*Article*

Identification of Donors for Fresh Seed Dormancy and Marker Validation in a Diverse Groundnut Mini-Core Collection

Deekshitha Bomireddy [1,2], Vinay Sharma [1], Ramachandran Senthil [1], Mangala Reddisekhar [2], Priya Shah [1], Kuldeep Singh [1], Devarapalli Mohan Reddy [3], Palagiri Sudhakar [2], Bommu Veera Bhaskara Reddy [3] and Manish K. Pandey [1,*]

[1] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad 502324, India; deekshitha.bomireddy@icrisat.org (D.B.); vinay.sharma@icrisat.org (V.S.); senthil.ramachandran@icrisat.org (R.S.); priya.shah@icrisat.org (P.S.); kuldeep.singh@icrisat.org (K.S.)
[2] Department of Genetics & Plant Breeding, S. V. Agricultural College, ANGRAU, Tirupati 517502, India; mreddisekhar11@gmail.com (M.R.); sudhakarpalagiri@gmail.com (P.S.)
[3] Institute of Frontier Technology, RARS, ANGRAU, Tirupati 517502, India; dmrgene@gmail.com (D.M.R.); bvbreddy68@gmail.com (B.V.B.R.)
* Correspondence: manish.pandey@icrisat.org; Tel.: +91-40-3071-3305

Abstract: Domestication and extensive selection in the development of modern, high-yielding commercial groundnut cultivars have resulted in the selection of an undesirable trait known as in situ germination, which is also referred to as the pre-harvest sprouting of seeds. This is particularly prevalent in regions where humid weather coincides with the harvest season. Delayed harvesting and pre-sprouting can cause production losses and increase the chances of aflatoxin contamination, thereby impeding the quality and kernel yield. Breeding early maturing groundnut cultivars with 2–3 weeks of fresh seed dormancy, particularly in Spanish-type cultivars, enhances the sustainability of agriculture. In this context, we conducted a comprehensive evaluation of a groundnut mini-core collection, a major resource for genetic diversity, for fresh seed dormancy using an in vitro germination assay for two seasons, viz., rainy 2022 and post-rainy 2022–2023 at ICRISAT (Hyderabad). To enhance the effectiveness and accuracy of traditional breeding methods via the use of markers for marker-assisted selection, we performed molecular screening of the mini-core accessions using two allele-specific markers. The *GMFSD1* marker was successfully validated by effectively differentiating dormant and non-dormant genotypes. By employing phenotypic and marker data, we identified a set of accessions, viz., ICG 5827 (Virginia Runner), ICG 11457 (Virginia Runner), ICG 7000 (Virginia Bunch), and ICG 11322 (Virginia Bunch) of sub spp. *hypogaea* var. *hypogaea* and ICG 9809 (Spanish Bunch) of sub spp. *fastigiata* var. *vulgaris* that exhibited a fresh seed dormancy period ranging from 2 to 3 weeks. These identified accessions hold potential as donors in breeding programs that are designed to address the groundnut production needs in various cropping systems across different countries. The validated marker, particularly *GMFSD1*, demonstrated considerable potential for facilitating faster breeding of groundnut cultivars with the desired dormancy using marker-assisted selection. This research provides a foundation to expediting groundnut breeding programs and offers opportunities to mitigate pre-harvest sprouting, ultimately improving seed quality and productivity in groundnut-producing regions.

Keywords: fresh seed dormancy; in vitro germination; mini-core; pre-harvest sprouting; validation

1. Introduction

Groundnuts (*Arachis hypogaea* L.) are prominent edible oilseed crops belonging to the Fabaceae or Leguminosae family, and they are cultivated in over 100 countries worldwide. Groundnuts gained popularity due to their versatile and multifaceted use in human nutrition, serving purposes such in the manufacture of cooking oil and serving as confectionery

products, dietary food, and livestock fodder. Based on the branching pattern, flower arrangement, kernel, and pod attributes, cultivated groundnuts have been categorized into three major market types, i.e., Virginia (subsp. *hypogaea* var. *hypogaea*), Spanish (subsp. *fastigiata* var. *vulgaris*), and Valencia (subsp. *fastigiata* var. *fastigiata*) [1–3]. Generally, Virginia genotypes are late-maturing with seeds exhibiting varying durations of dormancy, whereas the Spanish and Valencia types exhibit shorter maturity durations and lack seed dormancy [4]. Spanish varieties, which are extensively cultivated in the semi-arid regions of Asia and Africa, contribute to 60% of the world's groundnut production [5]. However, untimely rains before harvest can lead to in situ germination (pre-harvest sprouting) of seeds, resulting in a 10–20% yield reduction and potentially reaching 50% in Spanish types. Such losses adversely affect kernel quality, market prices, and increase the susceptibility to pathogen infection and aflatoxin contamination [5,6]. On the other hand, prolonged seed dormancy can delay germination when freshly harvested seeds are used for sowing, affecting the cropping season duration and breeding generations per year [7,8]. It has been shown that growth inhibitors such as maleic hydrazide can be applied to the leaves of Spanish-types to increase their dormant [9], but this is not a cost-effective method, especially for rain-fed farming. A long-term solution to this problem relies on equipping the early maturing popular groundnut varieties with 2–3 weeks of fresh seed dormancy to allow tolerance to the detrimental effects of rain between maturity and harvest [5,8]. Farmers greatly benefit from these varieties as they enable delayed crop harvesting in the scenario of unexpected rainfall, preventing pre-harvest sprouting (PHS) losses.

Though seed dormancy or germinability are genetically inherited traits, they are also highly influenced by environmental effects (light, moisture, humidity, temperature) present during seed development and maturity [10]. Endogenous hormones such as abscisic acid (ABA) positively regulate dormancy, whereas ethylene and gibberellins (GA) tend to break seed dormancy. In various segregating populations (F_2), the observed inheritance ratio for dormant to non-dormant lines was documented as 3:1, signifying the dominance of the dormancy allele with monogenic or qualitative inheritance [3,11]. In contrast, data from various F_2 populations showed a segregation ratio of 15:1 (non-dormant/dormant), indicating that two duplicate recessive genes quantitatively regulate the trait [4,5]. These discrepant and conflicting findings make dormancy a complex agronomic trait involving monogenic, digenic, multigenic, and epistatic effects, and addressing this trait thus requires a clear understanding [12]. The determination of seed dormancy levels in germplasm accessions, cultivars, parents, and progenies would be useful in developing a clear understanding of the trait and for breeding programs. A groundnut mini-core set was developed from the ICRISAT germplasm collection [13] with a relatively high level of genetic diversity encompassing *hypogaea*, *hirsuta*, *fastigiata*, *peruviana*, *vulgaris*, and *aequatoriana* botanical varieties. A few mini-core accessions have been frequently requested by groundnut breeders for use in breeding programs. Therefore, the evaluation of a mini-core collection for fresh seed dormancy will help in identifying useful parents for crop improvement programs with the enhanced use of genetic resources for improving quantitative traits.

