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Research Articles

Genotypic variability studies and identification of pre-harvest sprouting tolerant wild *Vigna*

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ABSTRACT

Seed dormancy is the main component of domesticated traits, loss of which results into rapid and uniform germination, synchronous maturity and ease in harvesting. However, breakdown or loss of fresh seed dormancy (FSD) has made seeds vulnerable to pre-harvest sprouting (PHS). Though, seeds of *Vigna* species are protected by pod, these are still susceptible to PHS. Identification of donor(s) having short duration of FSD (30-45 days) has become utmost important to transfer the trait to high yielding varieties. The wild progenitors and wild relatives of the cultivated *Vigna* offer a source of several useful traits; therefore, the experiment was initiated to understand the level of variation in PHS and fresh seed germination (FSG) in wild accessions of *Vigna* species. PHS and FSG among 59 *Vigna* accessions ranged between 0-100%, with a mean of 55.86% and 61.19%, respectively. Five accessions (IC276983, IC349701, Trichy Local 1, LRM/13-33 and LRM/13-26) of *Vigna trilobata* and one accession (IC251440) of *Vigna sublobata* recorded no pod loss due to PHS with 0% FSG (2.5% in *V. sublobata*). Seed size showed a positive association with PHS, where smaller seeds (100-SW <1 g) recorded comparably low PHS (30.82%) than bold seeds (69.06 and 62.94% with 100-SW between 1-2 g and > 2 g, respectively). The accessions identified, especially *V. sublobata* (crossing compatible) could be used as a source of PHS tolerance to transfer dormancy in *Vigna* species, particularly in mungbean and urdbean.

Keywords: Dormancy, Fresh seed germination, Pre-harvest sprouting, *Vigna* species

Quality seed is a vital input for successful crop establishment and agricultural production. Quality seed comprises genetic and physical purity, viability, germination, vigour, seed health and physical appearance like colour, size, shape and weight (Hampton 2002). Each of these seed quality parameters are influenced by climatic variables prevailing during the crop growth period, particularly during reproductive growth, maturity and during storage (Hampton *et al*. 2013, Maity *et al*. 2016, Rashid *et al*. 2018, Lamichaney *et al*. 2019). Among these climatic variables, rainfall at the time of crop maturity can result into pre-harvest sprouting (PHS) and could affect the quality and quantum of seed/grain produced. PHS refers to germination of mature seeds within pods/fruits while still attached to mother plant. In legumes, despite that seeds are placed safely inside pods, PHS occurs frequently, especially in *Vigna*. Sporadic rain coupled with high RH at the time of crop maturity contributes to PHS, resulting into reduced yield and quality of seed (Kulwal *et al*. 2012). Durga and Kumar (1997) had reported that PHS can reduce mungbean yield by 60–70%. Therefore, development of cultivars having a short duration of dormancy (30-45 days) has become imperative to prevent the loss incurred by PHS, for which identification of donors having fresh seed dormancy (FSD) is vital. Earlier, number of mungbean and urdbean germplasm has been screened for PHS tolerance (Anupama *et al*. 2012, Lamichaney *et al*. 2018). Selection was made consciously or unconsciously for traits that favoured early germination (lack of dormancy) for ease in cultivation and uniform establishment. As a result, cultivated crops germinate faster than the wild progenitors. Therefore, the wild *Vigna* may be used for introgression of limited dormancy in cultivated lines with an aim of developing climate resilient cultivars (Pratap *et al*. 2015). A comprehensive analysis of fresh seed germination, hard-seededness, tolerance to PHS and their level of variation in wild *Vigna* species is currently lacking in the literature. Thus, the purpose of this study was to assess variation in fresh seed germination and tolerance to PHS among diverse collections of wild *Vigna* species.

MATERIALS AND METHODS

Fifty nine accessions of 11 Asiatic *Vigna* species (10 accessions of *V. radiata*: 18 of *V. sublobata*, 5 of *V. mungo*, 15 of *V. trilobata*, 3 of *V. umbellata*, 2 each of *V. dalzelliana* and *V. setulosa*, 1 each of *V. pilosa*, *V. silvestris*, *V. khandalensis* and *V. aconitifolia*) were used in this experiment (Table 1) conducted during 2017-18 at ICAR-Indian Institute of Pulses Research, Kanpur. Phenotyping for PHS and FSG was done

