Development of mungbean (Vigna radiata L. Wilczek) genotypes for shattering tolerance and correlation analysis with biochemical and morphological factors governing Pre harvest sprouting

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Article

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Abstract

Development of pre-harvest sprouting (PHS) tolerant varieties is imperative to minimize yield losses due to viviparous germination. F_2 seeds derived from the crosses DGGV-2 x Pant Moong-1 were irradiated with 60 kR gamma rays for the creation of desirable variability. In F_2M_2 generation, segregants with small pod beak and angle, thick pod wall, hard seededness and higher epicuticular wax and lignin content were isolated. Desirable mutants conferring tolerance to PHS were selected from 206 progenies consisting of 4812 plants. During the subsequent selfing generations the desirable plants with less than five percent PHS were stringently characterized by morphological and biochemical indicators.

Due to incessant heavy rain fall (462.2mm) during pod development and maturity coupled with high humidity weather was conducive for viviparous germination, resulting in higher PHS. Correlation analysis between pre harvest sprouting and biochemical and morphological parameters revealed that negative correlation of PHS was observed for epicuticular wax (r = -0.983), phenol (r = -0.892), lignin content (r = -0.981) and hard seed percentage (r = -0.942). Significant negative correlation was observed for pod wall thickness (r = -0.570). Pod beak length recorded highly significant positive correlation (r = 0.911) to PHS. Genotypes with inherent tolerance to seed shattering hold a promise to minimize yield losses due to viviparous germination.

Introduction

A prolonged rainy period at maturation often results in poor seed quality due to fungal infestation, sprouting of seeds within pods and discoloration of seeds. Premature sprouting is a serious problem in mungbean in the tropics (Fernandez and Shanmugasundaram 1988). Seed shattering is primarily a genetically controlled trait; however, it is significantly influenced by environmental conditions, management practices and their interactions, especially in agro-ecosystems (Maity et al 2021). Small pod beak and angle, thick pod wall, low rate of moisture absorption by pod wall, hard seededness and higher cuticular wax content on pod wall were found to impart resistance to pre-harvest sprouting (Naidu et al. 1996). A moderate level (15–20 days) of hard seediness may be useful in contributing to tolerance to weather damage. Transient hard-seediness is common in mungbean; the level of hard seededness in mungbean has been observed to be the highest at harvest, and it declines with storage. It has been observed that the hard-seeded character in the wild progenitor of mungbean, V. *radiata*var. Sublobata, is governed by a dominant gene Hd_1Hd_1 (Singh et al. 1983). Most of the mungbean genotypes are prone to shattering. The indeterminate flowering habit of this crop leads to a spread of flowering and pod maturity on a single plant over the entire reproductive phase. Consequently, pods which develop at the earliest flower may shatter prior to 100% pod maturity. To avoid shattering, often the pods are hand-picked. Therefore, it may be desirable to identify donors and incorporate gene(s) for non-shattering. ‘Pant Moong-1’ is tolerant to shattering. Its harvesting can be delayed by 7–10 days, so as to allow the maturity of pods from second flush of flowers (Singh and Sharma 1984 The average productivity of this crop is low and uncertain due to neglected management and poor adoption of the production technology due to the risk of pre-harvest sprouting. Sometimes losses due to pre-harvest sprouting will be as high as 60–70%. (Sharma *et al.,* 2018). High yielding varieties developed/identified in recent years, despite their high yield potential, could not increase/stabilize the yields of this crop due to lack of resistance to pre-harvest sprouting. Therefore an attempt was essentially made to develop tolerant varieties to pre-harvest sprouting in the current investigation.

Materials And Methods

Experimental site

A series of field experiments spanned over six years (2015-2020) were conducted at F block, AICRP on MULLaRP, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, India, geopositioned at 15° 26´ North latitude, 75° 07´ East longitude and at an altitude of 750 m above the Mean Sea Level and located in the North Western transitional agro-climatic zone of Karnataka, which receives an average annual rainfall of 718mm well distributed over the season. The average temperature and relative humidity ranged from 11 to 37°C and 40 to 85 per cent respectively. The ill – distributed rainfall during kharif 2019 was 892.2 mm, as against average rainfall of 718.23 mm. The maximum and minimum temperature during the crop season was 30.1 °C and 18.3°C respectively. Similarly, the relative humidity ranged from 57 to 96 per cent as against normal RH of 54.12 to 88.86 per cent. Due **to high rain fall in August** (462.2mm) during **pod development and maturity** coupled **with higher humidity across two years**

(*kharif* 2019 and 2020) weather parameters (Table 1 and Fig.1) were highly conducive for viviparous germination, resulting in higher percent of pre harvest sprouting.

