

## QTL MAPPING OF ADULT-PLANT RESISTANCE TO LEAF RUST IN CHINESE WHEAT CULTIVAR LANTIAN 9

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### Abstract

Chinese wheat cultivar "Lantian 9" showed a stable high yield in the field. Together with its other desirable traits such as tolerance to cold, drought, leaf rust and stripe rust, made it a good source for wheat breeding. In our preliminary test, "Lantian 9" showed a typical adult resistance phenotype (susceptible at seedling stage but high resistance at adult stage) to most of the major Chinese leaf rust pathotypes. To clarify the adult-plant resistance (APR) genes in this cultivar, a population with 197 F<sub>2:3</sub> lines was generated by crossing "Lantian 9" with susceptible line "Huixian Hong". The population was phenotyped in the field over three years (year 2012, 2013 and 2014) by a mixture of three leaf rust pathotypes (THTT, THTS and THTQ). A total of 1232 simple sequence repeat (SSR) markers were used to screen the parental lines. Polymorphic ones were further applied on the population. Linkage mapping analysis showed that one QTL from "Lantian 9" was located on chromosome 2BS, which was relative stable among the data from the year 2012 and 2013 with phenotypic variations of 6.0% and 9.1%, respectively. Three other QTLs from "Lantian 9" on chromosome 4BS, 3A and 1BL were detected. We also identified one QTL from "Huixian Hong" on chromosome 1BL. All these identified wheat leaf rust resistance QTLs with their closely linked molecular markers will greatly facilitate genetic improvement of wheat resistance to leaf rust in China.

**Key words:** Wheat, Leaf rust, Resistance gene, QTL mapping.

### Introduction

Wheat leaf rust, caused by *Puccinia triticina* (*Pt*), is widely distributed at wheat cultivation area around the world (Line & Chen, 1995). Leaf rust is a historically severe diseases in northern China with eight epidemics reported since 1980. A recent epidemic of leaf rust in five provinces of China including Gansu, Sichuan, Shanxi, Henan and Anhui resulted in significant yield losses (Zhou *et al.*, 2013). Development and deployment of wheat resistance cultivars is still the most efficient, economic and eco-friendly way to control this disease.

There are two types of resistance to leaf rust in wheat. The former is race-specific resistance conferred by a single resistance gene. The race-specific resistance is easy to loss due to rapidly merged virulent *Pt* races. The latter is adult-plant resistance (APR) or slow rusting resistance. APR is normally controlled by quantitative trait locus (QTL) and shows resistance only at adult-plant stage, which is also known as "field resistance" (Das *et al.*, 1992. Ribeiro *et al.*, 2001). Genetic study on QTLs is relative difficult due to the phenotypic variation caused by environmental changes. Compared with race-specific resistance, APR showed reduction of selection pressure on pathogen and more durable resistance (Masood *et al.*, 2005). At present, more than 100 wheat leaf rust resistance genes for both race-specific and APR have been reported, with 72 of them officially named (Herrera-Foessel *et al.*, 2014). In China, wheat cultivars carrying race-specific resistance genes *Lr9*, *Lr19*, *Lr24* and *Lr38* have been widely used, which lay a potential risk for future epidemics (Li *et al.*, 2010). Especially, wheat

cultivar "Pavon76" with multiple leaf rust resistance genes showed a good field resistance since 1976 (Wang *et al.*, 2006). There is an urgent need in China for the rational utilization of APR genes such as *Lr34* (Dyck, 1977), *Lr46* (Singh *et al.*, 1998), *Lr67* (Mcintosh *et al.*, 2011) and *Lr68* (Herrera-Foessel *et al.*, 2012), Rasheed *et al.* (2012). Further research on the identification of domestic wheat cultivars with APR will greatly facilitate wheat breeding program in China.

In our previous study, we've found Chinese wheat cultivar "Lantian 9" showed a resistance phenotype to most of the major Chinese leaf rust pathotypes at adult plant stage (Li *et al.*, 2010). Together with its other desirable traits such as high yield, tolerance to cold, drought, leaf rust and stripe rust, made it a good source for wheat breeding (Qamar *et al.* 2014). Our identification of wheat leaf rust resistance QTLs and their closely linked molecular markers will greatly facilitate genetic improvement of wheat cultivars in China.