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following Lamichaney *et al*. (2018). For PHS, experiment was conducted in 3 replications each of 5 pods placed in Petri dishes, lined with water-soaked Whatman No. 1 filter paper. After culturing the pods, the Petri-dishes were incubated at 25°C and seed germination in a pod (%) was recorded after 4 days of incubation. Germination of seed inside a pod was used as a measure for PHS tolerance. Number of germinated seeds was recorded in each replication and percentage in a pod was calculated. Higher the PHS value, higher is the susceptibility to PHS. For FSG, three replications each of 50 seeds were placed in Petri dishes lined with water-soaked Whatman No. 1 filter paper in an incubator at 25°C. FSG (%) was recorded after 7 days of incubation as per ISTA (2011). For the calculation of germination index (GI), seeds were examined daily for germination and were considered germinated when seedling reached approximately 2mm length. GI was calculated following the equation given by Basra *et al*. (2005);

$$GI = \sum_{n=1}^{13} \left(\frac{Ni}{Di} \right)$$

where, Ni represents mean number of seeds germinated on i^{th} day while, Di represents number of days of germination.

PHS and FSG data were arcsin transformed and the single factor ANOVA was done using freely available online software OPSTAT (Sheoran *et al*. 1998). Principal component analysis was conducted using PAST (Paleontological statistics) version 3.02.

RESULTS AND DISCUSSION

The analysis of variance revealed significant differences for pre-harvest sprouting, fresh seed germination, germination index and 100-seed weight among 59 accessions under study indicating sufficient variability for the traits under study among accessions. Diverse *Vigna* accessions recorded a wide variability in relation to seed germination in pod (PHS value). PHS and FSG in 59 accessions ranged between 0.00–100%. Among the 59 Asiatic *Vigna* accessions, five accessions (IC276983, IC349701, Trichy local 1, LRM/13-33 and LRM/13-26) of *V. trilobata* and one (IC251440) of *V. sublobata* were tolerant to PHS as they recorded no pod loss due to PHS (Table 1). All these accessions also recorded no FSG even after 13 days of incubation, except for *V. sublobata* (IC251440) which recorded FSG of 2.5%. Proportion of PHS for *trilobata* and *sublobata* accessions were 33.33% (5/15 accessions) and 5.55% (1/18 accession), respectively. Maximum pod loss due to PHS was recorded in the accession W21 belonging to the species *V. radiata*. Likewise, 10 accessions, W95, W53, W59 (*V. sublobata*), W107 (*V. khandalensis)*, W15 (*V. mungo*), and W96, W18, W24, W32, W20 (*V. radiata*) recorded maximum germination (100%). Significant positive correlation was found between PHS value and FSG with a correlation coefficient of 0.869. GI and FSG also recoded very high and positive correlation with a correlation coefficient of 0.832. During domestication, selection was made for traits those were useful to mankind for food, fuel, fibre, fodder, *etc*., which led to improvement in traits like reduction in toxic compounds, bigger seed size, early and uniform germination, non-shattering habit, synchronous maturity, better nutritive value, *etc*. However, all these traits were the plant/seed defensive traits, and improvement of these traits for human welfare has somehow made plants/seeds susceptible to other biotic or abiotic stresses. For example, reduction in toxic compounds (tannins, phenols) has made plants/seeds susceptible to diseases and pests. Likewise, complete loss

Table 1 Wild *Vigna* accessions and their distribution in terms of PHS susceptibility

Number	Species	IC No./Collection No.	FSG (%)	PHS (%)	100-seed weight (g)	GI
W50	*Vigna sublobata*	248343	72.50	72.17	0.40	8.09
W26	*Vigna trilobata*	251416	85.00	80.45	0.54	40.00
W107	*Vigna khandalensis*		100.00	71.67	0.63	22.81
W85	*Vigna trilobata*	LRM/13-38	20.00	19.69	0.67	4.59
W86	*Vigna trilobata*	LRM/13-36	25.00	25.68	0.70	2.95
W108	*Vigna umbellata*		17.50	32.69	0.75	3.07
W43	*Vigna trilobata*	210580	90.00	90.29	0.75	18.31
W94	*Vigna trilobata*	NSB 007	45.00	30.77	0.78	20.28
W63	*Vigna trilobata*	276983	0.00	0.00	0.82	0.00
W65	*Vigna trilobata*	331454	22.50	11.52	0.84	2.76
W62	*Vigna trilobata*	349701	0.00	0.00	0.85	0.00
W68	*Vigna sublobata*	251440	2.50	0.00	0.85	1.25
W100	*Vigna trilobata*	Trichy Local 1	0.00	0.00	0.88	0.00
W84	*Vigna trilobata*	LRM/13-37	40.00	13.57	0.88	4.31
W64	*Vigna trilobata*	331454	17.50	27.54	0.91	1.98
W58	*Vigna sublobata*	LRM/13-30	40.00	47.89	0.92	9.35
W82	*Vigna trilobata*	LRM/13-33	0.00	0.00	0.92	0.00

Contd.

