



RESEARCH ARTICLE

Assessing the relationship between hardseedness and seed morphometrics in Blackgram

C Menaka¹, S Pozhilarasi^{2*}, A Yuvaraja³, M Djanaguiraman⁴, C Vanitha⁵ & K Raja⁵

¹Department of Seed Science and Technology, Krishi Vigyan Kendra, Vamban, Pudukkottai 622 303, Tamil Nadu, India

²Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

³Department of Plant Breeding and Genetics, Krishi Vigyan Kendra, Vamban, Pudukkottai 622 303, Tamil Nadu, India

⁴Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

⁵Seed Centre, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Email: poz hilarasi7@gmail.com



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Abstract

Seed dormancy in black gram (*Vigna mungo*) was studied about seed size (weight, length, width) and their impact on hard seed formation and imbibition rate during the summer and *Kharif* seasons at NPRC, Vamban, Pudukkottai. The seeds of blackgram obtained during the summer season showed a significant negative correlation with hard seed occurrence ($r = -0.639$, $P \leq 0.01$) and a positive correlation with imbibition rate ($r = 0.451$, $P \leq 0.05$). Whereas seed width had a weaker negative correlation with hard seed occurrence ($r = 0.357$) and a positive correlation with imbibition rate ($r = 0.273$). In addition, the strongest correlation was found between hard seed occurrence and imbibition rate ($r = 0.802$, $P \leq 0.01$). The seed length of the blackgram harvested during the *kharif* season had a stronger negative correlation with hard seed occurrence ($r = -0.790$, $P \leq 0.01$) and a positive correlation with imbibition rate ($r = 0.555$, $P \leq 0.01$). Seed width also showed significant correlations with hard seed occurrence ($r = -0.673$, $P \leq 0.01$) and imbibition rate ($r = 0.613$, $P \leq 0.01$). At the same time, the obtained seed weight correlations were generally weak. The results also proved a seasonal difference in the hard seedness of blackgram, which confirmed the occurrence of hard seed during the summer compared to the *Kharif* season. Thus, the study's findings highlight the importance of seed morphometrics and imbibition in predicting the presence of hard seeds in blackgram.

Keywords

hard seed; imbibition rate; *kharif*; summer season

Introduction

Pulses have a high ranking in Indian agriculture because they are a rich source of protein (17 to 25 %) compared to cereals (6 to 10 %) and their fat content is much lower than that of other protein-rich food crops. Legumes are flowering plants classified under the Fabaceae family within the order Fabales. Traditionally, this family has been categorized into three subfamilies: Mimosoideae (acacia subfamily), Caesalpinoideae (bird-of-paradise subfamily) and Faboideae or Papilionoideae (bean subfamily). Black gram is one of the most important pulse crops in the leguminous family and a good source of dietary proteins and fibres (1). Seed dormancy, particularly hard seedness, is a significant trait in leguminous crops like black gram (*Vigna mungo* L. Hepper) to ensure quality. Hardseededness refers to the condition

where seeds have a hard seed coat impermeable to water, leading to delayed or non-uniform germination (2). While this trait is advantageous in natural ecosystems for preventing premature germination, it poses challenges in agricultural practices where uniform and timely germination is essential for optimal crop yield and management.