### Materials and Methods

**Plant materials and *Puccinia triticina* (*Pt*) pathotypes:** A population with 197 F<sub>2:3</sub> lines derived from a crossing of wheat cultivar "Lantian 9" with "Huixian Hong" was generated for mapping the leaf rust resistance QTLs. "Lantian 9" is susceptible to most of Chinese leaf rust pathotypes at seedling stage, but high resistance at adult stage. "Huixian Hong" is susceptible to some of Chinese *Pt* pathotypes at both seedling and adult stages. The population was phenotyped by a mixture of three different *Pt* pathotypes (THTT, THTS and THTQ).

**Field trials:** The whole population and their parental lines were evaluated for leaf rust resistance for three consecutive years from 2012 to 2014 in the field at Baoding, Hebei, China. Field trials were raised in randomized complete blocks with two replicates. Each plot consisted of a single 1.5 m row with 30 cm distance between rows. Every tenth row was separated with a highly susceptible line “Zhengzhou 5389”, which was served as susceptible control and would aid the spread of spores within trial. Additional rows of “Zhengzhou 5389” were planted perpendicular and adjacent to the test rows. Epidemic was initiated each year by inoculating susceptible control at tillering stage using 0.05% Tween solution with urediniospores from *Pt* pathotypes THTT, THTS and THTQ. Disease severity was scored for 2 or 3 times each year at weekly intervals from 4 weeks post inoculation according to the modified Cobb scale (Peterson *et al.*, 1948). When the susceptible controls reached the highest disease severity, the percentage of leaf infection area for each line was recorded as Final disease severity (FDS).

**Map construction and QTL analysis:** A total of 1232 simple sequence repeat (SSR) markers were used to screen the parental lines. Polymorphic ones were further applied on the population. Phenotypic and genotypic data were combined together to map the adult plant resistance QTLs in “Lantian 9” and “Huixian Hong” using software Map Manager QTXb20 (Manly *et al.*, 2001). Genetic distances between markers were estimated using Kosambi mapping function (Kosambi, 1944). QTL analysis was conducted using ICIM-ADD function of the software QTL IciMapping 3.1 (Li *et al.*, 2007). A logarithm of odds (LOD) threshold of 2.5, calculated from 1,000 permutations at a probability of 0.05, was used for declaring the definitive QTL.

## Results

**Phenotypic evaluation:** Continuous distribution was observed in the distribution frequency of FDS data from 197  $F_{2:3}$  lines in each of the three years (Fig. 1), which indicated a polygenic inheritance controlling the resistance phenotype. The FDS of the  $F_{2:3}$  lines ranged from 5% to 100% over the three years, and the mean of which was 48.9%, 51.5% and 59.1%, respectively. For the two parental lines, mean FDS of “Lantian 9” was 5%-10% over the three years, whereas “Huixian Hong” was 80%-100% (Fig. 1).

**Construction of linkage maps:** A total of 1232 SSR markers were screened between parental lines “Lantian 9” and “Huixian Hong”, and 208 (16.9%) of them showed polymorphism. The latter were used to genotype individual  $F_{2:3}$  lines, and 91 (7.4%) of them showed linkage with the phenotype.

**QTL for leaf rust resistance:** Phenotypic and genotypic data of the  $F_{2:3}$  lines were combined together to map the adult plant resistance QTLs (Table. 1). Five QTLs from “Lantian 9” were detected in the population. QTL *QLr.hbau-2BS* from “Lantian 9” on chromosome 2BS was very stable among the data from the Year 2012 and 2013 with phenotypic variations of 6.0% and 9.1%, respectively (Fig. 2). Another QTL *QLr.hbau-1BL.2* on chromosome 1BL was consisted over two years of 2013 and 2014 with

phenotypic variations of 7.7% and 10.7%, respectively (Fig. 3). Other three QTLs from “Lantian 9” were only identified in one year, including *QLr.hbau-1BL.1* on chromosome 1B in year 2012 (Fig. 3), *QLr.hbau-4BS* on chromosome 4B in year 2012 (Fig. 4) and *QLr.hbau-3A* on chromosome 3A in year 2012 (Fig. 5) with phenotypic variation of 11.7%, 8.5% and 5.6%, respectively. We also observed one QTL *QLr.hbau-1BL.1* from “Huixian Hong” on chromosome 1BL in year 2012 (Table. 1).

