

QTL mapping for the resistance to yellow rust race CYR34 in triticale

Fangyuan Zhao¹, Dongmei Li¹, Xinhui Tian¹, and Wenhua Du^{1*}

¹Gansu Agricultural University, College of Grassland Science, 730070, Lanzhou, China.

*Corresponding author (duwh@gsau.edu.cn).

Received: 3 January 2020; Accepted: 24 April 2020; doi:10.4067/S0718-58392020000300405

ABSTRACT

Yellow rust is an important destructive fungal disease caused by *Puccinia striiformis* in small grain cereals, and the prevalent Chinese yellow race CYR34 has recently become widespread in China. To detect quantitative trait loci (QTLs) responsible for resistance to CYR34 in triticale (*xTriticosecale* Wittm.), 520 F₂ plants derived from the cross between cv. Gannong No. 1 (susceptible parent) and cv. Gannong No. 2 (resistant parent) were used as mapping population. Fourteen inter-simple sequence repeat (ISSR) markers were used for constructing the linkage map. The obtained results indicated that 92 loci have been mapped on seven linkage groups (LG1-LG7). The total map length was 542.9 cM with an average of 6.95 cM per marker. Six QTLs (*qdr1*, *qdr3*, *qdr4*, *qdr5-1*, *qdr5-2*, and *qdr6*) related to the resistance to CYR34 have been detected. The contribution of these QTLs varied from 5.1% to 11.2%. Moreover, *qdr5-1* was the main QTL responsible for CYR34 resistance.

Key words: Genetic linkage map, inter-simple sequence repeat marker, quantitative trait loci, *xTriticosecale*, yellow rust race CYR34.

INTRODUCTION

Triticale (*xTriticosecale* Wittm.), a man-made wheat (*Triticum aestivum* L.)-rye (*Secale cereale* L.) hybrid, is considered a promising crop because of its high genetic variation for several agronomically important traits (Alheit et al., 2014). It has been developed into a multi-purpose grain-forage species as a substitute for winter wheat, and can be used as a winter pasture crop followed by a grain crop (Baron et al., 2015). There is an increasing interest in triticale cultivation because it can adapt better to adverse environmental conditions and use nutrients more efficiently than wheat (González et al., 2005). Triticale is an excellent forage crop because of its high biomass, easily digestible protein, and good palatability (Li et al., 2016). Because of its strong cold resistance, triticale may be broadly grown in the alpine pastoral areas of the Qinghai-Tibet Plateau, China (Song et al., 2016).

Yellow (stripe) rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most destructive diseases of triticale in this region. In recent years, the race CYR34 exhibited the most extensive distribution and the highest prevalence in China (Huang et al., 2019). It was firstly detected on the wheat 'Chuanmai 42' and then spread to different areas in 65 counties of 9 provinces of China in 2015. Liu et al. (2017) reported that CYR34 race is becoming the predominant yellow race national wide. Infected plants exhibit reduction in plant height, biomass and forage quality (Song et al., 2016). Fungicides may be used to prevent yellow rust, but their application is costly, creates health problems for users, and adversely affects the environment (Chen, 2005; Chedli et al., 2018). Thus, developing new resistant varieties to yellow rust represents the most effective and safe method for sustainable triticale cultivation in this region.

Conventional breeding methods are the most popular to develop new varieties, but they have some disadvantages including the considerable time required, the high cost, the low selection efficiency as well as the selection of target traits

is affected easily by the environment. Compared with the conventional breeding, molecular breeding by using quantitative trait loci (QTL) mapping and marker-assisted selection (MAS), appears to be more effective and precise (Khan, 2015). The application of molecular markers offers numerous advantages over conventional phenotype-based options as these markers are stable and detectable in all tissues regardless of growth, differentiation, development, or defence status of cells. Moreover, the markers are not confounded by the environment or pleiotropic and epistatic effects (Agarwal et al., 2008). Therefore, combining conventional breeding and marker-assisted selection breeding may accelerate the development of a new variety and increase the breeding efficiency (Kujur et al., 2013).

