Plant Physiology and Biochemistry 229 (2025) 110539

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Genomic analysis reveals the interplay between ABA-GA in determining fresh seed dormancy in groundnut

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ARTICLE INFO

Keywords:
ABA and GA pathway
Candidate genes
KASP markers
Multi-locus GWAS

ABSTRACT

Groundnut is an important oilseed crop; however, its productivity and seed quality are frequently reduced due to lack of fresh seed dormancy (FSD). To address this challenge, a mini-core collection of 184 accessions was phenotyped to identify potential donors in each agronomic type. In addition, whole genome re-sequencing and multi-season phenotypic evaluations were analyzed to identify stable marker-trait associations (MTAs) linked to FSD. Phenotypic analysis revealed substantial variability in dormancy durations, with days to 50 % germination (DFG) ranging from 1 to 30 days. Multi-locus genome-wide association study (ML-GWAS) identified 27 MTAs in individual seasons and 12 MTAs in pooled seasons data, respectively. Key candidate genes identified included *Cytochrome P450 superfamily proteins, protein kinase superfamily proteins,* and *MYB transcription factors* involved in the Abscisic acid (ABA) pathway, as well as *F-box interaction domain proteins, ATP-binding ABC transporters,* associated with the Gibberellic acid (GA) pathway. SNP-based KASP (Kompetitive Allele-Specific Polymerase chain reaction) markers were developed for 12 SNPs, of which four markers (snpAH00577, snpAH00580, snpAH00586 and snpAH00588) were found useful for the selection of FSD. The combination of favourable allele from four genes namely *Ahfsd1 (Arahy.8B3CAL), Ahfsd2 (Arahy.NRZN5K), Ahfsd3 (Arahy.JJ8FJQ),* and *Ahfsd4 (Arahy.PI0JM6)* provides stable 24–30 days of dormancy. Incorporating favourable dormant alleles into breeding programs could enable the development of high-yielding cultivars with a dormancy period of more than two weeks.

1. Introduction

Groundnut (*Arachis hypogaea* L.) is a globally important legume crop with significant economic and nutritional value. It is well-known for its rich nutritional content, offering a valuable source of protein, healthy unsaturated fatty acids, and essential vitamins (Taheri et al., 2024). In 2023, the Food and Agriculture Organization (FAO) reported that the worldwide groundnut harvest area exceeded 32.7 million hectares, resulting in total production of >54 million tons. Seeds plays central role in plant growth and development, acting as the primary carriers of genetic traits, passing on essential characteristics from one generation to the next (Wang et al., 2019). The transfer of genetic factors ensures

This article is part of a special issue entitled: Modern technologies for Crop Resilience published in Plant Physiology and Biochemistry.

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https://doi.org/10.1016/j.plaphy.2025.110539
Received 30 June 2025; Received in revised form 26 August 2025; Accepted 17 September 2025
Available online 18 September 2025

producing high-quality crops with uniform trait expression in the farmer's field, though most of these factors influenced by environmental and agronomic conditions.

Cultivated groundnut is classified into four agronomic types (Spanish bunch, Valencia bunch, Virginia bunch and Virginia runner), belonging to two subspecies (spp. *hypogaea* and *fastigiata*) - based on developmental patterns, flower arrangement, kernel and, pod traits (Upadhyaya and Nigam, 1999). Valencia bunch and Spanish bunch types have a shorter maturity duration, and lack fresh seed dormancy (FSD), while the Virginia bunch and Virginia runner types generally exhibit longer maturity duration and have seeds with variable dormancy durations (Naganagoudar et al., 2016; Bomireddy et al., 2024). Among these, Spanish varieties, which account for ~60 % of global production, are mainly grown in Africa and semi-arid regions of Asia. The major problem arises in crop production when there is early rainfall before harvest that often triggers pre-harvest sprouting (PHS), causing seeds to germinate prematurely in the soil. This can lead to 10–20 % reduction in yield, and in Spanish varieties, losses may exceed 50 %. Beyond yield reduction, PHS increase the risk of pathogen infections, elevates the aflatoxin contamination, lower market values, and degrades kernel quality (Vishwakarma et al., 2016).

Seed dormancy is defined as the inability of a viable seeds to germinate even under favourable conditions (Bewley, 1997). The concept of "germination" refers to the metabolic activation of a seed upon absorbing water, which causes the radicle to emerge through the seed coat, this process is also known as "chitting." In seed technology, germination is further defined as the process in which the embryo develops into a seedling that displays the essential structures necessary for normal growth, thereby indicating its potential to establish into a healthy plant under favourable conditions (Parihar et al., 2014). Dormancy is regulated by a complex interaction of hormonal, molecular, and environmental factors. In most plant species, abscisic acid (ABA) serves as the primary regulator for dormancy induction and maintenance, whereas gibberellic acid (GA) promotes dormancy release and germination (Finkelstein et al., 2008). In modern groundnut breeding, achieving an optimal dormancy period of 2–3 weeks requires the appropriate combination of genetic factors. ICRISAT groundnut breeding programs currently deploys genomics-assisted breeding (GAB) strategies, utilizing diagnostic markers and advanced molecular tools to enhance key traits such as oleic acid content and resistance to rust and late leaf spot (Pandey et al., 2020). The availability of diagnostic genetic markers will also help in performing early generation selection for FSD through GAB.

Considering above facts, introducing 2–3 weeks has emerged as an effective strategy to reduce PHS losses by allowing farmers to delay harvest during unexpected rainfall. Thus, developing high-yielding cultivars with FSD is a major breeding objective to improve sustainability and resilience in groundnut production (Vishwakarma et al., 2016). Various studies have demonstrated that the seed dormancy is influenced by many factors, including internal (embryo, seed coat, and endogenous inhibitors) (Gianinetti and Vernieri, 2007) and environmental factors (temperature, light and, air etc.) (Mohanty, 2022).

Genome-wide association study (GWAS) has proven to be an effective approach for uncovering genetic variants linked to complex traits of interest in different crop species. These studies help to bridge the gap between genetic information and its practical applications by identifying the genetic factors that influence various traits and behaviors (Uffelmann et al., 2021). By leveraging historical recombination events within diverse natural populations, GWAS enables the identification of quantitative trait loci (QTLs) and genes underlying important agronomic traits (Guo et al., 2024; Pandey et al., 2014). By tapping into the genetic diversity present in these populations, we can uncover valuable insights into the genetic underpinnings of traits that are often influenced by multiple factors (Varshney et al., 2021). Unlike traditional QTL mapping, which is restricted by limited genetic parental diversity, GWAS leverages natural populations that exhibit a wide range of genetic variation, allowing identification of a broader spectrum of alleles linked to complex traits (Liu et al., 2016), although are less useful to detecting very rare alleles for a trait. This approach enhances mapping accuracy by utilizing historical recombinations and linkage disequilibrium across diverse germplasm, facilitating the discovery of multiple quantitative trait nucleotides (QTNs) that contribute to the desired traits (Cui et al., 2017). Additionally, GWAS is adept at identifying small-effect QTLs that might be missed in biparental populations, offering a more comprehensive view of the genetic architecture underlying various traits.

GWAS has successfully identified MTAs for seed dormancy and PHS resistance in crops like wheat and rice. For instance, in wheat significant MTAs and putative candidate genes linked to *ABA-responsive proteins*, *protein kinases*, and *MAP-kinase-like proteins* have been reported (Rehman Arif et al., 2012). Similarly, in rice QTLs associated with seed dormancy and PHS resistance, include the *GA20-oxidase gene* located within a PHS associated genomic region (Li et al., 2004; Lu et al., 2018). Moreover, in barley, genes such as *mitogen-activated protein kinase kinase 3* (*MKK3*) and *alanine aminotransferase (AlaAT)* have been linked to seed dormancy regulation (Nakamura et al., 2011). In wheat, the mother of *FT* and *TFL1 (MFT)* and *Phs1* genes have also been recognized as key regulators of seed dormancy (Nakamura et al., 2011; Torada et al., 2016). While PHS poses a significant challenge in groundnut cultivation, research efforts to map the relevant FSD QTLs have been limited (Vishwakarma et al., 2016; Kumar et al., 2020; Bomireddy et al., 2022). Few QTLs associated with seed dormancy have been identified in this crop, highlighting a critical knowledge gap compared to wheat, rice, and barley (Kulwal et al., 2012; Lin et al., 2016). Nevertheless, the success of GWAS in other cereals underscores its potential to advance the genetic dissection of PHS and dormancy traits in groundnut, ultimately supporting the development of targeted breeding strategies for improved resilience and grain quality.

Several statistical models have been employed for association mapping, utilizing various methodologies (Kaler et al., 2020; Wang et al., 2016). Traditional single-locus genome scans using ordinary mixed models not fully effective in accounting for loci with large effects. As a solution, multi-locus genome-wide association study (ML-GWAS) models have been proposed (Rakitsch et al., 2013; Segura et al., 2012). These ML-GWAS models are recognized for their efficiency and reliability in mapping genomic regions, as they estimate the effects of all markers simultaneously. Unlike single-locus genome-wide association study (SL-GWAS), ML-GWAS do not necessitate stringent multiple testing corrections, which often lead to the dismissal of significant associations (Zhang et al., 2019). Furthermore, ML-GWAS models has proven more effective and reliable than single-locus GWAS in identifying significant MTAs (Wang et al., 2016).

To address the existing knowledge gaps and leverage advancements in association mapping analysis, this study employed GWAS models on a diverse mini-core collection of groundnut to identify significant MTAs associated with FSD. By integrating whole genome re-sequencing (WGRS) data with multi-season phenotypic data, we aimed to uncover candidate genes that regulate FSD, ultimately facilitating the development and validation of molecular markers for use in breeding. This information and knowledge greatly enhances our understanding of the genetic basis of FSD and contributes to the development of resilient groundnut varieties.