Conventional breeding relies on the visual screening of large segregating populations and breeding lines for fresh seed dormancy during harvesting. Performing phenotypic selection is often challenging under field conditions, as every selection/breeding cycle might not receive rain during the maturity/harvesting stages. In practice, breeding materials are selected and forwarded based on in vitro germination tests carried out under controlled conditions [14]. Based on the germination test, the chances of rejecting a large number of lines at advanced stages are higher, leading to wasted resources and time. Moreover, as this trait is highly influenced by climatic factors, screening and selection of cultivars based on phenotype is challenging. The availability of genomic resources and advanced breeding techniques, such as marker-assisted selection (MAS) [15], rapid generation advancements [16], and genomic selection [17], has accelerated groundnut breeding programs. Additionally, the availability of reference genome assemblies for diploid progenitors and tetraploid cultivated groundnuts [18–20] combined with advancements in next-generation

sequencing, such as genotyping-by-sequencing [21], and the utilization of high-density 'Axiom_*Arachis*' 58 K single-nucleotide polymorphism (SNP) arrays [22] have significantly reduced the overall cost of sequencing and made candidate gene discovery and marker development more precise and reliable. A QTL-seq approach was utilized in the RIL population developed from the cross ICGV 00350 (non-dormant) × ICGV 97045 (dormant) and identified RING-H2 finger protein and zeaxanthin epoxidase as the candidate genes responsible for inducing fresh seed dormancy [23]. Furthermore, allele-specific markers were also developed from the identified candidate genomic regions. Deploying these molecular markers associated with fresh seed dormancy in marker-assisted early generation selection (MEGS) could accelerate the development of improved varieties resistant to pre-harvest sprouting.

In this study, we aimed to evaluate a groundnut mini-core collection to identify accessions with 2–3 weeks of fresh seed dormancy and validate two allele-specific markers, developed and reported by Kumar et al. [23]. This research contributes to the quantitative improvement of this important trait, enhancing the utilization of genetic resources in crop improvement programs.

2. Materials and Methods

2.1. Plant Materials

The experimental material consisted of 184 accessions of a groundnut mini-core collection developed at ICRISAT, Hyderabad [13]. The mini-core collection included six botanical varieties, viz., *hypogaea*, *fastigiata*, *hirsuta*, *peruviana*, *vulgaris*, and *aequatoriana* with varying maturity durations. Seeds from all 184 accessions were obtained from the Genebank of ICRISAT, and detailed information was provided in Table S1 and at http://genebank.icrisat.org/IND/Minicore?Crop=Groundnut (accessed on 21 September 2023). The experiment was conducted during rainy 2022 and post-rainy 2022–2023 seasons (in the same experimental field) at ICRISAT, Hyderabad (17°31′48.00″ N latitude, 78°16′12.00″ E longitude, and 545 m altitude) with 184 accessions laid out in a randomized block design (RBD) with two replications. Each accession was planted in a 4 m row plot with a plant-to-plant spacing of 10 cm and a row-to-row spacing of 60 cm. The experiment was conducted in red soils with pH values ranging from 6.0 to 6.3. The weather conditions were recorded for both years; in the rainy 2022 season, the maximum temperature was 31.26 °C, the minimum temperature was 20.38 °C, and there was 198.2 mm of rainfall with 154.24 mm evaporation and 87.60% relative humidity. In the post-rainy 2022–2023 season, the maximum temperature was 33.25 °C, the minimum temperature was 18.61 °C, and there was 65.5 mm of rainfall with 98.75 mm evaporation and 82.70% relative humidity. Standard agronomical practices for groundnut cultivation were followed during both the growing seasons.

2.2. Phenotyping Protocol and Parameters Estimated for Fresh Seed Dormancy

Based on the maturity duration, accessions of the mini-core collection were categorized as early, mid-, and late-maturing types and harvested in three sets (95–105; 110–120; and 120–135 days after sowing). The maturity of the pods was determined according to the development of black pigmentation inside the shell [24]. Freshly harvested mature seeds were used for phenotyping via an in vitro germination assay [3]. Twenty good-quality and uniformly sized seeds from the two replicates of each accession were selected, treated with fungicides (Mancozeb and Carbendazim), and placed on moist germination paper within Petri plates. The Petri plates were kept in complete darkness and watered regularly at 24 h intervals to maintain moisture conditions. The number of germinated seeds was recorded daily for a period of 30 days (Figure 1a).

Following the method of Kumar et al. [25], fresh seed dormancy was characterized by its intensity and duration. The number of days needed for an accession to achieve 50% germination was used to calculate the duration of dormancy. At seven days following sowing, the percentage of non-germinated seeds was used to measure the intensity of fresh

seed dormancy. The percentage of germinated seeds for each accession was calculated using the following formula:

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds plated}} \qquad (1)$$
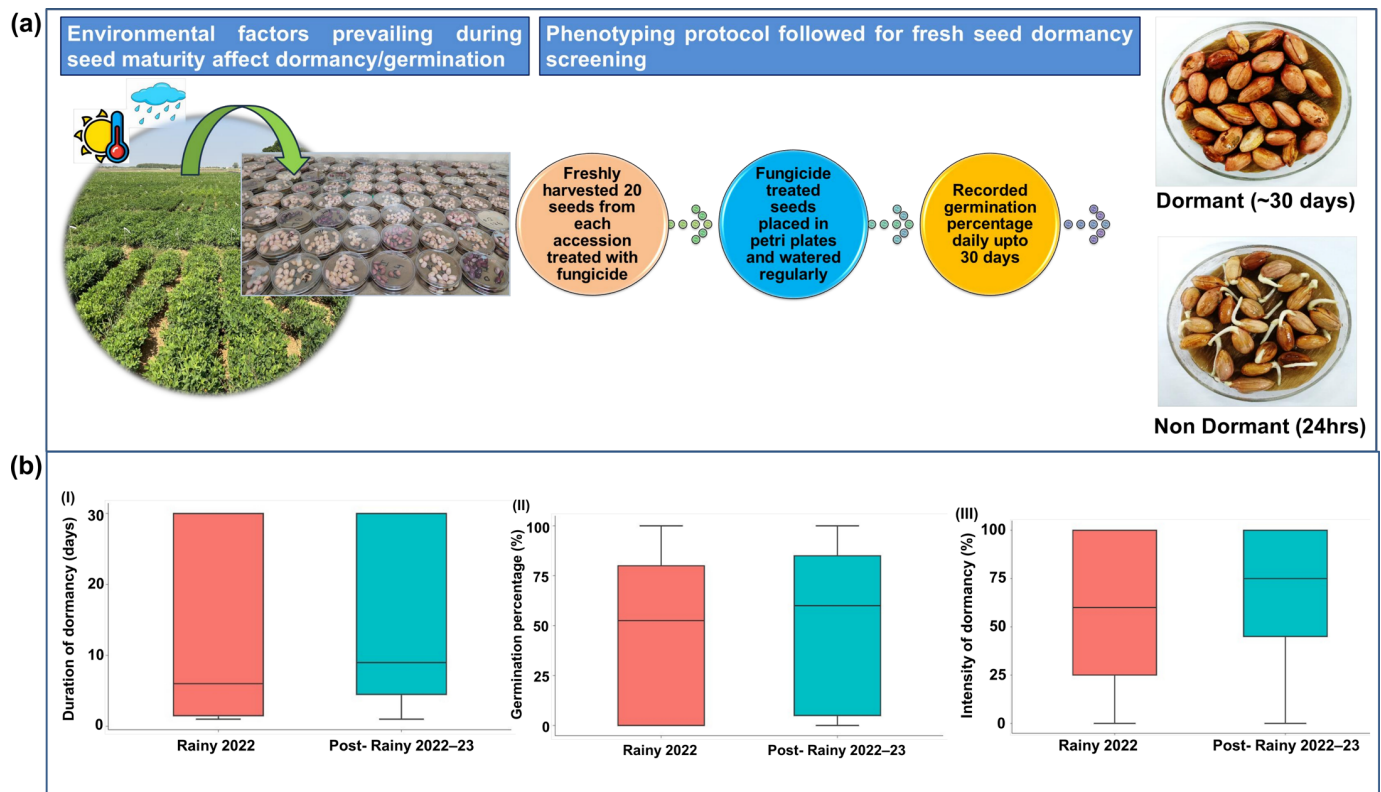

Figure 1. The phenotyping protocol and phenotypic variation for fresh seed dormancy. (**a**) The phenotyping protocol followed for the screening of the groundnut mini-core accessions for fresh seed dormancy. (**b**) The phenotypic variation in the mini-core accessions for seed dormancy traits: Boxplots for (**I**) the duration of dormancy (days); (**II**) the germination percentage (%); and (**III**) the intensity of dormancy across the seasons (Rainy 2022 and Post-Rainy 2022–2023). Box plots represent the interquartile range; the thick line in the middle of each box represents the median; and the whiskers represent 1.5 times the interquartile range.

2.3. Statistical Analysis

The replicated data collected on germination percentage, days to 50% germination (dormancy duration), and intensity of dormancy from both seasons were analyzed and subjected to statistical analysis following a randomized block design (RBD) as per the method described by Wang et al. [26]. An analysis of variance was performed using the statistical package 'variability' (v.0.1.0) of R programming.

2.4. Validation of Allele-Specific Markers Associated with Fresh Seed Dormancy

Tender leaf samples from 25-day-old plants of the groundnut mini-core accessions (comprising the *hypogaea*, *fastigiata*, *hirsuta*, *peruviana*, *vulgaris*, and *aequatoriana* botanical varieties) were collected for DNA isolation. The DNA was extracted using the Nucleospin Plant II kit (Macherey-Nagel, Duren, Germany). DNA quality and quantity were assessed using 0.8% agarose gel and a Nanodrop 8000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), respectively. Two allele-specific markers developed from the A09 and B05 chromosomes, previously reported in a QTL-seq study on the RIL population (ICGV 00350 × ICGV 97045) [23], were used to validate the marker efficacy for fresh

seed dormancy in the mini-core accessions. PCR amplification was carried out using the procedure described in Kumar et al. [23]. The 15 μL PCR mixture included 1X PCR buffer, 5 ng of DNA template, 2.5 mM of each dNTP, 3 pmoles each of forward and reverse primers, and 0.12 μL of Taq polymerase. The PCR amplification cycling conditions were as follows: initial denaturation at 94 °C for 4 min, followed by 5 cycles of denaturation at 94 °C for 20 s, annealing at 62 °C for 30 s, and extension at 72 °C for 30 s. This was followed by 30–35 cycles of denaturation at 94 °C for 20 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C. After PCR amplification, alleles were scored based on the presence or absence of bands on 2% agarose gel.

3. Results and Discussion

3.1. Analysis of Variance

The analysis of variance revealed highly significant differences among the accessions for days to 50% germination (dormancy duration), germination percentage, and intensity of dormancy (Table 1). However, no significant difference between the two replications was observed. A similar study that evaluated a U.S. groundnut mini-core collection for seed dormancy also identified significant variability among the accessions [27]. Although a plant's genetic makeup primarily determines the depth of its dormancy, environmental factors also play a role during seed development and maturation [10]. The high F values and very low p-values (*** $< 2 \times 10^{-16}$) of the "Genotype" factor showed that genetic variation among different accessions significantly contributed to the observed differences in the traits. The multi-location evaluation of the Spanish-type groundnut genotypes for dormancy duration and intensity under field conditions revealed highly significant variations in the G × E interaction [28]. In support of this, the present study also revealed the existence of highly significant genotype × year interaction differences for all the measured traits except for days to 50% germination. The existence of an interaction effect suggests that the germination percentage varies from year to year, which could be due to environmental factors such as temperature, rainfall, humidity, solar radiation, and other non-genetic variables. This explains the need for evaluating accessions of different botanical groups in multiple locations to conclude the impact of environmental conditions in inducing dormancy.

