Molecular Genetics and Genomics
https://doi.org/10.1007/s00438-018-1511-9

ORIGINAL ARTICLE

Genetic imprints of domestication for disease resistance, oil quality, and yield component traits in groundnut (*Arachis hypogaea* L.)

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Received: 27 January 2018 / Accepted: 8 November 2018
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Abstract

Ploidy difference between wild *Arachis* species and cultivated genotypes hinder transfer of useful alleles for agronomically important traits. To overcome this genetic barrier, two synthetic tetraploids, viz., ISATGR 1212 (*A. duranensis* ICG 8123 × *A. ipaensis* ICG 8206) and ISATGR 265-5A (*A. kempff-mercadoi* ICG 8164 × *A. hoehnei* ICG 8190), were used to generate two advanced backcross (AB) populations. The AB-populations, namely, AB-pop1 (ICGV 91114 × ISATGR 1212) and AB-pop2, (ICGV 87846 × ISATGR 265-5A) were genotyped with DArT and SSR markers. Genetic maps were constructed for AB-pop1 and AB-pop2 populations with 258 loci (1415.7 cM map length and map density of 5.5 cM/loci) and 1043 loci (1500.8 cM map length with map density of 1.4 cM/loci), respectively. Genetic analysis identified large number of wild segments in the population and provided a good source of diversity in these populations. Phenotyping of these two populations identified several introgression lines with good agronomic, oil quality, and disease resistance traits. Quantitative trait locus (QTL) analysis showed that the wild genomic segments contributed favourable alleles for foliar disease resistance while cultivated genomic segments mostly contributed favourable alleles for oil quality and yield component traits. These populations, after achieving higher stability, will be useful resource for genetic mapping and QTL discovery for wild species segments in addition to using population progenies in breeding program for diversifying the gene pool of cultivated groundnut.

Keywords DArT markers · Genetic map · Trait mapping · Introgression lines · Wild crop relatives · Groundnut

Introduction

The ancestral polyploidy in plants has long been recognized as a strong evolutionary force for speciation, adaptation, and dominance of plants (Jiao et al. 2011). Cultivated groundnut (*Arachis hypogaea* L.) or peanut is an allotetraploid ($2n = 4x = 40$) oilseed legume crop with AABB genome. It has been postulated that the tetraploid groundnut was originated from a single hybridization event followed by chromosome doubling between two wild diploid species, i.e., *A. duranensis* (A-genome) and *A. ipaensis* (B-genome), about 3,500 years ago (Kochert et al. 1996). Therefore, the primary gene pool of groundnut includes mainly tetraploids such as cultivars, advanced breeding lines, and landraces of *A. hypogaea*, along with *A. monticola*. The affiliates of this gene pool are cross compatible among each other, thus producing fertile hybrids. The secondary gene pool, on the other hand, consists of wild diploid species ($2n = 2x = 20$ or $2n = 2x = 18$) often called as crop wild relatives (CWR), which encompasses > 80 species in genus *Arachis* grouped into nine sections based on their cross compatibility and morphology (see Stalker 2013). Molecular markers studies conducted in the recent past have demonstrated ample genetic variation in the primary and secondary gene pools (Koppolu et al. 2010; Khera et al. 2013a; Pandey et al. 2017; Vishwakarma et al. 2017a). However, this genetic

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00438-018-1511-9) contains supplementary material, which is available to authorized users.

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Published online: 22 November 2018

variation could not be translated into improved varieties, because the ploidy difference between the cultivated and wild groundnut has been the major genetic bottleneck for natural hybridization. This has restricted introgression of useful alleles directly from wild species to the cultivated groundnuts (Pandey et al. 2012; Sharma et al. 2013, 2017; Kumari et al. 2014).

To overcome this genetic bottleneck, developments of synthetic hexaploid and tetraploids were proposed for gene introgression into cultivated groundnut *A. hypogaea* L., (Simpson 1991). However, transfer of useful gene(s) to cultivated groundnut involves multiple backcrossing and phenotyping at every stage. This cycle can be curtailed if we smartly deploy linked molecular makers through genomics-assisted breeding (GAB) (Varshney et al. 2009a, 2013). The pre-requisite to this deployment is quantitative trait locus (QTL) discovery for the trait of interest, which can be achieved by developing appropriate mapping populations, generating genotyping and phenotyping data followed by construction of genetic maps, and then performing the QTL analysis (Pandey et al. 2016; Pandey and Varshney 2018). The first tetraploid genetic map in groundnut involving an amphidiploid was constructed using restriction fragment length polymorphism (RFLP) markers on a BC_1 population (cultivated 'Florunner' × synthetic 'TxAG-6') (Burrow et al. 2001). The 'TxAG-6' is an amphidiploid from the cross [*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)], wherein *A. batizocoi* contributed B-genome, while *A. cardenasii* × *A. diogoi* contributed A-genome. Recently, using the advanced backcross-QTL (AB-QTL) approach in BC_3 generation of the above-mentioned population, seven QTLs were identified for root-knot nematode resistance (Burrow et al. 2014). Another genetic map based on simple sequence repeat (SSR) markers was constructed using the cross between the cultivated variety 'Fleur 11' and an amphidiploid derived from *A. ipaënsis* × *A. duranensis* containing 298 mapped loci (Foncéka et al. 2009). This was the first attempt wherein genetic map involving most probable progenitors were involved. The same genetic population was phenotyped and used for QTLs discovery for pod and seed morphology, plant architecture, and yield components under well-watered and water-limited conditions (Fonceka et al. 2012a). Recently, in the recombinant inbred line (RIL) population of the same cross, a genetic map containing 772 mapped loci (406 SSRs and 366 SNPs) was developed covering a total length of 1487.3 cM (Bertioli et al. 2014a). From the cross between a cultivar ICGS 76 and a synthetic ISATGR 278-18, a genetic map with 128 SSR marker loci spanning a length of 1103.2 cM with average distance of 8.62 cM was constructed and used for discovery of QTLs for foliar disease resistance in addition to identifying several lines with good agronomic features (Kumari 2013; Kumari et al. 2014). Similarly, Paratwagh and Bhat (2015) reported the development of 78 introgression lines from three crosses (ICGS 76 × ISATGR 278-18; DH86 × ISATGR 278-18 and DH86 × ISATGR 5B) showing high level of resistance for foliar fungal diseases. These studies proved that the synthetics are a good source for diversifying the cultivated gene pool and bringing the desired traits including the robust resistance for disease and agronomic traits.

