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Identification of two major quantitative trait locus for fresh seed dormancy using the diversity arrays technology and diversity arrays technology-seq based genetic map in Spanish-type peanuts

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With 3 figures and 3 tables

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Abstract

Seed quality for both germination in the next generation and for human consumption is adversely affected due to preharvest sprouting in peanut. It also makes seeds more vulnerable to infection by a number of pathogens. Therefore, it is desirable to have 2–3 weeks of fresh seed dormancy (FSD) in the peanut varieties. In this context, one F_2 population was developed from a cross between non-dormant (ICGV 00350) and dormant (ICGV 97045) genotypes. Phenotyping of this population showed control of the trait by two recessive genes. In parallel, genotyping of the population with Diversity Arrays Technology (DArT) and DArT-seq markers provided a genetic map with 1152 loci covering a map distance of 2423.12 cM and map density of 2.96 cM/loci. Quantitative trait locus (QTL) analysis identified two major QTLs, namely *qfsd-1* and *qfsd-2* explaining 22.14% and 71.21% of phenotypic variation, respectively. These QTLs, after validation in different genetic backgrounds, may be useful for molecular breeding for FSD in peanut.

The peanut or groundnut (*Arachis hypogaea* L.) is an important legume crop cultivated throughout the tropical, subtropical and temperate regions covering more than 100 countries of the world (FAO 2014). This crop is well known for its multiple uses such as for food, oil, confectionary and dietary for human consumption including as a fodder for livestock (see Pandey et al. 2012, Janila et al. 2013). Most of the peanut cultivation in Africa and Asia is under rainfed environments, where untimely rains prior to harvest cause the seeds to germinate inside the soil resulting in reduced pod yield and poor seed quality. Such pre-sprouted seeds provide low market price to the farmers, besides predisposing them to infection by other pathogens and contamination by mycotoxins and aflatoxin that are potent carcinogens. Thus, the lack of dormancy in these varieties always makes farmers anxious about untimely rain and puts more pressure on farmers to harvest crops at the right stage in a very less time period manually. The delay in harvesting could lead to 10–20% yield loss due to sprouting in the field (Gautreau 1984), even higher in Spanish types (Khalfaoui 1991), and in some situations, the loss can be as high as 50% (Varman and Raveendran 1991). Therefore, it is very important to equip all the popular varieties with 2–3 weeks of fresh seed dormancy (FSD) to avoid such losses. Foliar application of maleic hydrazide (diethanolamine salt of 1.2-dihydroxy-3, 6, pyridazine-dione), a growth inhibitor, has been reported to induce dormancy in Spanish Bunch types of peanuts (Gupta et al. 1985). However, this is not an economically feasible option for rainfed peanut cultivation; on the other hand, the genetic option of varieties with short periods (2–3 weeks) of FSD is sustainable and cost-effective. Such varieties besides reducing preharvest sprouting losses also allow farmers to harvest the crop at a later stage in the case of unseasonal rains. Thus, breeding for FSD is an economically important objective in peanut.

The cultivated peanut, based on branching pattern, flower arrangement on the main axis and pod and kernel features, is divided into three major botanical types, that is Spanish (subsp. *fastigiata* var. *vulgaris*), Valencia (subsp. *fastigiata* var. *fastigiata*) and Virginia (subsp. *hypogaea* var. *hypogaea*). The Spanish and Valencia genotypes have non-dormant seeds and short maturity duration, whereas Virginia genotypes have dormant seeds for variable periods and long maturity duration (Upadhyaya and Nigam 1999). In the semi-arid regions of Asia and Africa, which cover almost 60% of the world's peanut production area, generally Spanish peanut varieties are cultivated.

Studies on methods of dormancy testing and inheritance pattern (Khalfaoui 1991, Faye et al. 2010), physiochemical mechanisms and influential factors (Nautiyal et al. 2001, Hu et al. 2010) of dormancy in peanut were reported. It was reported that ethylene and an inhibitor, possibly the abscisic acid, may interact to control dormant peanut seed germination (Carmen et al. 2009). Some variability for dormancy among botanical types and cultures within a botanical type were also reported (Wang et al. 2012). Screening of segregating populations, as well as breeding lines for FSD, was often carried out visually at the harvesting stage, which compels the conventional breeders to generate and maintain a large number of breeding materials till harvest stage to perform a selection. Further, it is very difficult to perform phenotypic selection under field conditions as every breeding and selection cycle/season may not receive rain during the harvesting stage. In practice, the dormancy test is conducted on promising breeding lines that were selected and forwarded based on other important selection parameters over the generations. Based on dormancy test, large numbers of promising breeding lines get rejected at advanced stage, leading to wastage of time and resources. Genomics-assisted breeding (GAB) has proven to be a very successful approach to improve not only simply inherited traits with high precision and accuracy such as improvement of rust resistance (Varshney et al. 2014b) and high oleate trait (Janila et al. 2016) in peanut, enhancement of resistance to fusarium wilt and ascochyta blight in chickpea (Varshney et al. 2014a) but also complex traits such as drought tolerance in chickpea (Varshney et al. 2013b). Identification of genomic regions/genes controlling FSD and the development of user-friendly

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markers is a prerequisite for deploying GAB in improving peanut genotypes. The availability of linked markers for FSD in peanut would enable to track FSD trait among the segregating lines resulting in optimal utilization of time and resources. However, to date, no study has been conducted to identify genes/markers associated with FSD in peanut.