2. Materials and methods

2.1. Plant material and phenotyping for fresh seed dormancy

In present study, the experimental material comprised of 184 accessions of the groundnut mini-core collection developed at ICRISAT (Upadhyaya et al., 2002). This mini-core set represents 1.29 % of the full collection and 10.8 % of the core collection, respectively. The mini-core subset attempts to represent the overall genetic diversity of the ICRISAT gene bank collection. The 184 accessions in the mini-core set represents

different agronomic types of groundnut, including 33 accessions of Virginia runner, 48 of Virginia bunch, 57 Spanish bunch and 35 Valencia bunch. The significant genetic diversity of this mini-core collection makes it an informative panel for genetic dissection of complex traits in groundnut. It can also be utilized for molecular characterization, enabling the selection of parents and maximizing diversity in groundnut breeding programs to enhance the heritability and genetic gain.

The groundnut mini-core collection was sown during two post-rainy seasons (PR2020-2021 and PR2022-2023) and one rainy season (R-2022) at ICRISAT, Hyderabad, (17°31′48.00″ N latitude, 78°16′12.00″ E longitude, and an altitude of 545 m). Additionally, during the 2021 rainy season, the accessions were also planted at the SV Agricultural College farm in Tirupati, Andhra Pradesh (13°54′ N latitude, 79°54′ E longitude, and 182.9 m altitude). The experiment was conducted in a randomized block design (RBD), two replications, and with two plant and row spacing of 10 cm and 60 cm, respectively, under recommended agronomic conditions for groundnut.

For evaluation of FSD, fully matured seeds were freshly harvested and evaluated using an in-vitro germination assay (Upadhyaya and Nigam, 1999). From each accession, 20 seeds with uniform size were selected from two replicates. Seeds were treated with fungicides (Mancozeb and Carbendazim) and placed on moist germination paper in sterilized petri dishes. Plates were stored in total darkness and watered every 24 h. Germination data were recorded daily for 30 days, and the number of days required for an accession to reach 50 % germination was documented as its dormancy duration.

2.2. DNA extraction, sequencing and SNP calling

DNA was extracted from 100 mg of fresh leaf tissue from each accession following the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany) (Pandey et al., 2020). The leaf samples were first homogenized in 500 μL of lysis buffer, and 10 μL of RNase was added to remove RNA contaminants. The sample was placed into a water bath at 65 °C for 1 h. After incubation, the sample was centrifuged for 20 min at 6000 rpm, and the supernatant was collected and transferred. The supernatant and 450 μL of binding buffer were placed through a NucleoSpin Plant MN column. The column was centrifuged at 6000 rpm for 1 min, and the flow-through was discarded. The pellet was washed twice, first with 400 μL of buffer PW1 and then centrifuged. The pellet was then washed with 700 μL buffer PW2 to ensure the pellet was free of any contaminants. A warm elution buffer (65 °C) of 50 μL was added to the membrane filter of the column and incubated for 5 min at 65 °C. The elution was performed by centrifugation for 1 min at 6000 rpm. Following extraction, the concentration and quality of the extracted DNA was assessed using a Nanodrop 8000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and electrophoresis in a 0.8 % agarose gel.

Sequencing was performed on Illumina platform and 10X coverage was achieved for each accession. Post sequencing, adapter sequences were trimmed from sequences, and low-quality reads were filtered out. These reads were considered low-quality if they contained more than 20 % of bases that were low-quality (quality value ≤ 7) and more than 5 % "N" nucleotides; we used SOAP2 to clean the completed sequences to retain only reads with high-quality sequencing data for downstream analysis (Li et al., 2009). Next, we aligned cleaned reads to the reference genome for the cultivated tetraploid variety "Tifrunner" using the same software with parameters of "-m 300 -x 600 -s 35 -l 32 -v 5 -p 4." After alignment with the reference genome, we calculated the likelihood for all possible genotypes for each sample with SOAPsnp3, which takes into account the maximum likelihood estimates of allele frequencies in the population. To refine the dataset, low-quality variants were filtered based on strict criteria: sequencing depth greater than 10,000 and less than 400, mapping quality scores exceeding 1.5, and overall quality scores below 20. Only loci with estimated allele frequencies that were not equal to 0 or 1 were kept. We removed all remaining SNPs with more than 50 % missing data per genotype to ensure that all downstream evaluations were derived from high-quality SNP.

2.3. Single and multi-locus genome-wide association studies analysis and identification of candidate genes

WGRS data from 184 mini-core accessions were analyzed. A working subset of 2,55,144 filtered SNPs with 0.25 heterozygosity, and 0.05 minor allele frequency (MAF) using TASSEL v.5 software (Bradbury et al., 2007). To identify MTAs, GWAS analysis was performed using the Genome Association and Prediction Integrated Tool (GAPIT) package in R (Tang et al., 2016). To pinpoint genetic regions linked to our traits of interest, we conducted a comprehensive GWAS analysis. This involved combining detailed multi-season observations with extensive genetic data – specifically, 255,144 SNPs from mini-core collection. Employing both single- and multi-locus statistical models, including CMLM, BLINK, MLMM, and FarmCPU using the GAPIT package (Lin et al., 2016). To minimize false positives, and ensure reliable associations, population structure and kinship were accounted by incorporating the first three principal components (PCs) and a kinship matrix. The significant threshold for MTAs was determined using Bonferroni correction, resulting a *p*-value of 6.7×10^{-7} which can be calculated from the negative log conversion of α/n (where n = total SNPs for GWAS analysis) (Alqudah et al., 2020). Candidate genes were identified within 200 kb genomic region window, (100 kb upstream and 100 kb downstream) of each significant MTA. Peanut base genome browser (https://Peanutbase.org/) was used to identify the candidate genes using GBrowse v1 (cultivated peanut) using gene ID.

2.4. Kompetitive Allele-Specific PCR marker development and validation

In this study, association mapping identified 39 SNPs significantly linked to FSD, of which 17 were prioritized for the development of Kompetitive Allele-Specific PCR (KASP) assays. These SNPs represented genomic regions located near candidate genes distributed across 11 chromosomes. To ensure the development of user-friendly and cost-effective markers, 300-bp upstream and 300-bp downstream flanking sequences were incorporated for SNP conversion into KASP markers (He et al., 2014). Each KASP assay included two allele specific forward primers and one common reverse primer (Intertek Pvt. Ltd.) The validation panel consisted of 37 genotypes, including 16 dormant, 5 moderately dormant, and 16 non-dormant lines (Supplementary Table 7). The developed KASP markers were validated across contrasting germplasm as well as breeding lines from ICRISAT. A complete list of the KASP markers used in this study is provided in Table 2.

3. Results

3.1. Phenotypic variation among different agronomic types for fresh seed dormancy in mini-core collection

In this study, FSD was estimated based on the number of days required for an accession to reach 50 % germination. The GWAS panel exhibited considerable variation in dormancy duration, ranging from 1 to 30 days. Among the different agronomic types, Virginia bunch and Virginia runner (var. *hypogaea*) accessions displayed the longest dormancy period, lasting 16–30 days, whereas Spanish bunch (var. *vulgaris*) and Valencia (var. *fastigiata*) accessions showed dormancy durations ranging from 1 to 25 days. The mini-core collection represented diverse geographic origins, as illustrated in Fig. 1a. The mean performance and phenotypic distribution of the accessions across multiple seasons were analyzed from two replications, with results depicted in Fig. 1b and Supplementary Table 1. These findings highlight the significant variability in FSD among the mini-core accessions, providing a strong basis for further genetic studies and association mapping.

Significant variation in FSD was observed across seasons among a diverse panel of 184 groundnut mini-core collection. Analysis of