The availability of molecular markers via genome sequencing of both the diploid progenitors (Bertioli et al. 2016; Chen et al. 2016) facilitated development of different types of genetic markers and genotyping platforms (Pandey et al. 2017; Vishwakarma et al. 2017a; Zhao et al. 2017). The low polymorphism rate (≤ 10%) (Pandey et al. 2017; Vishwakarma et al. 2017b) triggered the groundnut research community to develop large number of SSR, single-nucleotide polymorphic (SNP) markers (Pandey and Varshney 2018), and *AhMITE1* markers (Gayathri et al. 2018). However, very few SSR or SNP markers were available at the start and during the course of this study that can be successfully used to develop genetic maps for mapping populations derived from synthetic tetraploids. Therefore, diversity arrays technology (DArT) markers as an alternative high-throughput and cost-effective marker system were used for genotyping in this study (http://www.diversityarrays.com/). In the case of groundnut, ICRISAT and DArT Pty Ltd, Australia have jointly developed DArT arrays with 15,360 features. These arrays have been successfully used for developing high-density genetic maps for QTL analysis in groundnut (Vishwakarma et al. 2016; Shasidhar et al. 2017).

Efforts towards diversifying the primary gene pool of groundnut at ICRISAT resulted in the development of 17 autotetraploid/amphidiploid groundnuts (Mallikarjuna et al. 2011a; Sharma et al. 2017). Genetic populations developed by crossing cultivated and synthetic autotetraploid/amphidiploid genotypes are very useful resources for selecting promising introgression lines with important traits and QTL discovery through AB–QTL analysis approach (Tanksley and Nelson 1996).

In view of above, this study reports the development of two populations derived from one amphidiploid (allotetraploid) namely ISATGR 1212 [*A. duranensis* (ICG 8123) × *A. ipaensis* (ICG 8206)] and one autotetraploid, ISATGR 265-5A [*A. kempff-mercadoi* (ICG 8164) × *A. hoehnei* (ICG 8190)], identification of resistant lines for two foliar fungal diseases (late leaf spot and leaf rust), two genetic maps, marking of several wild species segments in the genetic background of cultivated groundnut and 50 QTLs for disease resistance, oil quality, and agronomic traits.

Materials and methods

Mapping populations and DNA isolation

Synthetic genotypes were developed through tetraploid route, i.e., crossing between wild species to develop

diploid hybrids and subsequently treating the diploid hybrid pollen with colchicine. If the synthetic tetraploid had different genomes, it was termed as allotetraploid, while if it had same genome combination, it was termed as autotetraploid. Two synthetics, namely, ISATGR 1212 (allotetraploid with AABB genome) and ISATGR 265-5A (autotetraploid with AAAA genome), were used to construct two populations (Sharma et al. 2013, 2017). The allotetraploid 'ISATGR 1212' was developed from the cross between two wild species, i.e., A-genome (ICG 8123, *A. duranensis*) and B-genome (ICG 8206, *A. ipaensis*). The ISATGR 1212 was crossed with a popular cultivar, ICGV 91114, to develop AB-pop1 population. The autotetraploid 'ISATGR 265-5A' was developed from the cross between ICG 8164 (*A. kempff-mercadoi*) and ICG 8190 (*A. hoehnei*) both containing A-genome and this autotetraploid was crossed with a cultivar ICGV 87846 to develop AB-pop2 population. Both the populations (BC_2F_1s) were developed by backcrossing with the synthetic tetraploid parents. ICGV 91114 is Spanish bunch with short duration and drought tolerant variety while ICGV 87846 is Virginia bunch with medium duration and drought tolerant variety.

Total DNA was extracted from the young leaves using the modified CTAB-based method (Doyle and Doyle 1987); quality and quantity of DNA were performed as per Mace et al. (2003).

Genotyping with DArT and SSR markers

Both the populations were genotyped using DArT arrays consisting of 15,360 features, of which 3653 polymorphic markers were considered for filtering and construction of genetic map (Suppl Table 1). The genotyping method of DArT was followed as per Pandey et al. (2014b) and Vishwakarma et al. (2016).

In addition to DArT markers, a total of 100 SSR markers were selected covering all the 20 linkage groups (LGs) based on the equal distribution of markers onto the international consensus genetic map developed by Shirasawa et al. (2013). For tracking the flow of SSR allele, the grandparents of both the synthetic tetraploid genotypes, i.e., ICG 8123 and ICG 8206 (parents of ISATGR 1212) and ICG 8164 and ICG 8190 (parents of ISATGR 265-5A), were also used along with parents. PCR reactions were performed following a touchdown PCR profile in an ABI thermal cycler (Applied Biosystems, USA) in a 5 µl reaction volume. SSR marker genotyping and allele scoring was performed as per procedure explained in Varshney et al. (2009b) and Pandey et al. (2014b).

Phenotyping

All the 186 individuals from each of these two backcross populations were phenotyped for traits related to oil quality, biotic stress resistance, and yield component traits at one or two locations during the year 2011 and 2012. The phenotyping methods used are briefed as follows:

Phenotypic screening for late leaf spot (LLS) and rust diseases

The LLS disease is caused by the pathogen *Cercosporidium personatum*, while the rust is caused by *Puccinia arachidis*. Phenotyping of both the mapping populations were carried out at two locations, i.e., ICRISAT-Patancheru and University of Agricultural Sciences (UAS)-Raichur, India. At ICRISAT-Patancheru, the phenotyping was done during the rainy season of 2011 and 2012, while in UAS-Raichur, it was done only during rainy 2012. Artificial disease environment was created using "spreader row technique" for LLS and rust disease screening. The two genotypes, namely, 'TMV 2' (susceptible to LLS) and '28-2' (susceptible to rust but resistant to LLS), were used as spreader rows for both the diseases. To effectively maintain the inoculum load, the spreader rows were planted at every tenth row and around the border of the plot. All the recommended practices were adopted for cultivation of a healthy groundnut crop. The LLS/rust conidia/urediniospores were extracted by soaking and rubbing the infected leaves in water for about half an hour. After 35 days of sowing, at least 20,000 conidia/urediniospores per ml concentration were used to spray on the plants. Disease scoring was done at 90 days after sowing for LLS/rust using the modified 9-point scale method, resistant being on scale 1 to highly susceptible for scale 10 (Subbarao et al. 1990).

Phenotypic screening for peanut bud necrosis disease (PBND)

The peanut bud necrosis virus (PBNV) is the causal organism for PBND and is transmitted by thrips. Usually, PBND is screened in the endemic areas under natural conditions and UAS-Raichur was identified as one of such hotspots for the PBND screening (Basu 1995). Therefore, these populations were screened for PBND only at UAS-Raichur during rainy 2012. Screening was done based on infector row technique wherein the backcross lines were sown in single row of 5 m length and, at every fourth row, a susceptible check 'KRG-1' was planted with a spacing of 45 cm × 10 cm. To have uniform infestation of the disease, prior to sowing of test entries, five lines of 'KRG-1' were sown along the border before 15 days. A screening block with cowpea seedlings (C-152) was planted for confirming the presence of PBNV.

