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Research Note

Trait interrelationships for pre-harvest sprouting tolerance in greengram: A correlation and path analysis approach

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Abstract
Pre-harvest sprouting (PHS) in greengram (*Vigna radiata* L.) is a complex trait influenced by seed physiological properties and pod morphology, affecting yield and seed quality. This study employed correlation and path coefficient analyses to examine the relationships among seed and pod traits, water imbibition, and α-amylase activity contributing to PHS tolerance in 64 genotypes. Results showed that positive association of PHS with fresh seed germination, water imbibition by seeds and pods, α-amylase activity, indicating that rapid moisture uptake and early enzymatic activation enhance sprouting. Conversely, percentage of hard seed and epicuticular wax content exhibited negative correlations, highlighting their protective role in restricting water entry and delay in germination. Path analysis revealed pod surface area as the most influential trait directly promoting PHS, while pod length, diameter and pod wall thickness exerted strong indirect effects *via* surface area. Water imbibition, α-amylase activity and wax content also contributed indirectly, particularly in genotypes with lower hard seed percentages or favorable pod morphology. Traits exhibiting negative direct effects, such as higher percentage of hard seeds and stronger pod structure, suggest inherent physiological and morphological mechanisms that contribute to reduced susceptibility to pre-harvest sprouting. These findings demonstrate that both seed physiological characteristics and pod architecture jointly regulate PHS and provide practical selection criteria for breeding greengram genotypes with improved sprouting tolerance while maintaining high germination potential.

Keywords: Greengram, pre-harvest sprouting, correlation analysis, path analysis

Greengram (*Vigna radiata* L. Wilczek) is one of the most important pulse crops of South and South-East Asia, valued for its high protein content, short duration and ability to fix atmospheric nitrogen (Nivethitha *et al.,* 2023). It plays a crucial role in dietary security and crop diversification under both rainfed and irrigated ecosystems. However, seed yield and quality in greengram are frequently challenged by abiotic and biotic stresses, among which pre-harvest sprouting (PHS) has emerged as a serious constraint in humid and rain-prone environments. Pre-harvest sprouting refers to the germination of seeds within the pod prior to harvest, usually triggered by unseasonal rainfall and prolonged humidity at physiological maturity. PHS is also known as weather damage. The consequences of PHS include poor seed viability, reduced storability, loss of vigor and diminished market value.PHS tolerance in greengram is a complex, quantitative trait controlled by several morphological, physiological and biochemical factors. Important contributors include high seed dormancy along with high hard seed percentage, epicuticular wax content, low seed coat permeability, pod wall thickness, time to pod dehiscence, surface area, and enzymatic activity such as α-amylase. Since, these traits act in a complementary and often interdependent manner, correlation analysis becomes an essential statistical approach to understand their association and to identify reliable selection indices for breeding tolerant genotypes.

Recent research has highlighted substantial variability among greengram genotypes for PHS-related traits. Gupta *et al.* (2024) reported wide genetic variation for PHS in 83 greengram genotypes, where water imbibition by pods and seed germination percentage in pods were strongly correlated with sprouting intensity. Pramod *et al.* (2024) studied 30 greengram genotypes and observed high heritability for hard seed percentage and α-amylase activity, with yield showing positive association with days to maturity but negative correlation with epicuticular wax and pod wall thickness. Similarly, Reddy *et al.* (2025) demonstrated that genotypes with strong seed dormancy and delayed pod dehiscence exhibited reduced sprouting under humid conditions, confirming the role of these characters in tolerance. Parallel findings in related pulses, such as blackgram (*Vigna mungo*), further support the utility of such traits in resistance breeding (Verma *et al.,* 2024).

Despite these advances, systematic correlation studies integrating morphological, physiological and biochemical PHS traits in greengram remain limited. Understanding these associations is vital for identifying indirect selection criteria, enabling breeders to improve tolerance efficiently while sustaining yield and quality. Therefore, the present investigation was undertaken to assess the correlation, direct and indirect effects among the traits associated with PHS tolerance in diverse greengram genotypes, with the objective of identifying key traits that can serve as reliable indicators for breeding sprouting-tolerant varieties.