Although simple sequence repeats (SSRs) have been used extensively for the construction of genetic maps and identification of genomic regions controlling agronomically important traits in cultivated peanut, genetic maps based on SSR markers have only 100–300 marker loci (see Pandey et al. 2012, Varshney et al. 2013a). The genome ploidy of cultivated peanuts allowed the identification and use of a limited number of good quality and informative single nucleotide polymorphisms (SNPs) in genetics and breeding studies (Khera et al. 2013). In such scenario, Diversity Arrays Technology (DArT) provides a good choice as high-throughput marker genotyping platform that can provide a relatively large number of polymorphic loci for constructing dense genetic maps in peanut. The other advantages of DArT markers include low cost, genome-wide profiling of a large number of SNPs and insertion/deletion polymorphisms (Kilian 2008). The DArT markers are currently being used in more than 55 species (http://www.diversityarrays.com/) and are more popular in crops with the unsequenced genome. More recently, the DArT markers were deployed for genome-wide association studies (GWAS) in peanut, leading to the identification of 524 marker–trait associations for 50 agronomical traits in peanut (Pandey et al. 2014). Therefore, the present study has deployed DArT and next-generation sequencing (NGS)-based DArT platform called as DArT-seq, for mapping FSD in the F_2 mapping population.

Materials and Methods

Plant materials and DNA isolation: One F_2 mapping population derived from the cross between contrasting parents, ICGV 00350 (non-dormant genotype) and ICGV 97045 (dormant genotype), was developed with 368 individuals. Both the parents are Spanish genotypes in which ICGV 00350 is released and cultivated in the states of Tamil Nadu and Andhra Pradesh in India under irrigated as well as in a rain-fed condition. Fig. 1 shows the parents used to make crosses and depicts the impact of lack of FSD. The F_1s from the cross ICGV 00350 × ICGV 97045 were sown along with the parents during 2011–12 postrainy season followed by the confirmation of hybridity using molecular markers. A total of 368 F_2 seeds were harvested from above-mentioned true F_1 plants. All the 368 F_2 plants were planted in the field during 2012 rainy season. For genotyping, leaf samples were collected, and genomic DNA was isolated from the F_2 plants following modified CTAB method described by Mace et al. (2003). After quantification, DNA samples were diluted to 100 ng/μl.

Phenotyping for fresh seed dormancy: All the 368 $F_{2:3}$ lines along with parents were phenotyped for FSD during 2012 rainy season following the methodology described by Upadhyaya and Nigam (1999). In brief, 30 mature seeds from each F_2 plant ($F_{2:3}$ seeds), shelled the next day after harvesting, were treated with fungicides captan and thiram at 0.2 g per 100 g seeds and placed on moist filter paper in a Petri dish. Regular watering at 24-h interval maintained the moisture level continuously. While watering the Petri dishes, care was taken not to expose the seeds to light. The Petri dishes were kept in the incubator that was set to 35 ± 3°C and complete darkness. The germination rate was recorded following three-day gap from 30th October to 20th November 2012. The progeny which did not show any germination symptoms even on 17th day were treated with 5% Ethrel (Ethrel is trade name of ethephon with a concentration of 0.75% w/w active ingredient) to check their viability.

Genotyping with DArT and DArT-seq markers: Selected F_2 lines along with their parents were genotyped with the DArT and DArT-seq features at the Diversity Arrays Technology Pty Ltd. (DArT P/L), Australia. All the polymorphic markers in the population were used for the construction of the dense genetic map. The method of genotyping with DArT is explained in detail in Pandey et al. (2014) and at http://www.diversityarrays.com/dart-application. The DArT-seq method next-generation sequencing (NGS) platform, deploys sequencing of the representations to achieve higher marker densities for conducting high-