For checking reaction of PBNV, enzyme-linked immunosorbent assay (ELISA) was also carried out on the randomly collected samples. The PBND incidence was calculated as percentage of number of plants infected with PBND by total number of plants per entry. Disease rating based on 0–5 scale was then computed for resistance/susceptible categorisation (Sunkad et al. 2001).

Estimation of oil quality traits

Ten seeds of each line were used for oil trait analysis. Fatty acids were extracted on a gas chromatography (GC) by saponification-trans-esterification method as described by Metcalf et al. (1996) and Shilpa et al. (2013). In brief, the fatty acid methyl esters (FAME) were separated by GC on GC-9, a GLC (gas liquid chromatography) unit with on board flame ionization detector, programmable oven, and electronic CR3-A integrator (Shimadzu corporation, Kyoto, Japan). In order to identify the fatty acids in the samples, a standard FAME mixture Nu Check 21 A (Nu Check Prep, Inc. MN, USA) was used, while, for recording the peak area of fatty acids and retention time, a Shimadzu C-R4 A Chromatopac Integrator (Shimadzu corporation, Kyoto, Japan) was used. Eight fatty acids were extracted, namely, oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20.0), gadolieic (C20:1), behenic (C22:0), and lignoceric (C24:0). Three important quality traits such as oleic to linoleic acid ratio (OLR), saturated fatty acids (SFA) percentage in a line, were obtained by adding the percentage contribution of six fatty acids namely palmitic, stearic, arachidic, gadolieic, behenic acid, and lignoceric acid and unsaturated fatty acids (UFA) percentage obtained by adding oleic and linoleic acid.

Measuring yield component traits

A total of four basic yield component traits were evaluated at ICRISAT-Patancheru during rainy 2012 season. The pod yield per plant (PYP) was calculated as average weight of pod per entry represented in grams. The sound mature kernels' (SMK) % was computed as the ratio of weight of SMK to the weight of total kernels. The total kernels include any immature and shrivelled kernels. The shelling percentage (SP) was computed as the percentage of the mass of seed in a given mass of pods, while the hundred seed weight (HSW) was measured as weight of hundred groundnut seeds.

Construction of genetic maps

Genotyping data from both the populations were used for carrying out genetic linkage analysis. In case of SSR markers which are co-dominant markers, the 1:1 ratio corresponds to the presence of homozygous allele to the presence of heterozygous allele, while, for DArT marker which is dominant marker, the ratio refers to absence of homozygous allele to the presence of heterozygous allele. The DArT markers were named with the prefix 'Ah_' where 'Ah' stands for *Arachis hypogaea*, followed by numbers corresponding to their unique clone ID. Markers with more than 10% of missing data were not used in the analysis. The software JoinMap version 3.0 (Van Ooijen and Voorrips 2001) was used for construction of linkage map.

All the markers were subjected to goodness-of-fit test to assess any deviations from the expected Mendelian segregation ratio of 1:1 at 5% level of significance. After preparing the data set, population node was created by the "create population node" and then ''locus genotype frequency'' function to compute the χ^2 values for all the markers. Regression mapping algorithm was applied for the construction of map with the factors such as recombination frequency smaller than 0.40, LOD larger than 3.0 and goodness-of-fit jump threshold for removal of loci as 5. Map distances were calculated using Kosambi mapping function (Kosambi 1944). For mapping optimum number of loci on to the genetic map, three rounds were allowed by setting the option "third round". Grouping of marker loci was performed under the "grouping (tree)" and the location of loci into linkage groups (LGs) was considered through selection of higher LOD groupings and ''create groups using the grouping tree'' commands. Map-Chart version 2.2 (Voorrips 2002) was used to draw final maps.

Assessment of wild genome introgression and preliminary quantitative trait analysis

The genetic map was used for the wild genome introgression analysis and calculating heterozygosity percentage using the CSSL Finder software (http://mapdisto.free.fr/CSSLFinder/). A subset of lines from the two BC_2F_1 populations were selected representing the optimal genome coverage of synthetic tetraploids into the cultivated parent. Target length of introgressed region was set at 20 cM for the overlapping of contiguous synthetic tetraploid segments for a given linkage group with the most conceivable background of the cultivated variety. Both the populations comprising of 186 individuals each were used for construction of genetic maps. The segregating heterozygous population is not good for conducting reliable trait mapping studies. Nevertheless, we performed phenotyping in BC_2F_1 and BC_2F_2 generations to identify potential lines for further evaluation and use in breeding program. To get an idea on the potential of these lines to serve as advanced backcross mapping populations once the populations are stabilized genetically, we performed preliminary QTL analysis for resistance to LLS, rust, PBND, OLR, SFA, UFA, PYP, SMK, SP, and HSW. The software Windows QTL Cartographer version

2.5 (Wang et al. 2012) was used for the identification of main-effect QTLs (M-QTLs). Composite interval mapping (CIM) approach was employed following parameters such as model 6, putative QTL with a window size of 10.0 cM and scanning intervals of 1.0 cM between markers, 500 permutations with 5% significance level, "Locate QTL" option and forward–backward stepwise regression as background control set by the number of marker cofactors.

Since the phenotyping was done for LLS and rust resistance traits in two years (2011 and 2012) and multiple locations (ICRISAT-Patancheru and UAS-Raichur), the consistency of QTL was interpreted by considering a QTL region as "consistent QTL" if the same QTL was identified and co-located in the same genomic region across different seasons (i.e. same QTL present in both ICRISAT_2011 and ICRISAT_2012 environment). However, if the same QTL was present in two locations (ICRISAT_2011 and/or ICRISAT_2012 and Raichur_2012), it was considered as a "stable QTL".

Results

Development of mapping populations

In case of AB-pop1, five F_1s were produced from the cross between ICGV 91114 and ISATGR 1212, which were backcrossed with ICGV 91114 to produce 202 BC_1F_1 seeds. The BC_1F_1 plants were raised and confirmed based on morphological features. These confirmed plants were used for making second backcross with the cultivated parent to produce 1809 BC_2F_1s. Similarly, in the case of AB-pop2, four F_1s were produced from the cross between ICGV 87846 and ISATGR 265-5A, which were backcrossed with ICGV 87846 to produce 109 BC_1F_1 seeds. After confirmation, these BC_1F_1 plants were backcrossed again with ICGV 87846 resulting in 734 BC_2F_1s. A total of 184 BC_2F_1 plants from each of the two populations were selected based on morphological variations. These two populations were then phenotyped during rainy 2011 and 2012. Several promising introgression lines have been identified with resistance to rust and LLS (Supp Table 1); many of them have resistance for both the foliar fungal diseases (Table 1).