Plant material

The Healthy and well dried F_2seeds derived from the crosses DGGV-2 x Pant Moong-1 were irradiated with 60 kR gamma rays for the creation of desirable variability. The gamma rays irradiated seeds were sown in augmented design during *kharif*-2017 along with their respective checks to grow the F_2M_1 generation. The individual plants were critically observed at maturity for morphological traits viz., small pod beak length and angle, thick pod wall, hard seededness that determine the tolerance to PHS. Desirable mutants conferring tolerance to PHS were selected from 806 progenies consisting of 24812 plants. Genotypes with short pod beak and angle, thick pod wall, hard seededness and higher epicuticular wax and lignin content were isolated in F_2M3 and F_2M4. Further a total of 49 advanced breeding lines along with a known susceptible check were screened and evaluated for tolerance to pre harvest sprouting.

Morphometric and biochemical parameters governing the pre harvest sprouting

Morphometric parameters

Pod beak length (cm)

The pod beak length was measured from five randomly selected plants from each genotype from the base of the beak to the tip of the beak as per the procedure given by Cheralu *et al.* (1999). The mean value was expressed as pod beak length.

Pod wall thickness (µm)

The pod wall thickness was measured from 10 randomly selected plants from each genotype with the help of digital micrometer as per the procedure given by Cheralu *et al.* (1999). The mean value was expressed as pod wall thickness.

Pod pubescence

Presence or absence of hairiness or pubescence on pods were recorded by following the DUS guidelines of PPV &FRA.

Biochemical parameters

Epicuticular wax (µg/cm2)

The epicuticular wax content of the pod wall was estimated by the gravimetric method. Cheralu *et al.* (1999).

Phenolic content

It was determined by Folin-Ciocalteu's method. The TPC was expressed as mg gallic acid equivalents (GA eq) per g of extract (Arun *et al.,* 2016)

Lignin content

Protein-free cell wall samples of greengram (20 mg) were used to estimate lignin content (Marwanto, 2007).

Percentage of sprouted pods per plant per plot

The number of plants per plot in which the sprouted pods were counted to the total number of plants per plot and the value was expressed as percentage of sprouted pods per plant per plot

Percentage of sprouted pods per plant

The number of pods with sprouted pods per plant to total number of pods per plant was counted from five randomly selected and tagged plants and the mean value was worked out and expressed as percentage of sprouted pods per plant.

Statistical analysis

The data recorded on each of five random plants for different quantitative traits were averaged and analyzed using WINDOSTAT version 9.3 and excel.

The data was statistically analyzed as per the method outlined by Gomez and Gomez (1984). The level of significance used in 'F' test was calculated at P = 0.05 in field studies and at P = 0.01 for seed quality studies in the laboratory.

All the methods were performed in accordance with the relevant guidelines/regulations/legislation.

Results

Analysis of variance

The Analysis of variances revealed significant variability for most of the characters. The stabilized mutant progenies of the cross derivative of DGGV-2 x Pant Moong-1 have shown significant variation for the characters like pod beak length and pod wall thickness , rate of moisture absorption by pod wall, hard seededness and cuticular wax content on pod wall (Data not shown) .The pod beak length differed significantly due to the genotypes. Longer pod beak length was recorded in DGGV-72 (0.245 cm), followed by kombhesaru (0.220 cm).While, shorter pod beak length was recorded by DGGV-125 (0.118 cm) followed by DGGV-79 (0.138 cm) (Table.2).

The pod wall thickness showed significant effect due to genotypes. Maximum thicker pod wall was recorded in DGGV-79 (0.654 µm) follo DGGV-67 (0.635 µm), DGGV-73 (0.631 µm) and DGGV-66 (0.628 µm), followed by DGGV-21 (0.585 µm). Whereas, thinner pod wall was recorded by DGGV-2 (0.341 µm), which was on par with DGGV-82 (0.348 µm), Kombhesaru (0.356 µm), DGGV-87 (0.356 µm), DGGV-81 (0.358 µm) and DGGV-213-1 (0.365 µm), followed by DGGV-184 (0.443 µm) (Data not shown).