Table 1 (*Concluded*)

Number	Species	IC No./Collection No.	FSG (%)	PHS (%)	100-seed weight (g)	GI
W66	*Vigna trilobata*	331456	47.50	23.14	1.01	8.87
W101	*Vigna trilobata*	Trichy local 2	25.00	17.44	1.03	4.87
W59	*Vigna sublobata*	TCR 512	100.00	72.78	1.09	49.38
W30	*Vigna setulosa*	277039	82.50	80.23	1.14	14.17
W52	*Vigna sublobata*	251436	85.00	76.67	1.18	21.72
W31	*Vigna silvestris*	277021	65.00	65.00	1.23	19.05
W80	*Vigna aconitifolia*	LRM/13-11	75.00	95.00	1.31	37.50
W53	*Vigna sublobata*	251438	100.00	95.00	1.40	47.81
W22	*Vigna sublobata*	349699	80.00	89.84	1.41	24.69
W10	*Vigna mungo*	251397	82.50	91.67	1.43	14.19
W41	*Vigna sublobata*	210575	97.50	39.22	1.45	30.69
W20	*Vigna radiata*	251427	100.00	89.45	1.55	50.00
W45	*Vigna dalzelliana*	203864	67.50	79.96	1.57	21.75
W72	*Vigna umbellata*	251445	100.00	54.33	1.71	24.17
W1	*Vigna radiata*	251432	97.50	94.00	1.72	30.42
W83	*Vigna trilobata*	LRM/13-26	0.00	0.00	1.72	0.00
W51	*Vigna sublobata*	251435	77.50	87.50	1.74	14.10
W39	*Vigna sublobata*	210563	67.50	92.48	1.82	4.66
W29	*Vigna sublobata*	277036	42.50	35.52	1.84	20.18
W44	*Vigna dalzelliana*	247408	60.00	85.19	1.86	9.66
W32	*Vigna radiata*	277014	100.00	85.85	1.94	39.17
W18	*Vigna radiata*	251426A	100.00	77.08	2.02	42.13
W23	*Vigna sublobata*	256158	30.00	58.44	2.02	5.49
W25	*Vigna sublobata*	247406	85.00	81.82	2.03	18.11
W42	*Vigna pilosa*	210576	60.00	68.72	2.03	15.89
W24	*Vigna radiata*	253924	100.00	81.90	2.05	37.02
W9	*Vigna sublobata*	251396	52.50	38.33	2.27	5.66
W36	*Vigna sublobata*	251423	17.50	18.52	2.30	2.58
W38	*Vigna sublobata*	247407	42.50	40.83	2.35	12.61
W49	*Vigna umbellata*	248326	40.00	47.54	2.40	3.47
W95	*Vigna sublobata*	TCR 279	100.00	65.64	2.41	49.38
W67	*Vigna sublobata*	TCR 513	42.50	56.45	2.42	3.68
W69	*Vigna setulosa*	251441	72.50	63.37	2.42	16.54
W3	*Vigna radiata*	251434	20.00	17.08	2.66	7.17
W5	*Vigna radiata*	57175	97.50	84.29	2.98	48.75
W21	*Vigna radiata*	251431	85.00	100.00	3.03	28.47
W19	*Vigna radiata*	251426B	85.00	91.88	3.21	40.00
W15	*Vigna mungo*	251390	100.00	74.76	3.36	23.47
W12	*Vigna mungo*	251385	57.50	37.90	3.41	21.23
W92	*Vigna mungo*	ZAP/10-7	97.50	72.50	3.55	45.63
W14	*Vigna mungo*	251387	95.00	68.57	3.61	24.55
W96	*Vigna radiata*	Mung Sel 4-I	100.00	76.11	4.35	25.00
		Mean	61.19	55.86	1.71	18.71
		Range	0.00-100.00	0.00-100.00	0.40-4.35	0.00-50.00
		SE(m)	7.14	8.81	0.05	1.93
		CV	18.62	39.21	3.78	36.42
		CD (0.5%)	20.27	24.56	0.13	5.47