DArT and SSR-based dense genetic maps

A total of 291 and 1,121 polymorphic DArT markers, alongwith nine and four polymorphic SSR markers were identified for the AB-pop1 and AB-pop2 populations, respectively (Supp Table 2). The genotyping data were used for construction of the genetic maps. In the AB-pop1 population, 258 markers (253 DArTs and 5 SSRs) were mapped spreading across 19 LGs with a total map length of 1,415.7 cM and an average marker density of 5.5 cM/loci. The map length of linkage groups (LGs) ranged from 8.33 to 172.9 cM with an average of 74.51 cM/LG (Table 2 and Supp Fig. 1). Similarly, in the AB-pop2 population, a total of 1,043 markers (1,035 DArTs and 8 SSRs) were mapped onto the 20 LGs covering a distance of 1,500.8 cM with an average marker density of one marker at every 1.44 cM. The total map length ranged from 8.1 to 178.4 cM with an average length of 75.04 cM/LG (Table 2 and Supp Fig. 2). Comparison of mapped markers between the AB-pop1 and AB-pop2 populations identified 61 common markers between these two genetic maps. Four LGs each in AB-pop1 (AhVI, AhXII, AhXVI, and AhXVII) and AB-pop2 (AhIV, AhXV, AhXVIII, and AhXIX) population did not have any common marker. In the AB-pop2 population, two LGs (AhI and AhVI) had maximum of 12 common markers each (Supp Table 3).

Table 1 Ten best BC_2F_2 introgression lines with resistance to rust and late leaf spot

S. No.	Introgression line	Pedigree	LLS score	Rust score
1	BC_2F_2-2031	(ICGV 87846 × ISATGR 265-5A) × ICGV 87846 × ICGV 87846	3	2
2	BC_2F_2-2090	(ICGV 87846 × ISATGR 265-5A) × ICGV 87846 × ICGV 87846	3	2
3	BC_2F_2-2091	(ICGV 87846 × ISATGR 265-5A) × ICGV 87846 × ICGV 87846	3	2
4	BC_2F_2-2104	(ICGV 87846 × ISATGR 265-5A) × ICGV 87846 × ICGV 87846	3	2
5	BC_2F_2-2149	(ICGV 87846 × ISATGR 265-5A) × ICGV 87846 × ICGV 87846	3	2
6	BC_2F_2-2279	(ICGV 87846 × ISATGR 265-5A) × ICGV 87846 × ICGV 87846	3	2
7	BC_2F_2-2360	(ICGV 87846 × ISATGR 265-5A) × ICGV 87846 × ICGV 87846	3	2
8	BC_2F_2-2372	(ICGV 87846 × ISATGR 265-5A) × ICGV 87846 × ICGV 87846	3	2
9	BC_2F_2-2389	(ICGV 87846 × ISATGR 265-5A) × ICGV 87846 × ICGV 87846	3	2
10	BC_2F_2-2424	(ICGV 87846 × ISATGR 265-5A) × ICGV 87846 × ICGV 87846	3	2

The disease was scored on 1–9 scale for both the foliar fungal diseases and the disease scores of 1–3 were rated as resistant lines

Table 2 Summary of the genetic maps constructed for the AB-pop1 and AB-pop2 populations

AB-pop1 (ICGV 91114×ISATGR 1212)				AB-pop2 (ICGV 87846×ISATGR 265-5A)			
Linkage group	Length (cM)	Mapped loci	Marker density (cM/loci)	Linkage group	Length (cM)	Mapped loci	Marker density (cM/loci)
AhI	113.6	56	2.0	AhI	178.4	317	0.56
AhII	97.8	24	4.1	AhII	120.6	231	0.52
AhIII	59.6	9	6.6	AhIII	35.3	6	5.88
AhIV	32.9	4	8.2	AhIV	40.0	5	8.00
AhV	144.0	29	5.0	AhV	42.3	3	14.10
AhVI	8.3	2	4.2	AhVI	130.7	200	0.65
AhVII	119.1	21	5.7	AhVII	39.1	5	7.82
AhVIII	172.9	33	5.2	AhVIII	163	103	1.58
AhIX	99.8	14	7.1	AhIX	56.9	22	2.59
AhX	67.4	11	6.1	AhX	144.5	9	16.06
AhXI	51.8	3	17.3	AhXI	8.1	3	2.70
AhXII	37.0	3	12.3	AhXII	116.1	73	1.59
AhXIII	31.3	2	15.7	AhXIII	91.3	16	5.71
AhXIV	39.3	8	4.9	AhXIV	35.7	7	5.10
AhXV	166.5	12	13.9	AhXV	52.0	4	13.00
AhXVI	16.4	3	5.5	AhXVI	26.7	3	8.90
AhXVII	66.8	17	3.9	AhXVII	119.8	24	4.99
AhXVIII	15.7	3	5.2	AhXVIII	65.9	7	9.41
AhXIX	75.5	4	18.9	AhXIX	8.6	2	4.30
–	–	–	–	AhXX	25.8	3	8.60
Total	1415.7	258	5.5		1500.8	1043	1.40

Assessment of wild genome introgression lines

The heterozygosity percentage in AB-pop1 population varied from 18.6 to 87.5% with an average of 46.48%, while, in the AB-pop2 population, it ranged from 8.80 to 60.23% with an average of 35.47%. The number of genomic segments from the donor genotype varied from 6 to 22 (average of 15.3) in the AB-pop1 population whereas it ranged from 8 to 27 (average of 16.6) in the AB-pop2 population. An optimal coverage of 20 cM of the wild genome between neighbouring lines was set to identify the most probable reoccurrence to the cultivated background. In the AB-pop1, a subset of 43 lines was identified with the segment length ranging between 27.72 and 93.15 cM (mean with 48.65 cM). Similarly, in the AB-pop2 population, 59 lines were identified with the segment varied from 17.22 to 94.45 cM with an average of 41.12 cM (Fig. 3).

Identification of linked markers with agronomic traits

QTL analysis using phenotypic data and genotypic data resulted in the identification of 50 QTLs for different traits in both the populations (Table 3). Interestingly, QTLs were located in the 7 genomic regions of 3 LGs in the AB-pop1 population (Fig. 1), while, in 11 genomic regions of 8 LGs in the AB-pop2 population (Fig. 2). The phenotypic variation explained (PVE %) by these QTLs ranged from 6.7% (*AB1qLLS1_2* and *AB1qSMK7*) to a maximum of 67.8% (*AB2qSMK13_3*) (Supp Table 4 and Supp Table 5). Of the total 50 QTLs (15 QTLs in AB-pop1 population and 35 QTLs in AB-pop2 population) for the traits in both the populations, 26 QTLs showed major effect (> 10% PVE). In the case of AB-pop1 population, only 6 QTLs showed major effect, i.e., 1 for rust resistance, 3 for PBND, and 2 for oil quality traits. However in the AB-pop2 population, 20 QTLs with major effect were identified for LLS (3 QTLs), rust (4 QTLs), oil quality (6 QTLs), and yield component (7 QTLs) traits.