A genetic map is a linear permutation graph of the relative positions of target genes or certain DNA sequences on the chromosome, and represents the basis for the preliminary determination of important plant traits (Rédei, 2008). Molecular markers used for mapping should have the following advantages: they are polymorphic and evenly distributed throughout the genome, their application requires only small amounts of tissue and DNA samples, no prior information about the genome is required, and the associated method should be simple, quick, and inexpensive (Agarwal et al., 2008).

Inter-simple sequence repeat (ISSR) markers are highly polymorphic DNA markers and beneficial in studying the genetic diversity, gene tagging and genome mapping (Reddy et al., 2002). Therefore, ISSR markers are favourable for constructing genetic linkage maps (Poudineh et al., 2018). Since the first constructed a genetic map of wheat (Kojima et al., 1998), ISSR markers have been used to construct the genetic map of several plant species.

Several F_2 mapping populations were developed to map QTLs and genes responsible for disease resistance in different crops. Barakat et al. (2010) developed F_2 mapping population in maize (*Zea mays* L.) and identified ISSR makers linked to northern corn leaf blight resistance *Ht1* gene. Wang et al. (2014) identified and mapped the leaf rust resistance gene in wheat line 5R618 by using F_2 mapping population and $F_{2:3}$ families between 5R618 and the susceptible wheat 'Zhengzhou 5389'. Takagi et al. (2013) identified QTLs for partial resistance to rice blast disease and seedling vigour in recombinant inbred lines and F_2 populations of rice using QTL-seq. Studies on the location of genes mediating yellow rust resistance have mainly focused on staple crops, the resistance to yellow rust race 'Warrior' in triticale has been reported (Losert et al., 2017), and there were no studies on triticale QTLs for resistance to yellow rust race CYR34.

Therefore, the objectives of this work were to construct a genetic linkage map of triticale, and to locate yellow rust resistant QTLs using F_2 mapping population. The identified QTL region with associated markers can be potentially for marker-assisted selection in the future for triticale breeding programs.

MATERIALS AND METHODS

Field conditions

A field experiment was conducted at the farm of the Lintao Agricultural College (35°37' N, 103°87' E; 1980 m a.s.l.), Gansu Province, China. The annual rainfall (562 mm) occurs predominantly in July, August, and September in this region, and the average temperature is 7.0 °C. The frost-free period is 153 d. Haplic Kastanozems soil dominates this region, and the soil fertility is uniform. The previous crop was maize (*Zea mays* L.)

Experimental materials and sampling

A total of 520 plants of F_2 population derived from a cross between \times *Triticosecale* Wittm. 'Gannong No. 1' (Chen et al., 2017) as female parent and 'Gannong No. 2' (Zhao et al., 2019) as pollen donor parent. Both parents were hexaploid. 'Gannong No. 1' was susceptible to CYR34 race, while 'Gannong No. 2' was resistant. All seeds were sown in single-row plots that were 1 m long, spaced at a distance of 20 cm, with plants separated by 10 cm, and a sowing depth of 3-4 cm. Fertilizer was applied according to the standard field practices in this region. Individual plants were marked using chopsticks at 20 d after emergence, then leaves of individual plants and both parents were collected (approximately 2 g). The samples were stored at -80 °C for a subsequent DNA extraction.

Inoculation of the rust fungus and disease evaluation

After collecting samples, artificial inoculations were conducted with race CYR34 produced by the Institute of Plant Protection, Gansu Academy of Agricultural Science, China, at the three-leaf stage. The inoculation solution was prepared by adding 1 L distilled water, 2 g fresh urediniospores of rust, and 4 drops of Tween 20 into a beaker. The solution was

thoroughly mixed, and the leaves of each plant were evenly sprayed with the suspension using a hand-held atomiser, after that the plants were covered with a plastic bag for 15 h to maintain humid conditions (Dakouri et al., 2013). Plants were frequently irrigated to provide favourable conditions for rust development. Disease reactions were scored according to the published methods (Line and Qayoum, 1992). The severity of the yellow rust infection was evaluated on a scale from 0 to 9, where 0 refers to completely healthy (non-infected plants), while 9 refers to completely infected plants. On the basis of the reaction of plants to yellow rust, all 520 F₂ plants were used for constructing the genetic linkage map.

