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Genetic Mapping and QTL Analysis for Disease Resistance Using F_2 and F_5 Generation-based Genetic Maps Derived from Tifrunner × GT-C20 in Peanut (*Arachis hypogaea* L.)

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Abbreviations: QTL, quantitative trait loci; PVE, phenotypic variation explained; TSWV, Tomato spotted wilt virus; LS, leaf spots.

Running Head: QTL analysis of disease resistance in peanut

Key words: Genetic map, Quantitative trait loci, thrips, leaf spots, Tomato spotted wilt virus

Abstract

One mapping population derived from Tifrunner × GT-C20 has shown great potential in developing high dense genetic map and identification of QTLs for important disease resistance, Tomato spotted wilt virus (TSWV) and leaf spot (LS). Both F_2 and F_5 generation-based genetic maps were constructed previously with 318 and 239 marker loci, respectively. Higher map density could be achieved with the F_2 map (5.3 cM/locus) as compared to the F_5 (5.7 cM/locus). QTL analysis using multi-environment phenotyping data from F_8 and higher generation for disease resistance identified 54 QTLs in the F_2 map including two QTLs for thrips (12.14 – 19.43% PVE), 15 for TSWV (4.40–34.92% PVE) and 37 for LS (6.61–27.35% PVE). Twenty-three QTLs could be identified in F_5 map including one QTL for thrips (5.86% PVE), nine for TSWV (5.20 – 14.14% PVE) and 13 for LS (5.95–21.45% PVE). Consistent QTLs identified in each map have shown higher phenotypic variance than non-consistent QTLs. As expected, the number of QTLs and their estimates of phenotypic variance were lower in the F_5 map. This is the first QTL study reporting novel QTLs for thrips, TSWV and LS in peanut, and thus, future studies will be conducted to refine these QTLs.

Introduction

Peanut has its global presence among growers and consumers with a total production of 37.7 Mton from 24.1 Mha in 2010 (FAO, 2012). The average yield was 1564 Kg/ha, and a wide gap exists between the genetic potential of the modern cultivars and their actual yield in the farmer's field. This gap has been heavily widened by several biotic and abiotic stress factors in the past and it may be even worse at the current scenario due to the fluctuating climatic and environmental conditions. Among the biotic stresses, early leaf spot (ELS) (caused by *Cercospora arachidicola*), late leaf spot (LLS) (caused by *Cercosporidium personatum*) and Tomato spotted wilt virus (TSWV) may cause significant yield loss (Nigam et al., 2012). TSWV is generally spread by thrips and the farmers try to control TSWV indirectly with insecticide spray. In 1997 and 1998, losses due to TSWV peanut was estimated ~ USD 40 million/year for Georgia alone in USA (http://www.caes.uga.edu/topics/diseases/tswv/peanut/intro.html). Despite several chemical treatments are available to control these diseases, host-plant resistance is the best control mechanism which has the advantage of being cost-effective and eco-friendly.

Conventional breeding has been the major force in providing modern cultivars to the farmers. Integration of genomics tools with conventional breeding has been successful in some of the crops but peanut lagged behind in terms of genetic and genomic resources required for such approach. However, the development in genetic and genomic resources in peanut in recent years has provided the possibility for improving peanut through marker-assisted selection to lead to the more rapid development of superior cultivars using informative markers linked to desired traits. Although, marker-assisted breeding has been applied on a limited scale (see Pandey et al., 2012), still peanut lacks availability of linked markers for important traits. Already marker-assisted breeding in peanut has successfully

demonstrated its utility by using available limited resources in conversion of peanut cultivar 'Tifguard' (Holbrook et al., 2008) into 'high oleic Tifguard' in 26 months (Chu et al., 2011).

Identification of linked markers is the base to improve peanut resistance for the important diseases through marker-assisted breeding, and a mapping population derived from the cross 'Tifrunner' × 'GT-C20' was developed for identification of linked markers. The parental genotypes have several contrasting traits such as Tifrunner with high level of resistance to TSWV, moderate resistance to early and late leaf spot (Holbrook and Culbreath, 2007) while GT-C20 is susceptible to these diseases but has resistance to aflatoxin contamination (Liang et al., 2005). Parental screening with ~5000 SSRs resulted in identification of 385 polymorphic loci which were genotyped on a set of 94 individuals of F_2 population. As a result, a genetic linkage map was constructed with 318 mapped loci distributed on 21 linkage groups with genome coverage of 1,674.4 cM and a marker density of 5.3 cM /locus (Wang et al., 2012). Meanwhile, this population was advanced to F_5 generation and used for development of another genetic map with 239 loci distributed on 26 linkage groups covering a total genome distance of 1,213.4 cM and average map density of 5.7 cM/locus (Qin et al., 2012). This population was then extensively phenotyped during the years for several important traits including three important diseases.

Thus, this study reports the use of genotyping data generated at F_2 and F_5 generation and phenotyping data generated at higher generations for identification of quantitative trait loci (QTL) for thrips, TSWV and leaf spots including early and late leaf spots in this study. The field phenotyping trials were conducted in multiple fields from 2010 to 2012. Late leaf spot was predominate pathogen in all three years. Also, comparison was made for the effects of identified QTLs and common genomic regions identified in the F_2 and F_5 maps.

MATERIALS AND METHODS

Mapping Population

A mapping population derived from the cross 'Tifrunner' × 'GT-C20' (referred to as T population) was developed through single seed descent method (SSD) at Crop Protection and Management Research Unit of USDA-ARS, Tifton, USA. The female parent, Tifrunner, is a runner market-type cultivar with high level of resistance to TSWV, moderate resistance to ELS and LLS, and has late maturity (Holbrook and Culbreath, 2007). The male parent, GT-C20, is a Spanish-type breeding line with high susceptibility to TSWV and leaf spots but has resistance to aflatoxin contamination (Liang et al., 2005). As of now, this mapping population consists of 248 RILs (recombinant inbred lines) and has been phenotyped for several agronomic traits including disease resistance.

DNA Isolation, Polymorphism and Genotyping

Initially the total genomic DNA was extracted from young leaflets of 94 F_2 plants along with the parental genotypes (Tifrunner and GT-C20). Parental polymorphism screening and population genotyping were conducted with SSRs markers available at UC-Davis and Tuskegee University, USA. Simultaneously, the generation advancement was done from F_2 to F_5 generation and again the DNA was isolated from a subset of 158 F_5 individuals in order to construct genetic map and use multiseason phenotyping data for QTL analysis. The details of PCR reactions and complete genotyping and map construction were early published for F_2 map (Wang et al., 2012) and F_5 map (Qin et al., 2012).

Phenotyping for Disease Resistance

The entire set of RILs with 248 individuals were phenotyped for several important traits including resistance to thrips, Tomato spotted wilt virus (TSWV) and leaf spots (LS) including both early and late leaf spots but late leaf spot was the predominate disease in all three years. Therefore, the general term of leaf spots was used in this study. The field trials were conducted using randomized complete block designs with at least 3 replications in 2010 at Dawson and Tifton, Georgia, and 2011 and 2012 at Tifton, Georgia. Late leaf spot was predominate pathogen in all three years.