For LLS resistance, a total of 3 and 7 QTLs were identified in the AB-pop1 and AB-pop2 population with PVE of up to 6.7–50.9%, respectively (Table 3; Supp Table 4; Supp Table 5). The ISATGR 1212 allele had an additive effect at all the genomic regions for LLS resistance except for '*AB1qLLS1_2*' which came from ICGV 91114. Likewise in the AB-pop2 population, the ISATGR 265-5A contributed all the resistance alleles with higher additive effect at all the genomic regions except for '*AB2qLLS6_4*'

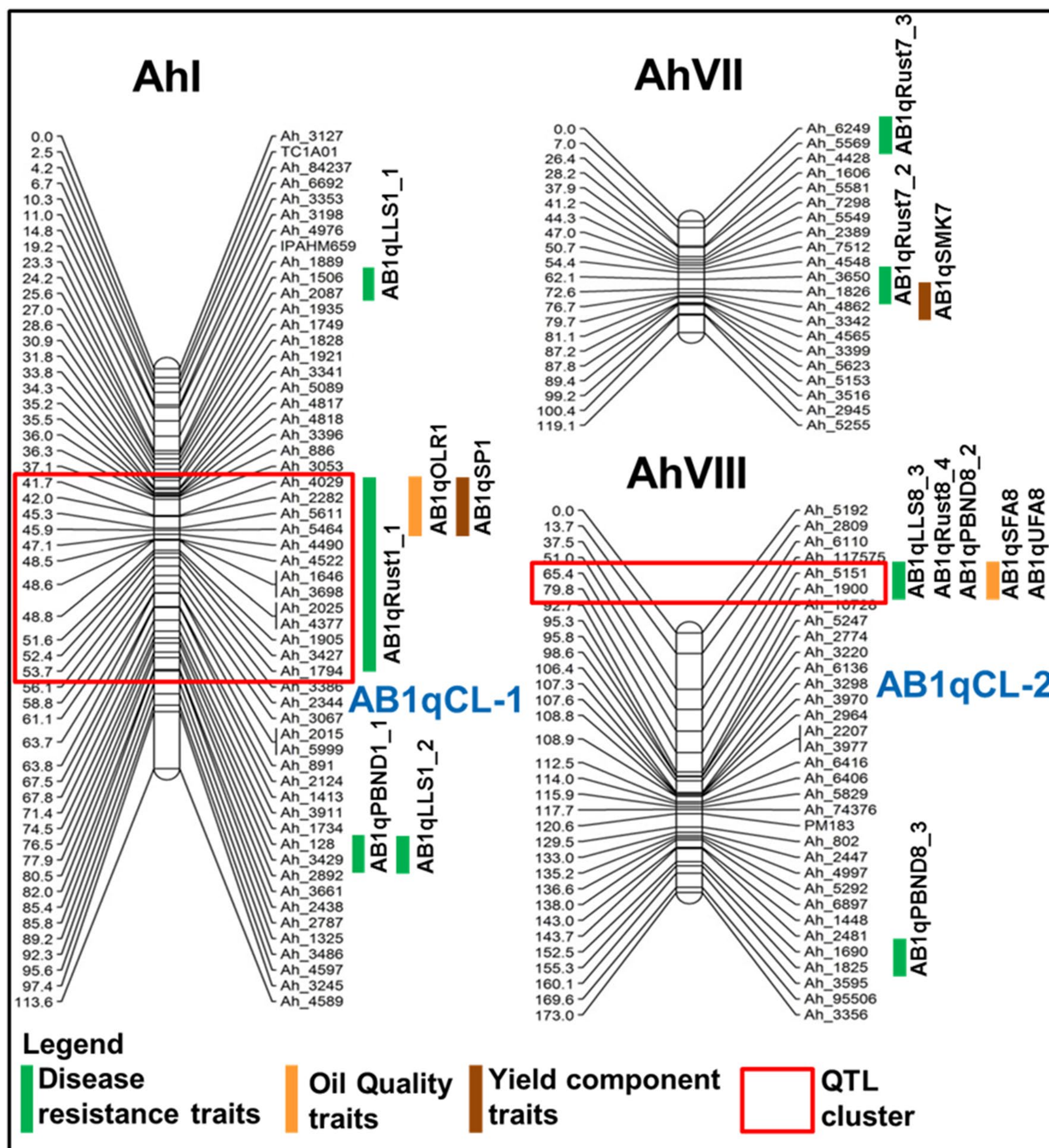


Fig. 1 Genetic map for the AB-pop1-population (ICGV 91114×ISATGR 1212) showing genomic regions with 15 QTLs for disease, oil quality, and yield component traits. This figure also shows two clusters containing QTL for multiple traits

which was contributed by ICGV 87846. For rust resistance, of the four QTLs detected in the AB-pop1 population, the QTL alleles of *AB1qRust1_1'* (AhI, 9.4% PVE) and *AB1qRust7_3* (AhVII, 7.2% PVE) had higher additive effect from the ISATGR 1212. In contrast, the QTL alleles of '*AB1qRust7_2'* (AhVII, 9.6% PVE) and *AB1qRust8_4* (AhVIII, 10.8% PVE) had higher additive effect from the ICGV 91114 (Supp Table 4). In the case of AB-pop2 population, of the seven QTLs identified (8.0–48.7% PVE), all the QTLs for rust resistance had contribution from the ISATGR 265-5A (Supp Table 5). For PBND resistance, three QTLs were detected in the AB-pop1 population (13.0–14.5% PVE). The resistance alleles for *AB1qPBND8_2* and *AB1qPBND8_3* were contributed by 'ISATGR 1212', while ICGV 91114 contributed for *AB1qPBND1_1* (Supp Table 4). In case of AB-pop2 population, only one QTL, namely, '*AB2qPBND'* (7.4% PVE) and the resistance allele for this QTL was contributed by the ISATGR 265-5A (Supp Table 5).

For oil quality traits (oleic to OLR, SFA content, and UFA content), the QTL analysis identified three QTLs, i.e., one for each quality traits in the AB-pop1 population