Seed quality is a critical factor in agriculture, influencing crop yield and quality. Among the various parameters that determine seed quality, hardseededness and seed size precisely, seed length and width are significant. Hardseededness can create considerable challenges in achieving synchronous germination. This trait leads to delayed or non-uniform germination, adversely affecting crop establishment and yield potential. Farmers may struggle to secure a good crop stand due to the variability in germination timing (3). While hard seeds can preserve seed viability over extended periods, they also pose challenges in achieving uniform crop establishment. Seed dormancy is primarily classified into different types: physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY) and a combination of physical and physiological dormancy (PD + PY) (3, 4). Physical dormancy, or hardseededness, is the second most common type worldwide. It has been found in 14 % of all species with known dormancy status (2000 species from 20 families) and is prevalent in many lineages such as Fabaceae, Cistaceae, Geraniaceae, Malvaceae, Rhamnaceae and Sapindaceae (4, 6-8). Physical dormancy occurs in seeds or fruits of 18 angiosperm families and is caused by a water-impermeable palisade cell layer(s) in the seed or fruit coats. Before germination, the seed or fruit coat must become permeable to absorb water. Research indicates that hard seeds have higher pectin, cellulose, hemicellulose, lignin and phenolic content than non-hard seeds. SEM analysis showed thicker cuticles (60.96 μm), a waxy layer and rough textures in hard seeds, while non-hard seeds had thinner cuticles (33.81 μm) and smoother surfaces, aiding permeability. It was reported that hard seed dormancy in black gram is due to an impermeable seed coat. GC-MS analysis showed higher levels of octadecanoic acid (27.48), 3-tert-butyl-4-hydroxyanisole (10.76) and hexadecanol (1.29) in hard seeds (10). SEM revealed thicker cuticles, denser palisade layers and rougher surfaces, restricting water uptake. These findings aid breeding and agronomic strategies to reduce dormancy and improve seed quality.

Understanding the relationship between hardseededness and seed morphometrics and the measurement of seed dimensions and physical characteristics is essential for developing effective agricultural strategies. Investigating this relationship in Black gram can help identify key morphometric traits associated with hardseededness, enabling the prediction and management of this trait in breeding programs. Seed size plays a crucial role in seed germination and seedling vigour, as larger seeds generally contain more reserves that support seedling growth under suboptimal conditions. However, the relationship between seed size and other seed quality parameters, such as hardseededness, is complex and varies across species and environmental conditions. Studies have indicated that a

reduction in seed size tends to increase hardseededness (11-13).

Additionally, previous research on mung bean has shown that smaller seeds contain a higher proportion of hard seeds than larger seeds, considering factors such as genetic variation, environmental conditions and their combined effects on seed germination and viability (14). Research indicates that seed size and colour are key characteristics for distinguishing between hard-seeded and soft-seeded lines (15, 16). With all this background, the study focuses on analyzing the impact of seed dimensions and imbibition on hardseededness in blackgram. The relationship between hardseededness and seed morphometrics in Black gram presents a complex but critical study area. By addressing the objectives outlined, this research aims to bridge the gap between seed biology and practical agriculture, paving the way for improved crop management and productivity in leguminous crops. Reducing hard seeds before germination has become difficult, resulting in reduced field emergence and thereby reducing crop performance.

Materials and Methods

Seed selection and preparation

Seeds of blackgram varieties were collected and raised in NPRC, Vamban, during summer & *kharif* season 2023. The National Pulses Research Centre (NPRC) in Vamban is approximately 10.424° N latitude and 78.751° E longitude. This places it near the town of Vamban in Tamil Nadu, India. A laboratory experiment was conducted at the Department of Seed Science & Technology, Coimbatore, with freshly harvested seeds from the field trial. The Tamil Nadu Agricultural University (TNAU) in Coimbatore is approximately 11.1176° N latitude and 76.9944° E longitude. In this experiment, twenty-five seeds were placed individually in 25 test tubes. The initial observations included measuring individual seeds' weight, length and width. Each seed was assessed separately to record these physical parameters, ensuring accurate data collection for further analysis. Single seeds were subjected to imbibition study by soaking them in 1 ml of distilled water uniformly. After soaking for 3 hours, the seeds were examined for weight, length and width changes to assess their water absorption and expansion. Each seed was labelled and numbered for individual tracking throughout the experiment.

Measurement of Seed Characteristics

Initially, the seeds' weight, length and width were measured individually. The weight was recorded using a digital precision balance of 0.01 mg. The morphological observations like Seed length and width were measured (Image Focus Plus V₂ software).

