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RESEARCH ARTICLE

Role of Macrosclereid Length in Seed Hardness and Dormancy in *Vigna* Species

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Abstract

Pulses are an important and affordable source of dietary protein, and the genus *Vigna* includes species with high agronomic and nutritional value. Seed dormancy, mainly controlled by seed coat hardness, is a key trait that affects germination, crop establishment, and management of genetic resources. In this study, we explored how macrosclereid length influences hard-seededness (Physical dormancy) in six *Vigna* species representing different levels of domestication: domesticated (*V. mungo*, *V. radiata*), semi-domesticated (*V. stipulacea*), and wild relatives (*V. sublobata*, *V. silvestris*, *V. setulosa*). We studied nineteen accessions for seed coat anatomy using a light microscope, and the data were analysed through ANOVA, Duncan's test, and correlation analyses. We found a clear domestication-related pattern: domesticated species had smoother testa with shorter macrosclereids, wild species showed rough, thick-walled testa with longer macrosclereids, and the semi-domesticated species was intermediate. Macrosclereid length and seed hardness were negatively associated with germination. These results shed light on the structural basis of dormancy and provide guidance for germplasm conservation and breeding for improved seed permeability in *Vigna*.

Keywords: Anatomy, Domestication, Macrosclereid length, Physical dormancy, *Vigna*

Introduction

The Fabaceae is one of the largest and most diverse families of flowering plants, comprising more than 770 genera and about 20,000 species, and representing nearly 5% of all known plant diversity (Lewis *et al.,* 2005). Members of this family hold great agronomic and ecological importance as they provide protein-rich seeds for human consumption, forage for livestock, and green manure that improves soil fertility. Their symbiotic association with nitrogen-fixing bacteria such as *Rhizobium* and *Bradyrhizobium* contributes to soil health and supports sustainable agricultural systems (Graham and Vance, 2003; Sprent, 2009; Peoples *et al.,* 2009).

Within Fabaceae, the genus *Vigna* is pantropical and highly diverse, comprising more than 200 species distributed mainly across Asia and Africa (Pratap *et al.,* 2014; Tripathi *et al.,* 2019). India serves as a major centre of origin and diversification for several cultivated taxa, including mungbean (*V. radiata*), urdbean (*V. mungo*), mothbean (*V. aconitifolia*), and rice bean (*V. umbellata*) (Bisht and Singh, 2005; Pradheep *et al.,* 2014). These crops are valued for their short duration, adaptability to marginal environments, and contribution to food and nutritional security, particularly in rainfed regions (Ali and

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Gupta, 2012). However, the effective utilization of *Vigna* germplasm is constrained by seed dormancy, especially in wild and semi-domesticated species.

Seed dormancy in legumes is predominantly physical, caused by a water-impermeable seed coat that prevents imbibition even under favourable conditions. While this trait enhances survival and persistence under natural habitats, it is undesirable in cultivation due to its adverse effects on germination and crop establishment (Singh *et al.,* 2020; Soltani *et al.,* 2021). The impermeability of legume seed coat is mainly associated with the palisade or macrosclereid layer, which is reinforced with cuticular deposits of lignin, suberin, and waxes (Cechová *et al.,* 2017; Hradilová *et al.,* 2017; Janská *et al.,* 2019). Variations in macrosclereid structure, seed coat surface morphology, and hilum characteristics are known to influence permeability and dormancy expression (Ignacimuthu and Babu, 1985; Umdale *et al.,* 2017; Paul *et al.,* 2024).

Despite advances in understanding the anatomical and physiological aspects of seed dormancy in legumes, comparative studies linking macrosclereid cell characteristics with hard-seededness in *Vigna* remain limited. Understanding these structural differences is crucial for explaining interspecific variation in seed hardness and dormancy. The present study was therefore undertaken to examine the relationship between macrosclereid length and seed hardness among selected domesticated, semi-domesticated, and wild *Vigna* species.