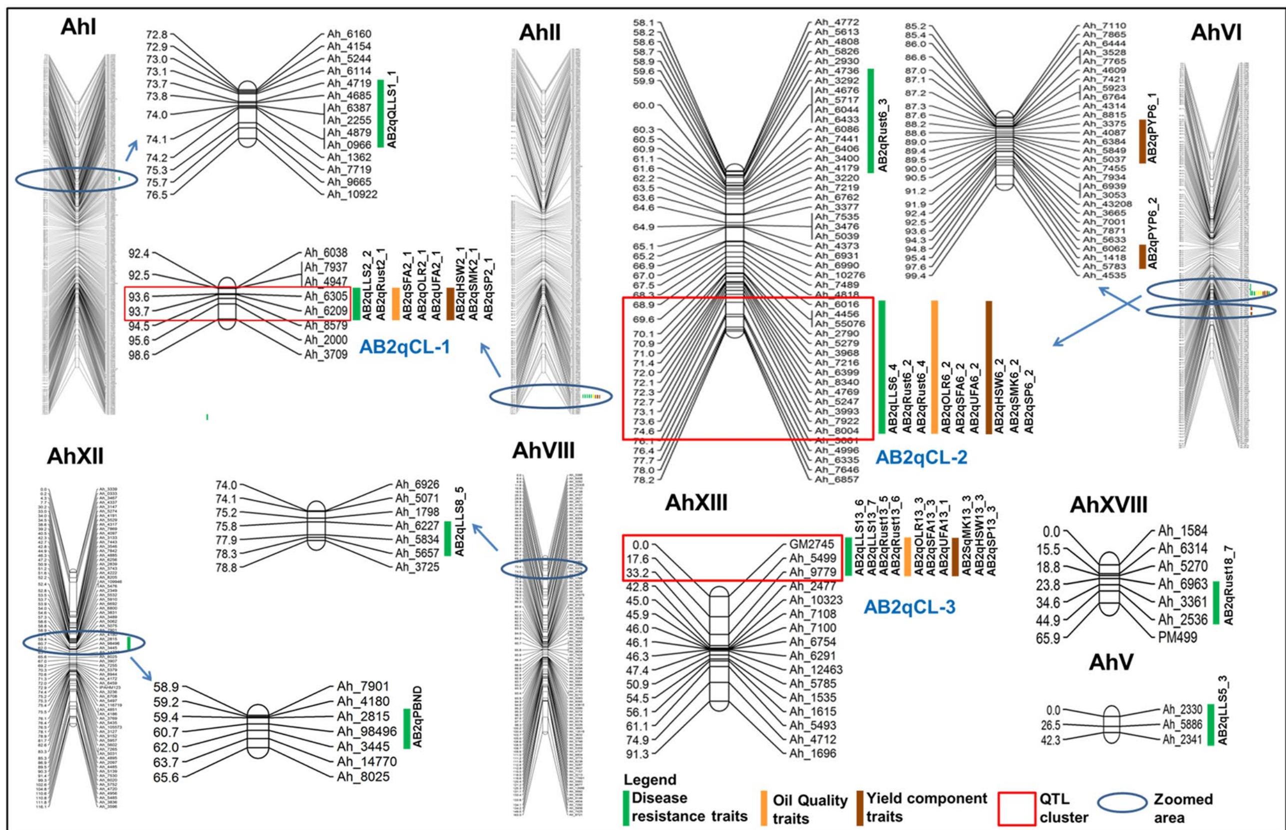


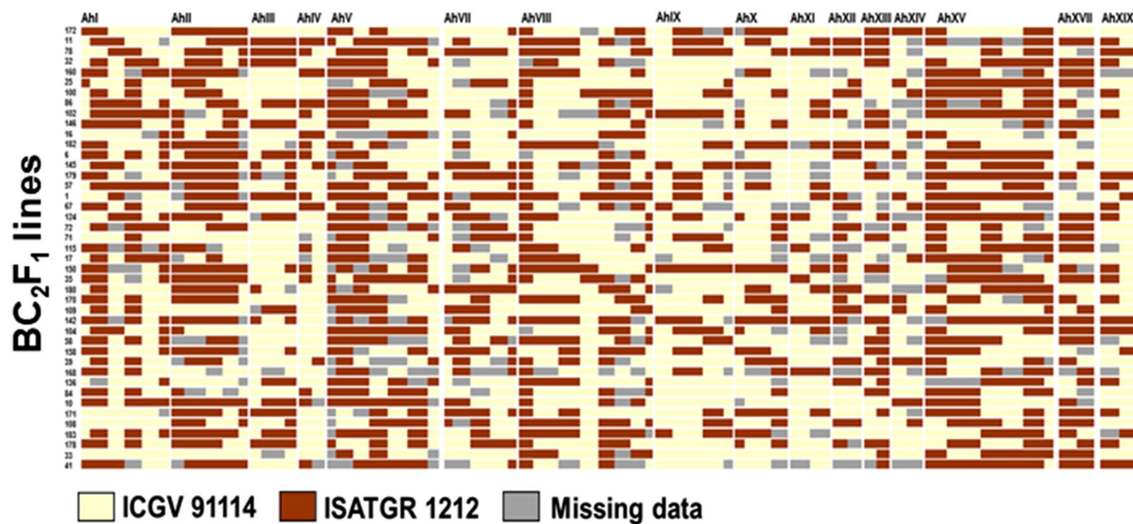
Fig. 2 Genetic map for the AB-pop2 population (ICGV 87846 × ISATGR 265-5A) showing genomic regions with 35 QTLs for disease, oil quality, and yield component traits. This figure also shows three clusters containing QTL for multiple traits

Table 3 Summary of QTLs identified in the AB-pop1 and AB-pop2 populations

Traits	AB-pop1 (ICGV 91114 × ISATGR 1212)				AB-pop2 (ICGV 87846 × ISATGR 265-5A)			
	QTLs identified	LOD value range	Phenotypic variance (%)	Additive effect (a0)	QTLs identified	LOD value range	Phenotypic variance (%)	Additive effect (a0)
Late leaf spot	3	2.5–2.9	6.7–9.7	1.42 to (−) 0.86	7	2.8–8.2	7.2–50.9	0.56 to (−) 3.18
Rust	4	2.6–3.4	7.2–10.8	1.38 to (−) 1.40	7	3.2–8.5	8.0–48.7	(−) 0.37 to (−) 2.50
Peanut bud necrosis disease	3	3.3–5.1	13.0–14.5	11.11 to (−) 10.21	1	2.9	7.4	(−) 7.06
Oil quality	3	3.0–3.5	9.6–14.8	0.71 to (−) 27.48	9	3.0–12.0	7.4–52.7	(−) 0.89 to (−) 74.38
Yield component traits	2	2.5–2.9	6.7–8.0	19.64 to (−) 25.31	11	3.0–60.6	7.6–67.8	(−) 5.91 to (−) 81.71
Total	15	2.5–5.1	6.7–14.8	0.71 to (−) 27.48	35	2.9–60.6	7.2–67.8	0.56 to (−) 81.71

(9.6–14.8% PVE), while nine QTLs, i.e., three for each quality traits were detected in the AB-pop2 population. Except for the genomic region on AhI, the higher allele effect was observed from the parent ‘ICGV 91114’. In the AB-pop2 population, the nine QTLs (7.4–52.7% PVE) interestingly co-located with QTLs from each of the three oil quality traits with contribution from ‘ICGV 87846’. The QTL analysis was also performed for yield component traits, namely PYP, SMK, SP, and HSW. In the AB-pop1 population, only two QTLs, i.e., one each for SP (8.0% PVE) and SMK (6.7% PVE), were detected with allele contribution from ISATGR 1212 and ICGV 91114,