Our results confirmed that leaf rust resistance of the “Lantian 9”×“Huixian Hong” population was controlled by QTLs. In year 2012, four leaf rust resistance QTLs were detected and the total phenotypic variation was 31.8%. Two QTL was detected in year 2013 with phenotypic variation of 16.8% and one QTL in year 2014 with phenotypic variation of 10.7%.

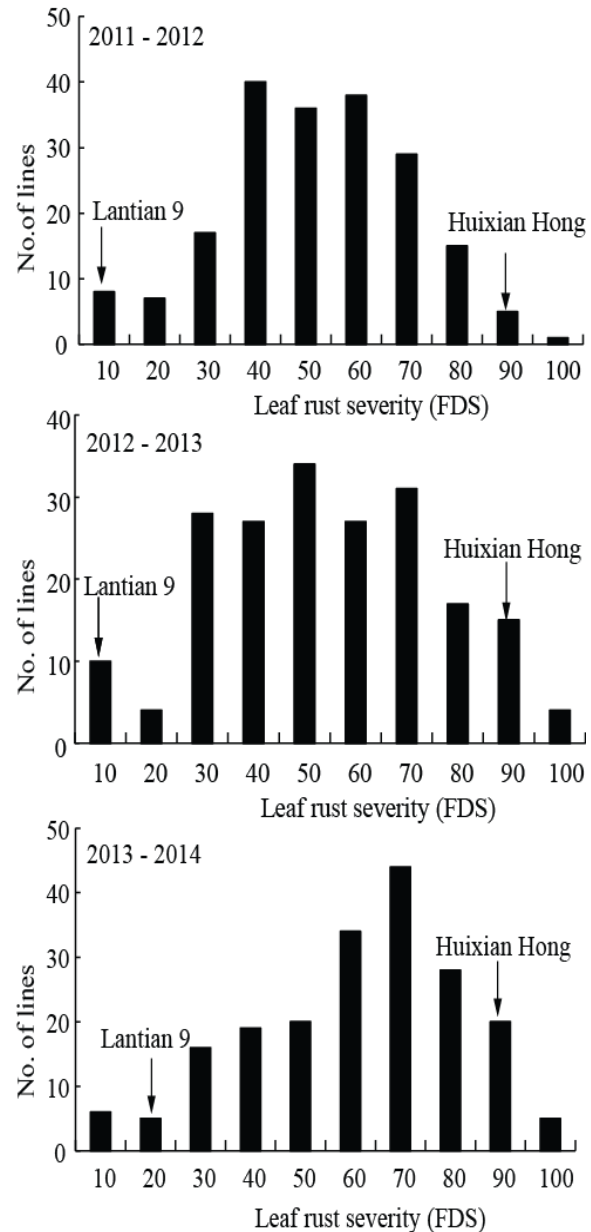


Fig. 1. Distribution for FDS in the “Lantian 9”×“Huixian Hong”  $F_{2:3}$  lines in three years.