DNA isolation and ISSR-PCR amplification

Total genomic DNA was isolated from leaf samples of 520 F₂ plants and both parents using the modified cetyltrimethylammonium bromide (CTAB) method (Allen et al., 2006). Total genomic DNA was purified, then quantified on 0.8% agarose gel electrophoresis and verified by UV-spectrophotometry. High-quality isolated DNA was stored at -20 °C.

DNA amplification was carried out using 14 ISSR primers screened in a previous study (Zhao, 2015) (Table 1). The primers were artificially combined by the Shanghai Biotech Bioengineering (Shanghai, China). The PCR reaction was conducted in 20 µL reaction mixture containing 50 ng template DNA, 0.65 µmol L⁻¹ primer, 1.9 mmol L⁻¹ Mg²⁺, 2 U Taq DNA polymerase and 0.2 mmol L⁻¹ dNTPs. All of MgCl₂, dNTPs and TaqDNA polymerase were purchased from Thermo Scientific (Waltham, Massachusetts, USA). PCR amplifications were carried out on an Applied Biosystems Veriti 96-well Thermal Cycler (Thermo Scientific) under the following conditions: one cycle of 94 °C for 5 min for primary denaturation; followed by 40 amplification cycles of 94 °C for 30 s for denaturation, 30 s for primer annealing, 1 min at 72 °C for extension; followed by 7 min at 72 °C for final extension. The PCR products were resolved by electrophoresis on 2% agarose gel, which was conducted using 1× TBE buffer over 90 min at 80 V. The amplified DNA fragments were visualized using a NuGenius gel documentation system (Bio-Rad, Irvine, California, USA).

Linkage map and QTL analysis

The statistical analysis of the phenotypic results was conducted using Statistica Software version 8.0 (StatSoft, Tulsa, Oklahoma, USA). The normal distribution of scores was verified by the Shapiro-Wilk test to validate the use of parametric tests. The effect of tested variable/variables was examined by a single-factor ANOVA. A *post hoc* comparison was conducted using Duncan's multiple range test ($P \leq 0.05$).

Genetic linkage map was constructed using JoinMap 4.0 software (Kyazma B.V., Wageningen, Netherlands) with the polymorphic ISSR markers between the parents. The recombination frequency was converted into genetic map distance (centiMorgan, cM) using the Kosambi mapping function. QTLs responsible for resistance to CYR34 race were identified with composite interval mapping using Windows QTL Cartographer (version 2.5) (Wang et al., 2007). The relationship between the segregation of a single marker and the studied trait was analysed using the Kruskal-Wallis test of the MapQTL 5.0 package (Van Ooijen, 2004). Threshold logarithm of odds (LOD) scores were calculated with 1000 permutations. The percentage of phenotypic variation was calculated with a single factor regression (R²).

Table 1. Inter-simple sequence repeat (ISSR) primer names, sequences, annealing temperature and melting temperature (T_m) value for each primer.

Primer	Sequences* (5'→3')	Annealing temperature (°C)	T _m (°C)
UBC807	(AG) ₈ T	52.3	52.2
UBC808	(AG) ₈ C	56.2	54.6
UBC810	(GA) ₈ T	50.7	52.2
UBC815	(CT) ₈ G	57.3	54.6
UBC822	(TC) ₈ A	52.3	52.3
UBC825	(AC) ₈ T	56.2	54.0
UBC826	(AC) ₈ C	58.0	50.3
UBC834	(AT) ₈ YA	52.3	50.3
UBC835	(AT) ₈ YA	54.4	56.2
UBC847	(CA) ₈ RC	56.2	52.8
UBC849	(GT) ₈ YA	56.2	53.9
UBC857	(AC) ₈ YG	56.2	54.5
UBC860	(TG) ₈ RA	54.4	53.2
UBC873	(GACA) ₄	50.7	51.6

*Y: Pyrimidine; R: purines.

