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Identification, Validation of a SSR Marker and Marker Assisted Selection for the Goat Grass Derived Seedling Resistance Gene *Lr28* in Wheat

Pallavi JK[1], Anupam Singh[1], Usha Rao I[2] and Prabhu KV[3]*

[1]*National Phytotron Facility, Indian Agricultural Research Institute, New Delhi-110012, India*
[2]*Department of Botany, University of Delhi-110007, New Delhi, India*
[3]*Joint Director (Research), Directorate, Indian Agricultural Research Institute, India*

Abstract

The goat grass (*Aegilops speltoides*) derived seedling leaf rust resistance gene *Lr28* is effective in providing resistance against infection to leaf rust including its most virulent strain, 77-5 (121R63-1) of the pathogen. A polymorphic SSR marker specific to *Lr28* was identified by employing bulk segregant analysis on an F_2 population derived from the cross between PBW343-*Lr28*, a leaf rust resistant near isogenic line of the most cultivated variety PBW343 and CSP44-*Lr48*, the Australian cultivar Condor derived CSP44 line carrying the APR gene *Lr48*. The marker amplified a polymorphic fragment which was particular to the presence of the seedling resistance gene and it was mapped at a distance of 2.9 cM from the *Lr28* resistance locus on chromosome 4AL. It was also validated on a set of 42 NILs which carried other potent leaf rust resistance genes of diverse origin. Such a polymorphic codominant SSR marker will be useful in wheat breeding programmes to differentiate plants homozygous at the *Lr28* locus from those that are heterozygous.

Keywords: Microsatellite markers; Seedling leaf rust resistance; Bread wheat

Introduction

Leaf rust disease caused by the fungal pathogen *Puccinia triticina* syn. *P. recondita* Rob. Ex. Desm. f.sp. *tritici* Eriks. & E. Henn is a significant threat to the yield of wheat crop in all major wheat growing parts of the world. Reports of yield loss in wheat due to damage by leaf rust range from 30-50% [1]. Plant breeders utilize the model of transferring leaf rust resistance genes (*Lr* genes) into the host in order to confer it with genetic resistance. However, the pathogen has been able to throw up physiological races to cause virulence against the deployed *Lr* genes and convert the resistant variety into a susceptible one. Since it is expected that *Lr* genes sourced from wild relatives are likely to be more durable, several have been transferred into wheat from its wild relatives and many of these have been documented as located on different chromosomes [2,3]. The gene *Lr28* is one such gene transferred from *Aegilops speltoides*, which is assigned into bread wheat through a chromosomal translocation T4AS.4AL-7S #2S located on chromosome 4AL [2]. *Lr28* is an effective gene for resistance from seedling stage through the entire lifespan of wheat crop in most parts of the world including the South Asian wheat regions [4]. There are more than 60 *Lr* genes available with varying degrees of resistance of which many are indistinguishable from each other in their phenotypic expression. Molecular markers serve the purpose by detecting only those plants that carry the distinct genes. In breeding populations, the phenotypic expression of resistance would be identical in plants which are either heterozygous or homozygous at the resistance locus but distinction between these categories is essential since the latter only are desirable to be carried forward. Dominant molecular markers such as RAPD, SCAR or AFLP markers also do not serve that purpose. The currently available *Lr28* linked markers are only dominant type markers [5]. Though reported a null allelic SSR marker; it cannot be useful for direct selection. Such a marker could only be used for confirmation or zygosity determination in those plants which are already identified as *Lr28* positive through phenotyping or marker assisted selection utilizing other dominant markers. It has been already proved by that the codominant STS marker reported by was actually not associated with *Lr2* [6-8]. Pyramiding resistance genes in combination is an effective way of thwarting the breakdown of resistance and in providing diversity that limits race evolution. The current investigation to identify a codominant SSR marker polymorphic for *Lr28* gene locus employs one F_2 breeding population targeted at combining APR gene *Lr48* with the seedling resistance gene *Lr28*. It is anticipated that combinations of effective seedling resistance genes with race non-specific APR genes may provide a longer lasting resistance [9].