In Tifton, Georgia, two separate field trials were conducted at the Belflower Farm in all three years. Soil type is Tifton loamy sand (Fine-loamy, siliceous, thermic Plinthic Kandiudult). In each year, one experiment was planted in April to maximize potential for development of spotted wilt epidemics (Li et al., 2012) and one was planted in May to reduce potential for spotted wilt epidemics and increase the likelihood of heavy leaf spot epidemics. Experiment plots were 6.0 m long, separated by 2.4 m alleys. Peanut seeds were planted in 91-cm-spaced twin-row plots.

Severity of TSWV was assessed using a 0 to 5 severity scale adapted from Baldessari (2008) based on visual determination of presence of symptoms and estimation of the degree of stunting (reduction in plant height, width, or both) for symptomatic plants. Leaf spot severity was evaluated using the Florida 1 to 10 scale (Chiteka et al., 1988) where 1 = no leaf spot; 2 = very few lesions on the leaves and none on upper canopy; 3 = very few lesions on upper canopy; 4 = some lesions with more on upper canopy with 5% defoliation; 5 = noticeable lesions on upper canopy with 20% defoliation; 6 = numerous lesions on upper canopy with 50% significant defoliation; 7 = numerous lesions on upper canopy with 75% defoliation; 8 = Upper canopy covered with lesions with 90% defoliation; 9 = very few leaves covered with lesions remain and some plants completely defoliated; 10 = plants dead.

This population was phenotyped for thrips for one season (TPS_DW10) at Dawson in

2010 while TSWV for four seasons i.e., at Dawson in 2010 (TSWV_DW10E1), at two locations of Tifton in 2010 (TSWV_TF10E2 and TSWV_TF10E3) and at Tifton in 2011 (TSWV_TF11E4). This population was screened for leaf spot for a total of 10 seasons which include screening at Dawson in 2010 (LS_DW10E1 and LS_DW10E2), at Tifton in 2010 (LS_TF10E3) and in 2011 (LS_TF11E4, LS_TF11E5, LS_TF11E6, and LS_TF11E7) while three in 2012 (LS_TF12E8, LS_TF12E9 and LS_TF12E10).

Nomenclature Uniformity between Genetic Maps

The genetic maps were constructed at two different institutions using the two different generations of the same cross i.e., using 94 F_2 individuals at Tuskegee University and 158 F_5 individuals at USDA-ARS (Tifton), and published in the same year i.e., late 2012 (Wang et al., 2012) and early 2012 (Qin et al., 2012), respectively. The panel of markers screened on parental genotypes was different, hence, some differences in nomenclature used for names of markers were found. This was more frequent with the naming of unpublished markers having long IDs e.g., the markers developed through BAC-end sequencing were named with prefix "ARS" in the F_5 map while with prefix "GNB" in the F_2 map. Here we retained the names as such for all the published markers used in these two maps while few changes were made to keep size of names manageable and better viewing such as 'pPGP….' and 'sPGP….' were abbreviated to 'seq…..' in order to bring uniformity with recently published high dense consensus genetic maps (Gautami et al., 2012; Shirasawa et al., 2013). The purpose of all the above exercise was to bring the genetic information in uniformity which has helped in comparison of genetic maps between each other and also with published consensus genetic map. It is important to mention that the genetic map information generated using F_5 population (Qin et al., 2012) was used for construction of both the consensus genetic maps (Gautami et al., 2012; Shirasawa et al., 2013) while F_2 genetic map could not be completed due to delay in screening large number of markers and genotyping.

Reproducing Genetic Maps and QTL Analysis

The method of genetic map construction for both maps was given in detail by Qin et al. (2012) and Wang et al. (2012). Here we made the nomenclature of both the genetic maps uniform in consensus with the published consensus genetic maps (Gautami et al., 2012; Shirasawa et al., 2013) where distinct linkage groups have been assigned to particular genomes. MapChart 2.2 (Voorrips, 2002) was used for reproducing both the genetic maps using uniform nomenclature with the genetic map information. The genotyping information generated on both the generations (F_2 and F_5) was also used here for conducting QTL analysis using software WinQTL Cartographer, version 2.5 (Wang et al., 2007). The composite interval mapping (CIM) approach, which is based on a mixed linear model, was used for detection of QTLs with LD more than 2.5. Parameters such as model 6, scanning intervals of 1.0 cM between markers and putative QTLs with a window size of 10.0 cM were used for conducting the CIM analysis.

RESULTS

Comparison of Both Genetic Maps with Reference Consensus Genetic Map

Upon the comparison of the corresponding linkage groups (LGs) between these two (F_2 and F_5) maps, 19 LGs of the F_2 map were found identical to 20 LGs of the F_5 map (Supplemental Table S1). Of the total 22 LGs of the F_2 map and 26 LGs of the F_5 map, three LGs (AhIII, AhXXI and AhXXII) and six LGs (LGT1, LGT12, LGT19, LGT22, LGT23 and LGT26) could not correspond to each other due to less number of mapped loci as well as lack of common loci, respectively. Two LGs of the F_5 map (LGT15 and LGT25) shared common loci with one LG (AhVIII) of the F_2 map. Upon comparing these two genetic maps with reference consensus genetic maps using the common marker loci, a total of 9 of the 10 LGs

from A-genome and 8 of the 10 LGs from B-genome could be assigned. In general the co-linearity has been observed for these two maps with each other and also with the reference consensus genetic map (Figure 1).

QTL Analysis for Biotic Stresses

The entire RILs with 248 individuals were phenotyped for thrips, TSWV and leaf spots (LS) in multiple fields and planting dates from 2010 to 2012 in Georgia. Late leaf spot was predominate pathogen in all three years. Therefore, the general term of leaf spots (LS) was used in this study, including both early and late leaf spots. These phenotyping data were used in combination with genotyping data based on F_2 and F_5 generation for identification of QTLs associated with each trait. A total of 77 QTLs could be detected for these three diseases using both the genetic maps. Of the 77 QTLs, 54 QTLs (two for thrips, 15 for TSWV and 37 for LS) were placed on the F_2 map (Supplemental Table S2, Figure 2) while 23 QTLs (one for thrips, nine for TSWV and 13 for LS) on the F_5 map (Supplemental Table S3, Figure 3) with phenotypic variance (PV) range of 5.86-19.43% (thrips), 4.40-34.92% (TSWV) and 5.20-21.45% (LS), respectively (Table 1). The LOD values ranged from 2.51 (TSWV, LS) to 5.92 (TSWV) in F_2 map while 2.50 (TSWV) to 6.38 (LS) in F_5 map.

QTLs Identified for Thrips

Total three QTLs could be identified for thrips using genetic mapping information of both the populations and phenotyping data generated for one season at Dawson during 2010. Of the three QTLs, two QTLs (*qF2TPS1* and *qF2TPS2*) were detected on F_2 map with PV ranging from 12.14% to 19.43% while only one QTL (*qF5TPS1*) with 5.86% PV on F_5 map. Among three QTLs, the *qF2TPS1* (IPAHM108-2 – AHGS0347) located on AhIX and *qF2TPS2* (GM2337 – TC42A02) located on AhX are the two major QTLs detected for thrips with 12.14% PV and 19.43% PV, respectively (Table 2).