RESULTS

Development of F₂ segregating population

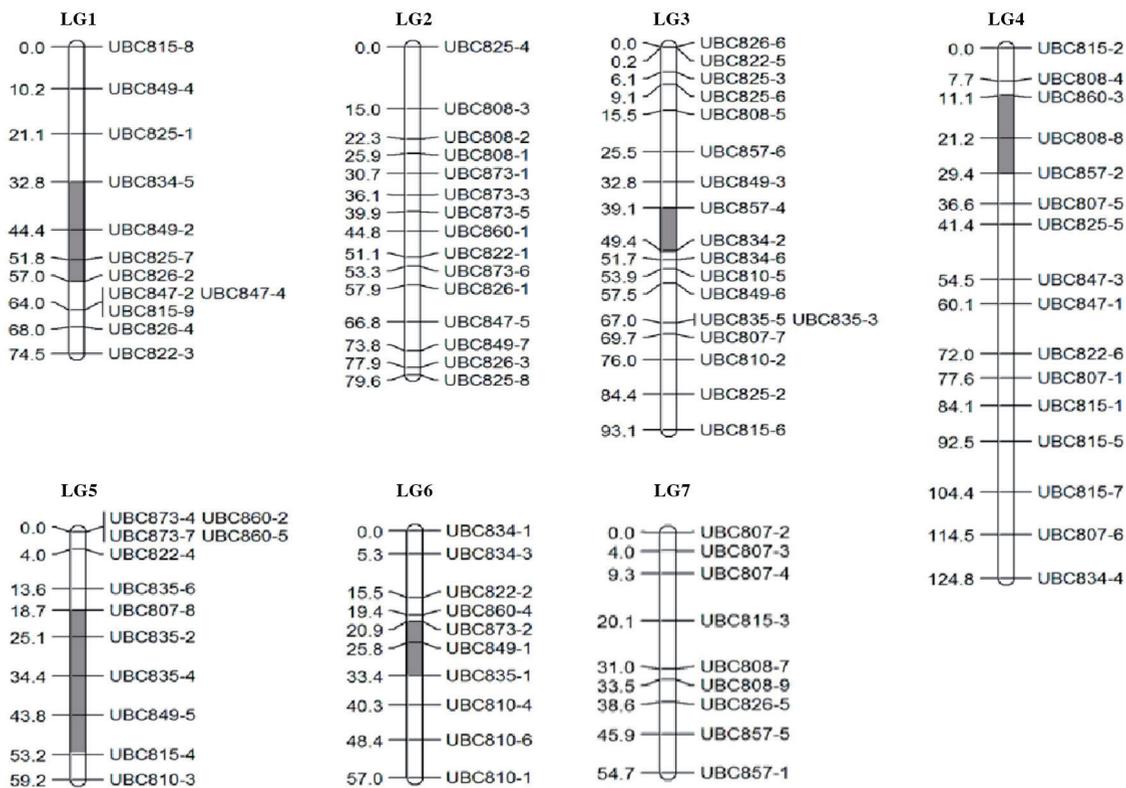
The two parental triticale varieties used for the crossing in the current study differ significantly in their resistance to CYR34 race. ‘Gannong No. 2’ is a resistant variety having score of 0 in the scale of 0-9 for disease resistance, while ‘Gannong No. 1’ is susceptible with score of 9. A population of 520 F₂ plants derived from the ‘Gannong No. 1’ × ‘Gannong No. 2’ cross was used. The yellow rust resistance score in the F₂ population ranged from 0 to 9 with an average of 4.46. The Z statistics was used to draw the frequency distribution curve using the disease resistance score and chi-square analysis was carried out to test the goodness of fit to a normal distribution.

Based on the phenotypic data, the results showed that the kurtosis of resistance and susceptibility to yellow rust of the F₂ population was 3, and the skewness was greater than 1. This result was consistent with a continuous normal distribution, and therefore, the population was suitable for constructing the triticale genetic linkage map.

Linkage map construction

The molecular data indicated that all 14 ISSR markers used in the current study generated polymorphic bands with polymorphism ratio of 100%. Genetic linkage map was constructed with the JoinMap 4.0 software, and the genetic distances were calculated using the Kosambi function. The map included 92 ISSR loci distributed on seven linkage groups (LG1-LG7), covering a genetic distance of 542.9 cM with an average of 6.95 cM per marker. Linkage group lengths ranged from 54.7 cM (LG7) to 124.8 cM (LG4). The number of markers per LG ranged from 9 (LG7) to 18 (LG3), and the number of intervals per LG varied from 8 (LG5 and LG7) to 16 (LG3). The average distance between markers on LGs ranged from 5.69 (LG2) to 8.32 cM (LG4) (Figure 1, Table 2).