The codominant SSR marker, *Xwmc497* which is being reported in this paper as linked to *Lr28* locus was used to select plants which carried homozygous *Lr28* resistance alleles. The two dominant flanking RAPD markers, $S3_{450}$ linked to the recessive resistance allele and $S336_{775}$ linked to the dominant susceptibility allele at the *Lr48* locus, which span a distance of 11.3 cM were employed to identify the plants carrying *Lr48* recessive resistant allele alone [10]. Wheat genotypes from diverse genetic backgrounds which have been testified to carry various other alien and native genes were included in the study for validating the marker for *Lr28*.

Materials and Methods

Plant material

An F_2 population developed from the cross between the most widely cultivated and successful Indian wheat cultivar PBW343 carrying the gene *Lr28* (PBW343-*Lr28*) developed at IARI, India and the Australian cultivar Condor derived CSP44 line (with WW80/2*WW1511Kalyansona parentage) carrying the gene *Lr48*

***Corresponding author:** Prabhu KV, Joint Director (Research), Directorate, Indian Agricultural Research Institute, New Delhi, India, Tel: +91-11-25843375; E-mail: jd_research@iari.res.in

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(CSP44-*Lr48*) was used for the study. *Lr28* is a seedling resistance gene thus conferring resistance in all stages of the plant and *Lr48* is an adult plant resistance gene, effective only from the time the plant reaches booting stage. The zygosity of each of the F_2 individual plants was established both by F_3 progeny testing and co-dominant molecular marker analysis. A set of 30 plants per each F_2 family were sown to erect the F_3 population. The experiments were conducted in the controlled conditions of National Phytotron Facility, IARI and New Delhi.

Pathotype of the fungal pathogen

The inoculum of the most virulent *Puccinia recondita* pathotype, 77-5 (121R63-1) was obtained from the Directorate of Wheat Research, Regional Station, Flowerdale, Shimla. Inoculation of the spores of the pathotype was done by spraying inoculum suspended in water fortified with Tween-20® (0.75 µl/ml) at an average concentration of 20 urediospores/microscopic field (10x × 10x).

DNA extraction

Young leaves from parents and individuals of the segregating population were collected, lyophilized and ground in liquid nitrogen using a pestle and mortar. DNA extraction was performed by the micro-extraction method described by Prabhu et al. [11]. Final concentration of DNA samples was maintained at 10 µg/µl for PCR reactions.

Seedling test

After sampling for DNA extraction, seedlings 8-10 days old at decimal code DC 11 stage were inoculated during the evening hours [12]. Prior to inoculation, the plants were sprayed with water to provide a uniform layer of moisture on the leaf surface. After inoculation, the seedlings were incubated for 36 h in humid glass chambers at a temperature of 23 ± 2°C and more than 85% relative humidity after which, the pots were shifted to muslin cloth chambers in the same green house. The disease reaction was recorded 12-14 days after inoculation, using the scoring method described by Stakman et al. [13].

PCR Amplification using molecular markers

Ten SSR markers specific to the 4A chromosome were selected from published data [14,15]. The SSR markers (custom synthesized at Biobasic Inc, Canada) were used to screen the parents (PBW343-*Lr28* and CSP44-*Lr48*), F_2 population (comprising homozygous resistant, homozygous susceptible and heterozygous plants) and bulks (resistant and susceptible).

PCR amplification was done following the protocol developed by Williams et al. [16]. The PCR reactions with SSR markers were performed in a 20 µl volume which consisted of 10 mM Tris HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTP (MBI Fermentas, Germany), 40 ng of each of the forward and reverse primers, 0.75 U Taq DNA polymerase (Banglore Genei Pvt. Ltd., India) and 50 ng template DNA. PCR amplifications for RAPD markers were performed in 20 µl reaction volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTP (MBI Fermentas, Germany), 0.2 µM of primer, 0.75 U Taq DNA Polymerase (Bangalore Genei Pvt. Ltd., India) and 10-15 ng of genomic DNA. The amplification reactions were carried in a PTC-200 thermal cycler (MJ Research, Las Vegas, NV, USA) with the following thermal profile – initial denaturation of 94°C for 10 min followed by 44 cycles of 94°C for 1 min (denaturation), 61°C and 36°C (for SSR markers and RAPD markers respectively) for 1 min (annealing), 72°C (extension) and a final extension step of 72°C for 10 min. This was followed by 4°C for 10 min.