Materials and Methods

A total of 19 accessions representing six species of the genus *Vigna* were selected for the study, including domesticated species (*V. mungo*, *V. radiata*), a semi-domesticated species (*V. stipulacea*), and wild species (*V. sublobata*, *V. silvestris*, *V. setulosa*). The accessions were obtained from the Division of Germplasm Evaluation, ICAR-NBPGR, New Delhi. Detailed information on the species, accession numbers, and passport data is provided in Table 1.

Histology of the seed coat

Histological studies were carried out to observe the internal structure of the seed coat and its relationship with physical dormancy in *Vigna* species. Mature, well-developed seeds were cleaned thoroughly to remove any surface impurities and then soaked in distilled water for 24 hours to soften the outer layers. The seed coats, including the hilum and micropylar regions, were carefully dissected under a stereomicroscope to ensure that the tissues remained intact for microscopic observation.

The excised seed coats were fixed in a formalin acetic acid alcohol (FAA) solution composed of 50% ethanol, 5% acetic acid, and 10% formalin, and kept for

Table 1: Passport Data of the *Vigna* species accessions

S.no	Species	Acc.no	Status	District	State
1.	*V. mungo*	IC650620	Domesticated	Kanpur	Uttar Pradesh
2.	*V. mungo*	IC651317	Domesticated	Sehore	Madhya Pradesh
3.	*V. mungo*	IC652065	Domesticated	Guntur	Andhra Pradesh
4.	*V. radiata*	IC650613	Domesticated	New Delhi	Delhi
5.	*V. radiata*	IC651263	Domesticated	Sehore	Madhya Pradesh
6.	*V. radiata*	IC652064	Domesticated	Guntur	Andhra Pradesh
7.	*V. stipulacea*	IC550532	Semi-domesticated	Vizianagaram	Andhra Pradesh
8.	*V. stipulacea*	IC622867	Semi domesticated	Tiruchirappalli	Tamil Nadu
9.	*V. stipulacea*	IC651911	Semi-domesticated	Bhubaneswar	Odisha
10.	*V. sublobata*	IC202643	wild	Thrissur	Kerala
11.	*V. sublobata*	IC331468	Wild	Jabalpur	Madhya Pradesh
12.	*V. sublobata*	IC322299	Wild	Thrissur	Kerala
13.	*V. sublobata*	IC406507	Wild	Palakkad	Kerala
14.	*V. silvestris*	IC585931	Wild	Mumbai	Maharashtra
15.	*V. silvestris*	IC618518	Wild	South Goa	Goa
16.	*V. silvestris*	IC622873	Wild	South Goa	Goa
17.	*V. silvestris*	IC622877	Wild	Uttara Kannada	Karnataka
18.	*V. setulosa*	IC251419	Wild	Unknown	Unknown
19.	*V. setulosa*	IC252003	Wild	Unknown	Unknown

24 hours at room temperature. After fixation, the samples were rinsed in 70% ethanol to remove residual fixative and stored at 4°C until further processing. Dehydration was carried out through a graded tertiary butyl alcohol (TBA) series, followed by infiltration and embedding in paraffin wax maintained at 58–60°C. Thin transverse sections, 10 µm thick, were obtained using a rotary microtome and mounted on adhesive-coated glass slides to prevent detachment during staining.

For histochemical staining, Toluidine Blue O (Sakai, 1973) was used to distinguish lignified tissues, while Ruthenium Red (Soukup, 2014) was employed to detect mucilage and pectic substances. The stained sections were mounted in DPX and examined under a compound microscope, Leica DM500, at magnifications ranging from 10X to 40X. Photomicrographs were captured and calibrated using a stage micrometre, and the measurements of the palisade layer and macrosclereid cells were taken with ImageJ software. These observations provided insights into the structural variations in the seed coat contributing to dormancy expression among *Vigna* species.

Measurement of seed weight

Randomly select ten mature, dry seeds from each accession. Weigh the seeds using a precision analytical balance (±0.001 g). Calculate the average seed weight (mg/seed).

Measurement of seed hardness

The Seed Hardness Machine, Parisa technology device, is used to determine the resistance of a seed to deformation or fracture. Select representative, clean seeds from each accession. Place individual seeds in the hardness machine. Record the force in Newton (N) required to crack or break each seed. Mean values calculated from the three replicates for each accession.