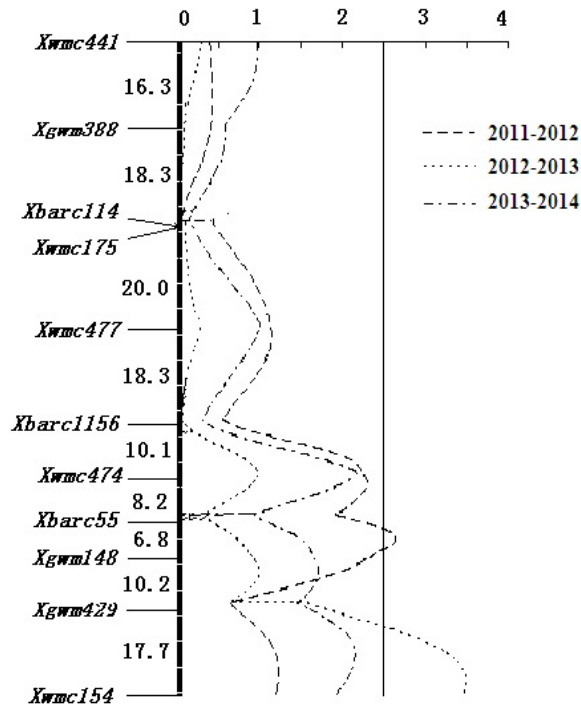


Fig. 2. The positions of the quantitative trait loci (QTLs) conferring adult-plant resistance to leaf rust on 2B.

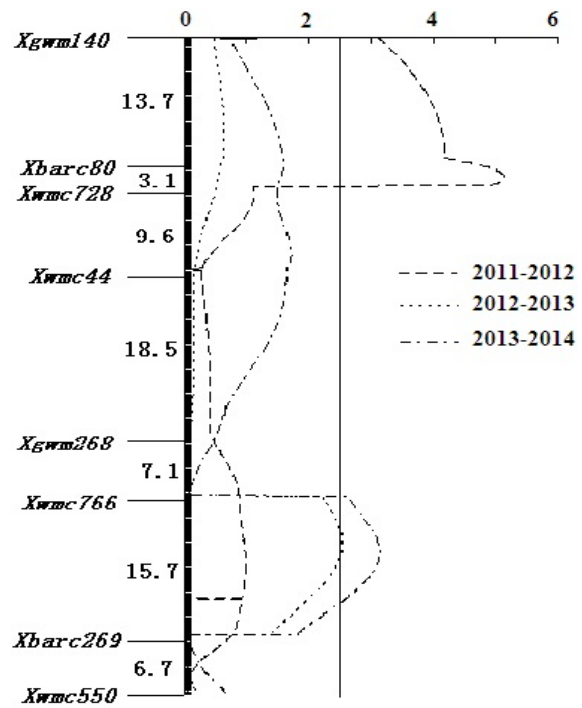


Fig. 3. The positions of the quantitative trait loci (QTLs) conferring adult-plant resistance to leaf rust on 1B.

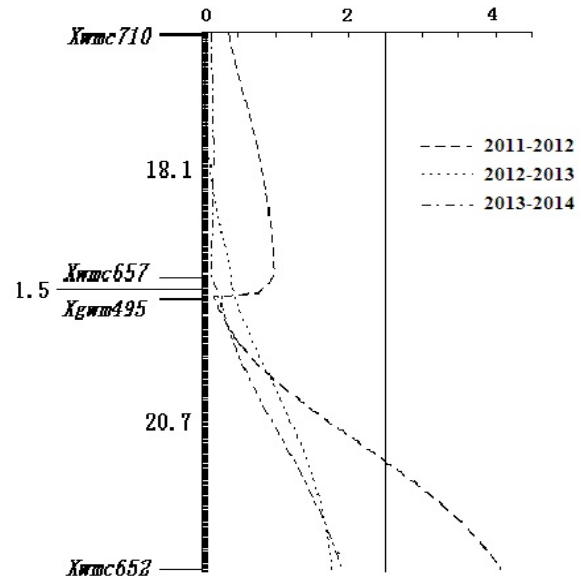


Fig. 4. The positions of the quantitative trait loci (QTLs) conferring adult-plant resistance to leaf rust on 4B.