The amplified products from SSR markers and RAPD markers were separated on a 3% Metaphor® agarose gel and 2% Agarose gel respectively, in 1X TAE buffer at 80 V for 3 hrs to separate the fragments. The gels were later stained with 10 mg/ml ethidium bromide and viewed in a digital gel documentation system (Alpha Innotech, San Leandro, CA, USA).

Bulked segregant analyses were done to identify the markers' linkage to the dominant resistance gene [17]. Ten randomly selected plants from the homozygous resistant and homozygous susceptible F_2 plants were used to prepare bulks. The bulks differentiated for the presence and absence of the leaf rust resistance gene *Lr28* (Figure 1).

Statistical Analysis

Segregation ratios were analyzed using a chi-square test. The individuals from the crosses that were scored as resistant and susceptible in the progeny populations were subjected to chi-square test for goodness of fit to test the deviation from the theoretically expected Mendelian segregation ratios. Mean and standard error of the grain yield of the F_2 plants was calculated on the basis of standard formulae. The linkage analysis was carried out using Mapmaker version 3.0 [18].

Results

The parent PBW343-*Lr28* showed resistance to the 77-5 (121R63-1) race of *Puccinia triticina* with a resistant infection type of 0; while the APR parent, CSP44 showed a typical seedling susceptibility with a reaction type of 33^+ (Growth stage 11 of Zadoks growth scale). 61 seedlings of the F_2 population showed susceptibility to the leaf rust infection while the remaining 193 plants remained resistant by expressing the seedling resistance conferred by the dominant resistance allele of the *Lr28* locus and the population followed a monogenic segregation ratio (P = 0.6645). All the susceptible F_2 derived F_3 families remained susceptible whereas only 67 out of the 193 resistant F_2 derived F_3 families were homozygous for resistance. The remaining 126 families were heterozygous thus distributing the F_2 genotypes into 1R:2R:1S monogenic segregation ratio (P = 0.6467). The phenotypic expression of adult plant resistance could not be examined due to the interference of the dominant seedling resistance gene *Lr28* in the same genetic background.

Out of ten SSR markers specific to the 4AL chromosome, only *Xwmc497* (Forward: 5'CCCGTGGTTTTCTTTCCTTCT3', Reverse: 5'AACGACAGGGATGAAAAGCAA3') with annealing temperature of 61°C was identified to be polymorphic between the parents. 10 randomly selected samples were taken from the resistant and susceptible plants to prepare bulks for bulk segregant analysis (Figure

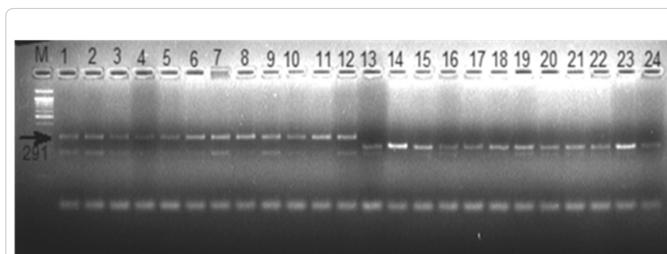

Figure 1: Screening of the SSR marker $Xwmc497_{291}$ on the bulked DNA constituent F_2 plants of the cross PBW343 X CSP44 for genetic linkage analysis. M: 100bp DNA ladder, Lanes1-10: F_2 seedling resistant individual plants, 11: Resistant Bulk, 12: Resistant parent, PBW343+ *Lr28*, 13-22: F_2 seedling susceptible individual plants, 23: Susceptible Bulk 24: Seedling susceptible parent, CSP44+ *Lr48*.

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1). The marker was found putatively linked to the *Lr28* locus. This polymorphic SSR marker was analysed on the 254 F_2 plants for linkage analysis with the *Lr28* locus. The marker *Xwmc497* was associated with the *Lr28* locus and was located at a distance of 2.9 cM from it. The PBW343-*Lr28* resistance allele linked SSR marker allele amplified a 291 bp fragment and the CSP44 susceptibility allele linked marker allele amplified a 226 bp fragment.