Measurement of different parameters of the seed-by-seed analyser

Seed morphology and colour characteristics were analysed using an automated seed image analysis system developed by Nachiket Kotwaliwale at the ICAR–Central Institute of Agricultural Engineering (CIAE), Bhopal. The system was operated with Smart Grain software (version 1.3). Before analysis, calibration was performed using standard reference scales for length and area to ensure measurement accuracy. Uniform and diffuse lighting was maintained during imaging to achieve consistent colour evaluation.

Clean and well-filled seeds were arranged on a flat, non-reflective surface to prevent glare and image distortion. Damaged or irregular seeds were excluded to maintain data reliability. High-resolution images were captured under controlled conditions, and the software automatically separated individual seeds from the background for measurement.

The software generated data for key geometric and colour parameters. Seed length was defined as the maximum dimension along the major axis, while seed breadth represented the maximum width along the minor axis. The extracted data were used for further analysis of morphological variation among *Vigna* species.

Measurement of hilum length and width with a vernier calliper

Hilum length and width were determined using a Vernier calliper to examine variation among seeds. Only mature, intact seeds with a clearly visible hilum were used for measurements. The calliper was calibrated to zero before each measurement. Hilum length was recorded from one end to the other, while width was measured at its widest point. For each accession, five seeds were measured, and the average values were calculated to ensure reliability and consistency of the data.

Data analysis

All recorded observations were analysed to assess variation among species and the relationships between traits. For each trait, descriptive statistics including mean, standard deviation, and coefficient of variation were calculated. One-way ANOVA was conducted to identify significant differences among species for morphological, physiological, and anatomical parameters. Pearson's correlation coefficients were used to explore the associations between seed size, hardness, germination, and macrosclereid length at significance levels of $P \leqslant 0.05$ and $P \leqslant 0.01$. Image-based measurements for anatomical study were obtained and verified using ImageJ (v1.54), and further statistical analyses were carried out using Microsoft Excel 2021 and SPSS 30.0 (IBM Corp., Armonk, USA).

Results

Analysis of variance (ANOVA) of seed traits for six Vigna species

The Analysis of Variance (ANOVA) shows that all seven seed parameters showed highly significant variation among the six *Vigna* species ($P \leqslant 0.05$ or $P \leqslant 0.01$), indicating that the species differ statistically in their seed characteristics (Table 2).

Table 2: Analysis of variance (ANOVA) of 7 seed traits for six *Vigna* species

Seed parameter	Sum of squares	Df	Mean square	F	Sig
Hundred seed weight (g)	87.92	5.00	17.58	58.40	0.00
Seed length (mm)	33.42	5.00	6.68	30.25	0.00
Seed width(mm)	14.71	5.00	2.94	19.80	0.00
Seed hilum length(mm)	4.87	5.00	0.97	10.91	0.00
Seed hilum width(mm)	1.49	5.00	0.30	5.20	0.00
Macroscleried length (µm)	42764.80	5.00	8552.96	30.69	0.00
Seed hardness (N)	100.05	5.00	20.01	42.46	0.00

Species-wise descriptive statistics

Seed traits varied notably among the six *Vigna* species studied (*V. mungo*, *V. radiata*, *V. setulosa*, *V. silvestris*, *V. stipulacea*, and *V. sublobata*) (Table 3).

The domesticated species, *V. mungo* (3.798 g) and *V. radiata* (3.402 g), had significantly larger seeds compared to the wild species, *V. setulosa* (0.732 g) and *V. sublobata* (0.956 g), while the semi-domesticated *V. stipulacea* (0.800 g) exhibited intermediate seed weight. Seed area, length, and width followed a similar trend, reflecting the influence of domestication on seed enlargement.