Influence of biochemical parameters associated with pre-harvest sprouting

Phenol content

There was significant difference in the phenol content among the genotypes, higher phenol content was recorded in DGGV-125 (9.43 mg GA eq/g) which was on par with DGGV-79 (9.37 mg GA eq/g) and followed by DGGV-73 (8.94 mg GA eq/g), while, lower phenol content was recorded in DGGV-96 (4.03 mg GA eq/g) and followed by DGGV-109 (4.37 mg GA eq/g).

Lignin content

While comparing the lignin content among the genotypes. Significantly, higher lignin content (6.42 mg/g) was recorded in DGGV-125, followed by DGGV-79 (6.10 mg/g) and lower lignin content (3.90 mg/g) was recorded in DGGV-72, followed by Kombhesaru (3.99 mg/g).

Pod epicuticular wax

The pod epicuticular wax differed among the genotypes. Significantly, higher pod epicuticular wax (13.90 µg/cm^2) was recorded in DGGV 81, followed by DGGV-125 (13.03 µg/cm^2) and DGGV-73 (12.85 µg/cm^2). Lesser pod epicuticular wax was recorded by Kombhesaru (0.95 µg/cm^2), followed by DGGV-109 (1.28 µg/cm^2)

Influence of morphometric parameters associated with pre-harvest sprouting

Pod beak length

Significantly, longer pod beak length was recorded in DGGV-72 (0.245 cm) and DGGV-125 (0.118 cm) recorded shorter pod beak length. This variation might be due to the genetic make of the genotype, which varies with individual genotype. In general, with increase in pod beak length the PHS percentage increased at varied level among the genotypes as the surface area get increased with longer pod beak length which helped the pod to absorb more rain water leading to higher PHS. Similar observation with increased PHS due to increase in pod beak length in green gram was earlier reported by Cheralu *et al.* (1999)

Pod wall thickness

The pod wall thickness differed significantly among the genotypes. The thicker pod wall was recorded by DGGV-79 (0.654 µm), while thinner pod wall was observed in kombhesaru (0.356 µm) (Table 2). This variation is due to the genetic make of the genotype, which varies with individual genotype. The thicker pod wall protects the seeds from PHS by avoiding or reducing the water entry into the pods. Cheralu *et al.* (1999) observed that thin pod wall increases PHS in mungbean.

Percentage of sprouted pods per plant per plot

The percentage of sprouted pods per plant per plot differed within the genotypes. Significantly, higher percentage of sprouted pods per plant per plot was recorded in Kombhesaru (34.27 %), followed by DGGV-182 (26.57 %) and lower percentage of sprouted pods per plant per plot was recorded in DGGV-79 (0.02 %) and DGGV-125 (0.02 %) which showed resistance to sprouting.

Percentage of sprouted pods per plant

The percentage of sprouted pods per plant varied among genotypes. Significantly, higher percentage of sprouted pods per plant (14.25 %) was recorded in DGGV-72 and followed by Kombheasru (13.36 %) and lower percentage of sprouted pods per plant (0.001 %) was recorded in DGGV-79 and DGGV-125 (0.0002 %), followed by DGGV-195 (0.9 %). (Table 2)

Influence of biochemical factors on pre harvest sprouting

The phenol and lignin content varied significantly due to genotypes. The higher phenol content was recorded in DGGV-125 (9.43 mg/g) and lower phenol content was recorded in DGGV- 96 (4.03 mg/g) similarly, higher lignin content was recorded in DGGV-125 (6.42 mg/g) and lower lignin content (3.90 mg/g) was recorded in DGGV-72.

From the correlation analysis it was observed that some of the pod characters and chemical content were correlated with sprouted pods per plant per plot either significant or non-significant positive correlation (r = 0.453) with pod length was observed. Significant negative correlation with pod wall thickness was recorded (r = -0.570) and significant positive correlation was recorded for pod beak length (r = 0.759). Sprouted pods per plant showed significantly higher positive correlation (r = 0.946) and other parameters showed highly significant negative correlation were, pod epicuticular wax (r = -0.983), phenol content (r = -0.892), lignin content (r = -0.981) and hard seed percentage (r = -0.942). Similarly, the percentage of sprouted pods per plant noted significant and non-significant correlation with other parameters. There was non-significant negative correlation with pod wall thickness was observed (r = -0.437), significant positive correlation was recorded in pod length (r = 0.623). Pod beak length showed highly significant positive correlation (r = 0.911 and highly significant negative correlation was recorded in pod epicuticular wax (r = -0.980), phenol content (r = -0.868), lignin content (r = -0.978) and hard seed percentage (r = -0.956). (Table .3 and Fig.3)

Discussion

Effect of weather

In the month of August-2019, a total of 443 mm of rainfall was received as against the normal rainfall of 101.73 mm, followed by sunshine and favourable temperature *i.e.* alternate wetting and drying process. This disrupted the crop during its grand growth period and led to pre-harvest sprouting of pods on mother plants.