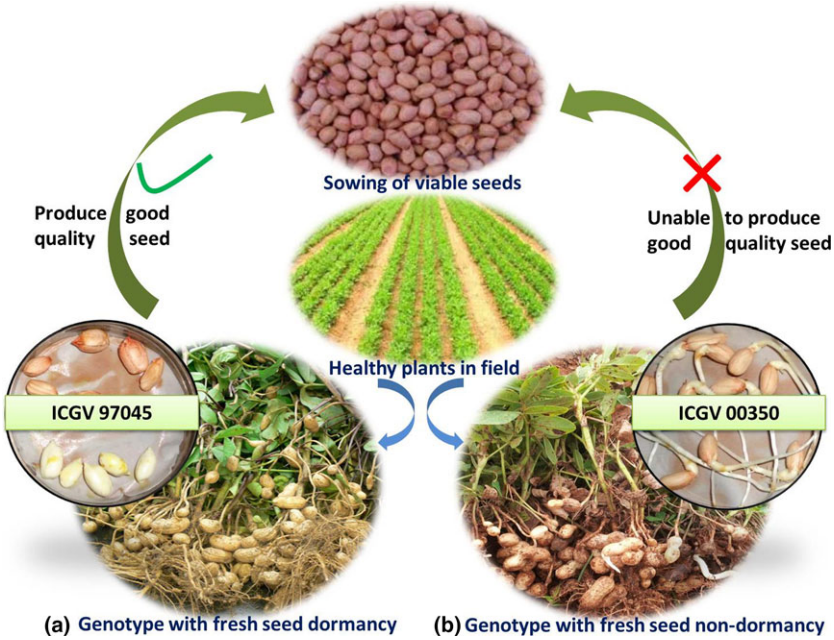

Fig. 1: Impact of fresh seed dormancy on seed quality. The pods are (a) healthy and of good quality in the case of dormant genotype ICGV 97045 which can be used, upon harvest, either for the consumption or for planting again in the field for cultivation. On the other hand, the pods are (b) unhealthy and of poor quality in the case of non-dormant genotype ICGV 00350 that is neither suitable for consumption nor for cultivation. The varieties with non-dormancy trait upon receiving uncertain rain at the harvest stage result in huge yield loss and quality deterioration of the produce. Such varieties despite having several good-quality traits are not accepted by the farming community

resolution genetic and trait mapping. Detailed genotyping procedure for DArT-seq is explained in Raman et al. (2014) and also at http://www.diversityarrays.com/dart-application-dartseq. Nevertheless, the method is briefly described below which will help in relating the results.

In the case of DArT markers, the first step involved complexity reduction in each DNA sample to obtain a 'representation' followed by the determination of sequence variation in the form of presence vs. absence through hybridization to DArT array consisting of a library from peanut. The fluorescent signal emitted from the hybridized fragments was recorded and analysed using the DArTsoft software. In the case of DArT-seq assay, DNA samples were digested/ligated primarily with two different adaptors accompanying to overhang by two different restriction enzymes (Raman et al. 2014). The Illumina flowcell attachment sequence, sequencing primer sequence and varying length barcode regions were included while designing the *Pst*I-compatible adapter. The flow cell attachment sequence and the *Mse*I-compatible overhang sequence contained the reverse adapter. The *Pst*I-*Mse*I fragments were amplified for 30 PCR cycles using the following reaction conditions: 94°C for 1 min, followed by 29 cycles of 94°C for 20 s, ramp 2.4°C/s to 58°C, 58°C for 30 s, ramp 2.4°C/s to 72°C, 72°C for 45 s. At last, amplicons were held at 72°C for 7 min and then at 10°C. All PCR amplicons from the 96-well multiplexed in equimolar amount and kept to c-Bot (Illumina) bridge PCR after that sequenced on Illumina Hiseq2000. A single lane sequencing was followed for all the amplicons; the single reads sequencing was run for 77 cycles. All the generated sequences from each lane were subjected to proprietary DArT analytical pipelines. Poor-quality sequences were filtered away from the FASTQ files in the primary pipeline. In the barcode region, more stringent selection criteria (≥Phred pass score of 30) were employed in comparison with the rest of the sequence. Resultantly, in the barcode split step, the sequences assignments to specific samples were very authentic. In marker calling, around 2 000 000 identified sequences per barcode/sample were used. Finally, identical sequences were broken into fastqcall files. In the secondary proprietary pipeline of DArT P/L, the fastqcall files were used for detecting presence/absence markers (PAM) through SNP calling algorithms (DArTsoftseq). The sequence data were then subjected to an analytical pipeline that identified all the polymorphic sequences of the DArT-seq markers generated from the parental lines of the F_2 population (ICGV 00350 and ICGV 97045) and scored polymorphic sequence variations were as present/absent (present = 1 vs. absent = 0). The nomenclature of DArT and DArT-seq markers was mentioned with 'Ah' and 'Ahs' prefix, respectively.

Construction of genetic map: The genotyping data for polymorphic markers were used to construct a genetic map using JoinMap 4.0 (Van Ooijen 2006). The highly distorted markers were removed from the genetic map construction, and only markers with the goodness of fit to 3 : 1 ratio were considered for map construction. The markers were grouped into linkage groups based on logarithm of odds (LOD) groupings and 'Create groups for mapping' command using the Kosambi map function (Kosambi 1944) and recombination frequency of 0.45. Marker order within linkage groups was established by 'Calculate Map' command, and the genetic map was redrawn using MapChart for Windows for better visuality (Voorrips 2002).

QTL analysis: The composite interval mapping model in the software WinQTL cartographer 2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) was used for QTL identification (Wang et al. 2007). The walking speed, window size and a number of control markers were set to 1 cM, 10 cM and 5 cM, respectively. The default genetic distance (5 cM) was used to define a QTL in a specific experiment. LOD thresholds were conducted to determine the significance of a QTL, and the thresholds were established with 500 permutations (Churchill and Doerge 1994).