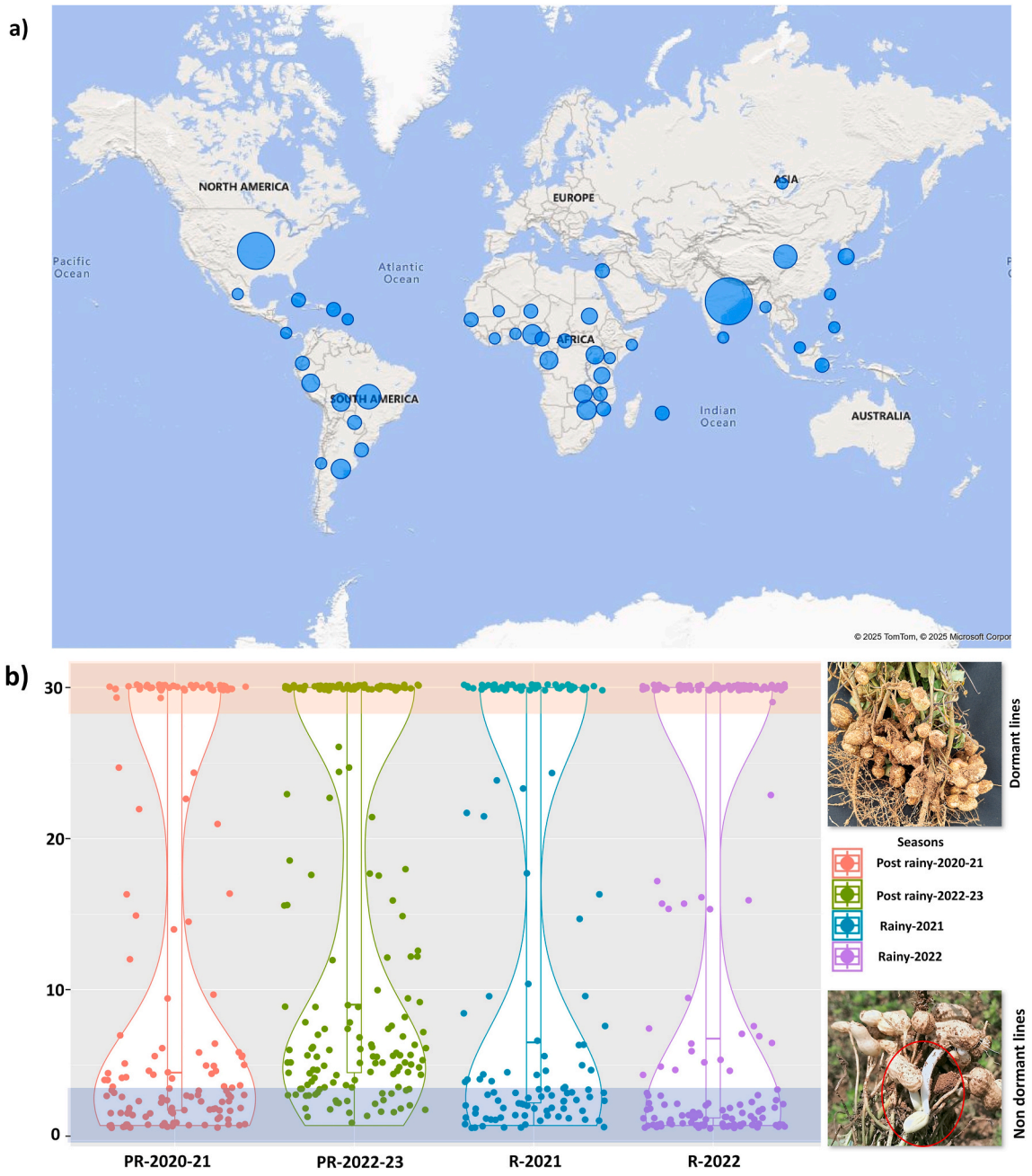

Fig. 1. Phenotypic variability for fresh seed dormancy in the mini-core accessions. a) The world map illustrates the geographical distribution of the groundnut mini-core collection globally. Countries are represented by circles of varying sizes, which indicate the presence and diversity of accessions. Larger circles denote a higher number of accessions, while smaller circles represent fewer accessions. **b)** The violin plot displays the dormancy characteristics of groundnut lines over four seasons: Post-rainy 2020-21, Post-rainy 2022-23, Rainy 2021; Rainy 2022. Each violin represents the distribution of data points for that season, with higher points indicating dormancy and lower points showing non-dormant lines. The shaded areas within the plot indicate the density of data points.

variance (ANOVA) indicated highly significant differences among accessions ($P < 0.01$), highlighting substantial phenotypic diversity within the panel. Violin plots depicted the distribution of trait values for each season, with wider sections of the violins indicated more frequent values, and reflecting the diversity among the accessions (Fig. 1b). Dormancy periods ranged from 1 to 30 days, with average durations of 12.54, 14.57, 14.96, and 15.72 days for four seasons, and standard errors of 0.52, 0.41, 3.01, and 2.98, respectively. High heritability (~90 %) further highlighted the genetic control of this trait across the seasons, emphasizing the broad variability within the mini-core collection. This genetic variability laid the foundation for conducting GWAS to identify significant MTAs associated with FSD.

3.2. Identification of significant MTAs associated with fresh seed dormancy through GWAS analysis

The analysis of SNP (Single Nucleotide Polymorphism) density across chromosomes revealed substantial variation in SNP distribution. Chromosomes Ah03, Ah19, and Ah04 exhibited the highest SNP densities, with 19001, 15758, and 14193 SNPs, respectively. In contrast, chromosomes Ah08 and Ah07 displayed relatively lower SNP densities. Notably, red-coloured regions indicated areas of high SNP density, particularly on chromosome Ah17. Additionally, white gaps observed on chromosomes Ah14, Ah15, and Ah16 suggest regions with low or undetected SNPs (Supplementary Figure 1).

A total 27 MTAs significantly associated with FSD were identified in GWAS analysis (Table 1). Among these, five MTAs (Ah04_98048339, Ah05_14439200, Ah05_25536212, Ah05_26459808, Ah20_126576288) were consistently identified across at least by two models (Fig. 2). Notably, MTAs on chromosomes Ah05 (Ah05_14439200) and Ah20 (Ah20_126576288) were consistently identified in all four models during PR2020–21 and R2022, with higher phenotypic variance explained (PVE %) of 69.61 and 66.54 respectively. Additionally, three MTAs (Ah04_79354879, Ah05_25536212, Ah05_26459808) were identified in at least three models, with the PVE (%) of 37.81, 30.59 and 13.24 during PR2020–21, R2021 and PR2022-23, seasons respectively. Furthermore, 17 MTAs were identified on chromosomes Ah01, Ah03, Ah04, Ah05, Ah11, Ah13, Ah14, Ah18, Ah19, and Ah20, with each chromosome revealing a single model (Table 1).

Moreover, the pooled data from two rainy and post rainy seasons identified significant MTAs that were not detected in individual seasons.

Table 1
Marker trait associations and corresponding candidate genes identified for fresh seed dormancy.

SNP	Chr. No	Position (bp)	Allele (Ref)	Allele (Alt)	P value	Models	PVE (%)	Gene ID	Annotations
Post-rainy – 2020-21									
Ah04_79354879	Ah04	79354879	G	T	3.16E-11	A, B, D	37.81	*Arahy.DC1G4E*	*dentin sialophospho protein*
								Arahy.MX2AUU	*Arabidopsis Inositol phosphorylceramide synthase*
Ah04_90180304	Ah04	90180304	C	T	2.81E-08	A	4.43	*Arahy.GP8RZD*	*Unknown protein*
								Arahy.D3VYYS	*isochorismate synthase*
Ah05_14439200	Ah05	14439200	A	T	2.13E-23	A, B, C, D	60.61	*Arahy.HLX2RH*	*zinc finger MYM-type protein*
Ah05_25536212	Ah05	25536212	A	T	1.40E-07	B, C	26.15	*Arahy.83PW9L*	*multiple C2 and transmembrane domain-containing protein*
								Arahy.DLDD1G	*DUF247 domain protein*
Ah11_49332078	Ah11	49332078	C	T	5.87E-08	A	17.68	*Arahy.83R1N2*	*trehalose-6-phosphate phosphatase*
								Arahy.MWX0EW	*cinnamyl alcohol dehydrogenase*
Ah11_56153560	Ah11	56153560	C	T	3.99E-09	A	18.4	*Arahy.2D5YI2*	*transmembrane protein*
								Arahy.511LEY	*callose synthase*
Ah20_141972519	Ah20	141972519	C	T	1.92E-07	A	19.43	*Arahy.08738Y*	*Cytochrome P450 superfamily protein*
								Arahy.8DF3UX	*receptor-like protein kinase*
Rainy – 2021									
Ah01_84852438	Ah01	84852438	A	G	8.32E-09	A	16.58	*Arahy.GJT3HI*	*kinesin light chain*
								Arahy.96ZSDT	*Protein kinase superfamily protein*
Ah04_104262336	Ah04	104262336	G	A	4.96E-15	D	44.08	*Arahy.R13DG3*	*Autophagy-related protein*
								Arahy.E6YGIA	*WRKY transcription factor*
Ah05_25536212	Ah05	25536212	A	T	1.28E-07	A, B, C	30.59	*Arahy.83PW9L*	*multiple C2 and transmembrane domain-containing protein*
								Arahy.DLDD1G	*DUF247 domain protein*
Ah05_14439200	Ah05	14439200	A	T	5.07E-23	C, D	51.41	*Arahy.HLX2RH*	*zinc finger MYM-type protein*
Ah14_27702154	Ah14	27702154	G	T	9.34E-10	A	3.02	*Arahy.C9BI3G*	*pumilio-family RNA-binding repeat protein*
								Arahy.KM341F	*metaxin-related*
Ah14_127271353	Ah14	127271353	C	T	8.00E-13	A	62.76	*Arahy.ZJK6AX*	*Unknown protein*
								Arahy.864M36	*phosphoenolpyruvate carboxykinase*
Ah18_125224726	Ah18	125224726	T	G	1.37E-08	A	12.30	*Arahy.X8VDXZ*	*transcription initiation factor TFIID*
								Arahy.XQA4CG	*Unknown protein*
Ah19_32252142	Ah19	32252142	T	C	1.27E-07	A	12.36	*Arahy.T0MTDZ*	*dolichol-phosphate mannosyltransferase*
Ah20_126576288	Ah20	126576288	T	C	9.04E-15	A, B	53.15	*Arahy.PI0JM6*	*uncharacterized protein*
Ah20_131946423	Ah20	131946423	T	C	1.19E-08	A	14.32	*Arahy.E1G8VG*	*CLAVATA3/ESR (CLE)-related protein*
Rainy – 2022									
Ah03_142676829	Ah03	142676829	A	T	5.12E-09	D	17.65	*Arahy.AH38KH*	*glucan endo-1,3-beta-glucosidase-like protein*
Ah04_98048339	Ah04	98048339	G	A	1.68E-13	A, B	28.26	*Arahy.T1PXLY*	*UPF0420 protein C16orf58 homolog*
								Arahy.JQ72AY	*alcohol dehydrogenase*
Ah05_9570817	Ah05	9570817	C	T	2.98E-08	D	11.13	*Arahy.C3EWW9*	*hypothetical protein*
								Arahy.0K456L	*Cytochrome P450 superfamily protein*
Ah05_88008131	Ah05	88008131	G	A	1.85E-09	D	20.72	*Arahy.83A3G6*	*NAC domain containing protein*
								Arahy.3X1GMP	*NAC domain containing protein*
Ah13_10515312	Ah13	10515312	G	A	8.66E-09	B	11.76	*Arahy.WD5AW4*	*Myc-type, basic helix-loop-helix (bHLH) domain transcription factor*
								Arahy.11KM4W	*Plasma membrane aluminum transporter*
Ah13_12248549	Ah13	12248549	C	T	1.89E-14	B	16.46	*Arahy.0SD0YX*	*cation/H+ exchanger*
Ah20_126576288	Ah20	126576288	T	C	2.90E-08	A, B, C, D	66.54	*Arahy.PI0JM6*	*uncharacterized protein*
Post-rainy – 2022-23									
Ah05_26459808	Ah05	26459808	C	T	9.74E-10	A, B, C	13.24	*Arahy.NUE0XS*	*cofactor assembly of complex C*
								Arahy.1ITW6E	*zinc finger MYM-type protein*
Ah15_135933419	Ah15	135933419	G	A	4.55E-08	D	21.99	*Arahy.JJ8FJQ*	*choline kinase*
Ah20_126576288	Ah20	126576288	T	C	1.86E-10	B, D	37.63	*Arahy.PI0JM6*	*uncharacterized protein*

A – FarmCPU, B – BLINK, C – CMLM, D - MLMM.