The present investigation was carried out during *Rabi,* 2023-2024 at Regional Agricultural Research Station, Lam, Guntur using 64 greengram genotypes (**Table 1**) in Alpha Lattice Design with three replications and eight blocks per each replication. Each genotype was grown in paired rows, where each row measured four meters in length with inter-row spacing of 30 cm x 10 cm. Fertilizers were applied at the rate of 20:50:0 NPK kg/ha, and necessary plant protection measures were taken to avoid insect and disease infestation. Irrigation was provided as per crop requirement, except during the maturity stage to simulate natural field conditions. To assess PHS tolerance, germination tests were conducted under laboratory conditions on physiologically matured pods and seeds using Complete Randomized Design (CRD) with three replications and data were recorded on different traits such as, pod length (cm), pod beak length (mm) using measuring scale; pod diameter (mm) and pod wall thickness (mm) were recorded using digital vernier calipers; water imbibition by pods (%), number of seeds germinated per pod (%) or pre-harvest sprouting (%) and number of hard seed in pods (%) were recorded from five pods of each replication; water imbibition by seeds (%), fresh seed germination (%), number of hard seed (%) were recorded from three sets of 50 seed samples from the bulk harvest of each plant. Epicuticular wax content on the pod wall (mg/g dry weight of pod) was estimated by a colorimetric assay (Ebercon *et al.,* 1977). Alpha-amylase activity in the seeds (mg of maltose released per gram of fresh tissue/minute) was estimated at harvest, 24 hours, 48 hours and 72 hours after germination, respectively according to (Rathi *et al.,* 2013). The statistical software used for analysis of the data is SAS JMP Statistics software version 17 and OPSTAT.

Pearson's correlation coefficients were estimated to determine the nature and magnitude of association among PHS and its associated traits (**Fig.1**). Seed hardness, a critical trait in preventing pre-harvest sprouting (PHS), is significantly influenced by seed coat characteristics. A strong positive correlation was observed between percentage of hard seeds and percentage of hard seeds within pods (r= +0.77***) suggests that the dormancy trait is expressed consistently at both seed and pod levels. This stability highlights the genetic basis of hard seed formation and its role in restricting water uptake, thereby reducing the risk of premature germination. Such a relationship confirms the importance of hard seed percentage as a reliable indicator trait for pre-harvest sprouting tolerance in greengram; percentage of hard seeds and epicuticular wax content (r = +0.33**), indicating that wax layer acts as a barrier, reducing water and gas permeability, thereby enhancing PHS tolerance. Conversely, negative correlations between hard seed percentage and pre-harvest sprouting (r = -0.74***), fresh seed germination (r = -0.99***), water imbibition by seeds (r = -0.70***), water imbibition by pods (r = -0.40***), alpha-amylase activity at 48 hours after germination (r = -0.35**) and alpha-amylase activity at 24 hours (r = -0.27*) confirms that increased seed hardness inhibits water uptake and metabolic activation during germination, mitigating PHS. Similar results were observed by Singh *et al.* (2012), Lamichaney *et al.* (2018), Lamichaney *et al.* (2023) in greengram and Verma *et al.* (2024) in blackgram.

Table 1. Name and origin of greengram genotypes employed in the study

S.No.	Genotype	Origin	S.No.	Genotype	Origin
1	Tripura Moong 1	Tripura	33	PUSA 1641	New Delhi
2	MH 125 (Basanti)	Haryana	34	PUSA 9072	New Delhi
3	HUM 12	Uttar Pradesh	35	SML 1115	Punjab
4	Shalimar Moong 2	Jammu & Kashmir	36	TARM 1	Maharashtra
5	Pairy Moong	Chattisgarh	37	MH 1142	Haryana
6	Pratap	Assam	38	MH 421	Haryana
7	Virat	Uttar Pradesh	39	PUSA 1431	New Delhi
8	IPM 2-14	Uttar Pradesh	40	VBN 2	Tamil Nadu
9	Vamban 1	Tamil Nadu	41	Soorya (IPM 512-1)	Uttar Pradesh
10	RMG 344	West Bengal	42	Meha (IPM 99-125)	Uttar Pradesh
11	RMG 975	West Bengal	43	PUSA M 19111	New Delhi
12	BM 4	Maharashtra	44	TMB 146	Mumbai
13	GM 6	Uttar Pradesh	45	VGG 15-30	Tamil Nadu
14	HUM 1	Uttar Pradesh	46	IPM 312-9	Kanpur
15	TARM 2	Maharashtra	47	LGG 668	Andhra Pradesh
16	PKV AKM 4	Maharashtra	48	LGG 657	Andhra Pradesh
17	Pragya	Chattisgarh	49	LGG 685	Andhra Pradesh
18	GM 4	Gujarat	50	LGG 450	Andhra Pradesh
19	HUM 16	Uttar Pradesh	51	LGG 410	Andhra Pradesh
20	DGGV 2	Karnataka	52	PM 110	Uttar Pradesh
21	MH 318	Haryana	53	WGG 42	Telangana
22	Sattya (MH 2-15)	Haryana	54	LGG 633	Andhra Pradesh
23	SGC 16	Assam	55	LGG 688	Andhra Pradesh
24	Ganga 8	Rajasthan	56	PM 1711	Pantnagar
25	TM 96-2	Maharashtra	57	PUSA VISHAL	New Delhi
26	ML 2506	Punjab	58	AKM 12-28	Akola
27	PUSA 1371	New Delhi	59	VBN 4	Vamban
28	PUSA 9531	New Delhi	60	LGG 713	Andhra Pradesh
29	Shikha (IPM 410-3)	Uttar Pradesh	61	LGG 716	Andhra Pradesh
30	LGG 460	Andhra Pradesh	62	LGG 717	Andhra Pradesh
31	Shalimar Moong 1	Jammu & Kashmir	63	LGG 706	Andhra Pradesh
32	HUM 6	Uttar Pradesh	64	AGG 35	Vamban