Dimensional parameters, including seed perimeter, equivalent diameter, axial and median lengths, and hilum size, were greater in cultivated species, with the longest hilum observed in *V. mungo* (2.166 mm). On the other hand, wild taxa displayed greater variability in these traits. Seed hardness was significantly lower in cultivated species (*V. mungo* 2.453; *V. radiata* 2.177) than in wild species (*V. setulosa* 6.108; *V. sublobata* 5.055). Similarly, macrosclereid length, which forms the palisade layer of the seed coat, was shortest in *V. mungo* (55.754 µm) and longest in *V. sublobata* (maximum 199.12 µm), indicating thicker seed coats in wild species.

Broadly, cultivated *Vigna* species produced larger, softer seeds with thinner coats, whereas wild species exhibited smaller, harder seeds with more robust palisade layers. These interspecific differences underscore the impact of domestication on seed morphology and dormancy-related traits.

Table 3: Description of variation in seed parameters among six *Vigna* species

Trait	*Vigna mungo*	*Vigna radiata*	*Vigna setulosa*	*Vigna silvestris*	*Vigna stipulacea*	*Vigna sublobata*
Hundred seed weight (g)						
Average	3.798	3.402	0.732	1.188	0.8	0.956
Max	4.32	4.8	0.85	1.71	0.85	1.59
Min	2.91	2.33	0.62	0.51	0.75	0.64
SD	0.642	1.038	0.101	0.415	0.034	0.367
CV (%)	16.908	30.516	13.869	34.923	4.239	38.392
Seed length (mm)						
Average	4.633	4.633	2.75	3.433	2.822	3.008
Max	5.2	5.6	2.9	4.1	3.1	3.6
Min	3.8	3.7	2.2	3	2.5	2.2
SD	0.561	0.7	0.274	0.35	0.186	0.507
CV (%)	12.113	15.108	9.959	10.191	6.576	16.858
Seed width (mm)						
Average	3.811	3.567	2.4	2.992	2.444	2.717
Max	4.3	4.3	2.8	3.6	2.8	3.4
Min	3	3	2.1	2.4	2.1	2.1
SD	0.434	0.406	0.276	0.396	0.27	0.432
CV (%)	11.395	11.389	11.487	13.253	11.036	15.917

Seed Hilum length (mm)						
Average	2.166	1.644	1.37	1.893	1.34	1.458
Max	2.61	2.01	2.17	2.19	1.49	2.04
Min	1.67	1.31	0.52	1.36	1.27	1.05
SD	0.332	0.254	0.54	0.269	0.065	0.282
CV (%)	15.321	15.426	39.394	14.202	4.851	19.329
Seed Hilum width (mm)						
Average	0.997	0.684	0.578	0.99	0.682	0.678
Max	1.34	1.05	1.07	1.31	0.9	1.67
Min	0.81	0.44	0.32	0.52	0.57	0.36
SD	0.147	0.171	0.271	0.256	0.107	0.348
CV (%)	14.738	25.021	46.902	25.828	15.749	51.26
Macroscleried length (µm)						
Average	55.754	83.099	111.227	115.323	83.081	136.998
Max	82.17	95.51	122.29	150.98	93.45	199.12
Min	41.06	77.93	103.26	97.5	64.97	116.23
SD	19.432	5.673	6.846	16.742	9.372	25.07
CV (%)	34.853	6.827	6.155	14.517	11.28	18.3
Seed hardness (N)						
Average	2.453	2.177	6.108	4.867	4.262	5.055
Max	2.922	2.86	7.877	6.644	4.577	7.185
Min	1.616	1.749	5.107	4.125	4.01	4.028
SD	0.486	0.36	1.101	0.708	0.203	0.915
CV (%)	19.797	16.545	18.021	14.546	4.761	18.104

Macrosclereid length

The macrosclereid length exhibited substantial interspecific variation among the six *Vigna* species evaluated (Table 4; Fig. 1). In the cultivated species *V. mungo* and *V. radiata*, the macrosclereid cells were relatively shorter, ranging from 41.06 µm to 82.17 µm and 77.93 µm to 95.51 µm, with mean lengths of 55.75 ± 19.43 µm and 83.10 ± 5.67 µm, respectively.

