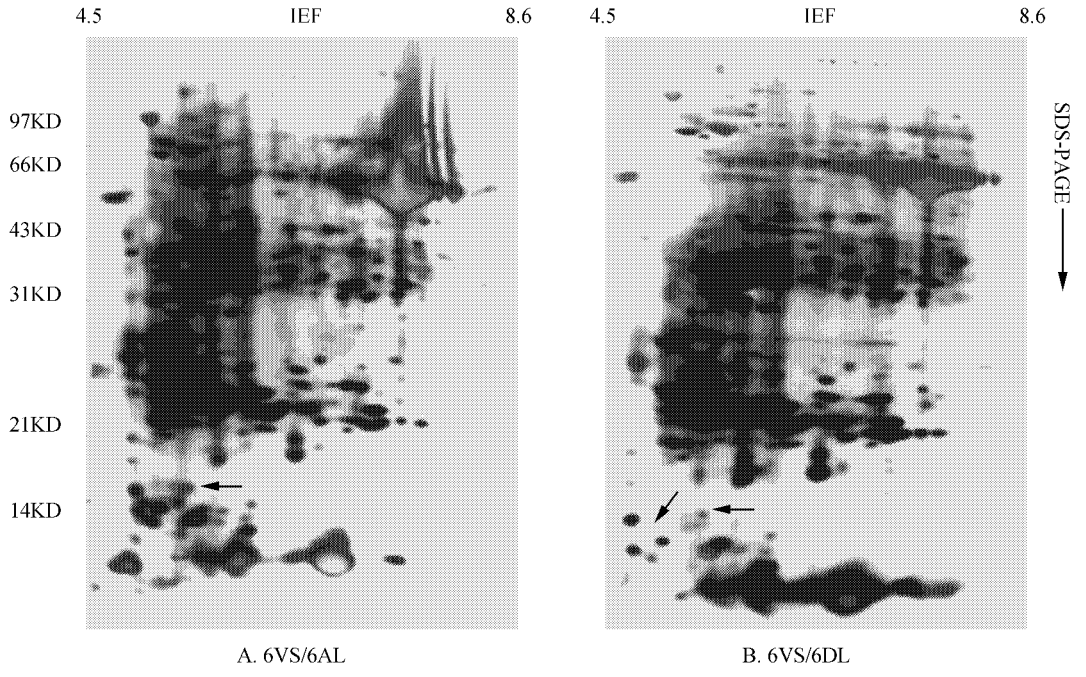

Fig. 3 2D-PAGE protein patterns of 6VS/6Al and 6VS/6DL

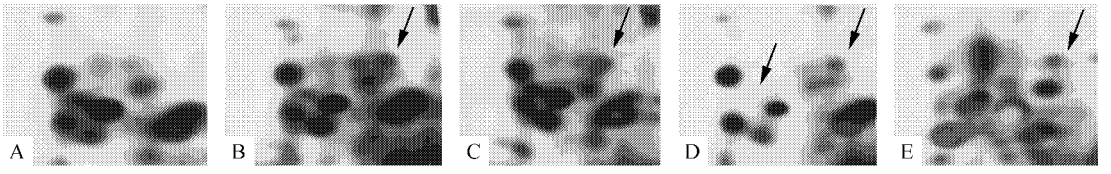

Fig. 4 The specific proteins existed in 6V/6A, 6VS/6AL, 6VS/6DL, and *H. villosa*(VV)
A. Cultivar wheat; B. 6V/6A; C. 6VS/6AL; D. 6VS/6DL; E. *H. villosa*(VV)

4 Discussions

Wheat-*Haynaldia villosa* 6V/6A chromosome substitution line and 6VS/6AL, 6VS/6DL translocation lines contained powdery mildew resistance gene *pm*21[2], which confers effective resistance to all current powdery mildew pathogens. Cytological and genetical investigation identified that *pm*21 was assigned to the short arm of the chromosome of *Haynaldia villosa*[1]. Powdery mildew, caused by *Erysiphe graminis f. sp. tritici*, is one of the most important wheat diseases in many regions of the world. Most of series gene origin are from *S*, *cereale*, *Triticum monocoocum*, *Aegilops*, *Triticum thaoudar* and have been overcome by new virulent *E. graminis* strains. Therefore, it is necessary to extend the search for the new sources of genetic resistance to powdery mildew for the wild relatives of cultivated wheat. In recent years, considerable progress has been made in the cloning of molecular marker and DNA fragments, which linked to powdery mildew resistance gene, using 6V/6A, 6VS/6AL, 6VS/6DL as the experimental materials. But there is a large difficulty for cloning powdery mildew resistance-related gene in wheat. Up to Now, no complete resistance – related gene has been cloned. Proteome concept was suggested by Wilkins[22]. Proteome analysis is a new technique for cloning genes. Damerval has successfully isolated a new transcriptional activator gene of opaques 2 near-isogene line in Zea Mays using this method[23]. Moon has also cloned several salt-resistance-related genes using similar techniques[24]. In this study, the specific protein (16 KD/pI5.0) was found in the substitution line 6V/6A, two translocation lines (6VS/6AL), (6VS/6DL) and *H. villosa* (VV), but not found in cultivar wheat (AABBDD). These results suggested the pro-

tein (16 KD/pI5.0) may be located in V chromosome or the short arm of V chromosome in *H. villosa*.