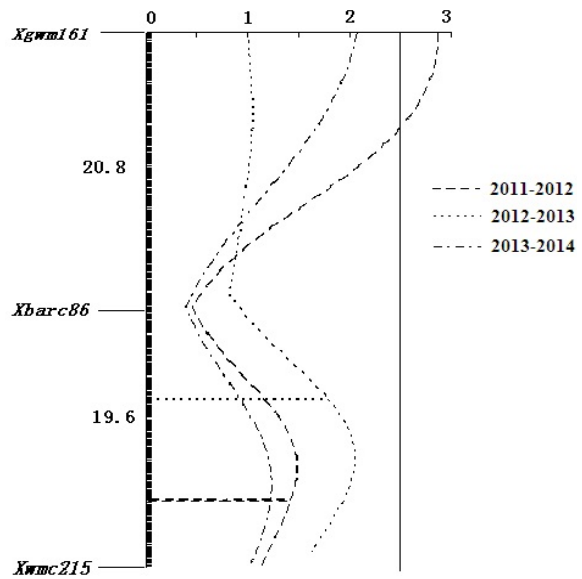


Fig. 5. The positions of the quantitative trait loci (QTLs) conferring adult-plant resistance to leaf rust on 3A.

Table 1. FDS-based composite interval mapping analysis of QTLs.

Year	QTL	Marker interval	Position	LOD	PVE (%)	Add	Dom
2012	<i>QLr.hbau-2BS</i>	<i>Xbarc55-Xgwm148</i>	96.0	2.6	6.0	-6.1	3.03
	<i>QLr.hbau-1BL.1</i>	<i>Xbarc80-Xwmc728</i>	16.0	5.1	11.7	6.5	-6.5
	<i>QLr.hbau-4BS</i>	<i>Xgwm495-Xwmc652</i>	40.0	4.0	8.5	-5.5	6.2
	<i>QLr.hbau-3A</i>	<i>Xgwm161-Xbarc86</i>	0.0	2.8	5.6	-3.1	6.6
2013	<i>QLr.hbau-2BS</i>	<i>Xgwm429-Xwmc154</i>	123.0	3.5	9.1	-8.7	3.4
	<i>QLr.hbau-1BL.2</i>	<i>Xwmc766-Xbarc269</i>	57.0	2.5	7.7	-1.0	-13.0
2014	<i>QLr.hbau-1BL.2</i>	<i>Xwmc766-Xbarc269</i>	58.0	3.1	10.7	-1.1	-14.9

## Discussion

Adult plant resistance (APR) or slow rusting normally retard rust development and save plant from significant yield loss. APR also showed a reduction of selection pressure on the pathogen side, which made this type of resistance more durable. Epidemiologically, APR has lower infection rate, longer incubation period, smaller uredinia size and fewer spores/uredinia (Caldwell, 1968). Genetic studies have shown that APR is a quantitative character and is normally controlled by several genes (Johnson & Law, 1973; Bjarko & Line, 1988; Das *et al.*, 1992). Designated APR genes, such as *Lr34/Yr18/Pm38/Sr57*, *Lr46/Yr29/Pm39/Sr58*, *Lr67/Yr46/Pm46/Sr55* and *Lr68*, normally showed a broad resistance to multiple pathogens (Li *et al.*, 2014). With APR to leaf rust and stripe rust, “Lantian 9” may have the potential resistance to wheat stem rust and powdery mildew. In terms of the QTL stability, QTL with greater and stable effects can be easily detected in different environment. QTL with minor effect is influenced by genetic background and prevalent external environment. Consequently, some QTLs are hard to be detected under certain environmental conditions. The QTLs detected in “Lantian 9” over the three years followed this phenomenon.

The current study detected a stable QTL on chromosomes 2B, *QLr.hbau-2BS*, from the resistance parental line “Lantian 9”. So far, designated adult-plant leaf rust resistance genes on chromosomes 2B were *Lr13*, *Lr35* and *Lr48*. According to the pedigree, “Lantian 9” was a common wheat cultivar without any exogenous modification, whereas *Lr35* was derived from *Aegilops*. *Lr13* and *Lr48* showed an adult-plant major resistance phenotype, whereas the phenotypic variation of *QLr.hbau-2BS* is minor. We speculated *QLr.hbau-2BS* as a minor resistance gene, but the relationship between this QTL and these two *Lr* genes required further study.