Results

Inheritance and variation for FSD

The phenotyping data generated on 368 $F_{2:3}$ plants of the cross ICGV 00350 × ICGV 97045 were used to study the inheritance of FSD. The F_1 seeds harvested on the female parent after hybridization did not show fresh seed dormancy, suggesting recessive nature. Of the total 368$F_{2:3}$ plant progeny evaluated for seed dormancy, only 23 were found dormant and the remaining plants did not possess FSD. Because a subset of this population consisting of 188 $F_{2:3}$ plant progeny were selected for further genotyping, only this subset was analysed for inheritance pattern separately through chi-square test. The chi-square test performed on the complete population as well as the subset revealed goodness of fit to 15 : 1 (non-dormant to dormant) ratio (Table 1), suggesting that FSD is controlled by two recessive duplicate genes (chi-square P-value for complete and the subset was 0.83 and 0.82, respectively).

DArT and DArT-seq based genetic map

With the 15,360 DArT array and DArT-seq approach, we found a total 1074 (6.9%) DArT and 3616 (23.5%) DArT-seq polymorphic markers in the population. After removing severely distorted markers, a total of 659 (4.2%) DArT and 794 (5.1%) DArT-seq polymorphic markers were used for the construction of genetic linkage map. Of the total 1453 polymorphic marker loci, 1152 marker loci were successfully mapped on to 20 linkage groups (LGs) covering a total map distance of 2423.1 cM and marker density of 2.96 cM/loci (Table 2, Fig. 2). Of the 1152 mapped loci, 563 and 589 loci were mapped onto 'A' and 'B' genomes with the average intermarker distance of 3.1 and 2.7 cM/loci covering 1184.7 and 1238.5 cM distance, respectively. The number of mapped loci ranged from 8 (A01) to 156 loci (B09); the individual LG length varied from 59.6 cM (A01) to 184.3 cM (A09), and marker density ranged from 0.7 cM/loci (B09) to 7.4 cM/loci (A01).

QTLs controlling fresh seed dormancy

Two QTLs for FSD were identified with the major phenotypic effect (>20%) using genetic mapping information and phenotyping data of the population. Of the two QTLs, the QTL '*qfsd-1'* (Ah2374-Ah4907) located on A05 explained 22.14% phenotypic variation (PV) (Table 3, Fig. 3a), while the QTL '*qfsd-2*' (Ahs4422I–Ahs4422II) explained 71.21% PV. The QTL with the largest effect (*qfsd-2)* was 1.4 cM interval between marker loci Ahs4422I–Ahs4422II on the linkage group 'B06' (Table 3, Fig. 3b). The LOD value of '*qfsd-1'* was 5.63 with the additive effect of −0.48 and dominance effect of 0.42, while '*qfsd-2*' had LOD value of 8.97 with the additive effect of -0.43 and dominance effect of 0.47.

Table 1: Assessment of genetic nature of FSD based on chi-square values and associated probability levels (P-value) in the segregating $F_{2:3}$ population (ICGV 00350 × ICGV 97045)

Trait	Observed numbers	Expected proportions	Expected numbers for 15 : 1 ratio	Chi-square	P-value
Entire population size = 368					
ND	344	15	345	0.046	0.83
D	24	1	23		
Subset size = 188					
ND	168	15	169	0.053	0.82
D	12	1	11		

ND, non-dormant; D, dormant.

Table 2: Features of the genetic map constructed for the F_2 population (ICGV 00350 × ICGV 97045)

Linkage groups	The number of mapped loci	Total map distance (cM)	Average inter-marker distance (cM/loci)	Linkage groups	The number of mapped loci	Total map distance (cM)	Average inter-marker distance (cM/loci)
A01	8	59.60	7.4	B01	75	142.4	1.9
A02	88	121.2	1.4	B02	13	81.30	6.3
A03	22	92.00	4.2	B03	59	149.6	2.5
A04	26	129.6	5.0	B04	40	118.3	3.0
A05	37	162.0	4.4	B05	33	106.2	3.2
A06	81	93.30	1.2	B06	35	116.1	3.3
A07	25	61.60	2.5	B07	73	146.8	2.0
A08	132	184.3	1.4	B08	59	184.9	3.1
A09	75	163.4	2.2	B09	156	111.6	0.7
A10	69	117.7	1.7	B10	46	81.30	1.8
Total	563	1184.7	3.14		589	1238.5	2.78

Discussion

Fresh seed dormancy is an economical and highly desirable trait in all the existing popular and new peanut cultivars to facilitate their better adoption in the farmer's field. In this context, the study successfully identified two major QTLs controlling FSD that will facilitate the development of breeder-friendly markers for their deployment in molecular breeding.