Figure 1. Genetic linkage map and quantitative trait loci (QTLs) for the resistance to the yellow rust race CYR34 in the F₂ population of triticale based on an inter-simple sequence repeat (ISSR) marker.



The genetic linkage map of triticale was constructed by using JoinMap 4.0 software according to the field phenotypic data of 520 F₂ individuals, the map included 92 ISSR loci which distributed on 7 linkage groups (LG1 ~ LG7) and covered a genetic distance of 542.9 cM with an average of 6.95 cM per marker. Six QTLs (shadows in the figure) were distributed on five linkage groups (LG1, LG3, LG4, LG5 and LG6).

QTLs mapping

Six QTLs (*qdr1*, *qdr3*, *qdr4*, *qdr5-1*, *qdr5-2*, and *qdr6*) associated with yellow rust resistance, with an LOD score ≥ 2.0 , were identified (Table 3, Figure 1). These QTLs were distributed on five linkage groups (LG1, LG3, LG4, LG5, and LG6), and they all contributed positively to the yellow rust resistance in triticale. The LOD values varied from 2.09 to 4.74, and the additive genetic effects ranged from -0.18 to 0.27. One QTL (*qdr6*) was located on LG6 at LOD 2.85 explaining 6.9% of phenotypic variance. This QTL was flanked by both ISSR markers UBC873-2 and UBC835-1. Another QTL (*qdr1*) was identified on LG1 at LOD 3.34 explaining 8% of phenotypic variance and flanked by both ISSR markers UBC834-5 and UBC826-2. The major QTL for yellow rust resistance in triticale described in the current study was *qdr5-1*, which mapped on LG5 with LOD score of 4.74 explaining 11.2% of the phenotypic variance.

Table 2. Characteristics of seven linkage groups identified in triticale using inter-simple sequence repeat (ISSR) markers.

Linkage group	Number of markers	Density	Number of intervals	Intervals range	Average interval	Length
		Markers cM ⁻¹			cM	cM
LG1	12	0.16	9	4.0-11.7	8.28	74.5
LG2	15	0.19	14	1.7-15.0	5.69	79.6
LG3	18	0.19	16	0.2-10.3	5.82	93.1
LG4	16	0.13	15	3.4-13.1	8.32	124.8
LG5	12	0.20	8	4.0-9.6	7.40	59.2
LG6	10	0.18	9	1.5-10.2	6.33	57.0
LG7	9	0.16	8	2.5-10.9	6.84	54.7
Total	92	0.17	79	-	6.95	542.9

Table 3. Quantitative trait loci (QTLs) related to the resistance to yellow rust CYR34 in the F₂ population of triticale.

QTL	Linkage group	Marker interval	Position	LOD	Variance	Add
			cM		%	
<i>qdr1</i>	LG1	UBC834-5~UBC826-2	44.38	3.34	8.0	-0.17
<i>qdr3</i>	LG3	UBC857-4~UBC834-2	39.07	2.09	5.1	0.21
<i>qdr4</i>	LG4	UBC860-3~UBC857-2	21.24	2.79	6.7	0.27
<i>qdr5-1</i>	LG5	UBC807-8~UBC835-4	25.07	4.74	11.2	-0.18
<i>qdr5-2</i>	LG5	UBC835-4~UBC815-4	43.76	2.70	6.5	-0.16
<i>qdr6</i>	LG6	UBC873-2~UBC835-1	25.76	2.85	6.9	0.24

LOD: A logarithm of odds; Add: additive effect.

DISCUSSION

Mapping population

For some plant species, different segregating populations might be used to construct a linkage map, with some advantages and disadvantages for each population (McCouch and Doerge, 1995). The mapping population used in different experiments mainly depends on the study purpose and plant species (Singh and Singh, 2015).

The F₂ population derived from F₁ hybrids possessed a considerable amount of genetic information and could be generated relatively quickly, making it the simplest type of mapping population for self-pollinated species (Collard et al., 2005). Yuste-Lisbona et al. (2011) constructed a linkage map for melon (*Cucumis melo* L.) using the F₂ population resulting from the hybridization between the multi-resistant genotype TGR-1551 and the susceptible Spanish 'Bola de Oro'. Takagi et al. (2013) found that QTL-seq applied to seedling vigour in rice demonstrated that this method successfully identifies QTL in an F₂ generation, which is a much earlier generation than the F₇ one that we used for conventional QTL analysis based on RILs. Therefore, the F₂ population could be used to construct a linkage map for triticale as a self-pollinated plant species. Mohan et al. (1997) concluded that the size of a population was crucial for the resolution and accuracy of a linkage map, with the population size generally ranging from 50 to 250; however, high-resolution mapping requires a larger population (Ledesma-Ramírez et al., 2018).