Image analysis measurement

Six replicates of ten seeds were placed on the lighting hood so that the embryo axis of the seed faced the image analysis system and the longitudinal axis of the seed ran parallel to the surface of the camera lens. Seeds were viewed with a video camera (DSP surveillance colour CCD camera (CVS

200/3300) using transmitted light so that a binary image of the silhouette of the seed was recorded by the Image Focus Plus V2 software (17). Before actual measurement, calibration was done by placing a transparent plastic ruler on the lighting hood illuminated from below. The ruler was aligned diagonally across the field of view and adjusting focus sharpened the image. Again, aperture adjustment was done until optimum colour and contrast were achieved. Input measurement was given in millimetres. All images and their data were saved in the document file and the interpreted data results and pictures were reported. The parameters studied are as follows.

Seed length - Length is measured in the vertical Y-axis.

Seed Width - Length is measured in horizontal X-axis

Length and Width: The dimensions were measured again using an Image analyzer to assess changes in size due to water uptake.

Imbibition experiment

The seeds were individually placed in separate containers with 1 ml of distilled water to assess the imbibition rate. The experiment was conducted at room temperature (approximately 25° C) under laboratory conditions. Measurements were taken at 0 and 3 hours after the seeds were placed in water. Seed Weight: Seeds were carefully removed from the water, blotted gently with filter paper to remove excess moisture and weighed immediately. (18). The imbibition percentage was calculated for each seed at each time point using the following Equation 1 formula:

Imbibition rate (%) =

$$\frac{\text{Seed weight at time} - \text{Initial seed weight}}{\text{Initial seed weight}} \times 100$$

(Eqn. 1)

The seed's hardness status was mentioned as 1 and non-hard seeds were mentioned as 0 in the results (Fig. 1).

Statistical analysis

Pearson's correlation analysis was performed to determine the direction and strength of the relationship between hard seed and non-hard seed traits and the measured variables. The results were interpreted based on the significance level ($p \leq 0.05$). The analysis was conducted using IBM SPSS software, providing insights into the interdependence of key parameters in the study.

Results

The results of Table 1 present a correlation matrix of the relationships between five different variables related to seeds: Seed weight (mg), Seed length (mm), Seed width (mm), Imbibition Rate % and Hard seed (1) and non-hard seed (0). Correlation coefficients that range from -1 to +1 are in the table. A positive correlation (closer to 1) indicates that as one variable increases, the other tends to increase. A negative correlation (closer to -1) suggests that as one variable increases, the other tends to decrease. A correlation close to 0 indicates little to no linear relationship between the variables.

The results indicate that seed weight has a positive but weak correlation with seed length (0.286) and seed width (0.191), suggesting that heavier seeds might be slightly longer and wider. However, the relationships are not strong or statistically significant. There is a weak negative correlation between seed weight and hard seed occurrence (-0.352), implying a slight tendency for heavier seeds to be less likely to develop hard coats. However, this relationship is also not strong. Interestingly, seed weight shows a positive but moderate correlation with imbibition rate (0.365), indicating that heavier seeds might absorb water at a higher rate. However, this correlation is not statistically significant either. Seed weight does not significantly influence hardseededness, as no notable differences in seed hardness were observed between different seed sizes within the same variety. Instead, the high percentage of hard seeds in the Harsha variety appears to be an inherent characteristic rather than related to seed weight.