Genetic control of fresh seed dormancy

Despite the availability of several reports on genetic studies of FSD in peanut, there was no clarity on its inheritance pattern. Lin and Lin (1971) reported it as a monogenic trait with complete dominance of dormancy over non-dormancy. In contrast, Hull (1937), John et al. (1948) and Nautiyal et al. (1994) reported it as a polygenic trait. Further, Stokes and Hull (1930) and Ramchandran et al. (1967) reported partial dominance in their study with Spanish × Virginia crosses. In another study involving a cross between two Spanish genotypes, dormancy was reported as a quantitatively inherited trait and genetically controlled by additive, dominance and digenic epistatic effects (Khalfaoui 1991). The present study revealed the goodness of fit to 15 : 1 ratio for non-dormant and dormant phenotypes, suggesting two dominant duplicate genes involved in governing non-dormant trait known as duplicate dominant epistasis. The dominant allele at either or both loci results in non-dormant phenotype. If we consider *d1* and *d2* as the recessive alleles responsible for dormancy phenotype, then eight gene combinations in F_2 population, *viz. D1D1 D2D2*, *D1D1 d2d2*, *D1D1 D2d2*, *D1d1 D2D2*, *D1d1 d2d2*, *D1d1 D2d2*, *d1d1 D2D2* and *d1d1 D2d2*, result in non-dormant phenotype. The dormant phenotype is expressed in the absence of the two dominant alleles (*d1d1 d2d2*). Therefore, the presence of any of these two dominant alleles will produce non-FSD, while the FSD condition occurs only when both these genes will be in a homozygous recessive condition (*d1d1 d2d2*). Both dominant and recessive genes controlling dormancy were reported in the literature depending on the parents used in the study. The above results suggest a role of more than one mechanism governing FSD in peanut. Further, it will be interesting to study whether different mechanisms governing FSD have effects on other seed quality parameters, particularly seed viability and seedling vigour.

Dense genetic linkage map

A moderately dense linkage map is essential for the detection of QTL and its phenotypic effect with higher precision. The alternative primer binding sites on homeologous chromosomes, competition for primers and dilution with correct annealing targets are the major constraints for using SSR and SNP marker system in a polyploid crop. In such circumstances, the use of DArT and DArT-seq genotyping platform seems to be a better option. The DArT and DArT-seq genotyping platforms manage the complexity reduction in the genome and therefore have been used in different range of crops for the genetic studies, such as rice (Jaccoud et al. 2001), barley (Wenzl et al. 2004), eucalyptus (Lezar et al. 2004), *Arabidopsis* (Wittenberg et al. 2005), cassava (Xia et al. 2005), wheat (Akbari et al. 2006), pigeonpea (Yang et al. 2006), sorghum (Mace et al. 2008), banana (Risterucci et al. 2009), tomato (Van Schalkwyk et al. 2012), rapeseed (Raman et al. 2012) and several fungal pathogens of chickpea (Sharma et al. 2014) and animals (Kilian 2008, www.diversityarrays.com).

The DArT array has been used recently in conducting genome-wide association studies using the 'Reference Set' developed by ICRISAT for several agronomic traits in peanut (Pandey et al. 2014). In the present study, we report dense linkage map using the DArT array and NGS-based DArT-seq for the first time in *Arachis* spp. using an intraspecific F_2 mapping population. In earlier studies on mapping in peanut, only SSR-based genetic maps were constructed using F_2 and RIL populations (see Pandey et al. 2012, Varshney et al. 2013a). Several linkage maps were constructed with the early-generation markers, viz. RFLP and RAPD, SSR and with SNPs (see Pandey et al. 2012, Zhou et al. 2014). In this study, 20 linkage groups were identified which corresponded to linkage groups of the previously published genetic linkage maps with the SSRs. In the area of genetic mapping in cultivated peanut, there are two recent studies on the development of intraspecific dense genetic maps using either SSRs and/or SNPs. In one study, the intraspecific dense genetic map was developed with 1261 (1241 SSR and 20 transposons) marker loci with a map density of one loci/1.14 cM (Shirasawa et al. 2013). The main drawback of this map was the use of small population size, that is 91 F_2s. In the other study, the genetic map has the highest number of mapped loci, that is 1685 loci (1621 SNPs and 64 SSRs) (Zhou et al. 2014). For developing this high-density genetic map, genotyping by sequencing technology (GBS) was used to achieve a higher number of mapped loci, and the technology has clearly emerged as the preferred genotyping platform. The current availability of reference genomes for both the diploid progenitors in the public domain will further catalyse the deployment of sequence-based genotyping platforms to enhance the precision in trait mapping and molecular breeding studies in peanut.