QTLs Identified for Tomato Spotted Wilt Virus (TSWV)

In the case of TSWV, a total of 24 QTLs were detected which include 15 QTLs from F_2 and nine QTLs from F_5 map with PV ranging from 4.40% to 34.92% and 5.20% to 14.14%, respectively (Table 1). All the 15 QTLs detected in F_2 map were located on eight genomic regions of six LGs (AhI, AhII, AhIX, AhX, AhXI and AhXII) (Table 2). The same names were given to all the QTLs if they were mapped with same genomic regions/marker interval. So in this case, 15 QTLs were mapped on eight genomic regions as *qF2TSWV1* to *qF2TSWV8* without referring to any season (Supplemental Table S2). The three genomic regions named seq5D5 – GM2744 (*qF2TSWV3*) on AhII, TC42A02 – GM2337 (*qF2TSWV6*) on AhX, and GNB2 – AHO116 (*qF2TSWV8*) on AhXII harbored three QTLs, while another genomic region named IPAHM108-2 – AHGS0347 (*qF2TSWV4*) on AhIX possessed two QTLs and these four genomic regions are referred as consistent QTLs across two or more different environments. The phenotypic variance shown by consistent QTLs were higher in general as compared to the non-consistent QTLs (which appeared in only one environment). Among four consistent QTLs, *qF2TSWV3* had higher PV range (5.14 – 34.92%) followed by *qF2TSWV8* (6.26 – 21.18% PV), *qF2TSWV4* (12.92 – 18.11% PV) and *qF2TSWV6* (10.78 – 16.56% PV) (Table 2). Among non-consistent QTLs, *qF2TSWV5* had the highest PV (23.02%) followed by *qF2TSWV7* (15.75%), *qF2TSWV1* (9.41%) and *qF2TSWV2* (4.40%).

Similarly, all the nine QTLs (5.20 – 14.14% PV) identified in F_5 map were located on seven genomic regions on seven different LGs named LGT1 (TC3H02-410 – seq14A7-300), LGT6 (TC11A02-300 – GNB523-500), LGT7 (GNB519-205 – GM1076-460), LGT9 (AC3C07-70 – RN35H04-1500), LGT11 (GNB619-340 – GM2607-90), LGT12 (seq14G03-500 – GM2808-400), and LGT25 (IPAHM167-130 – GM1555-1000). These genomic regions were named as *qF5TSWV1* to *qF5TSWV7*, respectively (Supplemental Table S3). Two

genomic regions i.e, *qF5TSWV4* (AC3C07-70 – RN35H04-1500) and *qF5TSWV7* (IPAHM167-130 – GM1555-1000) were consistent as both harbored two QTLs for TSWV which were located on LGT9 and LGT25 with PV range of 11.45 – 14.14% and 7.25 – 7.62%, respectively (Table 3). Among the five non-consistent QTLs, *qF5TSWV5* had high PV (10.80%) followed by *qF5TSWV6* (10.64%), *qF5TSWV1* (9.31%), *qF5TSWV2* (7.71%) and *qF5TSWV3* (5.20%).

Of the 15 QTLs detected in the F_2 map, 11 QTLs were contributed by Tifrunner while four QTLs were contributed by GT-C20 with additive effects, ranging from -0.443 (*qF2TSWV8*) to -1.250 (*qF2TSWV6*) and 0.797 (*qF2TSWV7*) to 1.347 (*qF2TSWV4*), respectively (Supplemental Table S2). Similarly in the case of F_5 map, five QTLs were contributed by Tifrunner while four QTLs were contributed by GT-C20 with additive effects, ranged from -0.235 (*qF5TSWV3*) to -3.860 (*qF5TSWV1*) and 0.332 (*qF5TSWV6*) to 0.401 (*qF5TSWV4*), respectively (Supplemental Table S3).

QTLs Identified for Leaf Spot (LS)

QTL analyses for ten different phenotyping data of LS led to identification of a total of 50 QTLs, which include 37 QTLs for F_2 map and 13 QTLs for F_5 map with PV ranging from 6.61% to 27.35% and 5.95% to 21.45%, respectively (Table 1). All the 37 QTLs detected in F_2 map were located on 12 genomic regions of nine LGs (AhII, AhV, AhVI, AhVIII, AhIX, AhX, AhXI, AhXII and AhXVIII). The same names were given to the QTLs if they are mapped with same genomic regions/marker interval. Thus, 37 QTLs mapped on 12 genomic regions on F_2 map were named as *qF2LS1* to *qF2LS12* without referring to any season (Supplemental Table S2).

The seven genomic regions namely GM2744 – seq5D5 (*qF2LS1*) on AhII, IPAHM108-2 – AHGS0347 (*qF2LS5*), TC5A07 – TC7G10 (*qF2LS6*) and TC42A02 – GM2337 (*qF2LS7*)

on AhIX, seq2G4 – PM499 (*qF2LS8*) and PM200 – AC2C05 (*qF2LS9*) on AhXI, and GNB2 – AHO116 (*qF2LS10*) harbored five, four, four, nine, four, two and four QTLs, respectively, thus, these seven genomic regions are referred as consistent QTLs (Table 2). The phenotypic variances explained by the consistent QTLs for LS were higher in general as compared to the non-consistent QTLs. Among seven consistent QTLs, three consistent QTLs namely *qF2LS5*, *qF2LS6* and *qF2LS7* contributed more or less equally as their PV ranged from 11.27 – 24.45%, 10.8 – 24.19% and 13.48 –24.85%, respectively, followed by *qF2LS10* (15.30 – 21.19%), *qF2LS8* (6.61 – 18.97%), *qF2LS1* (7.80 – 13.11%) and *qF2LS9* (10.29 – 11.51%) (Table 2). Similarly among the five non-consistent QTLs, *qF2LS11* had the highest PV (27.35%) followed by *qF2LS3* (12.56%), *qF2LS12* (11.59%), *qF2LS2* (8.22%), and *qF2LS4* (8.11%).

The 13 QTLs (5.95 – 21.45% PV) identified on F_5 map were located on 11 genomic regions of eight different LGs, LGT3, LGT5 , LGT6, LGT7, LGT14, LGT16, LGT17 and LGT18 (Supplemental Table S3). These genomic regions were named as *qF5LS1* to *qF5LS11*, respectively. Two genomic regions i.e, *qF5LS5* (TC7C06-170 – seq15D3-500) and *qF5LS10* (GM1254-160 – seq15C10-205) were consistent as both harbored two QTLs for LS, which were located on LGT6 and LGT10 with PV range of 7.61 – 11.20% and 7.50 – 9.08%, respectively. Among the nine non-consistent QTLs, *qF5LS1* had a PV of 21.45%, while the remaining eight QTLs (*qF5LLS2, qF5LS3, qF5LS4, qF5LS6, qF5LS7, qF5LS8, qF5LS9* and *qF5LS11*) had a low PV range of 5.95% (*qF5LS8*) to 8.98% (*qF5LS3*) (Table 3).

Of the 37 QTLs detected for LS in F_2 map, 20 QTLs were contributed by Tifrunner while 17 QTLs were contributed by GT-C20 with additive effects, ranged from -0.861 (*qF2LS1* at season TF11E4) to -2.921 (*qF2LS10* at season TF12E8) and 0.720 (*qF2LS8* at season TF11E6) to 4.629 (*qF2LS11* at season TF12E9), respectively (Supplemental Table S2).