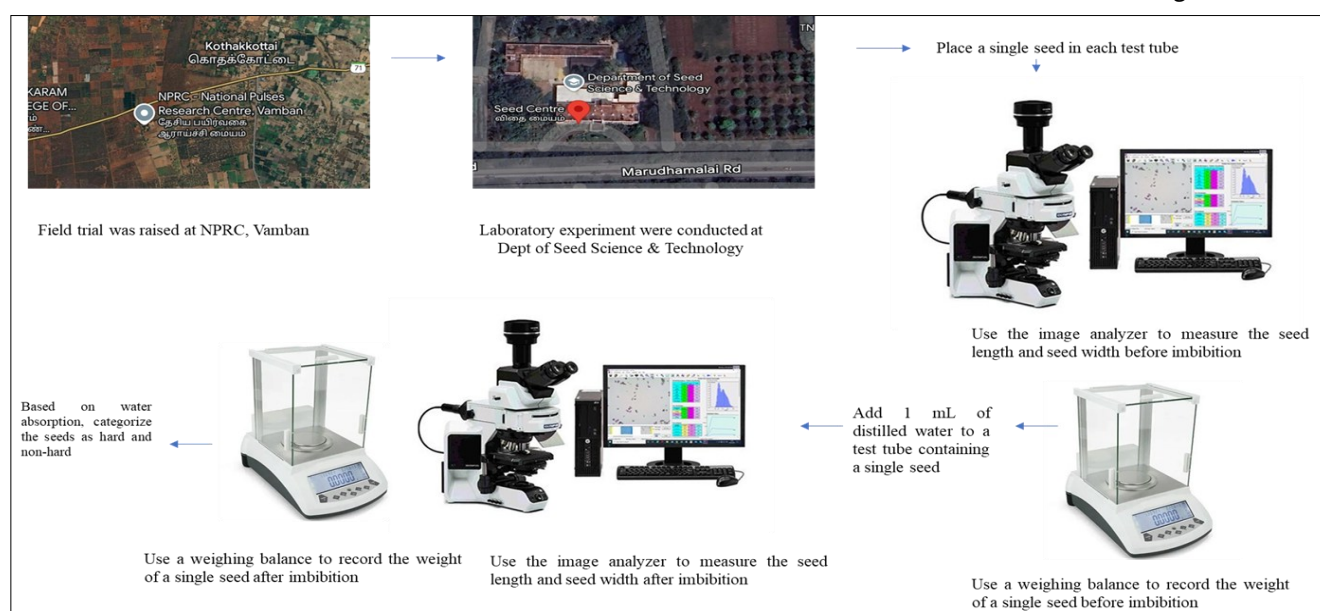


Fig. 1. Protocol for assessing the relationship between hard seeds and non-hard seeds.

Table 1. Correlation matrix of seed properties and hard seed phenomenon (Summer season)

Parameters	Seed weight	Seed length	Seed width	Hard seed	Imbibition rate
Seed weight	1				
Seed length	0.286	1			
Seed width	0.191	.503*	1		
Hard seed	-0.352	-.639**	-0.357	1	
Imbibition rate	0.365	.451*	0.273	-.802**	1

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

Seed length exhibits a moderate positive correlation with seed width (0.503), which is statistically significant, indicating that longer seeds tend to be wider. Notably, seed length shows a strong negative correlation with hard seed occurrence (-0.639), suggesting that longer seeds are significantly less likely to be hard. Additionally, seed length has a moderate positive correlation with imbibition rate (0.451), meaning that longer seeds absorb water more readily during imbibition and this correlation is also statistically significant. Phenotypic variation in seed coat permeability may result from maternal genetic and environmental influences (20). Hardseededness, characterized by strong water resistance, prevents seeds from absorbing water even under optimal conditions (21). Environmental factors such as temperature, moisture and soil conditions are crucial in determining hardseededness in leguminous crops (22). This trait can be advantageous for long-term seed viability, ensuring the availability of viable seed material for years (23).

Seed width positively correlates with seed length (0.503), confirming that longer seeds are generally wider. While seed width shows a weak negative correlation with hard seed occurrence (-0.357), this relationship is not statistically significant, suggesting that wider seeds may be slightly less prone to developing hard seed coats. Additionally, seed width has a weak positive correlation with imbibition rate (0.273), implying that wider seeds may absorb water more quickly, though this correlation is also not statistically significant. Research indicates a significant negative correlation (-0.584**) between seed size and hardseededness, while seed size in soybeans negatively correlated with seed water absorption and seed hardness (24, 25).