PHS is positively associated with fresh seed germination (r = +0.74***), water imbibition by seeds (r = +0.48***), water imbibition by pods (r = +0.42***) and alpha-amylase activity at 48 hours (r = +0.34**), suggesting that genotypes with high germination potential are more prone to sprouting under wet conditions. Singh *et al.* (2012), Ahmad *et al.* (2014), Singh *et al.* (2017), Lamichaney *et al.* (2018), Lamichaney *et al.* (2023), Gore *et al.* (2024) and Verma *et al.* (2024) reported similar reports on the positive association of PHS with the above traits in greengram and blackgram. These findings highlight the complex interplay between pod characteristics, seed physiology, and environmental factors in determining PHS susceptibility.

Pod architecture plays a crucial role in seed protection and pre-harvest sprouting (PHS). Pod surface area, positively correlated with pod dimensions like pod length (r = +0.84***), podwall thickness (r = +0.40**), pod diameter (r = +0.78***), reflects thicker pod walls that impede water penetration, thereby delaying germination processes. This study indicates that longer pods are generally associated with thicker pod walls and wider pod diameters, providing enhanced mechanical protection to developing seeds. Epicuticular wax content further contributes to PHS tolerance by hindering fresh seed germination (r = -0.34**) and alpha-amylase activity at 48 hours after germination (r = -0.28*). These findings collectively highlight the protective role of seed coat characteristics in modulating seed dormancy and preventing PHS.

The study reveals a significant positive correlation between fresh seed germination and water imbibition by

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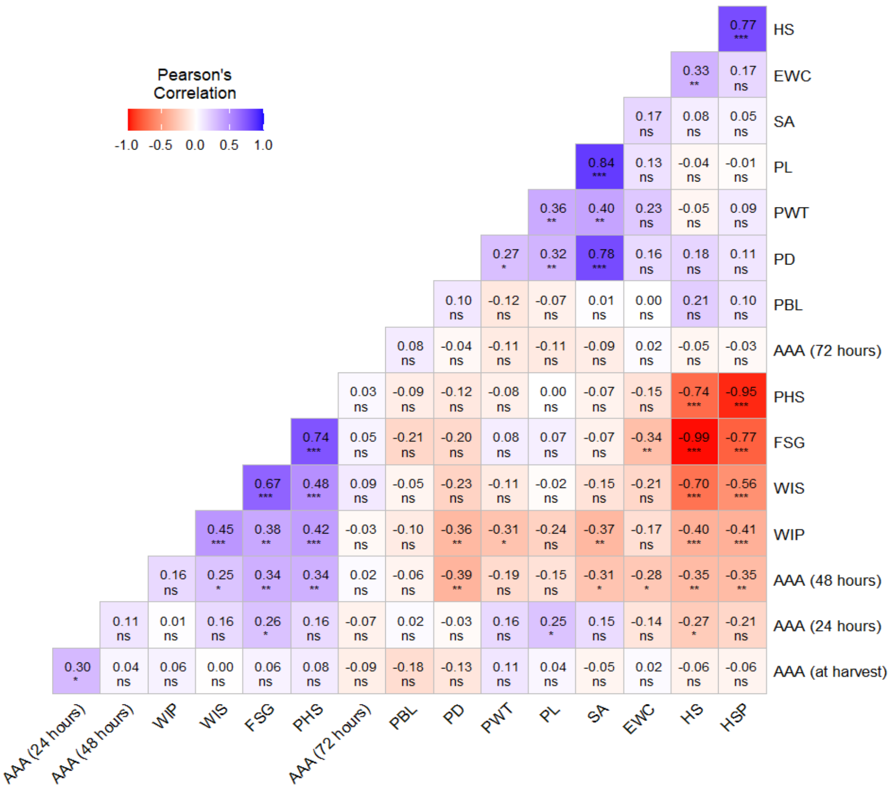