Two QTLs were detected on chromosome 1BL. QTL *QLr.hbau-1BL1* from the susceptible parental line “Huixian Hong” was located at the end of chromosome 1BL, which was also the location of designated APR gene *Lr46*. *Lr46* is widely used in CIMMYT wheat cultivars (Singh *et al.*, 2005), and has showed leaf rust resistance for 30 years (William *et al.*, 2006). Wheat cultivar “Huixian Hong” has been widely used as susceptible control for both stripe rust and leaf rust in China. The resistance effect of *QLr.hbau-1BL1* was relative small with phenotypic variation of 11.7%. This result is supported well by the idea that susceptible cultivars may also carry minor resistance genes during hybridizing desirable genotypes from other cultivars. The other QTL *QLr.hbau-1BL2* was located on a novel region of chromosome 1BL without any designated APR genes.

In our study, QTL *QLr.hbau-4BS* was located at the similar region of adult-plant major resistance gene *Lr49*. Although *QLr.hbau-4BS* was a minor resistance gene with relative low phenotypic variation, the relationship between *QLr.hbau-4BS* and *Lr49* required further validation. QTL *QLr.hbau-3A* was located on chromosome 3A near two previously reported leaf rust resistance QTLs *QLr.ubo-3A* and *QLr.sfrf-3AL*.

Although APR is much more durable than race-specific resistance, out of all the 72 officially named leaf rust resistance genes, only four of them belongs to this type. Therefore, there is an urgent need for identification of novel leaf rust resistance QTLs. Summarized APR QTLs for leaf rust, stripe rust and powdery mildew have been summarized to facilitate further research on the genetic improvement of wheat (Li *et al.*, 2014).

With several beneficial effects of APR, pyramiding and rational utilizing of wheat cultivars carrying these genes can prevent our field from multiple diseases at the same time. In our study, we have marked five leaf rust resistance QTLs from wheat cultivar “Lantian 9”, and discovered their tightly linked molecular markers, which could be used for both marker-assisted selection (MAS) breeding program and future QTL cloning research.

## Acknowledgments

This study was supported by National Natural Science Foundation (International/Regional Cooperation and Exchange Program) (No. 31361140367), the Joint Specialized Research Fund for the Doctoral Program of Higher Education (No. 20131302120004), the State Key Laboratory for Biology of Plant Disease and Insect Pests Open Project (No. SKLOF201513, SKLOF201606) and Science and Technology Program for Abroad Study in 2015 (No. C2015003033).

## References

- Bjarko, M.E. and R.F. Line. 1988. Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. *Phytopathology*, 78: 457-461.
- Caldwell, R.M. 1968. Breeding for general and/or specific plant disease resistance. In: *Proceedings of the Third International Wheat Genetics Symposium*. (Eds.): Finlay, K.W. and K.W. Shepherd. Canberra, Australia: Australian Academy of Sciences, 263-272.
- Das, M.K., S. Rajaram, C.C. Mundt, W.E. Kronstad and R.P. Singh. 1992. Inheritance of slow rusting resistance to leaf rust in wheat. *Crop Sci.*, 32: 1452-1456.
- Dyck, P.L. 1977. Genetics of leaf rust reaction in three introductions of common wheat. *Can. J. Genet. Cytol.*, 19: 711-716.
- Herrera-Foessel, S.A., J. Huerta-Espino, V. Calvo-Salazar, C.X. Lan and R.P. Singh. 2014. *Lr72* confers resistance to leaf rust in durum wheat cultivar Atil C2000. *Plant Disease*, 98: 631-635.
- Herrera-Foessel, S.A., R.P. Singh, J. Huerta-Espino, G.M. Rosewarne, S.K. Periyannan, L. Viccars, V. Calvo-Salazar, C. Lan and E.S. Lagudah. 2012. *Lr68*: a new gene conferring slow rusting resistance to leaf rust in wheat. *Theor. Appl. Genet.*, 124: 1475-1486.
- Johnson, R. and C.N. Law. 1973. Cytogenetic studies in the resistance of the wheat variety Bersée to *Puccinia striiformis*. *Cereal Rusts Bulletin*, 1: 38-43.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Annual Eugen.*, 12: 172-175.
- Li, H.H., G.Y. Ye and J.K. Wang. 2007. A modified algorithm for the improvement of composite interval mapping. *Genetics*, 175(1): 361-374.
- Li, Z.F., C.X. Lan, Z.H. He, R.P. Singh, G.M. Rosewarne, X.M. Chen and X.C. Xia. 2014. Overview and Application of QTL for Adult Plant Resistance to Leaf Rust and Powdery Mildew in Wheat. *Crop Sci.*, 54(5): 1907-1925.