(A) Introgression lines in AB- pop1 (ICGV 91114 x ISATGR 1212)



(B) Introgression lines in AB- pop2 (ICGV 87846 x ISATGR 265-5A)

Fig. 3 Graphical representation of selected BC_2F_1 genotypes in the AB-pop1 population. The linkage group is represented in each column, while the selected line in rows. **a** Maroon colour region indicates the heterozygous (wild/cultivated) segments (ISATGR 1212×ICGV 91114) and the orange colour the homozygous regions for cultivated alleles (ICGV 91114). The grey colour indicates missing data. **b** Maroon colour region indicates the heterozygous (wild/cultivated) segments (ISATGR 265-5A×ICGV 87846) and the orange colour the homozygous regions for cultivated alleles (ICGV 87846). The grey colour indicates missing data

respectively. Conversely in the AB-pop2 population, a total of 11 QTLs were detected, three each for SP (up to 52.0% PVE), SMK (up to 67.8% PVE), and HSW (up to 47.0% PVE), and two for PYP (up to 11.4% PVE). Of these QTLs, three common and consistent QTL genomic regions were identified one each on LG AhII (Ah_6305-Ah_8579 for LLS, rust, oil quality, SP and SMK), AhVI (Ah_6016-Ah_8004 for LLS, rust, oil quality, SP, HSW and SMK), and AhXIII (GM2745-Ah_9779 for LLS, rust, oil quality, SP, HSW, and SMK). It was interesting to note that all the alleles for higher contribution have come from ICGV 87846 only.

Clustering of the QTLs for multiple traits on the genome

All the 50 QTLs identified in both the populations were mapped in 7 genomic regions of 3 LGs in the AB-pop1 population and in 11 genomic regions of 8 LGs in the AB-pop2 population. By comparing the genomic locations of

the identified QTLs, some genomic locations with multiple QTLs were identified as a cluster (CL) if it harboured at least three QTLs controlling different traits. Using this parameter, a total of five CLs were identified, wherein various QTLs for disease resistance, oil quality, and yield component traits identified in this study were co-localized in the same genomic region of linkage group (Figs. 1, 2, box with solid line).

In the AB-pop1 population (Fig. 1, box with solid line), two clusters AB1qCL-1 and AB1qCL-2 were identified on LG AhI and AhVIII, respectively. The cluster AB2qCL-1 between markers Ah_4029 and Ah_1794 contained three QTLs, i.e., one each for rust, OLR and SP with a mean PVE of 9.0%. Similarly, in the AB-pop2 population, three clusters (Fig. 2, box with solid line) were detected possessing a total of 27 QTLs. The first cluster AB2qCL-1 located on LG AhII (Ah_6305-Ah_6209) contained 8 QTLs, i.e., 2 for disease resistance (one each for LLS and rust), 3 for oil quality (one each for OLR, SFA and UFA), and 3 for yield component traits (one each for HSW, SMK and SP). The PVE ranged from 7.2 to 8.8% with an average of 7.8%. The second cluster AB2qCL-2 located on the LG AhVI (Ah_6016-Ah_8004) had 9 QTLs with 14.1–16.9% PVE and an average of 14.9% PVE. Of the 9 QTLs in this cluster, three QTLs each for disease resistance (one for LLS and two for rust), oil quality (one each for OLR, SFA and UFA), and yield component traits (one each for HSW, SMK and SP) were clustered. The third cluster AB2qCL-3 (GM2745 and Ah_9779) possessed maximum number (10) of QTLs. Out of the 10 QTLs identified, 4 QTLs were for disease resistance (2 each for LLS and rust), 3 QTLs for oil quality traits (one each for OLR, SFA, and UFA), and 3 QTLs for yield component traits (one each for HSW, SMK, and SP). Apart from having the highest number of QTLs, the PVE was also found to be the highest ranging from 28.9 to 67.8% and mean of 49.7%.

Discussion

Pre-breeding in groundnut holds an utmost importance for imparting resistance to disease and pests as well as some quality traits (Sharma et al. 2013). We have made here an effort by developing useful genetic material in the form of advanced backcross mapping populations. This will help to diversify the gene pool as well as conduct genomics and breeding research in cultivated groundnut. This study led to identification of several resistant lines for both the foliar fungal diseases and thus provides a diverse source of resistance for use in breeding new varieties. Sharma et al. (2017) has reported generation advancement for these resistant lines and have further performed disease screening and identified resistant lines across seasons. It was also very clear that foliar disease resistance was much better recovered in the introgression lines of AB-pop1 population as compared to AB-pop2 population indicating the importance of resistance source from *A. kempff-mercadoi* × *A. hoehnei*.

The genetic map for AB-pop1 population had 258 mapped loci with average marker density of 5.49 cM/loci, while the genetic map for the AB-pop2 population had 1,043 mapped marker loci achieving marker density of 1.44 cM/loci. The high polymorphism in the AB-pop2 population as compared to the AB-pop1 population indicated higher diversity in ISATGR 265-5A (*A. kempff-mercadoi* × *A. hoehnei*) than the ISATGR 1212 (*A. duranensis* × *A. ipaensis)* with some level of diversity in cultivated genotypes. The previous study while studying the genetic diversity among the 17 synthetic tetraploids and 6 cultivated groundnuts by DArT markers showed that the genetic dissimilarity of 'ICGV 91114' with 'ISATGR 1212' was lower (0.342) as compared to 'ISATGR 265-5A'. i.e., 0.506 (Mallikarjuna et al. 2011a). A separate study using the population derived from the cross between the cultivated variety 'Fleur 11' (or 'Runner IAC 886') and an amphidiploid 'AiAd' (*A. ipaënsis* × *A. duranensis)* mapped 1261 loci (BAC end sequence-SSR and transposon marker) achieving the map density of 1.14 cM/loci (Shirasawa et al. 2013). The same population was also used in parallel to develop genetic map containing 722 SNP and SSR marker loci covering a total map distance of 1487.3 cM and map density of one loci every 2.06 cM (Bertioli et al. 2014a). Another population developed from the cross between a cultivated line 'Florunner' and 'TxAG-6', derived from the cross between *A. batizocoi* × (*A. cardenasii* × *A. diogoi*), had 370 mapped RFLP loci with map density of 5.97 cM/loci (Burrow et al. 2001). All these genetic populations derived from wide crosses showing high polymorphism rate as compared to populations derived from cultivated genotypes highlight the utility of such populations for the diversification of gene pool of the cultivated groundnut.