Conversely, the wild species exhibited considerably higher macrosclereid dimensions. *V. setulosa* and *V. silvestris* recorded mean lengths of 111.23 ± 6.85 µm and 115.32 ± 16.74 µm, respectively, with maximum values reaching 122.29 µm and 150.98 µm. While the semi-domesticated species *V. stipulacea* displayed intermediate values (mean 83.08 ± 9.37 µm, range 64.97–93.45 µm), *V. sublobata* possessed the longest macrosclereid cells, varying from 116.23 µm to 199.12 µm with a mean of 136.99 ± 25.07 µm.

The coefficient of variation (CV) was lowest in *V. radiata* (6.83%) and *V. setulosa* (6.16%), indicating greater uniformity within the cultivated and wild accessions, while *V. mungo* showed the highest variability (34.85%). A significant trend of increasing macrosclereid length was evident from cultivated to wild taxa, suggesting progressive enhancement of palisade cell elongation in wild *Vigna* species.

Table 4: Duncan's multiple range test of seed parameters across species

Species	Hundred seed weight	Seed length	Seed width	Hilum length	Hilum width	Macroscleried length	Seed hardness
V. mungo	3.798± 0.21 b	4.63±0.19c	3.811±0.14c	2.17±0.11c	0.997±0.05b	55.754±6.48a	2.453±0.16a
V. radiata	3.402± 0.35 b	4.63±0.23c	3.567±0.14c	1.64±0.08ab	0.684±0.06a	83.099±1.89b	2.177±0.12a
V. sublobata	0.956± 0.11 a	3.00±0.15ab	2.717±0.12ab	1.46±0.08a	0.678±0.10a	136.998±7.24d	5.055±0.26c
V. silvestris	1.188± 0.12 a	3.43±0.10b	2.992±0.11b	1.89±0.08bc	0.990±0.07b	115.323±4.83c	4.867±0.20bc
V. setulosa	0.732 ± 0.04 a	2.75±0.11a	2.400±0.11a	1.37±0.22a	0.578±0.11a	111.227±2.79c	6.108±0.45d
V. stipulacea	0.800 ± 0.01 a	2.822±0.6a	2.444±0.09a	1.34±0.02a	0.682±0.04a	83.081±3.12b	4.262±0.07b