- Li, Z.F., X.C. Xia, Z.H. He, X. Li, L.J. Zhang, H.Y. Wang, Q.F. Meng, W.X. Yang, G.Q. Li and D.Q. Liu. 2010. Seedling and Slow Rusting Resistance to Leaf Rust in Chinese Wheat Cultivars. *Plant Disease*, 94(1): 45-53.
- Line, R.F. and X.M. Chen. 1995. Success in breeding for and managing durable resistance to wheat rusts. *Plant Disease*, 79: 1254-1255.
- Manly, K.F., R.H. Cudmore and J.M. Meer. 2001. Map manager QTX, cross-platform software for genetic mapping. *Genome*, 12: 930-932.
- Masood, S., Y. Seiji, Z.K. Shinwari and R. Anwar. 2005. Mapping quantitative trait loci (QTLs) for salt tolerance in rice (*Oryza sativa*) using RFLPs. *Pak. J. Bot.*, 36(4): 825-834.
- Mcintosh, R.A., J. Dubcovsky, W.J. Rogers, C.F. Morris, R. Appels and X.C. Xia. 2011. Catalogue of gene symbols for wheat: 2011 supplement. *Annual Wheat Newsletter*, 57: 303-321.
- Peterson, R.F., A.B. Campbell and A.E. Hannah. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Can. J. Res.*, 26(5): 496-500.
- Qamar, M., S.D. Ahmad, M.A. Rabbani, Z.K. Shinwari and M. Iqbal. 2014. Determination of rust resistance genes in Pakistani bread wheats. *Pak. J. Bot.*, 46(2): 613-617.
- Rasheed A., A.S. Mumtaz and Z.K. Shinwari. 2012. Genetic characterization of novel *Lr* gene stack in spring wheat variety Chakwal86 and its effectiveness against leaf rust in rain fed areas of Pakistan. *Pak. J. Bot.*, 44(2): 507-510.
- Ribeiro, D.O., F.X. Vale, J.E. Parlevliet and L. Zambolim. 2001. Concepts in plantdisease resistance. *Fitopatologia Brasileira*, 26: 577-589.
- Singh, R.P., J. Huerta-Espino and H.M. William. 2005. Genetics and breeding for durable resistance to leaf and stripe rusts in wheat. *Turk. J. Agric. For.*, 29: 121-127.
- Singh, R.P., A. Mujeeb-Kazi and J. Huerta-Espino. 1998. *Lr46*: A gene conferring slow-rusting resistance to leaf rust in wheat. *Phytopathology*, 88: 890-894.
- Wang, Z.L., S.D. Liu, H. Wang, Z.H. He, X.C. Xia, X.M. Chen, X.Y. Duan and Y.L. Zhou. 2006. Advances of study on adult-plant resistance in bread wheat. *J. Triticeae Crops*, 26: 129-134.
- William, H.M., R.P. Singh, J. Huerta-Espino, G. Palacios and K. Suenaga. 2006. Characterization of genetic loci conferring adult plant resistance to leaf rust and stripe rust in spring wheat. *Genome*, 49(8): 977-990.
- Zhou, H.X., X.C. Xia, Z.H. He, X. Li, C.F. Wang, Z.F. Li and D.Q. Liu. 2013. Molecular mapping of leaf rust resistance gene *LrNJ97* in Chinese wheat line Neijiang 977671. *Theor. Appl. Genet.*, 126(8): 2141-2147.

(Received for publication 18 May 2015)