To improve the accuracy of the preliminary genetic mapping of triticale, 520 F₂ individuals were used to construct a linkage map. If the constructed map is to be used for a QTL study, the mapping population must be phenotypically evaluated before the QTL mapping (Collard et al., 2005). The field evaluations of the yellow rust resistance in 520 F₂ individuals were completed before the linkage map was constructed. The revealed continued normal distribution meant this population was suitable for detecting QTLs.

Construction of the triticale genetic linkage map

A previous study (Ji et al., 2017) concluded that the parents used for a hybridization must exhibit major phenotypic differences for their offspring to have considerable variations, which were appropriate for the screening of polymorphic markers and constructing a linkage map. The current two parents were both released varieties with significant phenotypic differences regarding their reaction to yellow rust. Consequently, the resulting segregating population exhibited considerable variability in their reactions to the yellow rust race CYR34, and were useful for the construction of a preliminary genetic linkage map. The ideal genetic linkage map generally consists of many molecular markers that are evenly distributed and close together, with the same number of linkage groups and chromosomes (Niedziela et al., 2014).

Genetic linkage maps can be used to identify and locate the QTLs related to main agronomical traits, and transfer the target genes into specific plants (Pennington et al., 2016). To date, the construction of genetic maps is mainly concentrated in food crops, including wheat, rice, and maize, while the research on forage is relatively lagging, moreover, there are few studies on the forage crops like triticale (Tyrka et al., 2011; Niedziela et al., 2014). In general, linkage mapping requires that the average interval between markers is less than 20 cM (Gabay et al., 2018), the map of triticale constructed in our study, which contained seven linkage groups, with an average distance of 6.95 cM, it was close to the result of Tyrka et al. (2011) making it useful for locating QTLs. The future research should assess the association between these seven linkage groups and chromosomes A, B, and R. Moreover, the ISSR marker should be combined with other markers to construct high-quality genetic maps with high density.

QTLs mapping for resistance to the yellow rust race CYR34

QTL mapping approaches can be employed to dissect the genetic architecture underlying complex traits and to identify QTL for marker-assisted selection programs (Alheit et al., 2014). Using genome wide association mapping in triticale, Losert et al. (2017) identified 10 QTLs for resistance to the yellow rust pathogen race ‘Warrior’ on leaves, seven of them were responsible for ear resistance. The exotic ‘Warrior’ race has conquered Europe in recent years. However, in China, the yellow rust pathogen race CYR34 is an increasing threat to triticale, so it is imperative to against it effectively. The data presented herein are crucial for the breeding of new triticale varieties resistant to CYR34. However, only six QTLs associated with CYR34 were identified, which is fewer than the QTLs related to ‘Warrior’ detected by Losert et al. (2017). In contrast to wheat with its much broader germplasm pool, triticale breeding can currently only exploit the Yr loci available within this man-made crop. An additional future source for resistance is, therefore, the targeted introgression of effective resistance loci into triticale germplasm, either through the generation of novel primary triticale or through crosses with wheat. Thus, more QTLs related to CYR34 will need to be identified in future studies.

CONCLUSIONS

In this study, a triticale genetic linkage map was constructed, which included seven linkage groups (LG1-LG7). The total genetic distance for the map was 542.9 cM, which included 92 loci, with an average distance between markers of 6.95 cM. And six quantitative trait loci (QTLs) (*qdr1*, *qdr3*, *qdr4*, *qdr5-1*, *qdr5-2*, and *qdr6*) were detected related to triticale resistance to yellow rust race CYR34. These QTLs were distributed on five linkage groups, and *qdr5-1* contributed the most to the resistance to CYR34.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (31760702, 31360577), the National Key R&D Program of China (2018YFD0502402-3), major projects on the improvement and utilization of forage genotypes (XZ201901NA03).