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Reference

1. Qi L L, Chen P D, Liu D J, et al. The Gene *pm*21-A new source for resistance to wheat powdery mildew [J]. Acta Agronmica Sinica, 1995, 21(3): 257 – 262.
2. Chen P D, Qi L L, Zhou B, et al. Development and molecular cytogenetic analysis of wheat-*Hayualdia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew [J]. TAG, 1995, 91: 1125 – 1128.
3. Liu Z Y, Sun Q X, Li H J, et al. Molecular identification and marker assisted selection of *pm*21 gene conferring resistance to powdery mildew in wheat [J]. Acta Genetica Sinica, 1999, 26(6): 673 – 682.
4. Liu S B, Tang Z H, You M S, et al. Identification, mapping and application of a chromosome-specific RAPD marker from *Haynaldia villosa* [J]. Acta Genetica Sinica, 2002, 29(5): 453 – 457.
5. Wang X Y, Chen P D, Zhang S Z. Pyramiding and marker-assisted selection for powdery milder resistance genes in common wheat [J]. Acta Genetica Sinica, 2001, 28(7): 640 – 646.
6. Fang Y D, Liu Y G, Wu H, et al. Construction of a transformation competent artificial chromosome (tac) library of wheat *Haynaldia villosa* translocation line [J]. Chinese Journal of Biotechnology, 2000, 16(4): 433 – 436.
7. Kong F J, Chen Y Y, Xin Z Y, et al. Microdissection of *Haynaldia villosa* telosome 6VS and cloning of species-specific DNA sequences [J]. Acta Botanica Sinica, 2002, 44(3): 307 – 313.
8. Peusha H, Enno T, Priilinn O. Chromosomal location of powdery mildew resistance genes and cytogenetic analysis of meiosis in common wheat cultivar [J]. Meri Hereditas, 2000, 132(1): 29 – 34.
9. Li W L, Farnis J O, Chittoor J E, et al. Genomic mapping of defense response genes in wheat [J]. TAG, 1999, 98: 226 – 233.
10. Li W L, Chen P D, Qi L L, et al. Isolation, characterization and application of a species – specific repeated sequence from *Haynaldia villosa* [J]. TAG, 1995, 90: 526 – 533.
11. Qi L L, Cao M S, Chen P D, et al. Identification, mapping and application of polymorphic DNA associated with resistance gene *pm*21 of wheat [J]. Genome, 1996: 191 – 197.
12. Cenci A, Dovidio Tanzarella, O A, Ceoloni C, et al. Identification of molecular markers linked to *pm*13, an Aegilops longissima gene conferring resistance to powdery mildew in wheat [J]. TAG, 1999, 98: 448 – 454.
13. Ma Z Q, Sorrels M E, Tandsley S D. RFLP markers linked to powdery mildew resistance genes *pm*1, *pm*2, *pm*3 and *pm*4 in wheat [J]. Genome, 1994, 37: 871 – 875.
14. Yu L, Niu J S, Ma Z Q, et al. Cloning, characterization and chromosome localization of two powdery mildew resistance-related gene sequences from wheat [J]. Acta Botanica Sinica, 2002, 44(12): 1438 – 1444.
15. Zhang H Q, Jia J Z, Zhang B S, et al. Chromosomal Localization of Powdery Mildew Resistance Gene *PmAeY*1 from *Ae. tauschii* Y219 [J]. Review of China Agricultural Science and Technology, 2006, 8(4): 19 – 22.
16. Chen X Z, Zhou R H, Jia J Z. Isolation, primary expression analysis of a full-length cDNA clone encoding small GTP-binding protein gene tarab5B in Wheat [J]. Acta Agronmica Sinica, 2005, 31(7): 897 – 901.
17. Sun D Z, Wang S G, Cheng Z F. Research advances of molecular breeding about resistance to powdery mildew in wheat [J]. J Shanxi Agric Univ. 2004, 24(2): 199 – 203.
18. Nabila Y, Payorm S, Robert D, et al. Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm*3*b* from hexaploid wheat [J]. The Plant Journal, 2004, 37: 528 – 538.
19. O'Farrell P H. High resolution of two-dimensional electrophoresis of proteins [J]. J Biochem, 1975, 250: 4007 – 4021.
20. Laemmli U K. Cleavage of structure proteins during the assembly of the head of bacteriophase T4 [J]. Nature (Lond), 1972, 227: 680 – 685.
21. Wary W, Boulikas T, Wary V P, et al. Silver staining of proteins in polyacrylamide gels [J]. Anal Biochem, 1981, 118: 197 – 203.
22. Wilkins M R, Sanchez J C, Gooley A A, et al. Progress with proteom projects: why all proteins expressed by a genome should be identified and how to do it [J]. Biotechnol Genet Eng Rev, 1995, 13: 19 – 50.
23. Damerval C, Le Guilloux M. Characterization of novel proteins affected by the 2 mutation and expressed during maize endosperm development [J]. Mol Gen Genet, 1998, 257(3): 354 – 361.
24. Moons A, Prinsen E, Bauw G, et al. Antagonistic effects of abscisic acid and jasmonates on salt stress-inducible transcripts in rice roots [J]. Plant Cell, 1997, 9: 2243 – 2259.