Table 1. ANOVA of the mini-core accessions phenotyped for fresh seed dormancy during the rainy 2022 and post-rainy 2022–2023 seasons at ICRISAT.

Traits	Source	Df	Sum Sq	Mean Sq	F Value	Pr(>F)	Significance
	Genotype	183	107,874	589.5	18.299	$<2 \times 10^{-16}$	***
Days to 50% germination	Replication	1	12	12.1	0.376	0.5398	
	Season	1	118	117.7	3.652	0.0565	
	Residuals	535	17,234	32.2			
	Genotype	183	946,243	5171	13.382	$<2 \times 10^{-16}$	***
Germination Percentage	Replication	1	41	41	0.105	0.746	
	Season	1	4735	4735	12.255	0.000503	***
	Residuals	535	206,716	206,716			
	Genotype	183	946,243	5171	13.382	$<2 \times 10^{-16}$	***
Intensity of dormancy	Replication	1	41	41	0.105	0.746	
	Season	1	4735	4735	12.255	0.000503	***
	Residuals	535	206,716	206,716			

Df: degrees of freedom; Sum Sq: sum of Squares; Mean Sq: mean sum of squares. Significant codes: 0 '***'.

3.2. Germination Percentage, Duration, and Intensity of Dormancy

At the end of the experiment, i.e., after 30 days, no germination was observed in 28 accessions, followed by 7 accessions with 5–10 percent germination; all of these belonged to the *hypogaea* subspecies, except for ICG 6654. The highest germination percentage (95–100) was observed in 11 accessions of the *fastigiata* subspecies during both seasons. The average germination percentage of the mini-core accessions ranged from 0 to 100%

during both the rainy 2022 and post-rainy 2022–2023 seasons. This large variation in the germination percentage could be due to genotypic differences among the accessions and the environmental factors present during seed maturation. Similar findings were reported earlier on groundnut seed germination [25,29–32].

The mini-core accessions showed different durations of dormancy ranging from 1 to >30 days. Sixty-three accessions from sub spp. *hypogaea* var. *hypogaea* and ICG 118 and ICG 6654 from sub spp. *fastigiata* var. *vulgaris* displayed dormancy durations of more than 30 days during the rainy 2022 and post-rainy 2022–2023 seasons. ICG 9809 of sub spp. *fastigiata* var. *vulgaris*, ICG 11457, and ICG 14705 of sub spp. *hypogaea* var. *hypogaea* had 2–3 weeks of dormancy during both seasons. During the post-rainy 2022–2023 season, ICG 9809, ICG 7000, and ICG 11322 of sub spp. *hypogaea* var. *hypogaea* had 2–3 weeks of dormancy, whereas these accessions exhibited more than 30 days of dormancy during the rainy 2022 season. Similarly, ICG 12879 and ICG 12921 of sub spp. *fastigiata* var. *vulgaris* and ICG 11426 of sub spp. *hypogaea* var. *hypogaea*, which were observed to be non-dormant during the rainy 2022 season, had 2–3 weeks of dormancy in the post-rainy 2022–2023 season. Ninety-three accessions of the mini-core set showed no signs of dormancy and germinated within 1–14 days during both seasons, with most of them belonging to the *fastigiata* subspecies, except ICG 188, ICG 6402, ICG 6703, ICG 7963, ICG 3982, and ICG 14710 from the *hypogaea* subspecies.