Effect of morphometric traits on PHS

The genotype DGGV 125 recorded shortest pod beak length (0.118cm),followed by DGGV 79 (0.138 cm) (Fig.2a). In general, with increase in pod beak length the PHS percentage also increased indicating that as the surface area increases with longer pod beak length, which facilitates the pod to absorb more rain water leading to higher PHS. Similar observation with increased PHS due to increase in pod beak length in green gram was earlier reported by Cheralu *et al.* (1999).

The pod wall thickness differed significantly among the genotypes. The thicker pod wall was recorded by DGGV-79 (0.654 µm), (Table 2 and Fig.2a). While the thinner pod wall was observed in kombhesaru (0.356 µm). The thicker pod wall protects the seeds from PHS by avoiding or reducing the water entry into the pods. Cheralu *et al.* (1999) and Anupama *et al.* (2012) also observed increase in PHS with decreased pod wall thickness. However, pod wall thickness alone may not account for minimum imbibition of water by pods.

Effect of biochemical traits on Pre harvest sprouting (PHS)

Higher wax content in pod wall of PHS tolerant genotypes might restrict water to come in contact with the seeds causing failure of seed germination and thereby making the genotypes PHS tolerant. So, significantly, higher epicuticular wax was recorded in DGGV-79 (13.90 µg/cm^2) and lesser epicuticular wax (0.95 µg/cm^2) was recorded in Kombhesaru (Table. 2). Higher epicuticular wax on the pod wall induces the impermeability to water on its surface and avoids the occurrence of PHS on mother plant. The similar findings were reported by Tekorny *et al.* (1980) in soybean and by William (1984) in mungbean. Baker 1974 also reported that an increase in the temperature, humidity and rainfall mainly reduces wax content.

The desirable plants with less than 5 percent PHS were characterized by morphological and biochemical indicators. Lower PHS was recorded in DGGV-79 (0.02 %), DGGV-125 (0.02 %). and DGGV 195 (0.91 %). (Table 2). Some genotypes showed resistant to pre-harvest sprouting mainly due to shorter pod beak length, thicker pod wall, higher pod epicuticular wax, phenol, lignin content and more number of hard seeds lead to development of hydrophobic thick coat of pod and seed, which, possess impermeable nature for water absorption and prevent pre-harvest sprouting of pods on mother plant under heavy and continues rainfall condition. Similar observation were reported by Cheralu *et al.* (1999).From the correlation analysis it was observed that some of the morphological and biochemical factors were correlated with pre harvest sprouting. Non-significant positive correlation (r = 0.453) with pod length was observed. Significant negative correlation with pod wall thickness was recorded (r = -0.570) while significant positive correlation was recorded for pod beak length (r = 0.759). Sprouted pods per plant showed significantly higher positive correlation (r = 0.946) and other parameters which showed highly significant negative correlation included pod epicuticular wax (r = -0.983), phenol content (r = -0.892), lignin content (r = -0.981) and hard seed percentage (r = -0.942). Pod beak length showed highly significant positive correlation (r = 0.911). The desirable plants with less than 5 percent pre PHS were characterized by these morphological and biochemical indicators. Lower PHS was recorded in genotypes DGGV-79 (0.02 %), DGGV-125 (0.02 %). and DGGV 195(0.91 %) hence testifying the observed correlations conferring shattering tolerance, while DGGV-191 and DGGV-95 were mild susceptible to PHS, DGGV-72 and Kombhesaru were susceptible to pre-harvest sprouting. Genotypes with inherent tolerance to seed shattering hold a promise in profitable mungbean cultivation.