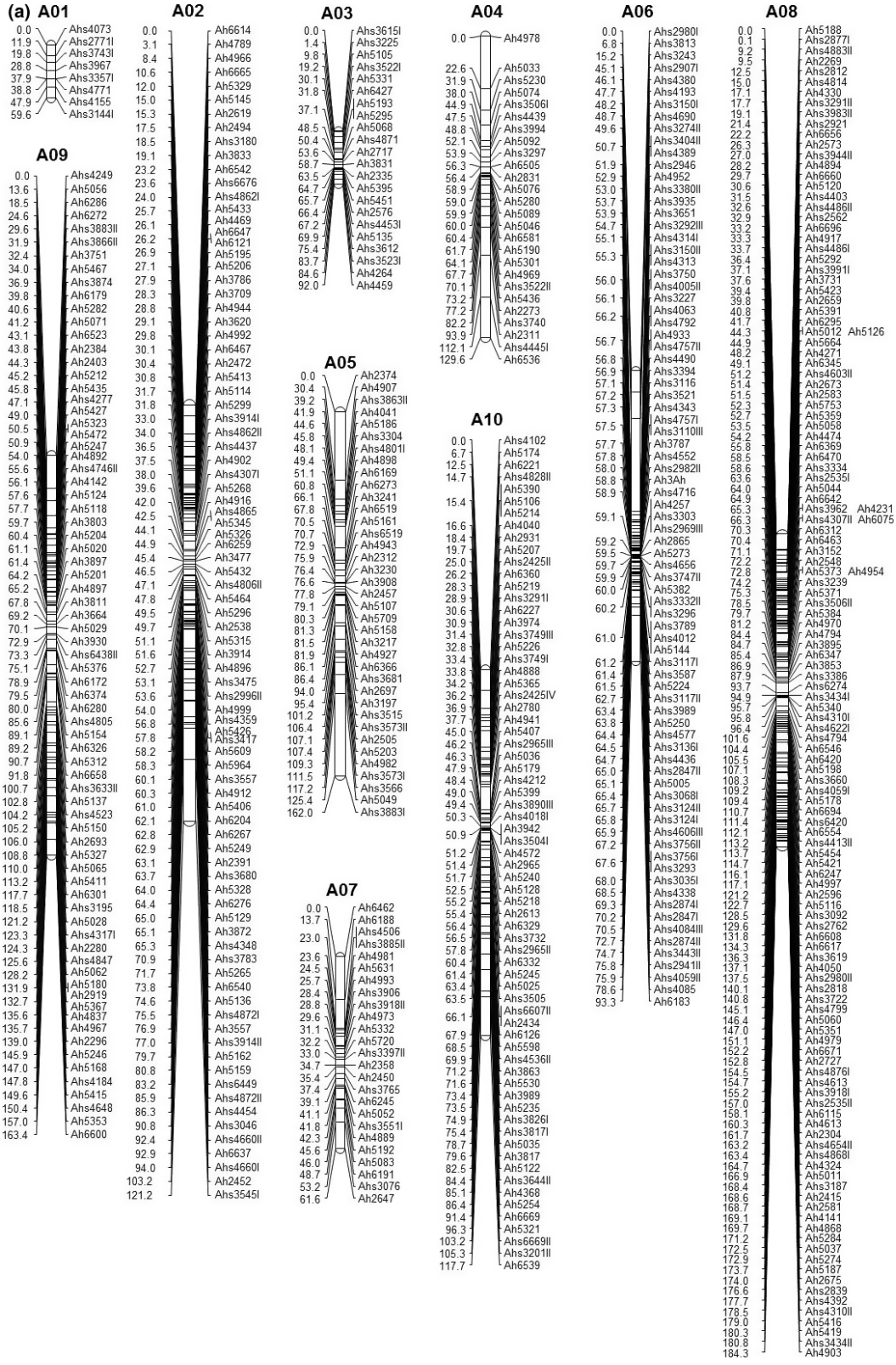

Fig. 2: Genetic map for ICGV 00350 × ICGV 97045 population. Genetic map was constructed on the basis of the F_2 population using DArT and DArT-seq markers. The mapped loci are present on the left side and their positions are mentioned on the right side of linkage groups

Two major QTLs for fresh seed dormancy

In addition to the being first DArT and DArT-seq based genetic map, this study also has the distinction of being the first study on the identification of QTLs for FSD in peanut. As a result, no comparison can be made for identified QTLs from the past studies in peanut. Further, it is important to mention here that both QTLs showed high phenotypic variation, negative additive effect