Table 2
List of validated KASP markers from candidate genes associated with fresh seed dormancy in groundnut.

KASP ID	SNP ID	Chr. No	Pos (bp)	Allele (Ref)	Allele (Alt)	Gene ID
snpAH00571	A09_29726644	A09	29726644	C	T	*Arahy.XE42X*
snpAH00572	B05_112344292	B05	112344292	A	G	*Arahy.J6XE1*
snpAH00573	B09_143731363	B09	143731363	A	G	*Arahy.CGP7E*
snpAH00577	Ah05_95779759	Ah05	95779759	G	A	*Arahy.J8ADIH & Arahy.UB3QJP*
snpAH00580	Ah08_17840995	Ah08	17840995	C	T	*Arahy.NRZN5K*
snpAH00582	Ah13_18020146	Ah13	18020146	T	G	*Arahy.VK2HD8 & Arahy.TA4VKK*
snpAH00585	Ah15_38301179	Ah15	38301179	A	G	*Arahy.A1GEBB & Arahy.GD66SW*
snpAH00586	Ah15_135933419	Ah15	135933419	A	G	*Arahy.JJ8FJQ*
snpAH00588	Ah20_126576288	Ah20	126576288	T	C	*Arahy.PI0JM6*

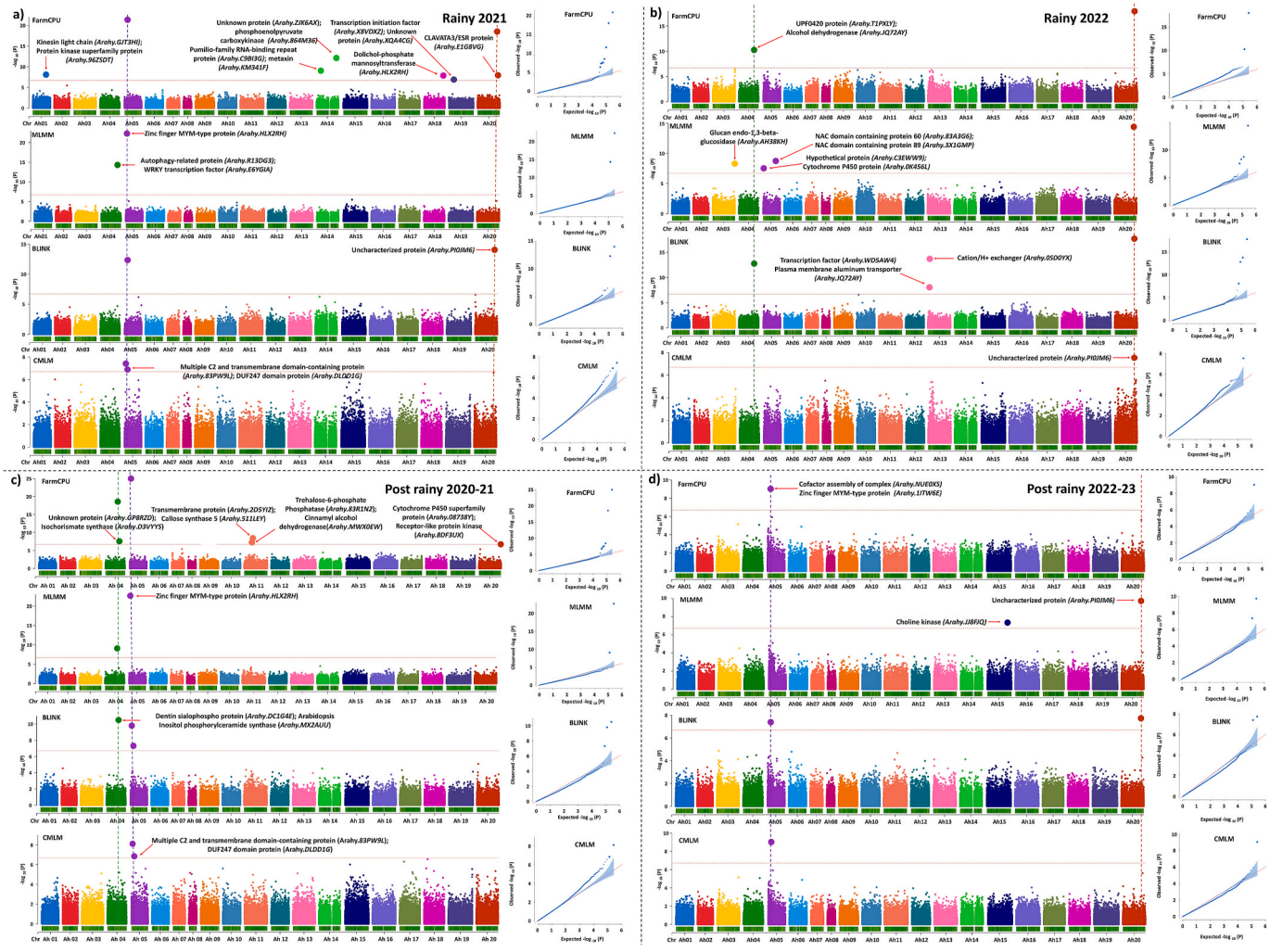

Fig. 2. The Manhattan and Q-Q Plot represent the genome-wide association study (GWAS) results for groundnut days to 50 % germination across four different models: FarmCPU, MLMM, BLINK, and CMLM. Data is shown for four growing seasons: Rainy 2021, Rainy 2022, and Post-rainy 2020-21, Post-rainy 2022-23. Each panel corresponds to a different model and illustrates the statistical significance of associations between genetic markers and days to 50 % germination. **a)** Manhattan and Q-Q plot for Rainy 2021 **b)** Rainy 2022 **c)** Post-rainy 2020-21 and **d)** Post-rainy 2022-23.

A total 12 MTAs were identified across six chromosomes, with PVE (%) from 6.04 to 71.94 (Supplementary Table 2). Interestingly, MTAs on Ah05 (Ah05_14439200) and Ah20 (Ah20_126576288), were consistently identified, in both pooled rainy and post rainy seasons. The MTA Ah20 (Ah20_126576288) was identified in both individual season analyses and pooled data (Supplementary Figure 2). These consistent findings across multiple models and datasets strongly suggest the potential of these MTAs for further exploration. This led to an in-depth investigation of candidate genes underlying these MTAs.

3.3. Identification of candidate genes for fresh seed dormancy

Across environments, we identified 44 candidate genes associated with the trait, based on 27 distinct MTAs. Specifically, seven MTAs were found in PR 2020–21, ten in R 2021, seven in R 2022, and three in PR 2022–23. These genes were involved in different cellular, molecular and biological functions related to the trait. The candidate genes encoded important genes like, *dentin sialophospho protein (Arahy.DC1G4E), Arabidopsis Inositol phosphorylceramide synthase (Arahy.MX2AUU), isochorismate synthase 2 (Arahy.D3VYYS), zinc finger MYM-type protein*

(Arahy.HLX2RH), multiple C2 and transmembrane domain-containing protein (Arahy.83PW9L), trehalose-6-phosphate phosphatase (Arahy.83R1N2), callose synthase 5 (Arahy.511LEY), receptor-like protein kinase 1 (Arahy.8DF3UX), Protein kinase superfamily protein (Arahy.96ZSDT), Autophagy-related protein (Arahy.R13DG3), WRKY transcription factor (Arahy.E6YGIA), Cytochrome P450 superfamily protein (Arahy.08738Y), alcohol dehydrogenase (Arahy.JQ72AY), NAC domain containing protein (Arahy.83A3G6), Myc-type, basic helix-loop-helix (bHLH) domain transcription factor (Arahy.WD5AW4), Plasma membrane aluminium transporter (Arahy.11KM4W), cation/H^+ exchanger (Arahy.0SD0YX), cofactor assembly of complex C (Arahy.NUE0XS) and *choline kinase (Arahy.JJ8FJQ)* were prominent genes known for their involvement in regulation dormancy/germination (Table 1).

Similarly, a total of 17 candidate genes were identified using 12 MTAs across both pooled seasons, specifically nine genes in the pooled rainy season and eight in the pooled post rainy season. The main genes are *zinc finger MYM (Arahy.HLX2RH), proteasome subunit beta type-7-A (Arahy.J8ADIH), calcium-transporting ATPase (Arahy.NRZN5K), homeobox-leucine zipper protein (Arahy.VK2HD8), RING-box (Arahy.A1GEBB), 5-formyltetrahydrofolate cyclo-ligase (Arahy.GD66SW), histone deacetylase (Arahy.GRNJ5W), ninja-family protein (Arahy.12DG6R)* and *Target of Myb protein 1(Arahy.IG89BU)* (Supplementary Table 2). These both individual season and pooled seasons obtained genes are directly or indirectly involved in the regulation of dormancy/germination pathway.