or reduction of FSD has made seed/grain susceptible to weathering damage and PHS. In legumes, hard-seededness is often considered undesirable, as it hampers water uptake resulting into difficulty in milling. Also, persistent hard-seededness may cause non-uniform germination in field affecting crop establishment and yield. Therefore, selection was made against the trait that favours hard-seededness. Hence, the wild types which had not undergone artificial selection have been reported to have a higher level of FSD than the cultivated types (Dorian and Robin 2009). In our previous experiment on PHS in mungbean, we observed that germplasm lines had better PHS tolerance than cultivated varieties (Lamichaney *et al*. 2018).

There was a positive correlation on PHS, FSG and GI value with seed size. The overall mean PHS, FSG and GI values were 55.86%, 61.19% and 18.71**,** respectively. Smaller seeds with 100-seed weight of <1 g recorded comparably lower PHS (30.82%), FSG (34.11%) and GI (8.23) values as compared to those accessions with larger seeds. Seeds with 100-seed weight between 1–2 g recorded PHS, FSG and GI value of 69.06%, 73.92% and 23.19, while, seeds with 100-seed weight >2 g recorded PHS, FSG and GI value of 62.94%, 70.47% and 22.70, respectively (Fig 1). In general small seeded *Vigna* accessions had better tolerance to PHS with low FSG and GI as compared to the accessions with large seeds. Williams (1989) reported higher occurrence of hard-seededness in small seeded than the large seeded mungbean and were in concurrence with our findings. Humphry *et al.* (2005) conducted QTL analysis to decipher the relationship between hard-seededness and seed weight in mungbean. They reported 4 QTLs for hard-seededness and 11 for seed weight while, two of the four QTLs for hard-seededness co-localised with loci conditioning seed weight. Development of medium-large and hard-seeded varieties has now become an important breeding objective of mungbean improvement programme and linkage between these two traits perhaps, explains the reasons for difficulty in maintaining hard-seededness in large seeded varieties. Highly significant positive association was observed between PHS value and FSG, with a pearson correlation coefficient of 0.869 (P< 0.01). Despite high correlation between the seed germination in a pod (PHS) and after removing from the pod (FSG), the germination % was higher after removing the seed from the pod, which could be attributed to mechanical or physical barrier provided by pod restricting the availability of water to the seeds, preventing its imbibition and consequently affecting the germination of seeds. The accessions (W5, W20, W53, W59, W95) having very high GI (Table 1) of more than 48 (48 seeds out of 50 germinated within 24 h) had PHS value ranging from 65–90% indicating the role of pod wall as a physical barrier. Lamichaney *et al.* (2018) had reported positive association between water holding capacity of pod with PHS in mungbean accessions, which is influenced by the wax content of pod (Vijay and Gupta, 2008) and pod wall thickness (Rao *et al*. 2007, Ahmad *et al*. 2014).

It was interesting to note that there was a significant

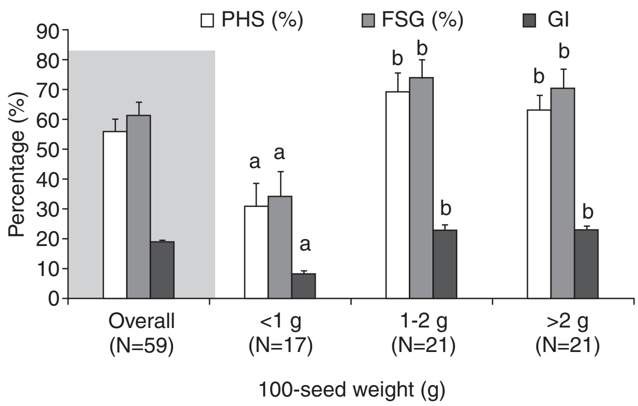

Fig 1 Differences in pre-harvest spouting (PHS), fresh seed germination (FSG) and germination index (GI) based on seed size. Data for FSG and PHS were recorded on 7th and 4th day of incubation respectively, at 25°C. For GI calculation, germination count was made daily up to 13 days. Bar represents standard error (±).

difference in PHS and FSG value due to differences in flowering and maturity time (Table 2). The accessions that flowered early during late July had the least value for PHS (21.34%) and FSG (21.25%) followed by accessions flowering in early August (60.98%, 65.08%) and middle or late August (65.96%, 76.18%). Likewise, accessions maturing during middle of August recorded least PHS and FSG value of 38.58 and 39.50% respectively, followed by accessions maturing during end of August (55.34%, 60.74%), and early to middle September (71.93, 81.17%). It was observed that the early maturing *Vigna* accessions had higher tolerance to PHS with high seed dormancy as depicted by low FSG then late maturing accessions. This may be due to higher selective pressure for PHS resistance as these months receives maximum rainfall.