Similarly in F_5 map, of the 13 QTLs detected for LS, five QTLs were contributed by Tifrunner while six QTLs were contributed by GT-C20 with additive effects, ranged from -0.1739 (*qF5LS4* at season TF11E6) to -2.430 (*qF5LS1* at season TF11E4) and 0.1311 (*qF5LS2* at season TF11E4) to 0.2733 (*qF5LS7* at season DW10E2), respectively (Supplemental Table S3).

Common QTLs Identified Among the Traits

Two common regions were identified in F_2 map for all the three diseases. The first common genomic region (GM2337 – TC42A02) was located on AhX which harbored one QTL for thrips (*qF2TPS2*), three QTLs for TSWV (*qF2TSWV6* for 3 seasons) and nine QTLs for LS (*qF2LS7* for 9 of the total 10 seasons). This genomic region is contributing 19.43% PV for thrips, 10.78 – 16.56% PV for TSWV and 13.48 – 24.85% PV for LS. In all the three traits, the phenotypic contribution came from the resistant parent, Tifrunner. The second common region (IPAHM108-2 – AHGS0347) located on AhIX harbored one QTL for thrips (*qF2TPS1*), two QTLs for TSWV (*qF2TSWV4* for 2 seasons) and four QTLs for LS (*qF2LS5* for 4 of the total 10 seasons). This genomic region is contributing 12.14% PV for thrips, 12.92 – 18.11% PV for TSWV and 11.27 – 24.45% PV for LS. Interestingly, for all the three diseases, the phenotypic contribution came from the susceptible parent, GT-C20, for this second common region.

Further, other four genomic regions harbored QTLs for both TSWV and LS. These four genomic regions are located on four different LGs of F_2 map i.e., on AhII (GM2744 – seq5D5), AhIX (TC5A07 – TC7G10), AhXI (seq2G4 – PM499), and AhXII (GNB2 – AHO116). The first genomic region (GM2744 – seq5D5) harbored three QTLs for TSWV (5.14 – 34.92% PV) and five QTLs for LS (7.80 – 13.11% PV) with the contribution from the resistant parent, Tifrunner. Similarly, the second genomic region (TC5A07 – TC7G10)

harbored a single QTL for TSWV (23.02% PV) and four QTLs for LS (10.08 – 24.19% PV) with the contribution coming from the susceptible parent, GT-C20. The third genomic region (seq2G4 – PM499) harbored a single QTL for TSWV (15.75% PV) and four QTLs for LS (6.61 – 18.97% PV) contributed by the susceptible parent, GT-C20. The fourth genomic region (GNB2 – AHO116) harbored three QTL for TSWV (6.26 – 21.18% PV) and four QTLs for LS (15.30 – 21.19% PV) contributed by the resistant parent, Tifrunner.

In contrast to the F_2 map, there was no common QTL for all three traits in the F_5 map. There was only one common genomic region located on LGT6 (TC11A02-300 – GNB523-500) harboring one QTL for TSWV (*qF5TSWV2*) with 7.71% PV and LS (*qF5LS4*) with 8.02% PV.

Common QTLs Identified Between Two Maps

There was one QTL controlling LS in F_2 map (AhXVIII) and one QTL controlling TSWV in F_5 map (LGT7) flanked by same markers i.e., GNB159 – GNB335. In the other case, even though the flanking markers were not same but the QTLs were found on the same linkage group. Such QTLs have been observed between corresponding LGs of both genetic maps, for example between AhII and LGT17, AhV and LGT16, AhVI and LGT11, and AhX and LGT6.

DISCUSSION

Due to the increased uniformity in marker nomenclature, the corresponding linkage groups between these two maps have been identified. Further, a total of 9 of the 10 LGs from A-genome and 8 of the 10 LGs from B-genome could be assigned after comparing these two genetic maps with the reference consensus genetic maps using the common marker loci (Gautami et al., 2012). In general, a good co-linearity has been observed for these two genetic

maps and with the reference consensus genetic map (Figure 1). This population has shown great potential not only for genetic mapping but also for identification of QTLs to several economically important traits such as morphological descriptors, oil quality, and disease resistance. Here, successful attempt was made to make use of both the genetic maps and the identified QTLs for the three resistance traits to thrips, Tomato spotted wilt virus (TSWV) and leaf spots (LS).

RIL population is a set of genotypes of highly inbred F_2 lines. RILs approach complete homozygosity for all loci as the number of generations of inbreeding approaches infinity. In practice, the convention is to use six to eight generations of inbreeding, resulting in ~99.84 to 99.96% homozygosity respectively. A major advantage of RILs is that the descendents of any one RIL are genetically identical, hence "immortal", allowing RILs to be marker-genotyped once and phenotyped repeatedly in multiple labs and experiments (Elnaccash and Tonsor, 2010). It is well understood that RIL-based QTL analysis is more reliable than the F_2-based mapping populations for identification of QTLs. Majority of the studies showed identification of large number of QTLs with overestimated phenotypic effect. However, none of the study was conducted at both the stages (F_2 and RIL) using the same population and thus, this study was focused on using genotyping data generated at F_2 and F_5 generation and phenotyping data generated at F_8 generation onwards on the same population. Phenotyping data generated on this population after F_8 generation was used for both the genetic maps to identify QTLs for the three traits, thrips, Tomato spotted wilt virus (TSWV) and leaf spots (LS). Therefore, a total of 77 QTLs were identified in these two maps, 54 QTLs in F_2 map (Figure 2) while 23 QTLs in F_5 map (Figure 3) with PV up-to 19.43% (thrips), 34.92% (TSWV) and 21.45% (LS), respectively.

We should therefore expect that the F_2 and the RIL populations might show high phenotypic variance and this effect will be exaggerated in RIL compared to the F2 because

all individuals are homozygous at virtually all loci, and large sample size in RIL reducing the variance of the mean and transgressive segregation and homozygosity increasing the mean's variance (Beavers, 1998). As expected, the phenotypic variance explained by QTLs detected in F_2 map showed relatively higher phenotypic variance as compared to F_5 map. Occurrence of more QTLs with relatively higher estimation of phenotypic effect in F_2 map than the F_5 map was due to presence of higher level of heterozygosity in F_2 generation. Nevertheless, this study has provided comparative QTL analysis using genotyping data generated at F_2 and F_5 generation on the same population and confirms the assumption established based on studies on different populations. Because of above two technical deficiencies (higher number of QTLs and high estimation of phenotypic variance) of using F_2 population for conducting QTL analysis, earlier studies support the use of RIL populations such as double haploids and RILs. These RIL populations have additional advantage of being useful for phenotyping the population for multiple season/location in order to identify consistent (across seasons) and stable (across locations) QTLs.

It was interesting to note that not only alleles of the resistant parent have contributed towards the total phenotypic variance but the susceptible parent also made significant contribution through favorable alleles. For thrips no study so far has been conducted while for TSWV, earlier using the same population, Qin et al. (2012) reported one QTL with 12.9% PV (*qtswv1*). Beside above QTL, no other QTL for TSWV has been reported so far in peanut. Therefore, all the QTLs identified in current study for thrips and TSWV are novel in nature and are of great importance for further study and their deployment in molecular breeding.