In the 200 kb genomic region, the identified significant MTAs by individual seasons, total of 149 genes were extracted (Supplementary Table 3) of these, previously reported studies had identified 57 of the genes as candidate regulators of the process of seed dormancy or seed germination regulating *via* ABA, GA and ethylene signalling pathways (Supplementary Table 4). The genes corresponding to MTA on chromosome A05 (Ah05_9570817) in same season includes *Cytochrome P450 superfamily protein (Arahy.0K456L), glucan endo-1,3-beta-glucosidase (Arahy.B7TVVM),* also chromosome Ah20 in PR2020-21 having *Cytochrome P450 superfamily protein (Arahy.08738Y).* Similarly, *myb transcription factor (Arahy.2BK3KU; Arahy.S1DRQT; Arahy.1X4EZ2)* from Ah14_127271353, Ah20_131946423 and Ah13_12248549 are recognized as promising genes involved in ABA biosynthesis.

MTAs from pooled season results yielded a total of 57 genes (Supplementary Table 5). Previous studies have extensively reported 23 genes as potential genes among these included *serpin-ZX- protein (Arahy.GKKZ7Q), serine carboxypeptidase (Arahy.N7SB2Q)* on Ah05 and Ah08 from pooled rainy, similarly, from Ah04 having *F-box and associated interaction domain protein (Arahy.TJ6H4J)* were identified to regulate SD as reported in various crops (Supplementary Table 6). These genes were further analyzed to understand their expression patterns and functional roles in seed dormancy and germination.

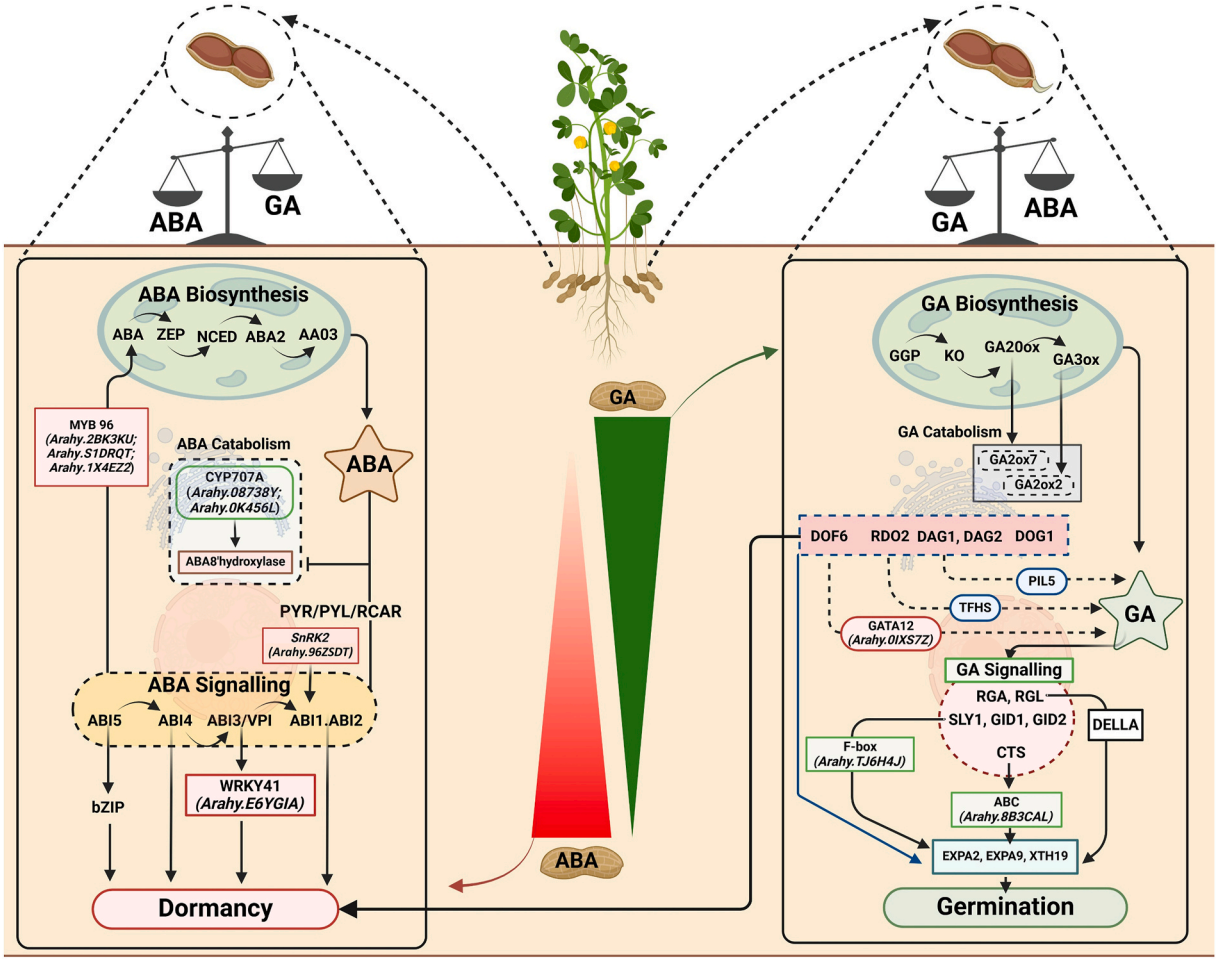

Fig. 3. The diagram represents the interaction between the abscisic acid (ABA) and gibberellic acid (GA) pathways, which play crucial roles in regulating seed dormancy and germination. ABA biosynthesis and signaling pathways, highlighted on the left side, promote seed dormancy, with key genes like *ABI5*, *ABI4*, and *ABI3/VPI* involved in signaling, while genes like *MYB96* and *SnRK2* are central to the dormancy process. In contrast, GA biosynthesis and signaling pathways, shown on the right side, promote germination through genes like *RGA*, *RGL*, *SLY1*, and *GID1*, with direct pathway factors such as *DOF6* and *DOG1*. The balance between ABA and GA is depicted, illustrating how their opposing actions determine the seed's transition from dormancy to germination. Note: The plant icons were taken from BioRender (https://www.biorender.com/).

3.4. Gene pathway for FSD regulated by ABA and GA

To explore the molecular mechanisms underlying dormancy and germination, we conducted an in-depth investigation into the functional roles of identified candidate genes involved in ABA and GA biosynthesis and signaling pathways. Among these candidates, the *Cytochrome P450 superfamily proteins (Arahy.08738Y*; *Arahy.0K456L)* were prominently recognized for their role in ABA catabolism. Additionally, several key players in ABA signaling were identified, including *WRKY family transcription factors (Arahy.E6YGIA)*, *protein kinase superfamily proteins (Arahy.96ZSDT)*, and *MYB transcription factors (Arahy.2BK3KU, Arahy.S1DRQT, Arahy.1X4EZ2)*. Furthermore, the *ATP-binding ABC transporter* (*Arahy.8B3CAL*), *F-box protein interaction domain protein (Arahy.TJ6H4J)* and *GATA transcription factor (Arahy.OIXS7Z)* emerged as a significant contributor to GA signaling. These findings highlight the intricate interplay between these hormones, providing valuable insights into the regulatory networks that govern seed dormancy and germination processes (Fig. 3). To further validate the identified MTAs, we examined their polymorphism in a diverse panel of groundnut accessions.

3.5. Development and validation of KASP markers for FSD

In relation to the phenotyping data, a representative of the mini-core diversity panel, with varied durations of dormancy, was chosen to evaluate the robustness of the identified MTAs. The allele calls of the selected accessions, for 27 significantly associated SNPs (identified season wise) and 12 associated SNPs (Pooled season), from WGRS and SNP array genotyping data based GWAS analysis were used for KASP assay design and validation. Among the 27 identified MTAs only nine showed polymorphism between dormant and non-dormant accessions in the mini-core collection, specifically for SNPs Ah03_142676829, Ah04_79354879, Ah05_9570817, Ah05_88008131, Ah11_56153560, Ah13_12248549, Ah15_135933419, Ah20_141972519 and Ah20_126576288. Additionally, from a pooled analysis of 12 MTAs, 6 SNPs exhibited polymorphism Ah05_95779759, Ah08_17840995, Ah13_18020146, Ah15_68637668, Ah15_38301179 and Ah20_126576288 (Supplementary Table 8). Notably, the SNP Ah20_126576288 consistently appeared across three growing seasons also in pooled seasons, indicating its reliability. So, a total of 14 different markers showing clear polymorphism and three SNPs namely; A09_29726644, B05_112344292 and B09_143731363 from the 58K panel were used for the validation.

KASP markers provide a cost-effective and efficient genotyping approach, enabling early-generation selection in breeding programs for indirect selection of target phenotypes. To develop and validate diagnostic markers for FSD, 14 SNPs were targeted for KASP marker development, distributed across multiple chromosomes (three SNPs each on Ah05 and Ah15, two SNPs each on Ah13 and Ah20, and one SNP each on Ah03, Ah04, Ah08, and Ah11). Markers for these 14 SNPs were successfully designed and validated using a diverse validation panel categorized by dormancy period. The validation panel was comprised of non-dormant lines with dormancy period ranging from 1 to 7 days, moderate dormant lines ranging from 9 to 12 days and dormant lines ranging from 15 to 30 days of dormancy (Supplementary Table 7).