Development of medium-large seeded varieties (3.5-4.2 g/100 seeds) is an important breeding objective considering its acceptance by the consumers. However, in the present scenario of changing climate with uncertainties in rainfall, especially during crop maturity stages, incorporation of hard-seededness (physical dormancy) has become utmost important. The identified accession, IC 251440 (W68) of *V. sublobata* (considering crossing compatible) may serve as a potential donor for FSD and incorporation of same to

Table 2 Average value of PHS and FSG based on flowering and maturity time

Flowering time	PHS (%)	FSG (%)
Late July (N=10)	21.34 ± 11.03^{a}	21.25 ± 10.22^{a}
Early August (N=30)	60.98 ± 5.11^{b}	65.08 ± 5.51^{b}
Mid to late August (N=19)	65.96 ± 5.23^{b}	76.18 ± 5.72^{b}
Maturity time		
Mid August (N=15)	38.58 ± 9.77^{a}	39.50 ± 8.95^{a}
Late August (N=27)	55.34 ± 5.47^{ab}	60.74 ± 6.25^{b}
Early to mid Sept (N=17)	71.93 ± 5.34^{b}	81.17± 5.94^{b}

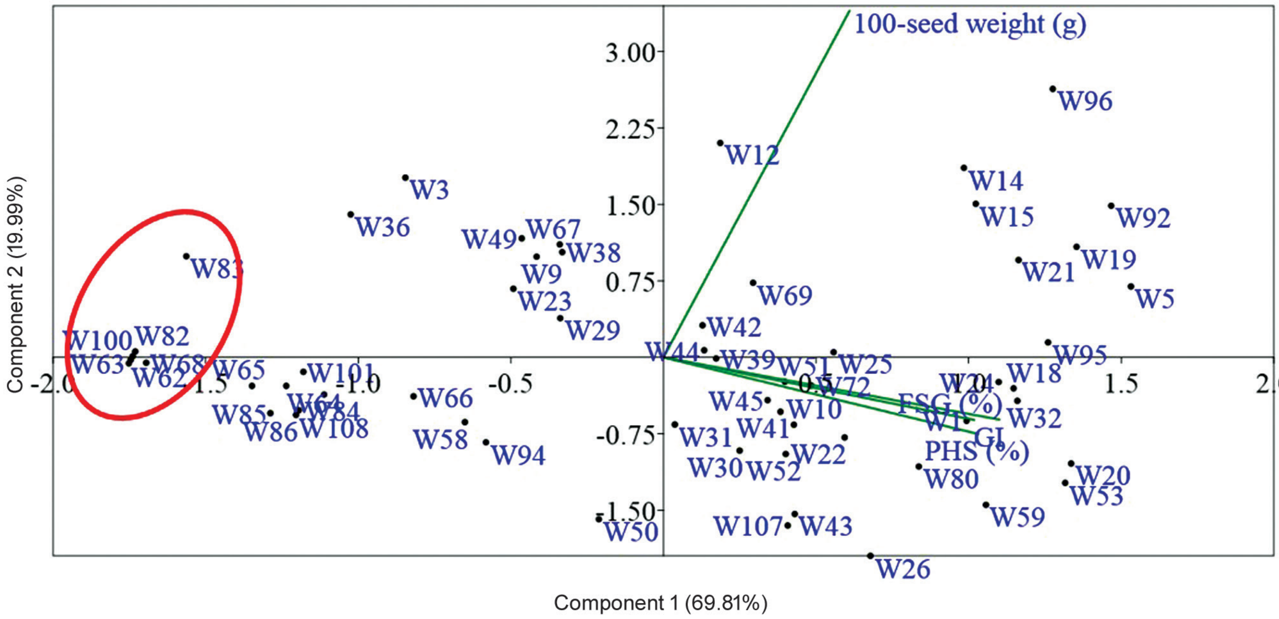

Fig 2 Scatter plot showing the distribution of 59 wild *Vigna* accessions based on PC1 and PC2 scores.

cultivated varieties of different *Vigna* species will help in reducing the loss incurred due to PHS.

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