According to Kumar et al. [25], the intensity of dormancy is defined as the percentage of seeds that are not germinated even after seven days of harvest. The intensity of dormancy ranged from 0% to 100% during the rainy 2022 and post-rainy 2022–2023 seasons. The results showed that around 37 accessions belonging to the *hypogaea* and *fastigiata* sub-species had 100% intensity of dormancy followed by 3 accessions with 90–95% during both seasons. From a practical standpoint, the genotypes with a higher intensity (>90%) of dormancy coupled with a 2–3-week duration are more effective in regions with erratic rainfall patterns during crop maturity [4]. Moreover, a prolonged seed dormancy of more than 3 weeks is undesirable in India, where the crop is cultivated during the rainy/post-rainy seasons as well. The results showed that ICG 5827, ICG 7000, and ICG 11322 had an average of 90–100% intensity of dormancy, with around 3 weeks of dormancy during the post-rainy 2022–2023 season but more than 30 days of dormancy during the rainy 2022 season. However, ICG 9809, ICG 11426, ICG 11457, ICG 12879, ICG 12921, and ICG 14705, which were noticed to have a dormancy duration of 2–3 weeks, had an intensity of dormancy in the range of 62.5–90%. Genetic variation among the accessions of the mini-core and environmental factors could be the reasons for this significant variation in the intensity of dormancy. These findings are consistent with those of Kumar et al. [25] and Kumar et al. [30], who also noted significant genetic variations in the intensity of dormancy. The box plots for the three traits, i.e., duration of dormancy, germination percentage, and intensity of dormancy, in the two rainy 2022 and post-rainy 2022–2023 seasons, are presented in Figure 1b. The season-wise mean values of all these three traits are presented in Table S2. On average, the accessions of Virginia Runner and Virginia Bunch (sub spp. *hypogaea* var. *hypogaea*) were observed to have higher intensity and duration of dormancy than the accessions from Valencia (sub spp. *fastigiata* var. *fastigiata*) and Spanish Bunch (sub spp. *fastigiata* var. *vulgaris*) (Figure 2). Among the various botanical varieties studied, the highest intensity of dormancy was observed among the accessions of sub spp. *hypogaea* var. *hypogaea* followed by sub spp. *hypogaea* var. *hirsuta.* The lowest intensity of dormancy was observed among the accessions of the sub spp. *fastigiata* var. *fastigiata*, followed by sub spp. *fastigiata* var. *peruviana*, sub spp. *fastigiata* var. *aequatoriana*, and sub spp. *fastigiata* var. *vulgaris.* Similar results were reported by Kumar et al. [27] when phenotyping U.S. mini-core accessions of groundnuts for fresh seed dormancy. The average germination percentage and intensity of dormancy of the mini-core accessions from various botanical varieties are presented in Figure 3a.
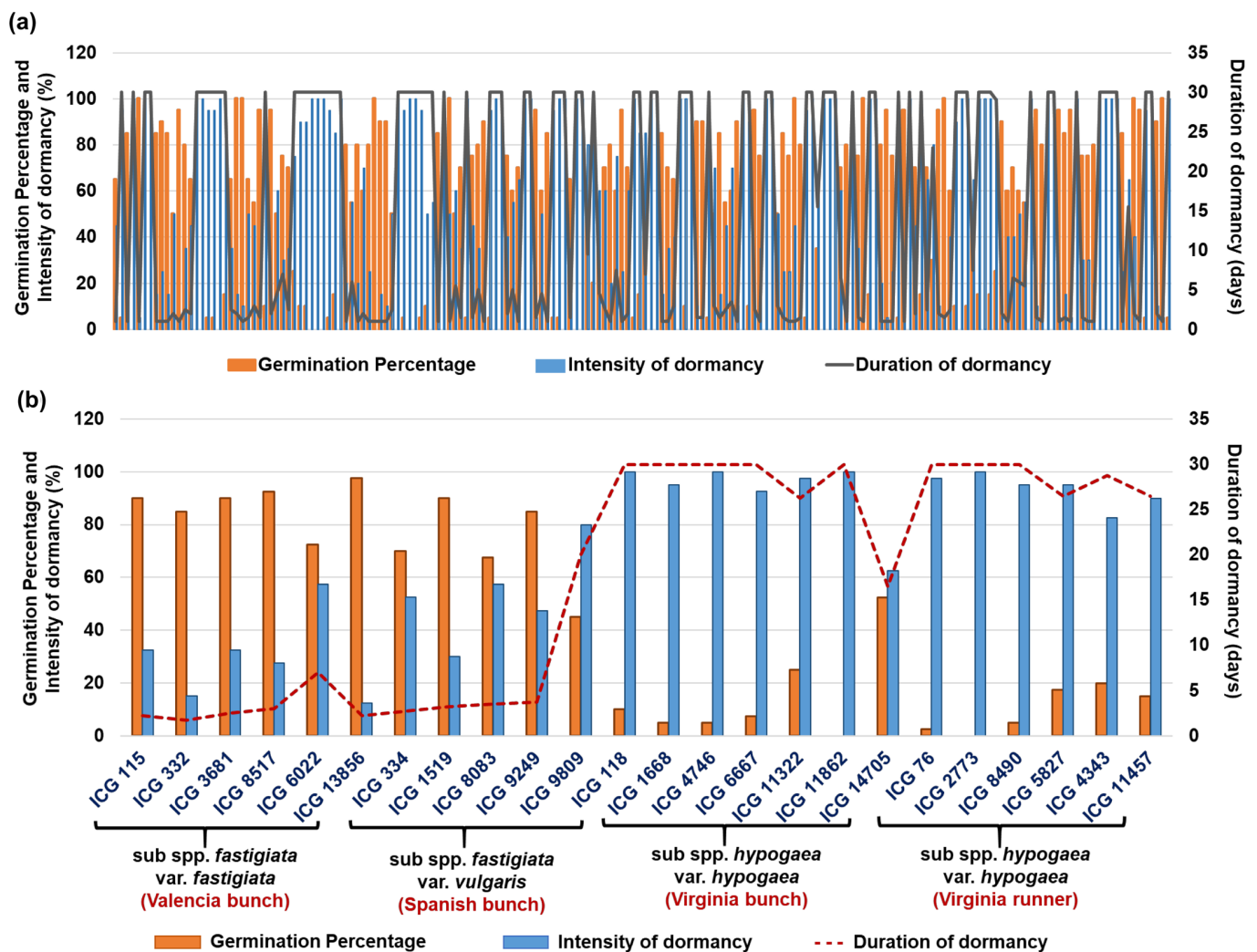
Figure 2. Average germination percentage, intensity, and duration of dormancy of (**a**) groundnut mini-core accessions from the rainy 2022 and post-rainy 2022–2023 seasons; (**b**) the set of mini-core accessions from various botanical varieties differing in germination percentage, intensity, and duration of dormancy.

3.3. The Validation of Mini-Core Accessions with Allele-Specific Markers Associated with Fresh Seed Dormancy

As the mini-core collection acts as the representative for most of the genetic diversity available in the germplasm, multi-season/location evaluation of the mini-core accessions will help in identifying stable accessions across the environments. However, the high influence of climatic cues and the selection of cultivars based on visual screening make fresh seed dormancy a difficult trait. Therefore, the use of molecular markers associated with fresh seed dormancy in marker-assisted selection can facilitate the development of cultivars with pre-harvest sprouting resistance. From this perspective, an evaluation of the mini-core collection via marker-assisted screening in addition to phenotypic screening were also performed in this study to identify accessions with 2–3 weeks of dormancy. Two allele-specific markers reported from an earlier QTL-Seq analysis [23] were employed to validate the accessions of the mini-core set for fresh seed dormancy. These markers, A09_115175289 and B05_111598196, were developed from the chromosomes A09 and B05, respectively (Table 2). The A09_115175289 marker failed to distinguish between the dormant and non-dormant accessions used in the validation panel, whereas the B05_111598196 marker, designated as *GMFSD1*, exhibited remarkable efficiency in distinguishing between non-dormant (Spanish bunch- subsp. *fastigiata* var. *vulgaris*; Valencia bunch- subsp. *fastigiata*