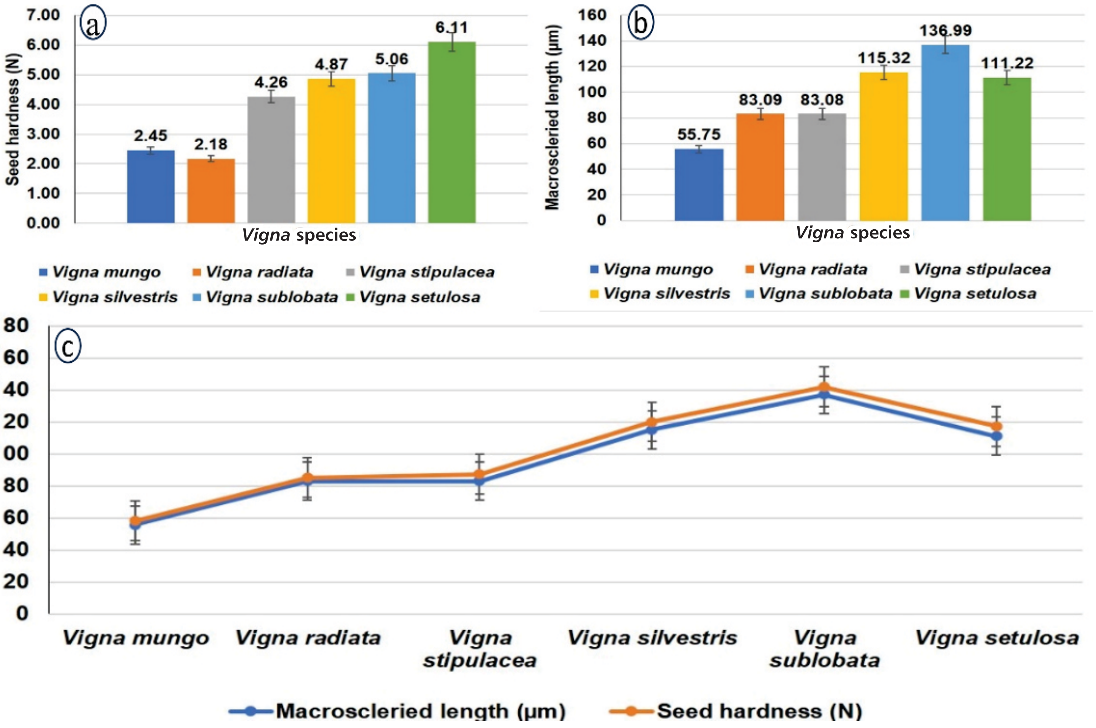

Fig. 1: (a) Seed hardness across *Vigna* species (N), (b) Macrosclereid length across *Vigna* species (µm), (c) Relationship between macrosclereid length and seed hardness across *Vigna* species.

In general, species with higher macrosclereid length (⩾ 120 µm), notably *V. sublobata* and *V. silvestris*, are likely to exhibit stronger physical dormancy compared to cultivated species such as *V. mungo* and *V. radiata*. The distribution and interspecific differences in macrosclereid length are illustrated in Fig. 1.

Seed hardness

Seed hardness varied widely among the six *Vigna* species studied, showing a clear interspecific pattern in seed coat strength. Overall values ranged from 1.62 to 7.88 N, reflecting considerable diversity in the mechanical resistance of the seed coat. The cultivated species showed lower hardness compared to their wild relatives. In *V. radiata*, seed hardness ranged from 1.75 to 2.86 N with a mean of 2.18 ± 0.36 N and a coefficient of variation of 16.55%. Similarly, *V. mungo* recorded values between 1.62 and 2.92 N, with a mean of 2.45 ± 0.49 N and a coefficient of variation of 19.80%. These two cultivated species formed the low-hardness group, indicating thinner and more permeable seed coats.

Among the wild species, *V. setulosa* showed the greatest hardness, ranging from 5.11 to 7.88 N with a mean of 6.11 ± 1.10 N and a coefficient of variation of 18.02% *V. silvestris* and *V. sublobata* exhibited intermediate to high values, with means of 4.87 ± 0.71 N and 5.06 ± 0.92 N, respectively. The semi-domesticated *V.stipulacea* occupied a moderate position, showing hardness values between 4.01 and 4.58 N with a mean of 4.26 ± 0.20 N and the lowest variability among species, with a coefficient of variation of 4.76%.

Across species, *V. stipulacea* showed the narrowest range of hardness values, suggesting a uniform seed coat texture, while *V. setulosa* displayed the widest range, indicating greater heterogeneity in seed coat resistance. When grouped by relative hardness, *V. mungo* and *V. radiata* represented the low class, *V. stipulacea* the moderate class, and *V. silvestris*, *V. sublobata*, and *V. setulosa* the high-hardness class. The general trend indicated a gradual increase in seed hardness from cultivated to wild taxa, reflecting progressive thickening and lignification of the seed coat during evolution. The interspecific differences in seed hardness are shown in Fig. 1.

Correlation analysis of seed traits

The correlation analysis revealed clear associations among the measured seed traits (Table 5). Hundred-seed weight showed a strong positive relationship with seed length (r = 0.893**), seed width (r = 0.815**), and germination percentage (r = 0.781**), indicating that larger seeds tended to germinate more efficiently. Conversely, hundred-seed weight was negatively correlated with macrosclereid length (r = –0.581**) and

Table 5: Correlation among seed traits in *Vigna* species

Seed trait	Hundred seed weight	Seed length	Seed width	Hilum length	Hilum width	Macroscleried length	Seed hardness	Germi-nation
Hundred seed weight	1							
Seed length	0.893**	1						
Seed width	0.815**	0.913**	1					
Hilum length	0.483**	0.484**	0.574**	1				
Hilum width	0.251	0.325*	0.382**	0.669**	1			
Macroscleried length	-0.581**	-0.516**	-0.464**	-0.374**	-0.165	1		
Seed hardness	-0.751**	-0.782**	-0.703**	-0.321*	-0.162	0.640**	1	
Germination	0.781**	0.682**	.609**	0.192		0.0185	-0.508**	-0.795**1

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed)

seed hardness (r = –0.751**), indicating that smaller, harder seeds tend to be lighter.