The highest PV explained by any QTL for leaf spot was 27.35% (*qF2LS11*) in present study, while earlier QTL analysis using extensive phenotyping data on two RIL populations (TAG 24 × GPBD 4 and TG 26 × GPBD 4) for 7–8 seasons and genotyping data (207 marker loci each) resulted in identification of a total of 28 QTLs for late leaf spot (LLS; 10.1 to

67.8% PV) (Khedikar et al., 2010; Sujay et al., 2012). These QTLs include a major QTL for LLS with upto 62.34% PV flanked by GM1573/GM1009 and seq8D09.

Plants possess a strong immune system and defense mechanism to prevent themselves from the pathogens. Thus common genomic regions controlling more than one disease may be even more important in order to improve plant resilience. Considering the above hypothesis, two common genomic regions (GM2337 – TC42A02 and IPAHM108-2 – AHGS0347) were identified in F_2 map for all the three diseases, while four common genomic regions (GM2744 – seq5D5, TC5A07 – TC7G10, seq2G4 – PM499 and GNB2 – AHO116) in F_2 map and one common genomic region (TC11A02-300 – GNB523-500) in F_5 map were identified for LS and TSWV. The presence of common QTLs has also been reported by Sujay et al., (2012) where in three genomic regions harbored QTLs from two populations for both leaf rust and late leaf spot. Thus, these common genomic regions may harbor genes which play major role in plant defense against several pathogens and hence can be used for improving resistance for more than one disease through increasing resistance.

In summary**,** through screening more than 5000 markers, genetic maps upto 329 marker loci have been developed. High DNA polymorphism and high phenotypic variability between parental genotypes have made the T-population a very good genetic material for identification of linked markers through QTL analysis to thrips, TSWV and LS. Common genomic regions controlling more than one disease has also been identified with significant contribution towards disease resistance. Thus, this population has shown great potential for dense genetic mapping and identification of QTLs controlling several disease and agronomic traits in peanut. In addition it was evident that the number of QTLs and the estimates of phenotypic variance were reduced in F_5 map. The identified QTLs, consistent or not, will be studied further through fine mapping for potential use in breeding for genetic improvement of disease resistance in peanut.

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511 **Figure Legends**

512 **Figure 1.** Co-linearity between two genetic maps of T-population and reference consensus
513 genetic map.

514 **Figure 2.** QTL locations for thrips, Tomato spotted wilt virus (TSWV) and leaf spots (LS) on
515 F_2 generation-based genetic map of T-population.
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517 **Figure 3.** QTL locations for thrips, Tomato spotted wilt virus (TSWV) and leaf spots (LS) on
518 F_5 generation-based genetic map of T-population.
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524 **Table 1. Summary on QTL analysis based on the F_2 and F_5 population for disease**
525 **resistance**

Traits/QTLs	Number of QTLs identified	LOD value range	Phenotypic variance range (%)	Additive effect (a0) range
Based on F_2 population				
Thrips	2	2.69 - 3.27	12.14 - 19.43	0.482 -0.608
Tomato spotted wilt virus (TSWV)	15	2.51- 5.92	4.40 - 34.92	1.347 - 0.526
Leaf spot (LS)	37	2.51 - 5.68	6.61 - 27.35	4.629 - 0.720
Based on F_5 population				
Thrips	1	2.51	5.86	0.0518
Tomato spotted wilt virus (TSWV)	9	2.50 - 4.61	5.20 - 14.14	0.400 - 0.249
Leaf spot (LS)	13	2.51 - 6.38	5.95 - 21.45	0.273 - 0.174

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533 **Table 2. Consistent QTLs detected for thrips, TSWV and leaf spots in F_2 T-population**

Traits/QTL name	Linkage group	Marker Interval	Seasons appeared	LOD value	Phenotypic variance (PV) %	Additive effect (a0)
Thrips						
qF2TPS1	AhIX	IPAHM108-2 - AHGS0347	DW10	2.69	12.14	0.482
qF2TPS2	AhX	GM2337 - TC42A02	DW10	3.27	19.43	-0.608
Tomato spotted wilt virus (TSWV)						
qF2TSWV1	AhI	GNB629 - TC31G11	TF10E2	2.62	9.41	-1.153
qF2TSWV2	AhI	GA161 - GNB154	TF10E3	2.51	4.40	-0.685
qF2TSWV3	AhII	seq5D5 -GM2744	TF10E2, TF10E3, TF11E4	2.79 - 5.92	5.14 - 34.92	(-)3.539 to (-) 0.526
qF2TSWV4	AhIX	IPAHM108-2 - AHGS0347	TF10E2, TF10E3	3.99 - 4.84	12.92 - 18.11	1.024 to 1.347
qF2TSWV5	AhIX	TC5A07 - TC7G10	TF11E4	4.42	23.02	1.120
qF2TSWV6	AhX	TC42A02 - GM2337	TF10E2, TF10E3, TF11E4	3.01 - 3.28	10.78 - 16.56	(-)1.250 to (-) 0.743
qF2TSWV7	AhXI	seq2G4 - PM499	TF11E4	2.93	15.75	0.797
qTSWV8	AhXII	GNB2 - AHO116	DW10E1, TF10E2, TF10E3	2.61 - 4.16	6.26 - 21.18	(-) 1.374 to (-) 0.443
Leaf spots (LS)						
qF2LS1	AhII	GM2744 - seq5D5	TF10E3, TF11E4, TF11E5, TF11E6, TF11E7	2.69 - 3.59	7.80 - 13.11	(-) 1.422 to (-) 0.861
qF2LS2	AhV	TC1B02 -TC4A02	TF12E9	2.54	8.22	1.399
qF2LS3	AhVI	GM2724 - GNB619	TF11E6	2.68	12.56	1.064
qF2LS4	AhVIII	PM36 - GM2137	TF12E9	2.78	8.11	-1.771
qF2LS5	AhIX	IPAHM108-2 - AHGS0347	DW10E1, DW10E2, TF11E6, TF11E7	2.51 - 5.68	11.27 - 24.45	1.188 - 2.262
qF2LS6	AhIX	TC5A07 - TC7G10	TF10E3, TF11E4, TF11E5, TF12E10	3.33 - 5.01	10.8 - 24.19	1.253 - 1.834

qF2LS7	AhX	TC42A02 - GM2337	DW10E1, DW10E2, TF10E3 TF11E4, TF11E5, TF11E6, TF11E7, TF12E8, TF12E10	2.51 - 4.82	13.48 - 24.85	(-)2.519 to (-) 0.978
qF2LS8	AhXI	seq2G4 - PM499	TF10E3, TF11E5, TF11E7, TF12E8	2.55 - 3.52	6.61 - 18.97	0.720 - 1.399
qF2LS9	AhXI	PM200 - AC2C05	TF11E4, TF11E5	2.51 - 2.70	10.29 - 11.51	0.738 - 1.347
qF2LS10	AhXII	GNB2 - AHO116	TF10E3,TF11E4, TF11E5, TF12E8	2.65 - 2.90	15.30 - 21.19	(-)1.208 - (-2.921)
qF2LS11	AhXVIII	GNB904 - GNB625	TF12E9	3.54	27.35	4.629
qF2LS12	AhXVIII	GNB159 - GNB335	TF12E9	3.11	11.59	-2.497