var. *fastigiata*) and dormant Virginia runner and Virginia bunch (subsp. *hypogaea* var. *hypogaea*). However, there were a few exceptions (16 accessions) wherein the marker data were not in accordance with the phenotyping data. The details of the dormancy status of the 184 accessions of mini-core based on both the phenotyping and *GMFSD1* marker data along with their deviations and SNP alleles are mentioned in Table S3. The representation of the *GMFSD1* marker differentiating a few dormant and non-dormant accessions of mini-core is demonstrated in Figure 3b, while others are provided in Figure S1. In confirmation with the marker data, ICG 5827 (Virginia Runner), ICG 7000 (Virginia Bunch), ICG 11457 (Virginia Runner), ICG 11322 (Virginia Bunch) of sub spp. *hypogaea* var. *hypogaea*, and ICG 9809 (Spanish Bunch) of sub spp. *fastigiata* var. *vulgaris* were identified as dormant accessions with 2–3 weeks of dormancy; therefore, they are desirable for use as donors in the breeding programs (Table 3). They can also be used to develop lines with the desired level of dormancy, high yields, and acceptable consumer quality by designing appropriate breeding strategies for the successful development and release of varieties.
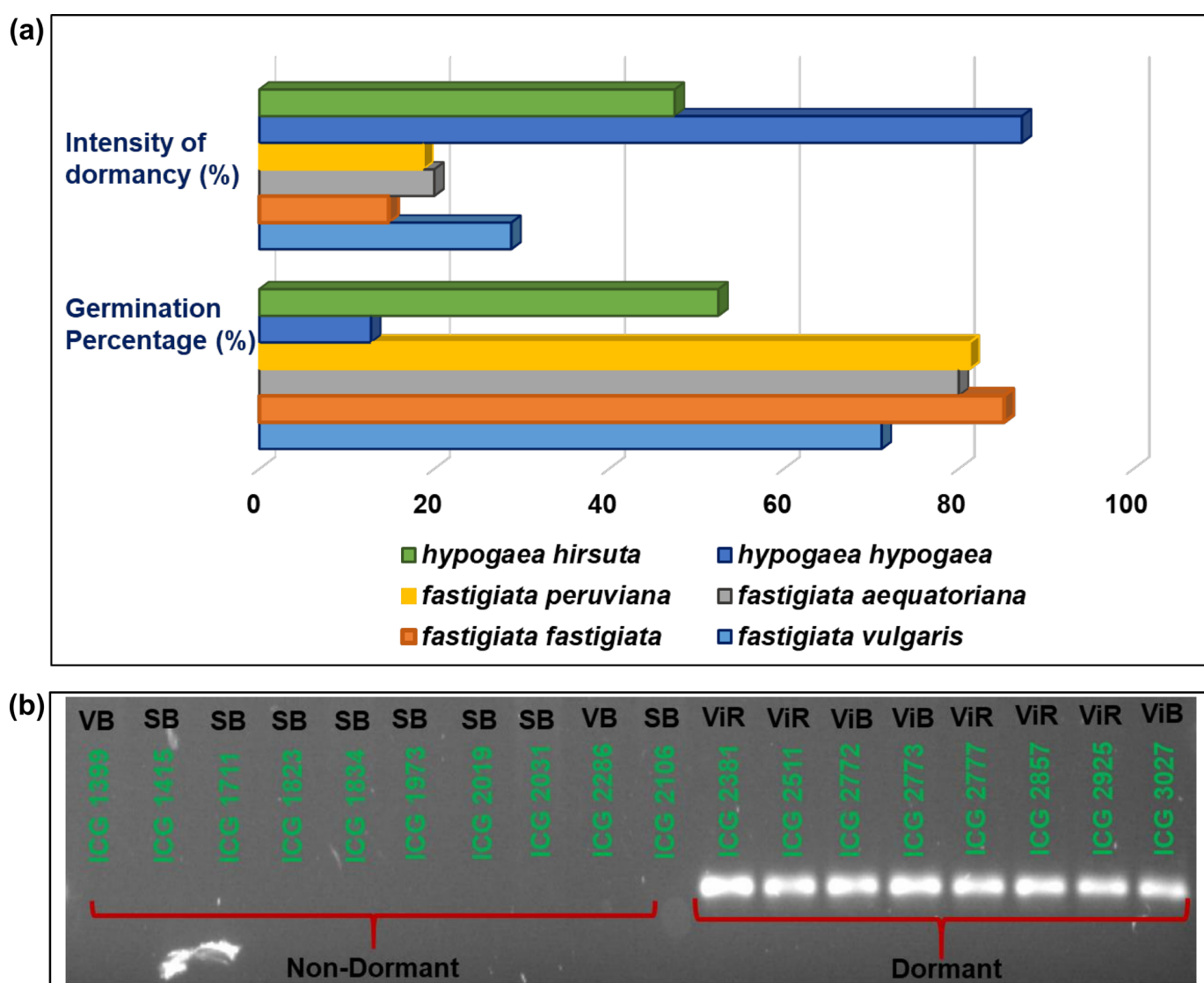

Figure 3. Comparison and validation of the groundnut mini-core accessions. (**a**) Average germination percentage and intensity of dormancy of groundnut mini-core accessions from various botanical varieties. (**b**) *GMFSD1* marker-differentiated non-dormant Valencia Bunch (VB) and Spanish Bunch (SB) from dormant Virginia Runner (VR) and Virginia Bunch (VB) of the groundnut mini-core accessions.

Table 2. Details of the allele-specific markers used for validating diverse germplasm.

Gene ID	SNP_Position	Function	F/R	Allele-Specific Primer Sequence	Length (bp)
Aradu.D94AQ.1	A09_115175289	Zeaxanthin epoxidase	F	CACATCTTCTAGTGAAAGCGGA	22
			R	CCATCTTTCTGATGGAACAACC	22
Intergenic	B05_111598196		F	TTTTCCTTAATATTTGAAAAATTATCCTAA	30
			R	CGTCTTTGCCAATTGTGTATAAC	23

Table 3. Details of the mini-core accessions with 2–3 weeks of fresh seed dormancy.

S. No.	Accessions	Agronomic Type	Rainy 2022			Post Rainy 2022–2023		
			DG	GP	DI	DG	GP	DI
1	ICG 5827	Virginia Runner	30	0	100	23	35	90
2	ICG 7000	Virginia Bunch	30	10	100	22	35	100
3	ICG 9809	Spanish Bunch	16	35	65	25	55	95
4	ICG 11322	Virginia Bunch	30	15	95	23	35	100
5	ICG 11457	Virginia Runner	23	30	80	30	0	100

DG—duration of dormancy; GP—germination percentage; DI—intensity of dormancy.