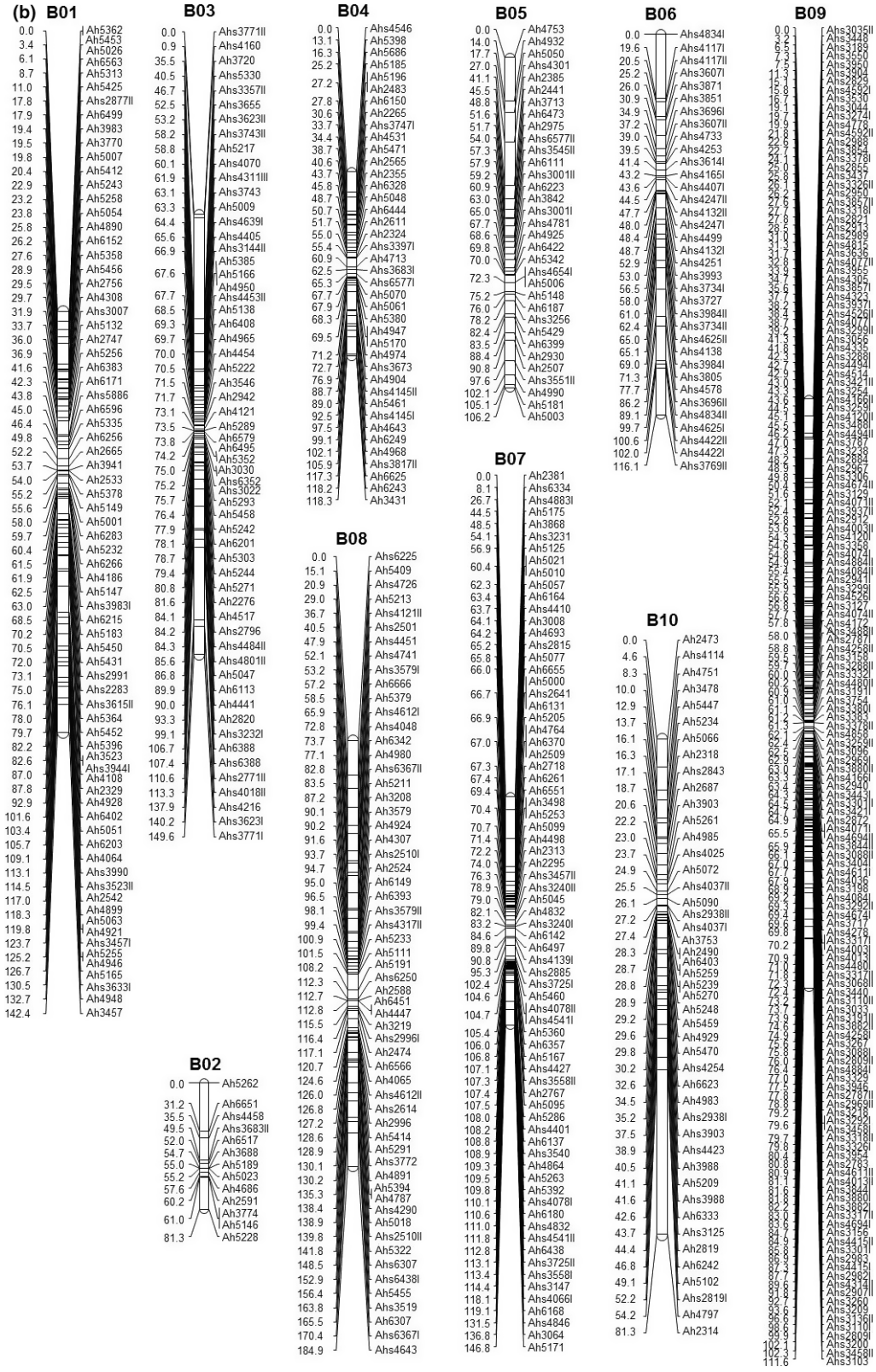

Fig. 2: Continued

and positive dominance effect. The non-detection of small-effect QTLs might be due to phenotypic scoring in the form of either present or absent for seed dormancy. Because of this reason, the phenotypic variation was discontinuous leading to the detection of only large-effect QTLs. The genetic control of preharvest sprouting or vivipary is a very important trait in many cereals and oilseed rape breeding programmes. QTL studies reported the identification of responsible gene/QTLs in other crop species for seed dormancy/preharvest sprouting (PHS) (rapeseed, rice, *Arabidopsis*, wheat, barley and sorghum) (Feng et al. 2009, Bent-

Table 3: Details of two major QTLs for fresh seed dormancy identified in ICGV 00350 × ICGV 97045 population

S. N.	QTLs	Linkage group	Position (cM)	LOD value	Phenotypic variance (PV) %	Nearest marker	Marker interval	Additive effect	Dominance effect
1	*qfsd-1*	A05	0.01	5.63	22.14	Ah2374	Ah4907-Ah2374	-0.48	0.42
2	*qfsd-2*	B06	102.0	8.97	71.21	Ahs4422I	Ahs4422II -Ahs4422I	-0.43	0.47

PV, phenotypic variation; QTL, quantitative trait locus.