The 291 bp fragment was specific to the *Lr28* resistance allele and did not amplify in other *Lr* genes carrying lines from other native and alien sources.

By employing the flanking RAPD markers $S3_{450}$ (5'CATCCCCTG3') and $S336_{775}$ (5'TCCCCATCAC3') linked respectively to the recessive resistance allele and dominant susceptible allele of the *Lr48* locus; plants which were homozygous for recessive APR gene *Lr48* were identified, as these two markers served as one co-dominant marker system capable of identifying both dominant and recessive alleles of heterozygous plants. 70 F_2 plants were found to possess the homozygous recessive resistance allele of *Lr48* out of the 254 plants (Table 1). Of these, only 14 plants were homozygous for the gene *Lr28* also and were identified to be carried forward as breeding lines.

The grain yield of each plant was recorded in order to advance only those which were comparable to PBW343 in mean yield/plant and displayed rust resistance imparted by both *Lr28* and *Lr48* (Table 1). PBW343 is a high yielding Indian cultivar and had a mean single plant yield of 9.50 gm while the APR parent CSP44 recorded a lower yield of 8.78 gm. The mean yield of the 14 plants homozygous for *Lr28*+ *Lr48* was 9.49 gm. These would be advanced as pyramided lines and followed for ear-to-row progeny analysis without elimination to select for high yielding recombinants through pedigree selection approach as the two genes are fixed in these progenies.

Discussion

Gene pyramiding holds its base on the concept that the probability of mutation at more than one avirulence gene locus in the pathogen is low for it to turn virulent for all the pyramided resistance genes. This enables a host variety which possesses more than one gene to remain durably resistant to the disease relatively for a long period compared to the single gene based resistance. In addition, when the added gene is from wild species the resistance is expected to last long as matching virulence is less likely to be present in the pathogen population. Further, if the resistance is race non-specific such as APR, there would be still less chance for virulence development for all the prevailing races. Thus a pyramided combination of alien seedling resistance and APR would be an ideal means to ensure durable resistance. In the past three decades, combinations of alien and APR genes such as *Lr16* and *Lr13, Lr13* and *Lr34*, *Lr13* and *Lr37*, *Lr34* and *Lr37* have been achieved through conventional means as there were available pathogen virulence differentials or phenotypic differences in reaction types to distinguish each gene [19,20]. However, in a case where the presence of both genes cannot be detected due to lack of such differences as in the case of *Lr28*, *Lr24*, etc, a selection process which employs molecular markers tagged to the genes is a reliable methodology as has been demonstrated by in pyramiding *Lr24* and *Lr48* in wheat by marker assisted selection utilizing dominant SCAR and RAPD markers in consecutive generations till homozygosity was achieved at both loci [10]. We were able to identify plants fixed for both genes *Lr28* and *Lr48* in F_2 generation itself owing to the codominant SSR marker in combination with the flanking RAPD marker set linked to both recessive resistance and dominant susceptibility alleles at the *Lr48* locus. Gene pyramiding is well utilized in rice breeding programmes also to develop plants carrying *Xa21* and *xa13* resistant to bacterial blight which has also led to commercial release of the pyramided variety in India. Marker assisted pyramiding is also reported against fungal blast (*Pi1* and *Pi2)* and brown plant hopper (*Qbph1* and *Qbph2)* [21]. This strategy is being followed in many other breeding programmes with various crops for a range of beneficial phenotypes.

Seedling resistance genes such as *Lr28* are important to control the pathogen infection during the entire crop duration. There are previous reports of identified markers tagged to *Lr28.* The SCAR marker $SCS421_{570}$ is being successfully employed in various wheat breeding programmes in India. A recent publication by has suggested the utility of two SSR markers, *Xbarc327* and *Xbarc343* to identify the presence of *Lr28* [5,22]. However, these two markers were found to be monomorphic amplifying the critical marker fragment in both the parents. A null allelic microsatellite marker, *Xgwm160* has also been reported to be specific to the *Lr28* gene. $Xgwm160_{196}$ and $Xwmc497_{291}$ are positioned at a distance of 144.9 cM and 149.9 cM respectively, from the centromere on the long arm of the 4A chromosome [6,14].