This marker can be validated on a much larger panel of breeding material to confirm its efficacy for use in marker-assisted selection (MAS) for cultivar development. Deploying this marker in MAS helps in identifying appropriate parental lines to be crossed and improves precision and selection efficiency through the reduction of labor-intensive and error-prone screening procedures. Moreover, the screening of these accessions with more gene-based dormancy markers will give an idea of the gene combinations required to maintain a variable degree of dormancy and will eventually assist in the molecular breeding of groundnut cultivars with the desired level of dormancy. Furthermore, the utilization of MAS with a speed breeding approach expedites the breeding procedure for developing new Spanish lines with 2–3 weeks of fresh seed dormancy. Nevertheless, proficiency in the field of technology, coupled with adequately equipped and complementing phenomics facilities, appropriate infrastructure, and continuous financial support for research and development are the essential factors for the implementation of speed-breeding techniques.

4. Conclusions

A short period of seed dormancy (2–3 weeks), specifically in the Spanish bunch type, is crucial for the mitigation of in situ germination losses resulting from unexpected rainfall during harvest or delayed harvesting. This study evaluated the ICRISAT mini-core collection of groundnuts over two seasons to investigate the genetic variability among the accessions for fresh seed dormancy. Notably, significant genetic variability was observed among the mini-core accessions for days to 50% germination, germination percentage, and intensity of dormancy. Utilizing two allele-specific markers used for molecular screening of the germplasm, the *GMFSD1* marker successfully validated dormant and non-dormant genotypes. The identified accessions, with 2–3 weeks of dormancy, hold promise for breeding commercial groundnut cultivars resistant to pre-harvest sprouting, thereby minimizing losses attributed to untimely rainfall. Although dormancy is a genetically controlled trait, it is also influenced by external factors such as rainfall, humidity, temperature, light, and other environmental factors present during crop maturity. Consequently, a multi-location evaluation of a mini-core collection for fresh seed dormancy helped in identifying useful donors for crop improvement programs with enhanced utilization of genetic resources for quantitative trait improvement. The present study not only highlighted the significance of seed dormancy but also emphasized the potential of the identified accessions for focused

breeding programs that aim to improve resistance to pre-harvest sprouting in commercially cultivated varieties.

References

1. Krapovickas, A. The origin, variability and spread of the groundnut (Arachis hypogaea). In *The Domestication and Exploitation of Plant and Animals*; Ucko, R.J., Dimbledy, W.C., Eds.; Greald Duckworth Co., Ltd.: London, UK, 1969; pp. 427–441.
2. Krapovickas, A. Evolution of the genus Arachis. In *Agricultural Genetics-Selected Topics*; Moav, R., Ed.; Wiley: New York, NY, USA, 1973; pp. 135–151.
3. Upadhyaya, H.D.; Nigam, S.N. Inheritance of fresh seed dormancy in peanut. *Crop Sci.* **1999**, *39*, 98–101. [CrossRef]
4. Naganagoudar, Y.B.; Kenchanagoudar, P.V.; Rathod, S.; Keerthi, C.M.; Nadaf, H.L.; Channappagoudar, B.B. Inheritance of fresh seed dormancy in recombinant inbred lines (RIL) developed for mapping population TAG 24 x GPBD 4 in groundnut (*Arachis hypogaea* L.). *Legum. Res.* **2016**, *39*, 844–846. [CrossRef]
5. Vishwakarma, M.K.; Pandey, M.K.; Shasidhar, Y.; Manohar, S.S.; Nagesh, P.; Janila, P.; Varshney, R.K. 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. *Plant Breed.* **2016**, *135*, 367–375. [CrossRef]
6. Zhang, C.; Selvaraj, J.N.; Yang, Q.; Liu, Y. A survey of aflatoxin-producing *Aspergillus* sp. from peanut field soils in four agroecological zones of China. *Toxins* **2017**, *9*, 40. [CrossRef] [PubMed]
7. Patro, H.K.; Ray, M. Seed dormancy in groundnut—A review. *Int. J. Trop. Agric.* **2016**, *34*, 31–37.
8. Wang, M.L.; Wang, H.; Zhao, C.; Tonnis, B.; Tallury, S.; Wang, X.; Clevenger, J.; Guo, B. Identification of QTLs for seed dormancy in cultivated peanut using a recombinant inbred line mapping population. *Plant Mol. Biol. Rep.* **2021**, *40*, 208–217. [CrossRef]
9. Gupta, R.K.; Singh, S.S.; Verma, M.M. Induction of dormancy in groundnut (*Arachis hypogea* L.) variety T−64 by maleic hydrazide. *J. Agric. Res.* **1985**, *19*, 82–86.
10. Footitt, S.; Douterelo-Soler, I.; Clay, H.; Finch-Savage, W.E. Dormancy cycling in Arabidopsis seeds is controlled by seasonally distinct hormone-signaling pathways. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 20236–20241. [CrossRef]
11. Yaw, A.J.; Richard, A.; Safo-Kantanka, O.; Adu-Dapaah, H.K.; Ohemeng-Dapaah, S.; Agyeman, A. Inheritance of fresh seed dormancy in groundnut. *Afr. J. Biotechnol.* **2008**, *7*, 421–424.
12. Zhang, M.; Zeng, Q.; Liu, H.; Qi, F.; Sun, Z.; Miao, L.; Li, X.; Li, C.; Liu, D.; Guo, J.; et al. Identification of a stable major QTL for fresh-seed germination on chromosome Arahy. 04 in cultivated peanut (*Arachis hypogaea* L.). *Crop J.* **2022**, *10*, 1767–1773. [CrossRef]
13. Upadhyaya, H.D.; Bramel, P.J.; Ortiz, R.; Singh, S. Developing a mini core of peanut for utilization of genetic resources. *Crop Sci.* **2002**, *42*, 2150–2156. [CrossRef]
14. Bomireddy, D.; Gangurde, S.S.; Variath, M.T.; Janila, P.; Manohar, S.S.; Sharma, V.; Parmar, S.; Deshmukh, D.; Reddisekhar, M.; Reddy, D.M.; et al. Discovery of major quantitative trait loci and candidate genes for fresh seed dormancy in groundnut. *Agronomy* **2022**, *12*, 404. [CrossRef]

15. Puppala, N.; Nayak, S.N.; Sanz-Saez, A.; Chen, C.; Devi, M.J.; Nivedita, N.; Bao, Y.; He, G.; Traore, S.M.; Wright, D.A.; et al. Sustaining yield and nutritional quality of peanuts in harsh environments: Physiological and molecular basis of drought and heat stress tolerance. *Front. Genet.* **2023**, *14*, 1121462. [CrossRef] [PubMed]