Conclusion

Pre-harvest sprouting is a complex trait and is controlled by many genes showing significant interaction with the environment. In this study, mungbean genotypes DGGV-79 DGGV-125 and DGGV 195with less than five percent of PHS have been identified. Resistance to pre-harvest sprouting is mainly due to shorter pod beak length, thicker pod wall, higher pod epicuticular wax, phenol, lignin content and more number of hard seeds (Figs. 2a,2b.2c and 2d), which have led to development of hydrophobic thick coat of pod and seed, which, possess impermeable nature for water absorption and prevent pre-harvest sprouting of pods on mother plant under heavy and continuous rainfall coupled with high humidity coupled with intermittent sunshine conditions. The Advanced Breeding Lines (ABLs) DGGV-79 (0.02 percent), DGGV-125 (0.02 percent) and DGGV 195 (0.91 percent) that recorded low pod shattering can also be used as donors for crossing with the agronomically superior genotypes to derive shattering tolerant high yielding genotypes. Hence these genotypes with inherent tolerance to seed shattering hold a promise in profitable mungbean cultivation.

Declarations

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Author contributions

SM and NKBP designed and conceived the experiments, and SM and Ranjita performed all the experiments and wrote the manuscript. GB assisted in analyzing the data. All authors read and approved the manuscript.

Conflict of interest.

The authors declare no competing interests

All data generated or analysed during this study are included in this published article (and its supplementary information file.

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Tables

Table 1

Heavy rainfall and high humidity favoring viviparous germination during *kharif 2019*

STD meteorological Week	Temp Max	Temp Min	Rainfall	Rainy Days	RH1	RH2
Jun 4-Jun 10	35.5	22.2	6.8	1	85	43
Jun 11-Jun 17	30.7	21.6	14.9	2	91	63
Jun 18-Jun 24	30.4	21.2	32.4	2	90	63
Jun 25-Jul 1	26.6	20.4	86.6	2	94	72
Jul 2-Jul 8	27.3	20.3	82.0	7	93	81
Jul 9-Jul 15	26.9	20.1	42.6	3	92	73
Jul 16-Jul 22	29.2	20.3	22.0	3	92	67
Jul 23-Jul 29	25.6	20.4	41.0	2	94	84
Jul 30-Aug 5	24.7	19.9	150.6	7	96	83
Aug 6-Aug 12	24.9	20.1	277.2	6	93	81
Aug 13-Aug 19	27.4	20.9	15.2	1	94	67
Aug 20-Aug 26	28.1	20.5	13.8	3	90	69
Aug 27-Sep 2	26.8	20.8	10.6	1	93	79

Table 2

Categorization of genotypes based on the Parameters related to pre-harvest sprouting

Treatments	Percentage of sprouted pods/plant/plot	Percentage of sprouted pods/plant	Pod length (cm)	Pod beak length (cm)	Pod wall thickness (µm)	Pod epicuticular wax (µg/cm^2)	Hard seed (%)	Phenol content (mg/g)	Lignin content (mg/g)
Resistant genotypes									
DGGV-79	0.02	0.00	7.57	0.138	0.654	13.90	41.88	9.37	6.10
DGGV-125	0.02	0.00	6.98	0.118	0.454	13.03	32.38	9.43	6.42
DGGV-195	0.91	0.91	8.37	0.150	0.461	12.33	27.38	8.83	5.90
Mean	**0.33**	**0.30**	**7.64**	**0.135**	**0.523**	**13.09**	**33.88**	**9.21**	**6.14**
Susceptible genotypes									
DGGV-191	13.42	5.09	6.96	0.150	0.46	8.40	9.75	7.10	5.29
DGGV-21	14.34	7.56	7.90	0.218	0.586	6.90	15.50	7.05	4.45
DGGV-95	11.24	9.30	8.88	0.235	0.546	7.53	20.00	7.09	5.19
Mean	**14.95**	**6.51**	**7.91**	**0.203**	**0.457**	**6.88**	**14.46**	**6.45**	**4.98**
Highly susceptible genotypes									
DGGV-72	25.68	14.25	8.68	0.245	0.451	1.63	7.88	4.50	3.90
Kombhesaru	34.27	13.36	7.99	0.220	0.356	0.95	6.25	4.47	3.99
DGGV-182	26.57	12.09	9.23	0.208	0.458	1.75	8.75	5.20	4.32
Mean	**28.84**	**13.23**	**8.63**	**0.224**	**0.421**	**1.44**	**7.63**	**4.72**	**4.07**

Table 3

Correlation analysis of Biochemical and Morphological Indicators of Pre harvest sprouting in Mungbean