The microsatellite marker reported in this paper will be helpful for breeding purposes since it differentiates the presence of the gene in homozygous resistant and heterozygous resistant plants (Figure 2). It has been suggested by that the markers should be within 10 cM of the gene of interest for effective marker-assisted selection breeding [23,24]. The marker *Xwmc497* mapped at a distance of 2.9 cM will therefore be especially useful for those breeding programmes in wheat where pyramiding is performed to stack more than one resistant gene into a single background. In the current study, molecular markers were effectively used to identify pyramided single plants in the F_2 generation itself which otherwise would have needed a laborious and time consuming selection process consisting a combination of phenotype based selection and a dominant marker based selection till the F_5/F_6 generations.

Gene(s)	Generation	Marker(s) employed	Marker alleles	No. of plants	Mean yield
Lr28	F_2	Xwmc497§	R	62	9.32 ± 0.1842
		Xwmc497§	H	132	9.23 ± 0.1933
		Xwmc497§	S	60	9.40 ± 0.2028
Lr48	F_2	S3#	+	70	9.22 ± 0.2143
		S336¶	-		
		S3#	+	117	9.32 ± 0.1352
		S336¶	+		
		S3#	-	67	9.01 ± 0.2205
		S336¶	+		
Lr28 + *Lr48*	F_2	Xwmc497§	R	14	9.49 ± 0.1827
		S3#	+		
		S336¶	-		
PBW343-*Lr28*	Parent	Xwmc497§	R	25	9.50 ± 0.1314
CSP44-*Lr48*	Parent	S3¶	+	25	8.78 ± 0.0980
		S336¶	-		

Table 1: Mean grain yield of the F_2 plants pooled with reference to the segregation of the resistant alleles of the marker loci. §Codominant microsatellite marker; R: Homozygous resistant; ¶Dominant RAPD marker; H: Heterozygous resistant; S: Homozygous susceptible; +: Presence of RAPD marker fragment; -: Absence of RAPD marker fragment.

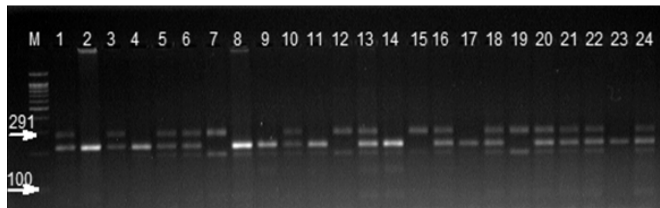

Figure 2: Segregation of the marker Xwmc497$_{291}$ in the heterozygous F$_2$ population. Individual F$_2$ plants amplifying the specific bands: Lanes 1, 3, 5, 6, 10, 13, 16, 18, 20, 21, 22, 24: heterozygous resistance, Lanes 2, 4, 8, 9, 11, 14, 17, 23: homozygous susceptibility, Lanes 7, 12, 15, 19: homozygous resistance; M: 100-bp DNA ladder.

The RAPD marker pair $S3_{450}$ and $S336_{775}$ which we used in the study had an advantage enabling us to successfully identify the plants which carried only the recessive adult plant resistance allele pair of the *Lr48* locus. From among 254 F$_2$ plants, we could select 14 plants carrying both the genes.

The grain yield of a plant follows a quantitative inheritance pattern and the expression of resistance is a qualitative character and there is no available information suggesting the influence of the leaf rust resistance loci on the grain yield of the plant. In this experiment we have also scrutinized the plants on the basis of their yield and only those plants with adequate grain number and with the presence of both the resistant genes were chosen. The 14 plants were comparable with PBW343 for mean yield/plant. The progeny of these plants will be carried forward through marker assisted pedigree breeding procedure.