Hard seed occurrence shows a strong negative correlation with both seed length (-0.639) and imbibition rate (-0.802), which are statistically significant. This indicates that longer seeds are less likely to develop hard coats and seeds that absorb water rapidly are far less likely to be complicated. These findings support the concept that hard seeds possess an impermeable seed coat, which restricts water uptake and induces dormancy. A hard seed coat in legumes (*Teramnus labialis*) is linked to poor water permeability (26). Similarly, research indicates that white seeds generally have a thinner seed coat with higher permeability, a lower content of lignans, phenols, tannins and unsaturated fatty acids and weaker adhesion between the seed coat and cotyledons. These characteristics facilitate faster water uptake but also increase the risk of imbibition damage, ultimately leading to quicker germination compared to colored seeds. Research indicates that seed lots containing hard seeds exhibited lower water uptake (28). At the same time, seed water absorption is

influenced by factors such as seed composition, the chemical properties of the seed coat and its structural characteristics, including thickness and permeability (29). Moreover, dormant and non-dormant seeds exhibit distinct water uptake patterns (30).

Overall, the analysis suggests that seed length is a more critical factor than seed weight or width in determining both the likelihood of hard seed development and the imbibition rate. Longer seeds are less likely to develop hard coats and more likely to absorb water quickly, which could be beneficial for germination. These insights could be valuable for breeding programs or treatments aimed at reducing the occurrence of hard seeds or enhancing germination rates, highlighting the importance of seed length and its relationship to other seed traits. The relationship map summarises the interactions between various seed properties, particularly on hard seeds. It highlights how key variables such as seed hardness (HS), imbibition rate (IR), seed length (SL), seed weight (SW) and seed width (WD) are interconnected. The nodes on the map represent these variables, with the size of each node indicating the frequency or importance of the variable in the dataset. The edges, or lines connecting the nodes, represent the strength or frequency of relationships between these variables, with thicker lines indicating stronger or more significant correlations (Fig. 2).

The map highlights the relationship between hard seeds and imbibition rate through a strong, thick connection, indicating a close association. Hard seeds generally exhibit a lower imbibition rate, as their impermeable seed coat restricts water absorption. Dormant and non-dormant seeds display distinct water uptake patterns (30). Additionally, a notable correlation exists between hard seeds and seed length, suggesting that longer seeds may be more susceptible to developing hardness. While seed weight and width also show some association with seed hardness, their influence appears weaker than seed length and imbibition rate. Hard seeds are characterized by the absence of pores in the epidermis and the presence of an impermeable seed coat composed of suberin layers. These traits are influenced by environmental factors, seed size and genetic inheritance (31). The map further illustrates the interconnectedness of seed length, weight and width, as these physical dimensions naturally vary together. The thickness of the lines connecting these dimensions indicates the degree to which they influence each other. Moreover, the size of the nodes for imbibition rate and hard seeds suggests that these variables are more significant within the dataset, reflecting their strong association with seed hardness. Hard seeds benefit wild plant species where long-term dormancy is an environmental adaptation but problematic for crop species where rapid uniform germination is more valuable in alfalfa (32).