Seed length and width were closely related (r = 0.913**), and both showed positive associations with germination, confirming that seed size influences germination performance. Hilum length correlated positively with hilum width (r = 0.669**) and moderately with seed size traits, though the relationships were comparatively weaker.

Macrosclereid length showed significant negative correlations with seed size parameters, including hundred-seed weight, seed length, and seed width, but had a strong positive relationship with seed hardness (r = 0.640**). Similarly, seed hardness was negatively correlated with seed length (r = –0.782**), seed width (r = –0.703**), hundred-seed weight (r = –0.751**), and germination (r = –0.795**), confirming that seeds with thicker macrosclereid layers and greater hardness tend to be smaller and less permeable.

Relationship between Macrosclereid length and seed hardness

Significant variation was observed in macrosclereid length and seed hardness among the six *Vigna* species studied (Fig. 1). Domesticated species such as *V. mungo* (55.75 μm) and *V. radiata* (83.09 μm) had significantly shorter macrosclereid cells compared to wild species like *V. silvestris* (115.32 μm), *V. sublobata* (136.99 μm), and *V. setulosa* (111.22 μm). The semi-domesticated *V. stipulacea* recorded an intermediate value (83.08 μm). Seed hardness followed a similar pattern. The lowest values were recorded in cultivar species like *V. radiata* (2.18 N) and *V. mungo* (2.45 N), while the wild species like *V. silvestris* (4.87 N), *V. sublobata* (5.06 N), and *V. setulosa* (6.11 N) showed much higher seed hardness. The semi-domesticated species *V. stipulacea* showed intermediate hardness (4.26 N). The strong positive correlation between macrosclereid length and seed hardness (Fig. 1), with both parameters increasing progressively from domesticated to wild types. *V. sublobata* exhibited the highest values for both macrosclereid length (136.99 μm) and seed hardness (5.06 N), suggesting that thicker macrosclereid layers contribute to seed coat strength and dormancy in wild species, while domesticated species have evolved thinner, softer seed coats promoting faster germination.

Discussion

The six *Vigna* species (*V. mungo*, *V. radiata*, *V. setulosa*, *V. silvestris*, *V. stipulacea*, and *V. sublobata*) showed considerable variation in seed morphology, structure, and hardness, reflecting their differing domestication levels, ecological adaptations, and phylogenetic relationships. Analysis of Variance (ANOVA) revealed that all 7 seed traits differed significantly among the species ($P \leqslant 0.05$ or $P \leqslant 0.01$), indicating clear statistical divergence (Table 1). Duncan's multiple range test further confirmed distinct groupings for each trait (Table 4), highlighting pronounced interspecific differences.

Seed traits clearly distinguished domesticated and wild *Vigna* species. Cultivated species, *V. mungo* and *V. radiata*, produced larger seeds with higher 100-seed weight, greater area, length, and width, reflecting human selection for traits that enhance seedling vigour and crop productivity (Gepts, 2004; Fuller, 2007; Zohary *et al.,* 2012;). In contrast, wild species such as *V. setulosa*, *V. stipulacea*, and *V. sublobata* had smaller, compact seeds, which likely support persistence in natural habitats through efficient dispersal and dormancy (Koinange *et al.,* 1996; Tomooka *et al.,* 2002)

Seed hardness and dormancy characteristics further emphasised these differences between cultivated and wild species. Wild species exhibited harder seeds and longer macrosclereid cells, forming tougher seed coats that delay germination until environmental conditions are favourable. By contrast, cultivated species had softer seeds and shorter palisade layers, enabling rapid and uniform germination under agricultural management (Rolston, 1978; Smýkal *et al.,* 2014; Dissanayake *et al.,* 2016). Notably, the high variability in seed hardness in *V. sublobata* indicates untapped genetic potential for breeding programs targeting dormancy and stress resilience. Together, these findings reveal the domestication syndrome in *Vigna*, where seed size, shape, and dormancy traits have evolved under human selection, while wild species retain compact, robust seeds adapted to natural environments. Wild *Vigna* species thus represent valuable genetic resources for breeding programs aiming to combine agronomic performance with adaptive resilience (Fuller & Allaby, 2009; Gopinath *et al.,* 2021).