Among the 14 verified KASP markers, six markers (snpAH00577, snpAH00580, snpAH00582, snpAH00585, snpAH00586, and snpAH00588) exhibited the expected polymorphism, clearly distinguishing between dormant and non-dormant genotypes (Fig. 4). Additionally, three markers (snpAH00571, snpAH00572, and snpAH00573), located on chromosomes A09, B05, and B09, were included based on previous findings (Bomireddy et al., 2024) (Table 2), further strengthening their relevance for FSD studies. In total, nine KASP markers demonstrated high polymorphism among non-dormant, moderate, and dormant groundnut genotypes. These markers serve as potential diagnostic tools for selecting dormant seeds and screening segregating breeding material at early stages of varietal development.

3.6. Best allelic combination for achieving fresh seed dormancy

Among nine KASP markers validated from WGRS and the 58K 'Axiom_*Arachis*' array (Table 2), only four markers; snpAH00577 (Ah05_95779759), snpAH00580 (Ah08_17840995), snpAH00586 (Ah15_135933419), and snpAH00588 (Ah20_126576288) exhibited the best allelic combination ($A_1T_1G_3Y$), resulting in 24–30 days of dormancy. This combination includes all favourable alleles ($A_1T_1G_3Y$) for dormancy, whereas the unfavourable allelic combination ($G_4C_1A_3C_2$) for these three markers led to only 1–6 days of dormancy, also when we combine the Ah05_95779759, Ah08_17840995, and Ah15_135933419 favourable allelic combination ($A_1T_1G_3$), it gives 22–30 days of dormancy, while the unfavourable allelic combination ($G_4C_1A_3$) gives 1–8 days of dormancy. Conversely, the other combinations for the remaining marker allelic combinations did not show significant variation in dormancy duration (Fig. 5). The candidate genes associated with these three KASP markers were identified as *ATP-binding ABC transporter* (*Arahy.8B3CAL*) is involved in GA signalling, *calcium-transporting ATPase* (*Arahy.NRZN5K*), *uncharacterized protein (Arhay.PI0JM6)* involved in ABA pathway, and *choline kinase* (*Arahy.JJ8FJQ*) involved in both ABA-GA interaction. These associated genes are now named as *Ahfsd1, Ahfsd2, Ahfsd3* and *Ahfsd4,* respectively.

4. Discussion

Groundnut is a widely cultivated leguminous crop valued for its high protein content and rich supply of unsaturated oils. However, one of the significant challenges in its production is PHS, where the premature germination of mature seeds on the mother plant before harvest, typically triggered by untimely rainfall or high humidity during seed maturation, which causes considerable yield losses and quality degradation (Sohn et al., 2021). In groundnut, it is more severe as the pods/seeds are already inside the soil receiving conducive environment even for full plant growth. Genotypes exhibiting over 90 % dormancy for a duration of 2–3 weeks are better adapted to regions with unpredictable rainfall patterns during the maturation stage. Incorporating such traits into breeding programs is crucial for ensuring stable yields under varying environmental conditions. GAB provides a significant advantage over traditional breeding methods by enabling the efficient tracking of desirable alleles in segregating populations through the use of trait-linked molecular markers (Varshney et al., 2014). In this study, a mini-core collection was analyzed using WGRS data and multi-season phenotyping to identify genomic regions and key candidate genes associated with FSD and germination traits. To effectively translate the genetic insights into practical breeding applications, advanced statistical and genomic tools such as Genome-Wide Association Studies (GWAS) are essential for pinpointing the specific genetic loci and alleles controlling these traits. Therefore, this integrative approach offers valuable understanding of the genetic factors influencing FSD, which could contribute to the development of improved groundnut varieties with enhanced resistance to PHS.

GWAS has emerged as a robust method for identifying MTAs related to complex traits of interest. By leveraging genetic diversity within diverse germplasm, this approach has been widely utilized for uncovering genetic variation that influences various traits across crops (Bohar et al., 2020). Advanced GWAS models like CMLM, MLMM, FarmCPU, and BLINK used in the present study, significantly improve the reliability and precision of identifying genetic associations in plant genomics (Liu et al., 2016). For instance, CMLM effectively controls for population structure and kinship, thereby reducing spurious associations (Tekeu et al., 2023) and MLMM addresses overfitting issues, making it particularly valuable for analysing complex traits. On the other hand, FarmCPU incorporate both fixed and random effects, making it highly effective across various trait analyses (Tekeu et al., 2023). BLINK offers high computational efficiency for rapid SNP detection while minimizing false positives, a critical advantage for large genomic datasets (Adhikari

a)

KASP Assay	Non dormant																Moderate Dormant					Dormant															
	ICG10092	ICG15309	ICG332	ICG4670	ICG5609	ICG6375	ICGV02266	ICG11144	ICG115	ICG14106	ICG13982	ICGV15415	ICGV171010	ICG13603	ICG13858	ICGV87846	ICGV00440	ICGV15410	ICGV211089	ICGV181065	ICGV86564	ICGV97045	ICGV15402	ICG15427	ICGV98432	ICG13723	ICG13787	ICG9777	ICG9842	ICG9905	ICG9961	ICG10185	ICG11219	ICG13099	ICG14482	ICG13895	ICG14834
	1	1	1	1	1	1	1	2	2	2	3	3	4	5	5	7	9	10	10	11	12	15	16	18	20	24	25	30	30	30	30	30	30	30	30	30
snpAH00571	C:C	C:C	C:C	C:C	C:C	C:C	T:T	C:C	C:C	C:C	C:C	C:C	T:T	C:C	C:C	T:C	T:T	T:T	T:T	C:C	C:C	NA	T:T	C:C	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
snpAH00572	A:A	A:A	A:A	A:A	A:A	A:A	G:G	A:A	A:A	A:A	A:A	A:A	G:G	A:A	A:A	G:G	G:G	G:G	G:G	A:A	A:A	NA	G:A	A:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
snpAH00573	G:A	G:A	G:A	G:A	G:A	G:A	G:G	G:A	G:A	G:A	G:A	G:A	G:A	G:A	G:A	NA	G:G	G:G	G:G	G:A	G:A	G:G	G:G	G:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
snpAH00577	G:G	G:G	G:G	G:G	G:G	G:G	A:A	G:G	G:G	G:G	G:G	A:A	G:G	G:G	G:G	A:A	A:A	G:G	A:A	G:G	A:A	NA	G:G	A:A	A:A	A:A	A:A	G:G	A:A	A:A	A:A	A:A	A:A	G:G	A:A	A:A
snpAH00580	C:C	C:C	C:C	C:C	C:C	C:C	T:T	C:C	C:C	C:C	C:C	C:C	T:T	C:C	C:C	C:C	T:T	T:T	T:T	C:C	C:C	T:T	NA	C:C	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
snpAH00582	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	G:G	G:G	T:T	T:T	NA	G:G	G:G	G:G	T:T	G:G	NA	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
snpAH00585	A:A	A:A	A:A	A:A	A:A	A:A	G:G	A:A	A:A	A:A	A:A	A:A	G:G	A:A	A:A	A:A	G:G	G:G	G:G	A:A	A:A	G:G	G:G	A:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
snpAH00586	A:A	A:A	A:A	A:A	A:A	A:A	G:G	A:A	A:A	A:A	A:A	A:A	G:G	A:A	A:A	G:G	G:G	G:G	G:G	A:A	A:A	NA	G:G	A:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
snpAH00588	C:C	C:C	C:C	C:C	C:C	C:C	NA	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	NA	C:C	C:C	NA	C:C	NA	T:C	C:C	T:C	T:C	T:C	T:C	T:C	T:C	T:C	T:C	T:C	NA	T:C	T:C	T:C

b)