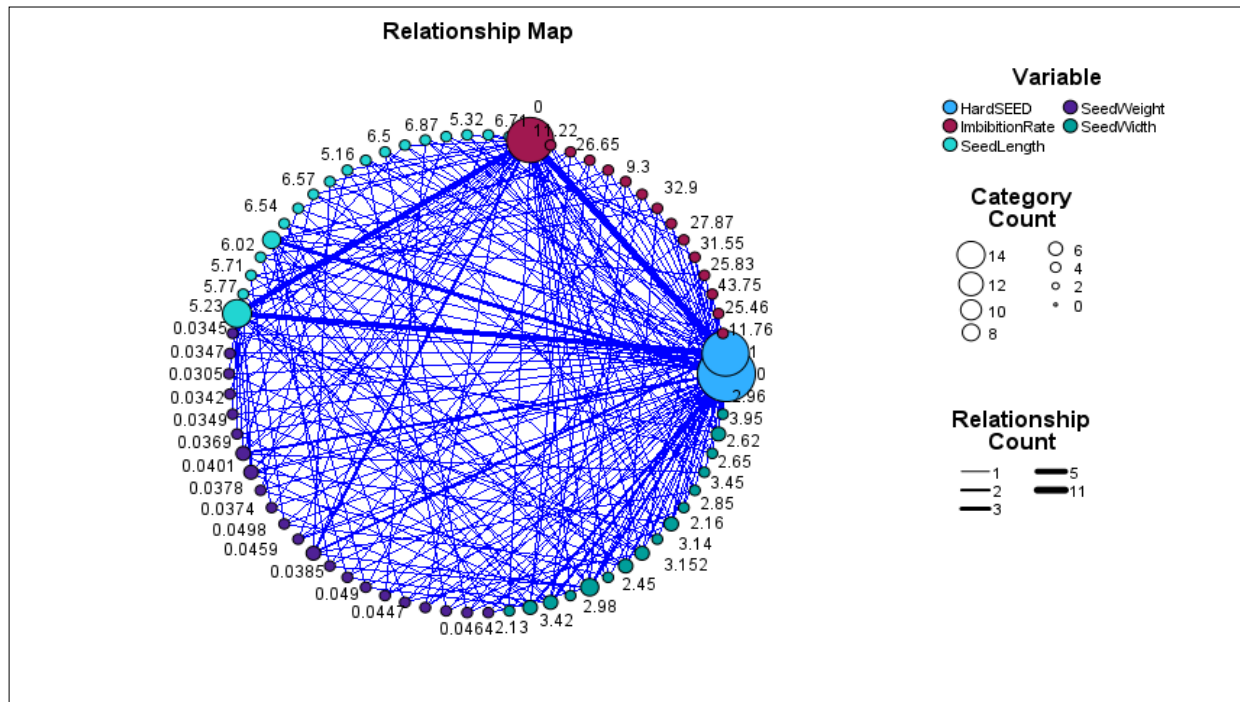


Fig. 2. Correlation and interaction among seed properties about hard seed phenomenon (Summer season) Variables (Nodes): hard seed (Blue): represents whether a seed is hard or not. Seed weight (Purple): represents the weight of the seed. Imbibition rate (Red): represents the rate at which seeds absorb water. Seed width (Teal): describes the width of the seed. Seed length (Cyan): means the length of the seed.

The relationship map underscores the critical role of imbibition rate and seed length in determining seed hardness. While other physical attributes like seed weight and width contribute, their impact is less pronounced. This visualization effectively captures the complex interactions between these seed traits, offering valuable insights for further analysis or hypothesis generation regarding seed dormancy and hardness. Table 2 presents a correlation matrix that shows the relationships between five variables related to seeds: Seed weight, Seed length, Seed width, Imbibition Rate % and Hard seed. This analysis examines the relationships between various seed traits, focusing on seed weight, length, width, hard seed occurrence and imbibition rate. Seed weight shows weak positive correlations with seed length (0.384) and seed width (0.331), suggesting that as seed weight increases, the length and width also tend to increase slightly, although the relationships are not strong. A weak negative correlation between seed weight and hard seed occurrence (-0.196) indicates little to no relationship between these traits. Similarly, the negative correlation between seed weight and imbibition rate (-0.041) is very weak, suggesting no meaningful connection between seed weight and the rate of water uptake.

Seed length, however, shows a moderate positive correlation with seed width (0.708), indicating that longer seeds tend to be wider. More notably, a strong negative correlation exists between seed length and hard seed occurrence (-0.790), implying that longer seeds are significantly less likely to be complicated. Additionally, seed length has a moderate positive correlation with imbibition

rate (0.555), showing that longer seeds tend to absorb water more quickly during imbibition. Research indicates that larger seed size is often associated with non-dormant seeds. Seed width is also positively correlated with seed length (0.708), reinforcing the relationship between these two dimensions. It strongly correlates negatively with hard seed occurrence (-0.673), indicating that wider seeds are less likely to be hard. A moderate positive correlation exists between seed width and imbibition rate (0.613), suggesting that wider seeds tend to imbibe water more rapidly.