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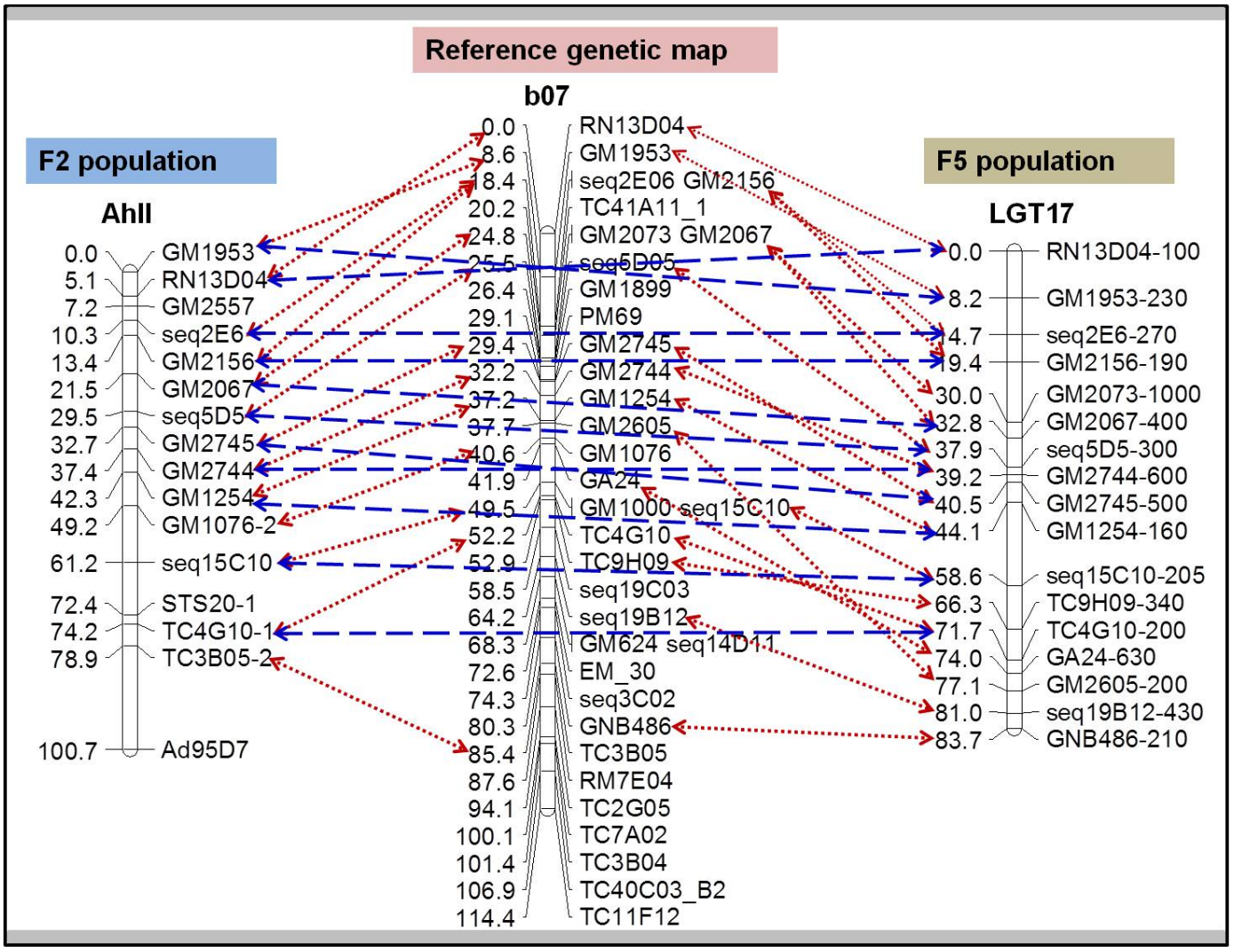
538 **Table 3. Summary of QTLs detected for thrips, TSWV and leaf spots in F_5 T-population**

Trait/QTLs	Linkage group	Marker Interval	Seasons appeared	LOD value	Phenotypic variance (PV) %	Additive effect (a0)
Thrips						
qF5TPS1	LGT2	RI1F06-410 - Ah26-195	DW10	2.51	5.86	0.0518
Tomato spotted wilt virus (TSWV) disease						
qF5TSWV1	LGT1	TC3H02-410 - seq14A7-300	TF10E2	3.56	9.31	-0.3860
qF5TSWV2	LGT6	TC11A02-300 - GNB523-500	TF11E4	3.50	7.71	-0.2486
qF5TSWV3	LGT7	GNB519-205 - GM1076-460	TF10E3	2.50	5.20	-0.2357
qF5TSWV4	LGT9	AC3C07-70 - RN35H04-1500	DW10E1, TF11E4	3.90 - 4.61	11.45 -14.14	0.335 - 0.401
qF5TSWV5	LGT11	GNB619-340 - GM2607-90	DW10E1	3.50	10.80	0.3453
qF5TSWV6	LGT12	seq14G03-500 - GM2808-400	TF10E3	3.40	10.64	0.3318
qF5TSWV7	LGT25	IPAHM167-130 - GM1555-1000	TF10E2, TF10E3	2.52 - 2.60	7.25 - 7.62	(-)0.347 to (-)0.274
Leaf spots (LS) disease						
qF5LS1	LGT3	TC1E06-370 - PM238-150	TF11E4	6.38	21.45	-0.2430
qF5LS2	LGT3	seq19G7-150 - GNB649-250	TF11E4	2.65	6.10	0.1311
qF5LS3	LGT5	GM1878 - GM637-240	TF11E6	3.25	8.98	-0.1835
qF5LS4	LGT6	TC11A02-300 - GNB523-500	TF11E6	3.35	8.02	-0.1739
qF5LS5	LGT6	TC7C06-170 - seq15D3-500	TF11E6, TF11E7	2.94 - 3.41	7.61 - 11.20	(-)0.169 to (-)0.199
qF5LS6	LGT6	TC3H07-500 - TC3H07-280	TF10E3	2.53	8.15	-0.1939
qF5LS7	LGT7	seq3B8-400 - GM1880-2000	DW10E2	2.69	7.35	0.2733
qF5LS8	LGT14	seq14D11-180 - IPAHM451-300	TF11E5	2.65	5.95	0.2218
qF5LS9	LGT16	GM678-300 - GM1742-1300	TF10E3	2.74	7.04	-0.1818
qF5LS10	LGT17	GM1254-160 - seq15C10-205	TF10E3, TF11E6	2.51 - 2.95	7.5 - 9.08	0.172 - 0.212
qF5LS11	LGT18	IPAHM229-170 - IPAHM219-155	TF11E7	3.70	8.71	0.1762