Correlation analysis revealed clear relationships among seed morphological and physiological traits in *Vigna* species. Larger seeds, indicated by higher hundred-seed weight, were strongly associated with greater seed length (r = 0.893**) and width (r = 0.815**), as well as higher germination percentages (r = 0.781**). This shows that bigger seeds, with more nutrient reserves, support better seedling vigour and improved germination (Baskin & Baskin, 2014). Seed weight was negatively correlated with macrosclereid length (r = -0.581**) and seed hardness (r = -0.751**), reflecting a trade-off between seed size and dormancy. Wild species, with smaller, harder seeds and thicker palisade layers, showed stronger physical dormancy, whereas domesticated species had larger, softer seeds that germinate quickly and uniformly (Fuller & Allaby, 2009; Smýkal *et al.,* 2014).

Seed length and width were strongly correlated with each other (r = 0.913**) and positively linked to germination, while negatively associated with seed hardness and macrosclereid length. This highlights the central role of seed size in water uptake, mechanical resistance, and germination behaviour (Kumar *et al.,* 2011; Dissanayake *et al.,* 2016). Hilum dimensions, though moderately correlated with seed size, were not significantly related to germination or macrosclereid length, indicating that hilum grows proportionally with seed size but does not independently affect dormancy or water permeability (Srinivasan et al., 2007; Bewley *et al.,* 2013; Tripathi *et al.,* 2019; Gore *et al.,* 2025). Collectively, these results showed how domestication has shaped seed traits in *Vigna*, favouring larger seeds with reduced dormancy to improve germination and seedling vigour, while wild species retain smaller, harder seeds with structural adaptations that enhance survival in natural environments. Macrosclereid length was negatively correlated with hundred-seed weight, seed length, and seed width, but positively associated with seed hardness (r = 0.640**) and negatively with germination (r = -0.508**), highlighting its critical role in enforcing physical dormancy in wild *Vigna* species. As the main structural component of the palisade layer, macrosclereids determine seed coat thickness and impermeability, directly influencing germination potential (Tomooka *et al.,* 2002).

Seed hardness similarly acted as a negative regulator of germination. Strong negative correlations with seed size traits and germination (r = -0.795**) indicate that hard seeds restrict water uptake and delay germination, a trait advantageous for survival in natural ecosystems. The positive association between seed hardness and macrosclereid length underscores the anatomical basis of this physiological barrier (Maxted *et al.,* 2001; Smýkal *et al.,* 2014). In general, the correlation matrix shows the balance between seed enlargement, dormancy, and germination strategies in *Vigna* species. Domestication has favoured larger seeds with reduced hardness and shorter macrosclereids, promoting rapid and uniform germination. Conversely, wild species retain dormancy-enhancing traits, such as high hardness and thick palisade layers, which limit germination but enhance seed longevity and environmental persistence.

Conclusion

This study reveals a clear domestication gradient in seed dormancy across *Vigna*. Domesticated species (*V. mungo*, *V. radiata*) are non-dormant, with seed coat traits favouring rapid germination, while wild relatives (*V. sublobata*, *V. silvestris*, *V. setulosa*) maintain strong physical dormancy through enhanced anatomical features. The semi-domesticated *V. stipulacea* exhibits intermediate traits. Reduced hard-seededness in domesticated species is linked to shorter macrosclereids, smoother testa, and thinner cell walls, showing a domestication syndrome. These findings have practical applications, like wild species require species-specific pre-sowing treatments, such as mechanical scarification, for genebank regeneration, and understanding dormancy anatomy aids breeding programs by enabling the use of wild germplasm without compromising germination. Future research should explore the genetic basis of macrosclereid development and cuticle deposition, as well as environmental influences on dormancy persistence and release.

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