Hard seed occurrence is strongly negatively correlated with both seed length (-0.790) and seed width (-0.673), emphasizing that shorter and narrower seeds are more likely to be hard. Additionally, hard seeds show a strong negative correlation with imbibition rate (-0.804), indicating that hard seeds are significantly less capable of absorbing water, which is consistent with the behaviour of seeds with impermeable seed coats. The seed coat is the primary barrier to water absorption in *Ormosia hosiei* seeds. It mainly consists of cuticles, palisade and thick-walled, impermeable cells (34). Hard seeds tend to be smaller (shorter and narrower), have a lower water absorption rate and are less likely to have a higher seed weight. Seed length and width are positively correlated, with longer seeds also tending to be wider. Imbibition rate is an important factor inversely related to the hardness of seeds. The seed coat of most hard seeds is exceptionally tough, primarily due to a well-developed stratum corneum, extensively developed palisade cells and bone stone cells (35).

Table 2. Correlation matrix of seed properties and hard seed phenomenon (Kharif season)

Parameters	Seed weight	Seed length	Seed width	Hard seed	Imbibition rate
Seed weight	1				
Seed length	0.384	1			
Seed width	0.331	.708**	1		
Hard seed	-0.196	-.790**	-.673**	1	
Imbibition rate	-0.041	.555**	.613**	-.804**	1

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

The imbibition rate is positively correlated with seed length (0.555) and seed width (0.613), suggesting that larger seeds in length and width tend to absorb water more quickly. The strong negative correlation between hard seed occurrence and imbibition rate (-0.804) further suggests that seeds classified as "hard" are less capable of water uptake. In the present study, the proportion of hard seeds was recorded higher in seed lots produced in summer compared to the *kharif* season in blackgram. The proportion of hard seeds was significantly lower in seed lots produced in the summer than in the *kharif* season in mung bean. The size of soybean and peanut seeds can impact germination and radicle emergence (37).

Overall, the analysis highlights that seed length and width are more closely related to the occurrence of hard seeds and the imbibition rate than seed weight, which shows weaker correlations with these traits. This suggests that seed weight alone may not predict whether a seed will be hard or how quickly it will imbibe water. The findings could be valuable in breeding programs or seed treatments aimed at reducing the occurrence of hard seeds or improving germination rates, as they pinpoint which seed traits are most closely related to these outcomes. The provided relationship map depicts the connections among various seed traits, specifically focusing on "Hard Seed," "Seed Weight," "Imbibition Rate," "Seed Length," and "Seed Width." The map is split into two clusters, each illustrating a network of relationships between these variables (Fig. 3).

The relationship map reveals two distinct clusters, each highlighting significant interactions among seed traits. The left cluster is larger and more densely interconnected, indicating a strong interaction among variables such as "Hard Seed" (blue), "Seed Weight" (purple) and "Imbibition Rate" (red). The right cluster, though smaller, still shows significant connections, particularly between "Hard Seed" and the physical dimensions of seeds, including "Seed Width" (green) and "Seed Length" (teal). The nodes on the map represent key variables, with the size of each node indicating the category count, reflecting the importance or frequency of the variable in these relationships. The lines between nodes represent relationship counts, with thicker lines indicating stronger or more frequent connections. In the left cluster, the "Hard Seed" node shows many strong

connections, especially with "Seed Weight" and "Imbibition Rate," suggesting a close relationship between hard seed formation and these two variables. The right cluster also highlights the influence of seed size on hard seed development, showing that structural properties of the seed coat could be important factors. Physical dormancy is problematic because it prevents timely and uniform seed germination (38).