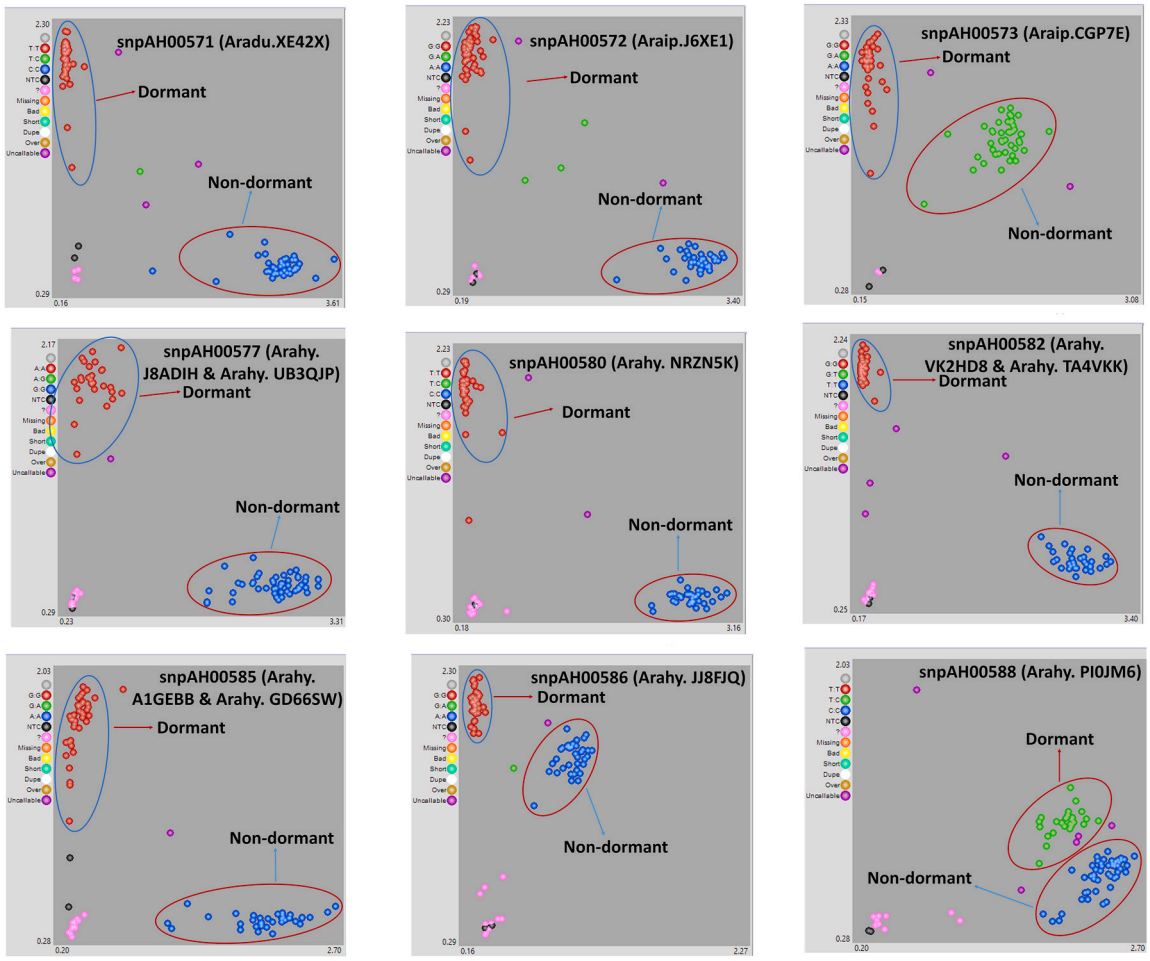

Fig. 4. Development and validation of Kompetitive allele specific polymerase chain reaction (KASP) markers from potential candidate genes identified for fresh seed dormancy. (a) Validation panel includes 16 non-dormant lines (1–7days), 5 moderate-dormant (9–12 days) and 16 dormant lines (15–30 days) (b) snpAH00571, snpAH00572, snpAH00573 KASP markers from 58K "Axiom_*Arachis*" array and snpAH00577, snpAH00580, snpAH00582, snpAH00585, snpAH00586 and snpAH00588 KASP markers from WGRS were developed and validated for FSD.

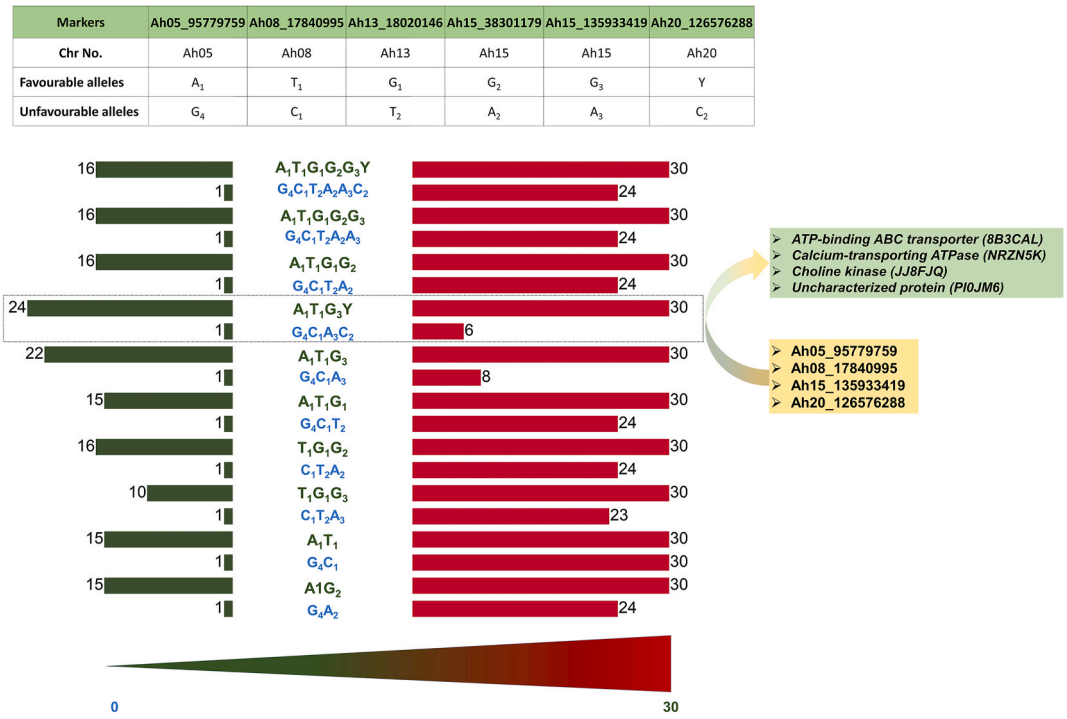

Fig. 5. The diagram illustrates the relationship between specific allelic combinations of KASP markers and the duration of seed dormancy in days. Green letters represent favourable allelic combinations associated with longer dormancy periods (ranging from 24 to 30 and 22–30 days), with the " $A_1T_1G_3Y$" and " $A_1T_1G_3$" combination within the dashed blue rectangle on markers Ah05_95779759, Ah08_17840995, Ah15_135933419 and Ah20_126576288 showing the strongest association with extended dormancy (24–30 and 22–30 days). Conversely, blue letters indicate unfavourable allelic combinations, such as "$G_4C_1A_3C_2$" and "$G_4C_1A_3$" for the same key markers, which result in significantly shorter dormancy (1–6 and 1–8 days). The associated candidate genes linked to these markers on chromosomes Ah05, Ah08, Ah15 and Ah20 are ATP-binding *ABC transporter, calcium-transporting ATPase*, *choline kinase*, and *uncharacterized protein* respectively, suggesting their potential roles in regulating the duration of dormancy. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

et al., 2023; Hu et al., 2018; Segura et al., 2012). Both FarmCPU and BLINK, as multi-locus models, increase statistical power by reducing false positives. Consequently, these approaches can be further utilized to explore the genetic basis of complex traits, such as seed dormancy and germination, through resequencing-based genotyping and GWAS.

Resequencing-based genotyping captures extensive natural variation, facilitating GWAS-driven discovery of functional genes and elite alleles to enhance breeding and improve crop performance (Yu et al., 2017; Zhao et al., 2018). In the current study, GWAS analysis of a mini-core collection identified 27 significant MTAs for the FSD trait across individual seasons and 10 significant MTAs from pooled seasonal data. Our previous findings through genetic mapping (QTL-Seq) reported two genomic regions on chromosomes B05 and A09 linked to FSD (Kumar et al., 2020). Also, a significant stable QTL linked with FSD was mapped on chromosome A04 (Zhang et al., 2022). Additionally, Bomireddy et al. (2022) deployed a 5K SNP assay in a bi-parental genetic mapping population and identified five major QTLs on chromosomes Ah01, Ah06, Ah11, Ah16, and Ah17, along with two minor QTLs identified on Ah04 and Ah15. Notably, the *qFSD_A04-1* (119 Mb) was found near to *qPD_A04-2* (Wang et al., 2024), on chromosome A04, and *qFSD_B05-1* (112 Mb) was located near to the region of interest on chromosome B05 (Kumar et al., 2020). These findings highlight the reliability of GWAS in pinpointing significant MTAs in proximity to previously reported genomic regions.

Identification of candidate genes associated with QTLs/MTAs provide perspectives into the molecular mechanisms regulating FSD. In particular, the roles of ABA, GA, and ethylene in regulating seed dormancy and germination have been well-documented across various crops (Nautiyal et al., 2023). In this study, genes identified through GWAS from both individual and pooled seasons were reviewed in existing literature to evaluate their functional roles in ABA and GA signalling pathways which elucidates how these genes contribute to the hormonal regulation of seed dormancy and germination process. Among them, *Cytochrome P450 gene* (*Arahy.08738Y*) identified is associated with ABA catabolism (Fig. 3). Similar gene found in *Arabidopsis* encodes an enzyme known as *8′-hydroxylase*, which plays a crucial role in the catabolism of ABA. A mutation in this gene, specifically the *cyp707a2* mutant, leads to a sixfold increase in ABA levels compared to wild-type plants. This elevated ABA concentration results in hyper-seed dormancy (Kushiro et al., 2004). This suggests that *CYP707A2* functions as a negative regulator of seed dormancy by reducing ABA levels during imbibition. Additionally, transcript analysis of the *cytochrome P450 superfamily* genes revealed high expression levels in the non-dormant cultivar ICGV 91114, indicating their positive role in germination (Bomireddy et al., 2022). This evidence underscores the importance of *CYP707A2* and related *P450* enzymes in modulating ABA levels, thereby affecting seed dormancy and germination dynamics. Thus, their suppression could be a strategy to increase seed dormancy.

MYB transcription factor (Arahy.2BK3KU; Arahy.S1DRQT; Arahy.1X4EZ2) identified in the current study and this class of transcription factor is known for their role in ABA signaling (Gosti et al., 1999). *The MYB96 transcription factor* is thought to play a crucial role in regulating seed dormancy by promoting the biosynthesis of ABA through the enhancement of *NCED* genes (Lee et al., 2015), the seeds from the *myb96-1* mutant germinated earlier than those with the wild-type *MYB96-1*. Conversely, seeds with an activated form of *myb96-1D* experienced delays in germination. This indicates that *MYB96* is key in fine-tuning the timing of seed germination.

Another candidate gene, *protein kinase superfamily protein (Arahy.96ZSDT)* found, is also involved in ABA signalling. In *Arabidopsis*, the kinases *SNF1-RELATED PROTEIN KINASE 2.2 (SnRK2.2)* and *SnRK2.3* are specific to ABA signalling and related to seed dormancy and

germination, respectively (Fujii et al., 2007). Double mutants lacking both *snrk2.2* and *snrk2.3* show reduced expression of several genes that are typically induced by ABA, indicating their essential function in promoting ABA signalling therby leads to increased SD. Additionally, redundant *ABA-activated SnRK2 kinases* have been recognized as key regulators of seed maturation and dormancy in *Arabidopsis* (Nakashima et al., 2009). This underscores the importance of *SnRK2 kinases* in the complex network of plant responses to environmental stresses and developmental cues.