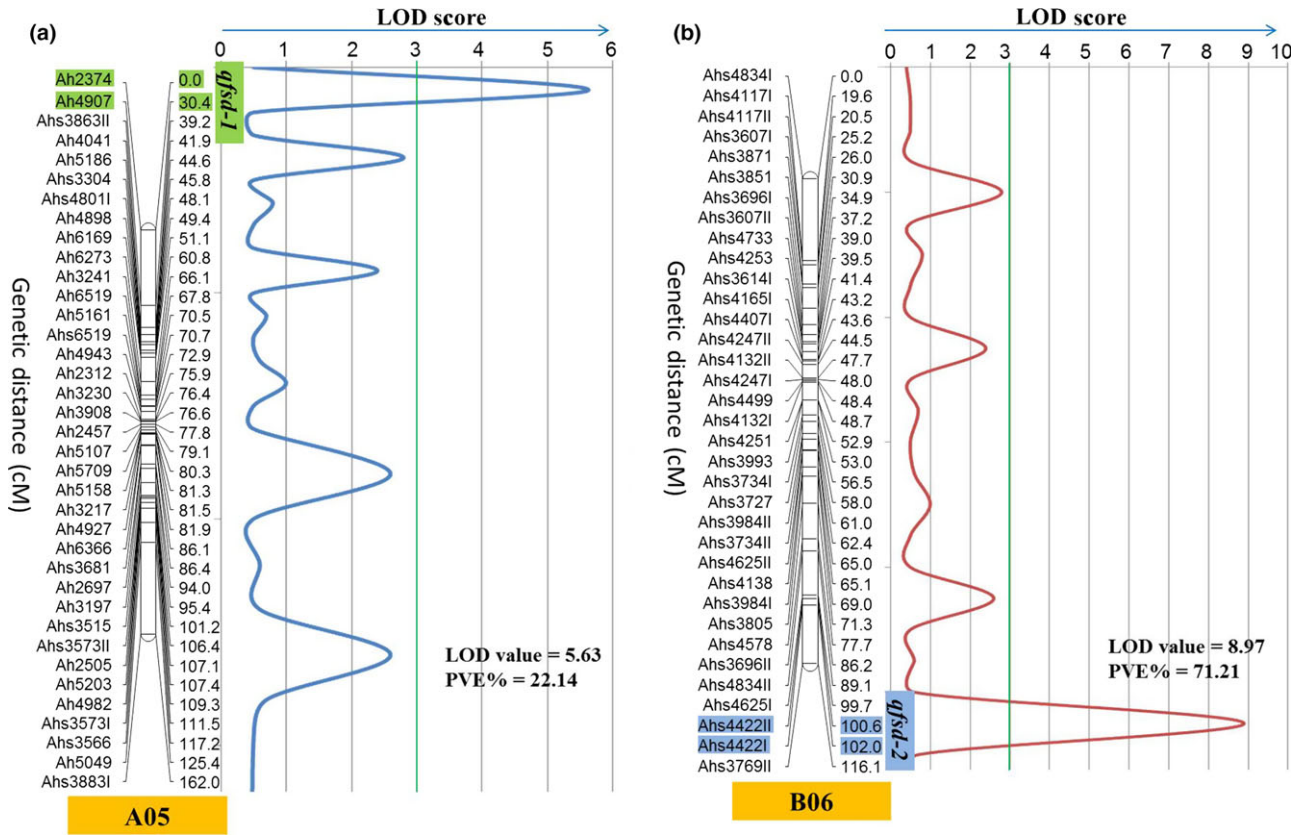

Fig. 3: Features of two major QTLs for fresh seed dormancy in ICGV 00350 × ICGV 97045 population. (a) The QTL '*qfsd-1*' identified on linkage group A05 explained 22.14% phenotypic variation (PV). (b) The QTL '*qfsd-2*' identified on linkage group B06 explained 71.21% phenotypic variation. The mapped loci are present on the left side and their positions are mentioned on the right side of the linkage groups. The vertical green line indicates the significant threshold (LOD = 3.0)

sink et al. 2010, Marzougui et al. 2012, Schatzki et al. 2013, Cabral et al. 2014).

In wheat, the PHS was reported as a polygenic quantitative trait and, so far, by using 35 bi-parental mapping populations, approximately 165 QTLs were identified for this trait (Jaiswal et al. 2012). For example, Chen et al. (2008) reported QTLs for PHS in wheat which explained up to 30.6% phenotypic variation and the QTL was found located on the long arm of chromosome 4A. In an another study in wheat, four consistent QTLs were identified for PHS on chromosome 4A that showed maximum 58.1% of the phenotypic variation (Cabral et al. 2014). Similarly in the case of barley, QTLs were identified on 4H, 5H and 6H and explained 25, 81 and 12% PV, respectively (Gong et al. 2014). In the case of rice, PHS has been reported as a complex and polygenic trait. More than 40 QTLs associated with PHS were identified across all the chromosomes of rice cultivars and wild relatives (Marzougui et al. 2012). In another study in rice, three putative QTLs with phenotypic variations ranging from 4 to 21% have been reported (Wan et al. 2005). This study also indicated that seed dormancy/PHS is affected by a number of minor genetic effects.

In *Arabidopsis*, QTL analysis revealed 11 QTLs for the 'seed dormancy-specific' loci, including the *DELAY OF GERMINATION* (*DOG*) genes (Bentsink et al. 2006, 2010). The *DOG1* gene is expressed in seeds during the maturation stage, and functional loss of *DOG1* results in a lack of seed dormancy (Bentsink et al. 2006). The phenotypic variance of these 11 QTLs identified in six populations ranged from 42 to 66% (Bentsink et al. 2010). Besides cereals, the QTL mapping led to the identification of 5 QTLs for total seed dormancy (TSD) in winter oilseed rape that together explained 42% PV (Schatzki et al. 2013). These studies highlight the genetic complexity of seed dormancy and preharvest sprouting in several crops. Realizing the importance of FSD trait in peanut in enhancing the quality and shelf life of seeds for the market value as well as seed production, the development of cultivars with dormancy up to

harvesting for a short time will be an important goal of future peanut breeding programmes.

In summary, this study reports the construction of dense genetic map for intraspecific cultivated genotypes based on DArT and DArT-seq-based genotyping platform and identification of two major QTLs for FSD. These results will further facilitate fine mapping and identification of candidate genes involved in FSD as well as the deployment of markers flanking the QTLs for the development of improved lines with dormancy up to harvesting through genomics-assisted breeding approaches.

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