REFERENCES

- Agarwal, M., Shrivastava, N., and Padh, H. 2008. Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports* 27:617-631.
- Alheit, K.V., Busemeyer, L., Liu, W., Maurer, H.P., Gowda, M., Hahn, V., et al. 2014. Multiple-line cross QTL mapping for biomass yield and plant height in triticale (*×Triticosecale* Wittmack). *Theoretical and Applied Genetics* 127:251-260.
- Allen, G.C., Floresvergara, M.A., Krasynanski, S., Kumar, S., and Thompson, W.F. 2006. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nature Protocols* 1:2320-2325.
- Barakat, M.N., El-Shafei, A.M., and Al-Doss, A. 2010. Molecular mapping of QTLs for resistance to northern corn leaf blight in maize. *Journal of Food, Agriculture and Environment* 8:547-552.
- Baron, V.S., Juskiw, P.E., and Aljarrah, M. 2015. Triticale as a forage. p. 189-212. In Eudes, F. (ed.) *Triticale*. Springer, Cham, Canada.
- Chedli, R.B.H., M'Barek, S.B., Yahyaoui, A., Kehel, Z., and Rezgui, S. 2018. Occurrence of *Septoria tritici* blotch (*Zymoseptoria tritici*) disease on durum wheat, triticale, and bread wheat in Northern Tunisia. *Chilean Journal of Agricultural Research* 78:559-568. doi:10.4067/S0718-58392018000400559.
- Chen, X.M. 2005. Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. *Canadian Journal of Plant Pathology* 27:314-337.
- Chen, L.X., Tian, X.H., and Du, W.H. 2017. Field studies on the drought resistance of *Triticale* wittmack 'Gannong No. 1'. *Acta Agrestia Sinica* 25:1079-1087.
- Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B., and Pang, E.C.K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* 142:169-196.
- Dakouri, A., Mccallum, B.D., Radovanovic, N., and Cloutier, S. 2013. Molecular and phenotypic characterization of seedling and adult plant leaf rust resistance in a world wheat collection. *Molecular Breeding* 32:663-677.
- Gabay, G., Dahan, Y., Izhaki, Y., Faigenboim, A., Ben-ari, G., and Elkind, Y. 2018. High-resolution genetic linkage map of European pear (*Pyrus communis*) and QTL fine-mapping of vegetative budbreak time. *BMC Plant Biology* 18:1-13.
- González, J.M., Muñoz, L.M., and Jouve, N. 2005. Mapping of QTLs for androgenetic response based on a molecular genetic map of *×Triticosecale* Wittmack. *Genome* 48:999-1009.
- Huang, L., Liu, T.G., Liu, B., Gao, L., Luo, P.G., and Chen, W.Q. 2019. Resistance evaluation of 197 Chinese wheat core germplasms to a new stripe rust race, CYR34. *Plant Protection* 45:148-154.
- Ji, G.S., Zhang, Q.J., Du, R.H., Lv, P., Ma, X., Fan, S., et al. 2017. Construction of a high-density genetic map using specific-locus amplified fragments in sorghum. *BMC Genomics* 18:51.
- Khan, M.A. 2015. Molecular breeding of rice for improved disease resistance, a review. *Australasian Plant Pathology* 44:273-282.
- Kojima, T., Nagaoka, T., Noda, K., and Ogihara, Y. 1998. Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. *Theoretical and Applied Genetics* 96:37-45.
- Kujur, A., Saxena, M.S., Bajaj, D., Laxmi, and Parida, S.K. 2013. Integrated genomics and molecular breeding approaches for dissecting the complex quantitative traits in crop plants. *Journal of Biosciences* 38:971-987.
- Ledesma-Ramírez, L., Solis-Moya, E., Ramírez-Pimentel, J.G., Dreisigacker, S., Huerta-Espino, J., Aguirre-Mancilla, C.L., and Mariscal-Amaro, L.A. 2018. Relationship between the number of partial resistance genes and the response to leaf rust in wheat genotypes. *Chilean Journal of Agricultural Research* 78:400-408. doi:10.4067/S0718-58392018000300400.
- Li, D.M., Tian, X.H., and Du, W.H. 2016. Study on the production performance of new forage triticale lines in irrigation area of Lintao, Gansu Province. *Grassland and Turf* 36:76-81.
- Line, R.F., and Qayoum, A. 1992. Virulence, aggressiveness, evolution and distribution of races of *Puccinia striiformis* (the cause of stripe of wheat) in North America, 1968-87. *USDA Technical Bulletin* 1788.
- Liu, B., Liu, T.G., Zhang, Z.Y., Jia, Q.Z., Wang, B.T., Gao, L., et al. 2017. Discovery and pathogenicity of CYR34, a new race of *Puccinia striiformis* f. sp. *Tritici* in China. *Acta Phytopathologica Sinica* 47:681-687.
- Losert, D., Maurer, H.P., Leiser, W.L., and Würschum, T. 2017. Defeating the Warrior: genetic architecture of triticale resistance against a novel aggressive yellow rust race. *Theoretical and Applied Genetics* 130:1-12.
- McCouch, S.R., and Doerge, R.W. 1995. QTL mapping in rice. *Theoretical and Applied Genetics* 11:482.
- Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R., et al. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding* 3:87-103.
- Niedziela, A., Bednarek, P.T., Labudda, M., Mankowski, D.R., and Aniol, A. 2014. Genetic mapping of a 7R Al tolerance QTL in triticale (*×Triticosecale* Wittmack). *Journal of Applied Genetics* 55:1-14.
- Pennington, H.G., Gheorghie, D.M., Damerum, A., Pliego, C., Spanu, P.D., and Cramer, R. 2016. Interactions between the powdery mildew effector BEC1054 and barley proteins identify candidate host targets. *Journal of Proteome Research* 15:826-839.
- Poudineh, Z., Fakheri, B.A., Sirosmehr, A.R., and Shojaei, S. 2018. Genetic and morphological diversity of fennel by using ISSR marker and biplot analysis. *Indian Journal of Plant Physiology* 23:564-572.