Fig.1.Correlation studies among PHS and its associated traits

*** Significant at 0.001 level, ** Significant at 0.01 level; * Significant at 0.05 level.
WIS-Water Imbibition by Seeds, FSG-Fresh Seed Germination, HS- Number of hard Seed, WIP-Water Imbibition by pods, HSP-Number of hard Seed in pods, EWC-Epicuticular Wax Content, AAA (Harvest)-Alpha amylase at harvest, AAA (24 h)-Alpha amylase at 24 hours after germination, AAA (48 h)-Alpha amylase at 48 hours after germination, AAA (72 h)-Alpha amylase at 72 hours after germination, PL-Pod Length, PBL- Pod Beak Length, PD-Pod Diameter, PWT-Podwall Thickness, SA-Surface Area, PHS-Pre-Harvest Sprouting value

seeds (r = +0.67***), pods (r = +0.38**), alpha-amylase activity at 48 hours after germination (r = +0.34**), 24 hours after germination (r = +0.26*). This suggests that rapid moisture uptake facilitates early germination by quickly hydrating the embryo and stimulating enzymatic activity for starch reserve mobilization. Similar results were observed by Lamichaney *et al*. (2018). Furthermore, a positive correlation between amylase activity at harvest and during germination indicates a consistent enzymatic potential across germination stages. These correlations collectively emphasize that rapid hydration and early enzymatic activation drive sprouting, while traits like hard seed percentage confer a protective influence. These findings underscore the importance of considering seed and pod characteristics in breeding strategies aimed at improving germination efficiency and pre-harvest sprouting tolerance.

Path analysis is a statistical approach employed to partition the direct and indirect effects of various traits on a dependent variable (Dewey and Lu, 1959) (**Table 2 and Fig.2**). A key component of path analysis is the assessment of residual effects, which represents the influence of other potential factors not considered in the study on the dependent variable. In the present study, observed residual value of path effect was 0.074. The direct effect of surface area (1.240) is positive and high on pre-harvest sprouting value. The fresh seed germination percentage (0.012), water imbibition by pods percentage (0.065), epicuticular wax content (0.012), alpha-amylase activity at 72 hours after germination (0.007), pod beak length (0.039) and podwall thickness (0.008) had a negligible direct effect on pre-harvest sprouting value. Water imbibition by seeds percentage (-0.124), percentage of hard seed in pods (-0.941), pod length (-0.807) and pod diameter (-0.719) exhibited negative direct effects on pre-harvest sprouting value. A high positive direct effect in path analysis indicates a strong causal influence of one variable on another, suggesting that an increase in the independent variable leads to a corresponding increase in the dependent variable. The direct effect of surface area on pre-harvest sprouting value indicates that surface area is a major determinant influencing sprouting behavior. A larger surface area increases the exposure of pods to