WRKY family transcription factor family protein (*Arahy.E6YGIA*) identified in this study was known to be involved in ABA signaling (Fig. 3). *SlWRKY37* is a *WRKY transcription factor* in tomato that plays a crucial role in regulating seed germination revealed that the expression of *SlWRKY37* significantly decreases during the germination process. Using CRISPR/Cas9 gene-editing technology, it was demonstrated that knocking out *SlWRKY37* enhances seed germination, whereas overexpressing this gene results in a delay of germination, playing an indirect role in dormancy (Wang et al., 2024). In *Arabidopsis*, seeds that lack *WRKY41* show a marked decrease in the expression of *ABI3*, (crucial gene for seed dormancy). Conversely, transgenic lines that overexpress *WRKY41* exhibit increased levels of *ABI3* expression (Ding et al., 2014). Further analysis of the double mutant *wrky41 aba2* indicates that both *WRKY41* and ABA work together to regulate *ABI3* expression and seed dormancy. According to this synergy, *WRKY41* regulates *ABI3* crucially, impacting the dormant phase in seeds (Fig. 3).

The *GATA-type zinc finger transcription factor family protein (Arahy.0IXS7Z)* identified in this study is involved in GA signalling. Furthermore, there is a novel interaction that involves *DOF6* and *RGL2*, which retains primary dormancy in *Arabidopsis* through *GATA transcription factors* (Ravindran et al., 2017). In *Arabidopsis thaliana*, the newly identified *RGL2-DOF6* complex is essential for activating the *GATA12* gene. It encodes a *GATA-type zinc finger transcription factor* and is a downstream target of *RGL2* expression. This molecular pathway provides a mechanism for how gibberellin (GA) signalling is repressed, which in turn helps maintain primary seed dormancy (Boccaccini et al., 2014; Ravindran et al., 2017; Stamm et al., 2012). Furthermore, the regulation of the *comatose (CTS)* gene, which is a part of GA signalling, a key factor in germination. When activated, *CTS* promotes germination by interacting with a peroxisomal protein from the *ATP-binding cassette (ABC) transporter* family *(Arahy.8B3CAL)* that utilizes energy to move substances across membranes. This process also involves the *F-box interacting domain protein* (*Arahy.TJ6H4J*), which is another essential component of the GA signalling pathway results in breaking seed dormancy and initiating growth (Footitt, 2002; Skubacz and Daszkowska-Golec, 2017). As an example, in rice, the *OsFbx352* gene, which encodes an *F-box protein*, regulates seed germination. When overexpressed, it lowered ABA levels by decreasing synthesis and increasing catabolism, thereby promoting germination. Conversely, knocking down *OsFbx352* raised ABA levels and prevented germination, showing its role in controlling ABA metabolism (Song et al., 2012). The genes discussed are promising candidates for controlling seed dormancy and germination due to their critical roles in the ABA and GA signalling pathways. Genes that promote ABA biosynthesis or its signalling pathway are known to enhance dormancy. Conversely, dormancy can be increased by using gene-editing tools like CRISPR-Cas to knock out genes that promote GA signaling, thereby shifting the hormonal balance toward ABA and suppressing germination to maintain seed dormancy.

The allelic variation from the representative panel of mini-core accessions for the identified significant MTAs revealed that accessions carrying all favourable dormant alleles exhibited longer dormancy durations. SNP association signals guided the evaluation of favourable alleles, bridging gene discovery with marker validation for use in marker-assisted selection in groundnut. In the current scenario, the diagnostic KASP markers are widely used for resistance to nematodes, leaf rust, and late leaf spot, as well as for enhancing oil quality through high oleic acid content (Bera et al., 2018, 2019; Chu et al., 2011; Deshmukh et al., 2020; Jadhav et al., 2021; Janila et al., 2016; Kolekar et al., 2017; Varshney et al., 2014).

Recognizing the importance of FSD in groundnut breeding, we developed and validated six KASP markers (snpAH00577, snpAH00580, snpAH00582, snpAH00585, snpAH00586 and snpAh00588) located on chromosomes Ah05, Ah08, Ah13, Ah15 and Ah20 (Table 2). These markers target key candidate genes, enabling the efficient selection of dormant plants. The validation panel for FSD includes 37 genotypes representing 16 dormant, 5 moderate dormant and 16 non-dormant genotypes. Among these markers, snpAH00577, snpAH00580 snpAH00586 and snpAH00588 should be prioritized, as their combinations lead to desired seed dormancy duration. Specifically, snpAH00577 is linked to an *ATP-binding ABC transporter (Arahy.8B3-CAL)* essential for GA signalling and snpAH00580 is near a *calcium-transporting ATPase (Arahy.NRZN5K)* that maintains calcium homeostasis, which is vital for regulating stress responses (Yadav, 2021). The marker snpAH00586 is located close to a *choline kinase (Arahy.JJ8FJQ)* gene, which is crucial for synthesizing phosphatidylcholine (PC), a primary component of new cell membranes required for early growth (Lin et al., 2015). Lastly, snpAH00588 is linked to an *uncharacterized protein (Arhay.PI0JM6)*, whose proximity to a prioritized marker suggests it may also have a relevant function. Therefore, the present findings indicate that the combination of these four KASP markers can reliably identify dormant lines with a dormancy period of 24–30 days (Fig. 5), outperforming earlier methods reported by Kumar et al. (2020) and Bomireddy et al. (2024), which were limited to detecting dormancy of up to 14 days. Furthermore, accessions carrying combinations of dormant and non-dormant alleles for these markers can be strategically used to achieve the desired dormancy duration, also enable precise, high-throughput identification of groundnut genotypes harboring favourable alleles for controlled dormancy. Their application in marker-assisted selection accelerates the development of cultivars with enhanced FSD, thereby mitigating PHS and ensuring seed quality and yield stability in breeding pipelines.

5. Conclusion

This study utilized WGRS data from a diverse mini-core collection, combined with phenotypic data collected over four seasons, to perform a GWAS analysis. We identified 27 MTAs in individual seasons and 12 MTAs pooled seasons for FSD. The analysis of these MTAs revealed potential candidate genes associated with FSD, highlighting the critical role of the ABA-GA balance in the indirect regulation of seed dormancy. The genes, *Cytochrome P450 superfamily proteins, protein kinase superfamily proteins, WRKY family transcription factors,* and *MYB transcription factors* involved in the ABA pathway, as well as *F-box interaction domain proteins, ATP-binding ABC transporters,* and *GATA-type zinc finger transcription factors* were identified as key contributors to FSD regulation and have been associated with the GA pathway. The genes; *Ahfsd1, Ahfsd2, Ahfsd3,* and *Ahfsd4* were identified to carry the favourable allelic combination $A_1T_1G_3Y$, and are linked with the KASP markers snpAH00577, snpAH00580, snpAH00586, and snpAH00588. This combination consistently conferred a desirable seed dormancy period of 24–30 days, while the $G_4C_1A_3C_2$ combination significantly reduced dormancy to 1–6 days, indicating its unfavourable impact. This study provides a foundation for the genetic improvement of groundnut seed dormancy, enabling the development of resilient cultivars to mitigate pre-harvest sprouting losses.

CRediT authorship contribution statement

D. Khaja Mohinuddin: Writing – original draft, Validation, Formal analysis. **Sunil S. Gangurde:** Writing – review & editing, Formal analysis. **Hasan Khan:** Writing – review & editing. **Deekshitha Bomireddy:** Methodology. **Vinay Sharma:** Writing – review & editing. **Priya Shah:** Writing – review & editing, Formal analysis. **U. Nikhil Sagar:** Writing –

review & editing. **Namita Dube:** Data curation. **Ramachandran Senthil:** Resources. **B.V. Tembhurne:** Writing – review & editing. **V. Hanumanth Nayak:** Writing – review & editing. **Ovais Hamid Peerzada:** Resources. **A. Amaregouda:** Writing – review & editing. **Kisan Babu:** Writing – review & editing. **Kuldeep Singh:** Writing – review & editing, Resources. **Pasupuleti Janila:** Resources. **Baozhu Guo:** Writing – review & editing. **Boshou Liao:** Writing – review & editing. **Rajeev K. Varshney:** Writing – review & editing. **Manish K. Pandey:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Informed consent statement

Not Applicable.

Data availability statement

The phenotypic data used in this work provided Supplementary Table S1. The sequencing data generated in this study is deposited in NCBI with bio-project ID PRJNA1002116, PRJNA490835 and PRJNA490832.

Institutional review Board statement

Not Applicable.

Funding

The authors are thankful to the Indian Council of Agricultural Research (ICAR) through ICAR-ICRISAT collaborative project, MARS Inc. USA, and Bill & Melinda Gates Foundation (BMGF), USA through Tropical Legumes III project.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Manish K Pandey reports financial support was provided by International Crops Research Institute for the Semi-Arid Tropics. Manish K Pandey reports a relationship with International Crops Research Institute for the Semi-Arid Tropics that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are thankful to GeneBank, ICRISAT for their support in providing seed material and assistance in phenotyping work. D. Khaja Mohinuddin is grateful to ICRISAT for providing the facilities to conduct the work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2025.110539.

Data availability

All the relevant datasets are made available. The phenotypic data is supplied in Supplementary Table S1, and sequencing data generated in this study is deposited in NCBI with bio-project ID PRJNA1002116, PRJNA490835, and PRJNA490832.

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