Reference genetic map
b07
F2 population
AhII
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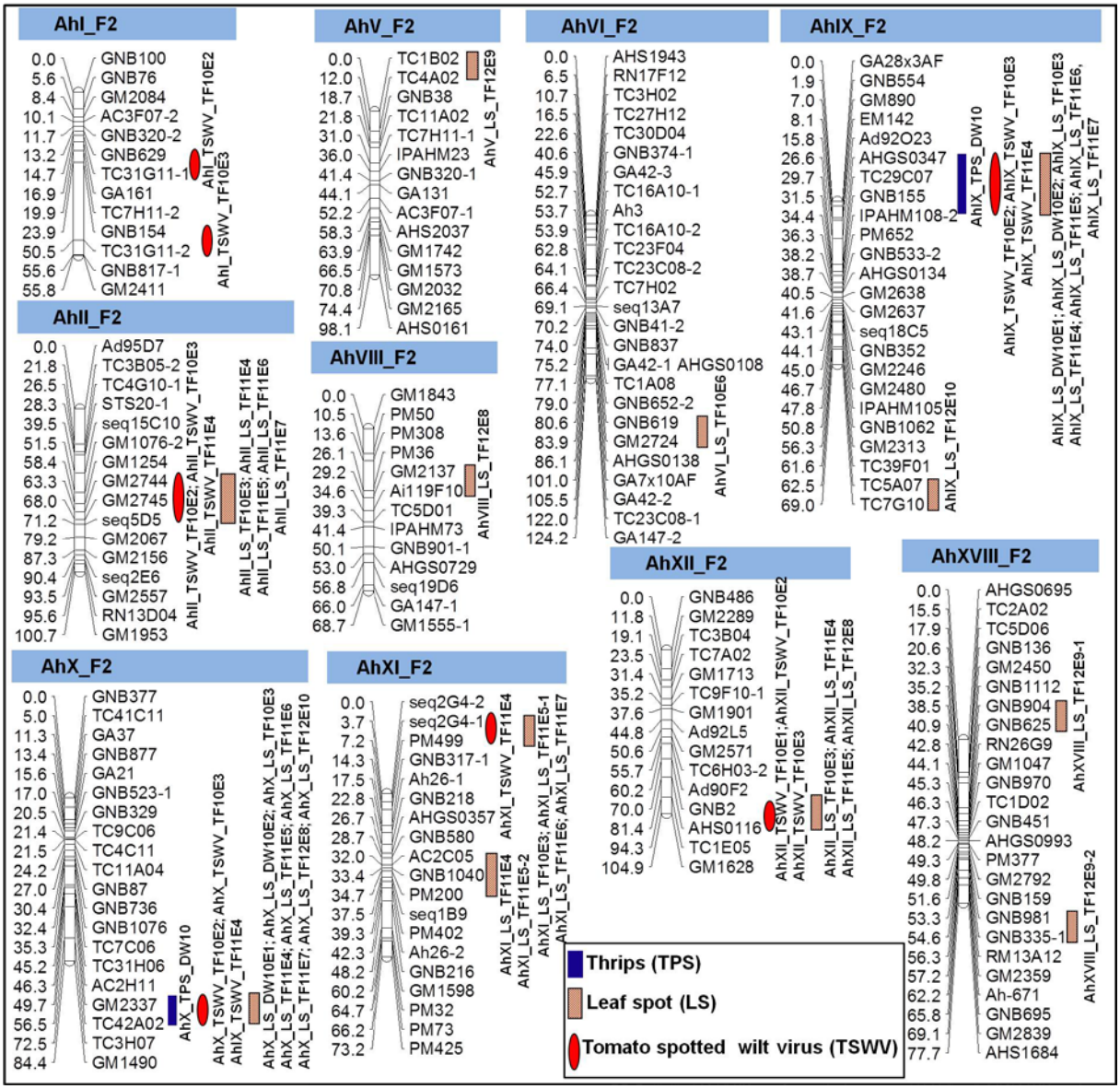
7.2 GM2557
10.3 seq2E6
13.4 GM2156
21.5 GM2067
29.5 seq5D5
32.7 GM2745
37.4 GM2744
42.3 GM1254
49.2 GM1076-2
61.2 seq15C10
72.4 STS20-1
74.2 TC4G10-1
78.9 TC3B05-2
100.7 Ad95D7
0.0 RN13D04
8.6 GM1953
18.4 seq2E06 GM2156
20.2 TC41A11_1
24.8 GM2073 GM2067
25.5 seq5D05
26.4 GM1899
29.1 PM69
29.4 GM2745
32.2 GM2744
37.2 GM1254
37.7 GM2605
40.6 GM1076
41.9 GA24
49.5 GM1000 seq15C10
52.2 TC4G10
52.9 TC9H09
58.5 seq19C03
64.2 seq19B12
68.3 GM624 seq14D11
72.6 EM_30
74.3 seq3C02
80.3 GNB486
85.4 TC3B05
87.6 RM7E04
94.1 TC2G05
100.1 TC7A02
101.4 TC3B04
106.9 TC40C03_B2
114.4 TC11F12
F5 population
LGT17
0.0 RN13D04-100
8.2 GM1953-230
14.7 seq2E6-270
19.4 GM2156-190
30.0 GM2073-1000
32.8 GM2067-400
37.9 seq5D5-300
39.2 GM2744-600
40.5 GM2745-500
44.1 GM1254-160
58.6 seq15C10-205
66.3 TC9H09-340
71.7 TC4G10-200
74.0 GA24-630
77.1 GM2605-200
81.0 seq19B12-430
83.7 GNB486-210

Figure 1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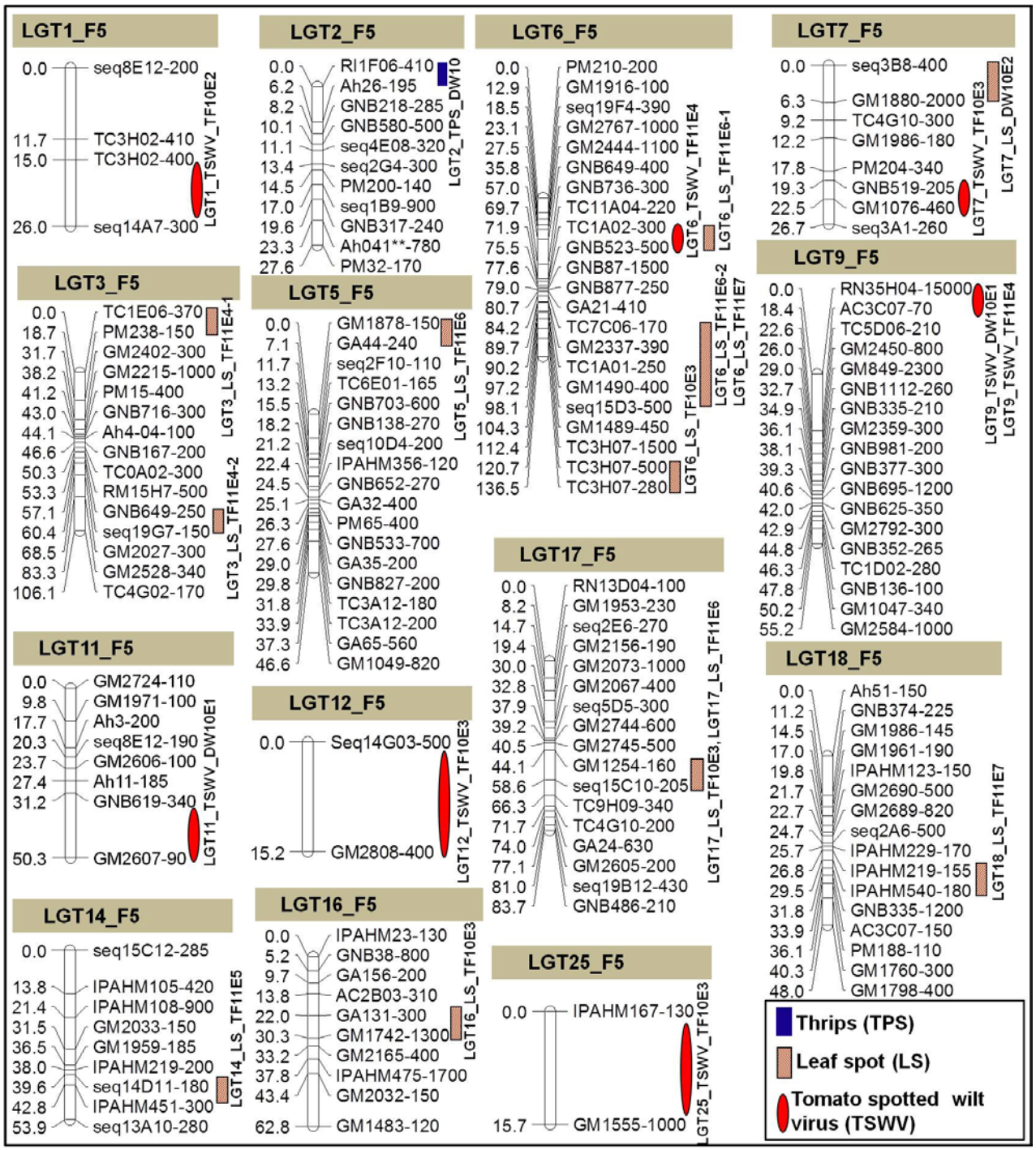
AhII_F2
AhV_F2
AhVIII_F2
AhVI_F2
AhIX_F2
AhX_F2
AhXI_F2
AhXII_F2
AhXVIII_F2
Thrips (TPS)
Leaf spot (LS)
Tomato spotted wilt virus (TSWV)