The dominance of the "Hard Seed" variable in both clusters indicates that hard seed formation is significantly influenced by multiple factors, particularly seed weight and imbibition rate. This suggests that seeds with higher weight and lower imbibition rates are more likely to develop into hard seeds, which could pose challenges for germination. The strong connection between "Hard Seed" and "Imbibition Rate" underscores the critical role of water absorption in seed dormancy, where a low imbibition rate may correlate with a higher likelihood of hard seeds. In the right cluster, the relationships between "Hard Seed," "Seed Length," and "Seed Width" suggest that the physical dimensions of seeds are also influential in the development of hard seeds, possibly due to the structural properties of the seed coat. Peripheral values around each cluster represent specific data points linked to the core variables, illustrating how individual measurements relate to the broader network. The variability in these traits could be key to understanding the genetic or environmental factors affecting seed dormancy.

In conclusion, this relationship map demonstrates the complex interactions between various seed traits and their influence on the development of hard seeds. The strong connections between "Hard Seed," "Seed Weight" and "Imbibition Rate" suggest that these factors are closely linked and critical in determining seed dormancy. Understanding these relationships is essential for developing strategies to overcome seed dormancy, mainly through targeted breeding or pre-treatment methods to enhance germination rates.

Conclusion

The study explored the relationships between seed properties viz., seed weight, length, width, imbibition rate and hard seed occurrence in Black gram (*Vigna mungo*) across summer and *Kharif* seasons. Key findings showed that seed length and imbibition rate were strongly linked to the development of hard seeds, with longer seeds being less likely to develop hard coats and more likely to absorb water quickly. Seasonal variations indicated a higher proportion of hard seeds in the summer compared to the *Kharif* season, suggesting environmental influences on seed hardness. Overall, seed length and imbibition rate were critical factors in seed hardness, offering valuable insights for breeding and seed treatment strategies. By understanding these dynamics, plant breeders and agricultural scientists can make informed decisions in breeding programs and seed technology applications, ultimately improving crop performance. The findings from this research will provide valuable insights into the management of seed dormancy in Black gram. By correlating seed morphometrics with hardseededness,

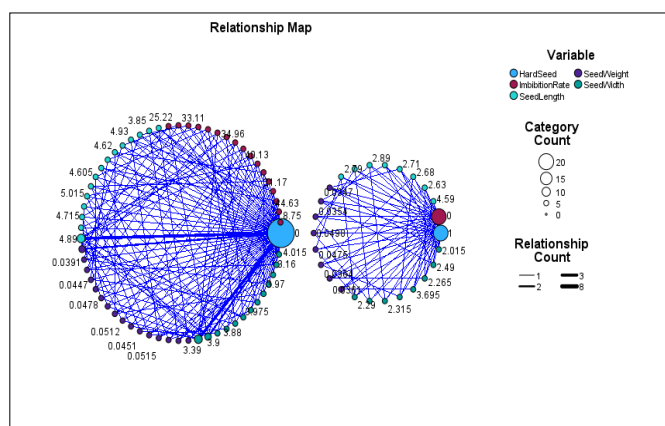


Fig. 3. Correlation and interaction among seed properties in relation to hard seed phenomenon (*Kharif* season) Variables (Nodes): The Left Cluster-"Hard Seed" (Blue), "Seed Weight" (Purple) and "Imbibition Rate" (Red). The Right Cluster-"Seed Width" (Green) and "Seed Length" (Teal).

breeders and farmers can adopt more informed practices, enhancing seed germination rates and improving crop yield and quality. This study contributes to the scientific understanding of seed dormancy in leguminous crops and supports the development of sustainable agricultural practices.

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Authors' contributions

MC contributed to the conceptualization, developed the research layout, took part in writing and editing and provided overall supervision throughout the study. SP was responsible for conceptualizing the research, gathering relevant literature and preparing the original manuscript draft. YA provided supervision, offered suggestions and made corrections. MD interpreted the results and contributed to writing the paper. VC also offered valuable suggestions, comments and corrections. RK provided suggestions, comments and corrections.

Compliance with ethical standards

Conflict of interest: Authors declare no conflict of interest

Ethical issues: None

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