Genetic mapping of the wild segments depicted the extent and level of donor wild genome in the cultivated background. Wide variation in terms of heterozygosity percentage from 8.80 to 87.5% was observed in the genome of the two AB-QTL populations at BC_2F_1 generation. However, the average overall heterozygosity percentage was 46.48% and 35.47% for the AB-pop1 and AB-pop2 population, respectively. Foncéka et al. (2009) reported a heterozygosity percentage in the range of 6.1–44.4% with an average of 22.2% in the population developed from the cross 'Fleur 11' and 'AiAd' (*A. ipaensis* × *A. duranensis*). The higher heterozygosity percentage in both the populations may be due to excess of donor alleles from synthetic tetraploids caused by the fixation of deleterious alleles in the cultivated genotypes as seen in the study by Burrow et al. (2001) in the cross between a cultivated groundnut 'Florunner' and a synthetic tetraploid 'TxAG-6'. However, this higher heterozygosity

percentage would drastically reduce when several rounds of backcrossing were performed as observed in the development of constitutive segment substitution lines (CSSL) in groundnut (Fonceka et al. 2012b). Nevertheless, the presence of conserved chromosomal blocks interpolated with capricious regions with relatively high retrotransposon frequencies cannot be ruled out in groundnut as seen from the comparison of gene clusters in the A and B genome (Bertioli et al. 2014b). With respect to the identification of introgression lines in groundnut, the very first study was done by Halward et al. (1993) in a diploid AA genetic map from the cross between *A. stenosperma* and *A. cardenasii*, wherein introgression was found in 10 of the 11 identified linkage groups. Later, Garcia et al. (1996) identified 46 introgression lines in the cross *A. cardenasii* × *A. hypogaea*. In another cross between ‘Fleur 11’ and AiAd (*A. ipaensis* × *A. duranensis*) by Foncéka et al. (2009) identified a subset of 59 lines at the BC_2F_1 generation having wild segment in the range of 2.3–46.9 cM. In our study, we identified a subset of 43 lines in the AB-pop1 population, whereas 59 lines in the AB-pop2 population. These introgression lines will be very useful for conducting further genetic and breeding studies in groundnut.

The CWR include all the genetically related wild species to cultivated crops (https://www.bioversityinternational.org/cwr/). The CWRs and landraces of cultivated crops are a promising source of trait diversity such as disease resistance, insect resistance, abiotic stress tolerance, yield component traits, and morphological and quality traits in a number of crops (Sharma et al. 2013). This study detected a total of 50 QTLs showing varied level of phenotypic variance for LLS (up to 50.9% PVE), rust (up to 48.7% PVE), and PBND (up to 14.5% PVE). In addition, the present study identified QTLs for SP (up to 52.0% PVE), SMK (up to 67.8% PVE), PYP (up to 11.4% PVE), and HSW (up to 47.0% PVE). In groundnut, several notable examples are available, wherein genes from CWRs have been successfully utilized for the groundnut improvement. This list includes resistance to root-knot nematode (*Meloidogyne* species) from the amphidiploid TxAG-6 (Burrow et al. 2014) and rust (*Puccinia arachidis* Speg.) disease resistance from the highly resistance variety GPBD 4 (Gowda et al. 2002). Furthermore, Fonceka et al. (2012a) reported several QTLs for yield component traits in the advanced backcross population of ‘Fleur11’ and amphidiploid ‘AiAd’ with PVE up to 20.6%. It was interesting to note that majority of the favourable alleles for disease resistance were contributed by the synthetic parents, while favourable alleles for remaining traits were mostly contributed by the cultivated parents.

The genetic study of wild and cultivated genotypes gives a rare insight into the molecular basis of crop evolution and plant domestication episodes. The phenotypic differences between the wild progenitors and their domesticated counterparts, i.e., the cultigens are termed as domestication syndrome (DS). Plants are dynamic in nature, and hence, WCR and cultivated genotypes share some traits while differ in many. It is now well known that there is a clear difference between the genetic variations linked with domestication and those as a result from crop diversification under domestication (Abbo et al. 2012; Olsen and Wendel 2013). It has been opined that a trait that show the presence–absence of phenotype between cultivated and wild genotypes such as hard seeded versus free germinating legumes represent primeval domestication phenomenon, while a trait which show phenotypic continuum such as seed size and seed weight are the traits that evolved under domestication (Abbo et al. 2014). The earlier study, by Fonceka et al. (2012a), considered traits related to size of seed and pod (width, length), 100 seed and pod weights, pod constriction, and plant growth habit to be involved in the groundnut domestication syndrome. However, under the realm of current opinion, these traits could well be characterized as traits that diversified under domestication showing continuous variation. In our study, we considered PYP, SMK, SP, and HSW traits that most probably be involved in the groundnut domestication. The QTL clusters identified in the current study explained an average phenotypic variance of 8.1%, 14.5%, and 55.6% in LG AhII, AhVI, and AhXIII. Interestingly, all the higher trait values were contributed by the allele ICGV 87846, the cultivated genotype. This suggests that, during the initial periods of groundnut domestication, conscious efforts were made to improve these yield component traits which led to the fixation of these alleles at QTL clusters. Furthermore, these three clusters also harboured QTLs for disease resistance and oil quality traits apart from yield traits. However, the allele for disease resistance came from the synthetic tetradiploid ISATGR 265-5A. This indicated the ignorance towards targeted selection of such traits during the domestication period. Therefore, the allelic arrangement in the cultivated genotypes favoured high yield, while, in the wild genotypes, disease resistance would be favoured for producing sufficient seeds to pass the DNA to its offspring. Our finding on the domestication-related QTL are in accordance with the previous studies in groundnut (Fonceka et al. 2012a) in groundnut, bean (Koinange et al. 1996), rice (Sweeney and McCouch 2007), maize (Briggs et al. 2007), and tomato (Grandillo et al. 1999). These findings on the contribution of wild genomic regions for disease resistance offer new opportunities in exploiting the CWR using these genetic populations for groundnut crop improvement.

In summary, these two genetic populations developed from wide crosses have shown high variability at molecular and phenotype level, providing further opportunity to deploy them in diversifying the cultivated gene pool. Despite the fact these QTLs need further validation once these genetic populations achieve more stability in their expression, these

results clearly show the importance of these new populations for use in further trait mapping and breeding studies. More importantly, such efforts are more frequently required to discover new sources of resistance and other desirable favourable alleles for diversifying the cultivated gene pool.

Acknowledgements We acknowledge the help and support from researchers of University of Agricultural Sciences- Dharwad and University of Agricultural Sciences-Raichur in conducting part of disease screening experiment. Funding support from Bill & Melinda Gates Foundation (Tropics Legumes I, II, and III) is greatly acknowledged. This work has been undertaken as a part of the CGIAR Research Program on Grain Legumes and Dryland Cereals (GLDC). ICRISAT is a member of CGIAR Consortium.

Author contributions RKV conceived experiment. RKV, MKP, and NM designed and supervised the experiments. MKP, MS, MR, PJ, SS, KS, NM, and HS performed the experiment. PK, MKP, RKV, and BG analyzed the data. PK, MKP, and RKV wrote the manuscript.

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