Table 2. Path coefficient analysis among PHS and its associated traits

	WIS	FSG	HS	WIP	HSP	EWC	AAA (h)	AAA (24 h)	AAA (48 h)	AAA (72 h)	PL	PBL	PD	PWT	SA	PHS
WIS	**-0.124**	0.008	0.059	0.029	0.530	-0.002	-0.001	-0.009	-0.003	0.001	0.013	-0.002	0.164	-0.001	-0.181	**0.484**
FSG	-0.083	**0.012**	0.085	0.024	0.728	-0.001	0.002	-0.014	-0.001	0.001	-0.054	-0.008	0.140	0.001	-0.085	**0.740**
HS	0.086	-0.012	**-0.085**	-0.026	-0.725	0.004	-0.002	0.015	0.005	-0.001	0.032	0.008	-0.127	-0.001	0.094	**-0.735**
WIP	-0.056	0.004	0.034	**0.065**	0.383	-0.002	0.002	-0.001	-0.002	-0.001	0.191	-0.003	0.262	-0.002	-0.456	**0.419**
HSP	0.069	-0.009	-0.066	-0.026	**-0.941**	0.002	-0.002	0.011	0.001	-0.001	0.011	0.004	-0.076	0.001	0.064	**-0.954**
EWC	0.025	-0.004	-0.028	-0.010	-0.160	**0.012**	0.001	0.007	0.004	0.001	-0.102	0.001	-0.112	0.001	0.215	**-0.150**
AAA (h)	0.001	0.001	0.005	0.003	0.053	0.001	**-0.016**	-0.001	-0.001	-0.029	-0.006	0.094	0.001	-0.064	-0.064	**0.084**
AAA (24 h)	-0.019	0.003	0.023	0.001	0.197	-0.001	0.012	**-0.057**	-0.001	-0.001	-0.205	0.001	0.017	0.001	0.184	**0.157**
AAA (48 h)	-0.031	0.004	0.030	0.010	0.331	-0.003	0.001	-0.006	**-0.014**	0.001	0.122	-0.002	0.277	-0.001	-0.379	**0.341**
AAA (72 h)	-0.011	0.001	0.004	-0.002	0.028	0.001	-0.003	0.004	-0.001	**0.007**	0.089	0.003	0.025	-0.001	-0.115	**0.029**
PL	0.002	0.001	0.003	-0.015	0.013	0.001	0.001	-0.014	0.002	-0.001	**-0.807**	-0.002	-0.233	0.002	1.046	**-0.001**
PBL	0.006	-0.002	-0.018	-0.006	-0.098	0.000	-0.007	-0.001	0.001	0.001	0.058	**0.039**	-0.070	-0.001	0.008	**-0.092**
PD	0.028	-0.002	-0.015	-0.023	-0.100	0.001	-0.005	0.001	0.005	-0.001	-0.262	0.003	**-0.719**	0.002	0.967	**-0.119**
PWT	0.014	0.001	0.003	-0.020	-0.086	0.003	0.004	-0.009	0.002	-0.001	-0.296	-0.004	-0.196	**0.008**	0.494	**-0.083**
SA	0.018	-0.001	-0.006	-0.024	-0.048	0.002	-0.002	-0.008	0.004	-0.001	-0.681	0.001	-0.560	0.003	**1.240**	**-0.065**

RESIDUAL=0.074

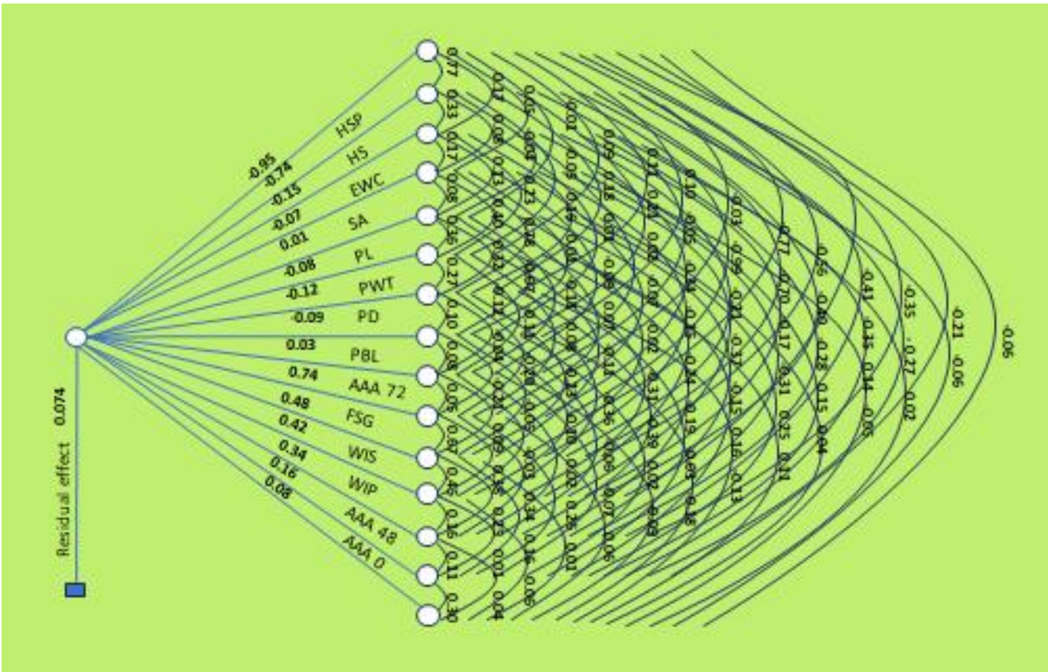

Fig.2.Direct and Indirect effects of PHS and its associated traits

WIS-Water Imbibition by Seeds, FSG-Fresh Seed Germination, HS- Number of hard Seed, WIP-Water Imbibition by pods, HSP- Number of hard Seed in pods, EWC-Epicuticular Wax Content, AAA (Harvest)-Alpha amylase at harvest, AAA (24 h)-Alpha amylase at 24 hours after germination, AAA (48 h)-Alpha amylase at 48 hours after germination, AAA (72 h)-Alpha amylase at 72 hours after germination, PL-Pod Length, PBL- Pod Beak Length, PD-Pod Diameter, PWT-Podwall Thickness , SA-Surface Area, PHS-Pre-Harvest Sprouting value