16. Xu, Y.; Luo, H.; Zhang, H.; Yung, W.S.; Li, M.W.; Lam, H.M.; Huang, C. Feeding the world using speed breeding technology. *Trends Plant Sci.* **2023**, *28*, 372–373. [CrossRef] [PubMed]

17. Hu, J.; Chen, B.; Zhao, J.; Zhang, F.; Xie, T.; Xu, K.; Gao, G.; Yan, G.; Li, H.; Li, L.; et al. Genomic selection and genetic architecture of agronomic traits during modern rapeseed breeding. *Nat. Genet.* **2022**, *54*, 694–704. [CrossRef] [PubMed]

18. Bertioli, D.J.; Jenkins, J.; Clevenger, J.; Dudchenko, O.; Gao, D.; Seijo, G.; Leal-Bertioli, S.C.M.; Ren, L.; Farmer, A.D.; Pandey, M.K.; et al. The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. *Nat. Genet.* **2019**, *51*, 877–884. [CrossRef] [PubMed]

19. Chen, X.; Lu, Q.; Liu, H.; Zhang, J.; Hong, Y.; Lan, H.; Li, H.; Wang, J.; Liu, H.; Li, S.; et al. Sequencing of cultivated peanut, *Arachis hypogaea*, yields insights into genome evolution and oil improvement. *Mol. Plant* **2019**, *12*, 920–934. [CrossRef] [PubMed]

20. Zhuang, W.; Chen, H.; Yang, M.; Wang, J.; Pandey, M.K.; Zhang, C.; Chang, W.-C.; Zhang, L.; Zhang, X.; Tang, R.; et al. The genome of cultivated peanut provides insight into legume karyotypes, polyploid evolution and crop domestication. *Nat. Genet.* **2019**, *51*, 865–876. [CrossRef]

21. Wang, J.; Yan, C.; Shi, D.; Zhao, X.; Yuan, C.; Sun, Q.; Mou, Y.; Chen, H.; Li, Y.; Li, C.; et al. The genetic base for peanut height-related traits revealed by a meta-analysis. *Plants* **2021**, *10*, 1058. [CrossRef]

22. Pandey, M.K.; Agarwal, G.; Kale, S.M.; Clevenger, J.; Nayak, S.N.; Sriswathi, M.; Chitikineni, A.; Chavarro, C.; Chen, X.; Upadhyaya, H.D.; et al. Development and evaluation of a high density genotyping 'Axiom_*Arachis*' array with 58 K SNPs for accelerating genetics and breeding in groundnut. *Sci. Rep.* **2017**, *7*, 40577. [CrossRef]

23. Kumar, R.; Janila, P.; Vishwakarma, M.K.; Khan, A.W.; Manohar, S.S.; Gangurde, S.S.; Variath, M.T.; Shasidhar, Y.; Pandey, M.K.; Varshney, R.K.; et al. Whole-genome resequencing-based QTL-seq identified candidate genes and molecular markers for fresh seed dormancy in groundnut. *Plant Biotechnol. J.* **2020**, *18*, 992–1003. [CrossRef] [PubMed]

24. Miller, O.H.; Burns, E.E. Internal color of Spanish peanut hulls as an index of kernel maturity. *J. Food Sci.* **1971**, *36*, 666–670. [CrossRef]

25. Kumar, A.S.T.; Gowda, M.V.C.; Nadaf, H.L. Seed dormancy in erect bunch genotypes of groundnut (*Arachis hypogaea* L.) I. Variability for intensity and duration. *J. Oilseeds Res.* **1991**, *8*, 166–172.

26. Panse, V.G.; Sukhatme, P.V. *Statistical Methods for Agricultural Workers*; Indian Council of Agricultural Research Publication: New Delhi, India, 1985.

27. Wang, M.L.; Chen, C.Y.; Pinnow, D.L.; Barkley, N.A.; Pittman, R.N.; Lamb, M.; Pederson, G.A. Seed dormancy variability in the US peanut mini-core collection. *Res. J. Seed Sci.* **2012**, *5*, 84–95. [CrossRef]

28. Kumar, N.; Ajay, B.C.; Dagla, M.C.; Rathnakumar, A.L.; Radhakrishnan, T.; Lal, C.; Samdur, M.Y.; Mathur, R.K.; Manivel, P. Multi-environment evaluation of Spanish bunch groundnut genotypes for fresh seed dormancy. *Indian J. Genet.* **2019**, *79*, 571–582. [CrossRef]

29. Faye, I.; Ndoye, O.; Diop, T.A. Evaluation of fresh seed dormancy on seven peanut (*Arachis hypogaea* L.) lines derived from crosses between Spanish varieties: Variability on intensity and duration. *J. Appl. Sci. Res.* **2009**, *5*, 853–857.

30. Kumar, N.; Ajay, B.C.; Rathanakumar, A.L.; Radhakrishnan, T.; Lal, C.; Samdur, M.Y.; Mathur, R.K.; Manivel, P.; Chikani, B.M. Genetic variability for fresh seed dormancy in Spanish bunch advanced breeding lines of groundnut (*Arachis hypogaea* L.). *J. Oilseeds Res.* **2017**, *34*, 119–124.

31. Kumar, N.; Ajay, B.C.; Dagla, M.C.; Rathanakumar, A.L.; Lal, C.; Radhakrishnan, T.; Samdur, M.Y.; Mathur, R.K.; Manivel, P.; Makwana, A.D.; et al. Evaluation of Spanish bunch advanced breeding lines for fresh seed dormancy in groundnut (*Arachis hypogaea* L.). *Front. Crop Improv.* **2018**, *6*, 9–12.

32. Kumar, N.; Ajay, B.C.; Dagla, M.C.; Rathanakumar, A.L.; Radhakrishnan, T.; Samdur, M.Y.; Mathur, R.K.; Manivel, P.; Lal, C.; Makwana, A.D.; et al. Identification of Spanish bunch advanced breeding lines having fresh seed dormancy in groundnut (*Arachis hypogaea* L.). *Progressive Res. Int. J.* **2018**, *13*, 19–23.