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References

1. McIntosh RA, Wellings CR, Park F (1995) In Wheat rusts: An Atlas Resistance Genes CSIRO Publishers, Australia pp. 1-20.
2. McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, et al. (2003) Catalog of gene symbols for wheat. Proceedings of the 10th International Wheat Genetics Symposium.
3. McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers J, Morris C (2008) Catalog of gene symbols for wheat. 11th International Wheat Genetics Symposium.
4. Tomar SMS, Menon MK (1998) Adult plant response of near isogenic lines and stocks of wheat carrying specific Lr genes against leaf rust. Indian Phytopathol 51: 61-67.
5. Cherukuri DP, Gupta SK, Ashwini C, Sunita K, Prabhu KV, et al. (2005) Molecular mapping of Aegilops speltoides derived leaf rust resistance gene Lr28 in wheat. Euphytica 143: 19-26.
6. Vikal Y, Chhuneja P, Singh R, Dhaliwal HS (2004) Tagging of an Aegilops speltoides derived leaf rust resistance gene Lr28 with a microsatellite marker in wheat. J Plant Biochem. Biotechnol 13: 47-49.
7. Prabhu KV, Gupta SK, Charpe A, Koul S, Cherukuri DP, et al. (2003) Molecular markers detect redundancy and miss-identity in genetic stocks with alien leaf rust resistance genes Lr32 and Lr28 in bread wheat. J Plant Biochem and Biotech 12: 123-129.
8. Naik S, Gill KS, Prakasa Rao VS, Gupta VS, Tamhankar SA, et al. (1998) Identification of a STS marker linked to the Aegilops speltoides derived leaf rust resistance gene Lr28 in wheat. Theor Appl Genet 97: 535-540.
9. Nazari K, Wellings CR (2008) Genetic analysis of seedling stripe rust resistance in the Australian wheat cultivar 'Batavia'. The 11th International Wheat Genetics Symposium proceedings. Sydney University Press.
10. Samsampour D, Maleki Zanjani B, Singh A, Pallavi JK, Prabhu KV (2009) Marker assisted selection to pyramid seedling resistance gene Lr24 and adult plant resistance gene Lr48 for leaf rust resistance in wheat. Indian journal of genetics and plant breeding 69: 1-9.
11. Prabhu KV, Somers DJ, Rakow G, Gugel RK (1998) Molecular markers linked to white rust resistance in mustard Brassica juncea. Theoretical and Applied Genetics 97: 865-870.
12. Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14: 415-421.
13. Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiological races of Puccinia graminis var. tritici, USDA-ARS-Bulletin E617
14. Torada A, Koike M, Mochida K, Ogihara Y (2006) SSR-based linkage map with new markers using an intra specific population of common wheat. Thoer Appl Genet 112: 1042-1051.
15. Roder MS, Victor K, Wendehake K, Plaschke J, Tixier MH, et al. (1998) A microsatellite map of wheat. Genetics 149: 2007-2023.
16. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA,Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.
17. Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci 88: 9828-9832.
18. Lander ES, Green P, Abrahamson J, Barlow A, Daley MJ, et al. (1987) MAPMAKER: an interactive computer package for constructing primary genetic maps of experimental and natural populations. Genomics 174-181.
19. Samborski DJ, Dyck PL (1982) Enhancement of resistance to Puccinia recondite by interaction of resistance gene in wheat. Canadian Journal of Plant Pathology 4: 152-156.
20. Kloppers FJ, Pretorius ZA (1997) Effects of combinations amongst Lr13, Lr34 and Lr37 on components of resistance in wheat to leaf rust. Plant Pathology 46: 737-750.
21. He Y, Li X, Zhang J, Jiang G, Liu S, et al. (2004) Proceedings of the 4th International Crop Science Congress.
22. Cakir M, Drake Brockman F, Shankar M, Golzar H, McLean R, et al. (2008) Molecular mapping and improvement of rust resistance in the Australian wheat germplasm. 11th International Wheat Genetics Symposium.
23. Timmerman GM, Frew TJ, Weeden NF, Miller AL ,Goulden DS (1994) Linkage analysis of er-1, a recessive Pisumsativum gene for resistance to powdery mildew fungus (Erysiphe pisi D.C.). Theor Appl Genet 85: 1050-1055.
24. Cheng FS, Weeden NF, Brown SK, Aldwinckle HS, Gardiner SE, et al. (1998) Development of a DNA marker for Vm, a gene conferring resistance to apple scab. Genome 41: 208-214.