Figure 2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11.7 TC3H02-410
15.0 TC3H02-400
26.0 seq14A7-300
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LGT2_F5
0.0 RI1F06-410
6.2 Ah26-195
8.2 GNB218-285
10.1 GNB580-500
11.1 seq4E08-320
13.4 seq2G4-300
14.5 PM200-140
17.0 seq1B9-900
19.6 GNB317-240
23.3 Ah041**-780
27.6 PM32-170
LGT2_TPS_DW10
LGT6_F5
0.0 PM210-200
12.9 GM1916-100
18.5 seq19F4-390
23.1 GM2767-1000
27.5 GM2444-1100
35.8 GNB649-400
57.0 GNB736-300
69.7 TC11A04-220
71.9 TC1A02-300
75.5 GNB523-500
77.6 GNB87-1500
79.0 GNB877-250
80.7 GA21-410
84.2 TC7C06-170
89.7 GM2337-390
90.2 TC1A01-250
97.2 GM1490-400
98.1 seq15D3-500
104.3 GM1489-450
112.4 TC3H07-1500
120.7 TC3H07-500
136.5 TC3H07-280
LGT6_TSWV_TF11E4
LGT6_LS_TF11E6-1
LGT6_LS_TF11E6-2
LGT6_LS_TF10E3
LGT6_LS_TF11E7
LGT7_F5
0.0 seq3B8-400
6.3 GM1880-2000
9.2 TC4G10-300
12.2 GM1986-180
17.8 PM204-340
19.3 GNB519-205
22.5 GM1076-460
26.7 seq3A1-260
LGT7_TSWV_TF10E3
LGT7_LS_DW10E2
LGT3_F5
0.0 TC1E06-370
18.7 PM238-150
31.7 GM2402-300
38.2 GM2215-1000
41.2 PM15-400
43.0 GNB716-300
44.1 Ah4-04-100
46.6 GNB167-200
50.3 TC0A02-300
53.3 RM15H7-500
57.1 GNB649-250
60.4 seq19G7-150
68.5 GM2027-300
83.3 GM2528-340
106.1 TC4G02-170
LGT3_LS_TF11E4-1
LGT3_LS_TF11E4-2
LGT5_F5
0.0 GM1878-150
7.1 GA44-240
11.7 seq2F10-110
13.2 TC6E01-165
15.5 GNB703-600
18.2 GNB138-270
21.2 seq10D4-200
22.4 IPAHM356-120
24.5 GNB652-270
25.1 GA32-400
26.3 PM65-400
27.6 GNB533-700
29.0 GA35-200
29.8 GNB827-200
31.8 TC3A12-180
33.9 TC3A12-200
37.3 GA65-560
46.6 GM1049-820
LGT5_LS_TF11E6
LGT9_F5
0.0 RN35H04-15000
18.4 AC3C07-70
22.6 TC5D06-210
26.0 GM2450-800
29.0 GM849-2300
32.7 GNB1112-260
34.9 GNB335-210
36.1 GM2359-300
38.1 GNB981-200
39.3 GNB377-300
40.6 GNB695-1200
42.0 GNB625-350
42.9 GM2792-300
44.8 GNB352-265
46.3 TC1D02-280
47.8 GNB136-100
50.2 GM1047-340
55.2 GM2584-1000
LGT9_TSWV_DW10E1
LGT9_TSWV_TF11E4
LGT17_F5
0.0 RN13D04-100
8.2 GM1953-230
14.7 seq2E6-270
19.4 GM2156-190
30.0 GM2073-1000
32.8 GM2067-400
37.9 seq5D5-300
39.2 GM2744-600
40.5 GM2745-500
44.1 GM1254-160
58.6 seq15C10-205
66.3 TC9H09-340
71.7 TC4G10-200
74.0 GA24-630
77.1 GM2605-200
81.0 seq19B12-430
83.7 GNB486-210
LGT17_LS_TF10E3, LGT17_LS_TF11E6
LGT11_F5
0.0 GM2724-110
9.8 GM1971-100
17.7 Ah3-200
20.3 seq8E12-190
23.7 GM2606-100
27.4 Ah11-185
31.2 GNB619-340
50.3 GM2607-90
LGT11_TSWV_DW10E1
LGT12_F5
0.0 Seq14G03-500
15.2 GM2808-400
LGT12_TSWV_TF10E3
LGT18_F5
0.0 Ah51-150
11.2 GNB374-225
14.5 GM1986-145
17.0 GM1961-190
19.8 IPAHM123-150
21.7 GM2690-500
22.7 GM2689-820
24.7 seq2A6-500
25.7 IPAHM229-170
26.8 IPAHM219-155
29.5 IPAHM540-180
31.8 GNB335-1200
33.9 AC3C07-150
36.1 PM188-110
40.3 GM1760-300
48.0 GM1798-400
LGT18_LS_TF11E7
LGT14_F5
0.0 seq15C12-285
13.8 IPAHM105-420
21.4 IPAHM108-900
31.5 GM2033-150
36.5 GM1959-185
38.0 IPAHM219-200
39.6 seq14D11-180
42.8 IPAHM451-300
53.9 seq13A10-280
LGT14_LS_TF11E5
LGT16_F5
0.0 IPAHM23-130
5.2 GNB38-800
9.7 GA156-200
13.8 AC2B03-310
22.0 GA131-300
30.3 GM1742-1300
33.2 GM2165-400
37.8 IPAHM475-1700
43.4 GM2032-150
62.8 GM1483-120
LGT16_LS_TF10E3
LGT25_F5
0.0 IPAHM167-130
15.7 GM1555-1000
LGT25_TSWV_TF10E3
Thrips (TPS)
Leaf spot (LS)
Tomato spotted wilt virus (TSWV)

Figure 3