- Reddy, M.P., Sarla, N., and Siddiq, E.A. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128:9-17.
- Rédei, G.P. 2008. *Encyclopedia of genetics, genomics, proteomics and informatics*. Springer, Dordrecht, Netherlands.
- Singh, B.D., and Singh, A.K. 2015. *Marker-assisted plant breeding: Principles and practices*. Springer, New Delhi, India.
- Song, Q., Tian, X.H., and Du, W.H. 2016. Studies on production performance of new forage Triticale lines in alpine pastoral area of Gansu. *Pratacultural Science* 33:1367-1374.
- Takagi, H., Abe, A., Yoshida, K., Kosugi, S., Natsume, S., and Mitsuoka, C. 2013. QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *The Plant Journal* 74:174-183.
- Tyrka, M., Bednarek, P.T., Kilian, A., Wędzony, M., Hura, T., and Bauer, E. 2011. Genetic map of triticale compiling DArT, SSR, and AFLP markers. *Genome* 54:391.
- Van Ooijen, J.W. 2004. *MapQTL®5*. Software for the mapping of quantitative trait loci in experimental populations. Kyazma B.V., Wageningen, Netherland.
- Wang, S., Basten, C.J., and Zeng, Z.B. 2007. *Windows QTL cartographer 2.5*. Department of Statistics, North Carolina State University, Raleigh, North Carolina, USA. Available at <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>.
- Wang, J., Shi, L., Zhu, L., Li, X., and Liu, D. 2014. Genetic analysis and molecular mapping of leaf rust resistance genes in the wheat line 5R618. *Czech Journal of Genetics and Plant Breeding* 50:262-267.
- Yuste-Lisbona, F.J., Capel, C., Sarria, E., Torreblanca, R., Gómez-Guillamón, M.L., Capel, J., et al. 2011. Genetic linkage map of melon (*Cucumis melo* L.) and localization of a major QTL for powdery mildew resistance. *Molecular Breeding* 27:181-192.
- Zhao, Y.J. 2015. Studies on the genetic diversity of Triticale and productivity in Lintao region, Gansu province. 19-23 p. MSc thesis. Gansu Agricultural University, College of Grassland Science, Lanzhou, China.
- Zhao, F.Y., Wang, W., Chen, P., and Du, W.H. 2019. Studies on the production performance of triticale in Yunnan-Guizhou Plateau. *Grassland and Turf* 39:43-47+53.