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external moisture, thereby enhancing water absorption and creating favorable conditions for seed germination before harvest. Similar results Singh *et al.* (2016) in greengram, Rigatti *et al.* (2019) in wheat, Ahmad and Belwal (2020) in greengram and Singh *et al.* (2022) in greengram.

The high positive indirect effect of water imbibition by seeds (0.530) on pre-harvest sprouting (PHS) through percentage of hard seed in pods suggests that increased water uptake enhances sprouting susceptibility when mediated by a reduced proportion of hard seeds. Hardseededness contributes to dormancy and resistance to premature germination. Therefore, genotypes with greater water absorption and lower percentage of hard seed are more prone to pre-harvest sprouting, highlighting the importance of seed coat permeability in determining sprouting tolerance.

Water imbibition by pods exhibited high and positive indirect effects on pre-harvest sprouting (PHS) through hard seed percentage (0.383), pod diameter (0.262) and pod length (0.191). This suggests that higher water absorption by pods indirectly increases PHS by influencing seed dormancy and pod morphology. Specifically, pods with lower percentage of hard seed are more prone to sprouting, while larger pod diameter and length may enhance moisture retention and create favorable micro-environments for germination. Similar findings have been reported in greengram, where pod traits and water imbibition were shown to significantly affect seed germination and PHS susceptibility (Pramod *et al.,* 2024). These results highlight that both pod physiological and morphological traits jointly mediate pre-harvest sprouting, providing important targets for breeding PHS-tolerant greengram genotypes.

Epicuticular wax content exhibited a positive indirect effect on pre-harvest sprouting (PHS) through surface area (0.215), indicating that genotypes with higher wax accumulation may indirectly influence sprouting by interacting with pod surface traits. Increased surface area, in combination with low epicuticular wax, can affect water retention, thereby promoting conditions favorable for pre-harvest sprouting.

Alpha-amylase activity at 24 hours after germination showed a positive indirect effect on pre-harvest sprouting (PHS) through percentage of hard seed in pods (0.197). This indicates that higher enzymatic activity can enhance sprouting susceptibility indirectly by interacting with the proportion of hard seeds, as seeds with lower hardseededness are more prone to germination. Similar studies in mungbean and greengram have reported that enzymatic activities, particularly alpha-amylase, are closely associated with seed dormancy and germination processes, influencing PHS susceptibility when coupled with pod traits like hard seed content (Reddy *et al.,* 2025). Similarly, alpha-amylase activity at 48 hours after germination showed positive indirect effects on pre-harvest sprouting (PHS) through pod length (0.122) and pod diameter (0.277). This suggests that higher enzymatic activity can promote sprouting indirectly by interacting with pod morphological traits, where larger pods may facilitate better water retention or create a favorable microenvironment for germination.

Pre-harvest sprouting (PHS) in greengram is a multifaceted trait governed by seed physiology and pod morphology. Correlation and path coefficient analysis reveal intricate relationships between pod and seed structure, physiological responses and enzymatic activity. A positive correlation exists between PHS and fresh seed germination, seed and pod imbibition, and α-amylase activity, indicating that rapid hydration and early enzymatic activation are key factors in field sprouting. Conversely, an inverse relationship between PHS and percentage of hard seeds, along with epicuticular wax content, emphasizes their protective role. Breeding strategies should therefore consider hard seed percentage, wax deposition, and pod structure as indirect selection criteria to enhance PHS tolerance while maintaining germination capacity. Path analysis identifies pod surface area as the most influential trait directly promoting PHS. While pod length, pod diameter and pod wall thickness exert strong indirect effects *via* surface area; water imbibition and alpha-amylase activity also contribute indirectly, particularly with reduced percentage of hard seed. Consequently, breeding programs should prioritize both seed physiological characteristics and pod morphological traits to develop PHS tolerant greengram genotypes.

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