	Percentage of sprouted pods/plant/plot	Percentage of sprouted pods/plant	Pod length (cm)	Pod beak length (cm)	Pod wall thickness (µm)	Pod epicuticular wax (µg/cm^2)	Phenol content (mg GA eq/g)	Lignin content (mg/g)	Hard seed (%)
Percentage of sprouted pods/plant/plot	1								
Percentage of sprouted pods/plant	0.946**	1							
Pod length (cm)	0.453	0.623*	1						
Pod beak length (cm)	0.759*	0.911**	0.721*	1					
Pod wall thickness (µm)	-0.570*	-0.437	-0.069	-0.150	1				
Pod epicuticular wax (µg/cm2)	-0.983**	-0.980**	-0.563*	-0.830**	0.534*	1			
Phenol content (mg GA eq/g)	-0.892**	-0.868**	-0.365	-0.702*	0.618*	0.906**	1		
Lignin content (mg/g)	-0.981**	-0.978**	-0.534*	-0.826**	0.539*	0.993**	0.911**	1	
Hard seed (%)	-0.942**	-0.956**	-0.540*	-0.879**	0.376	0.963**	0.879**	0.963**	1

Figures

Figure 1

View of Experimental plot affected to incessant rains

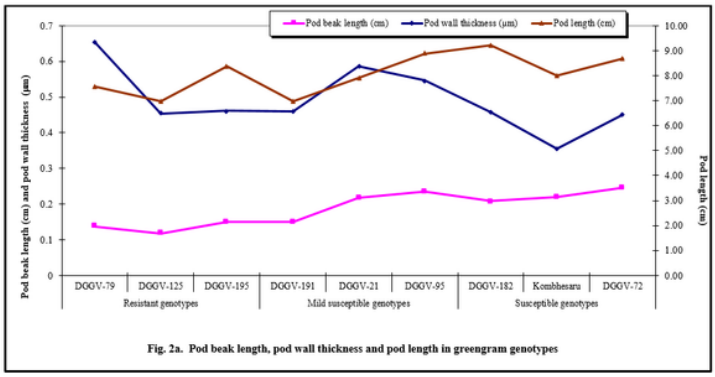

Fig. 2a. Pod beak length, pod wall thickness and pod length in greengram genotypes

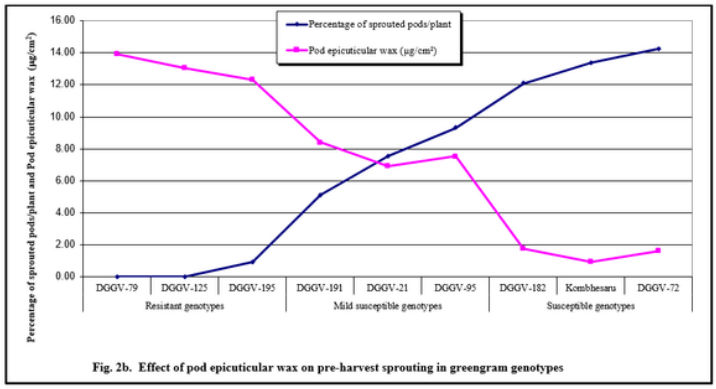

Fig. 2b. Effect of pod epicuticular wax on pre-harvest sprouting in greengram genotypes

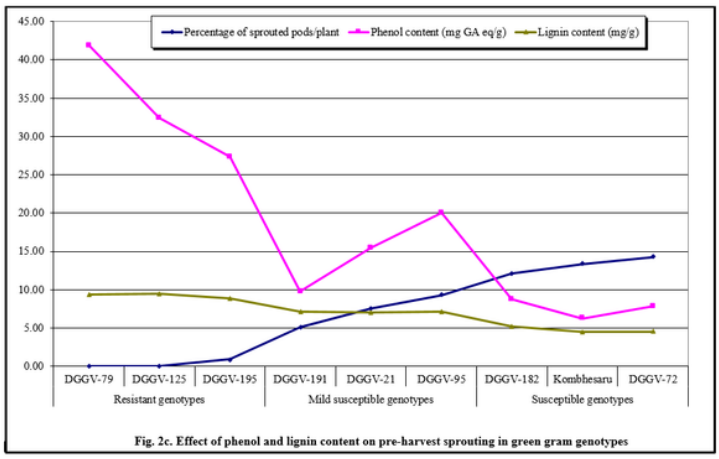

Fig. 2c. Effect of phenol and lignin content on pre-harvest sprouting in green gram genotypes

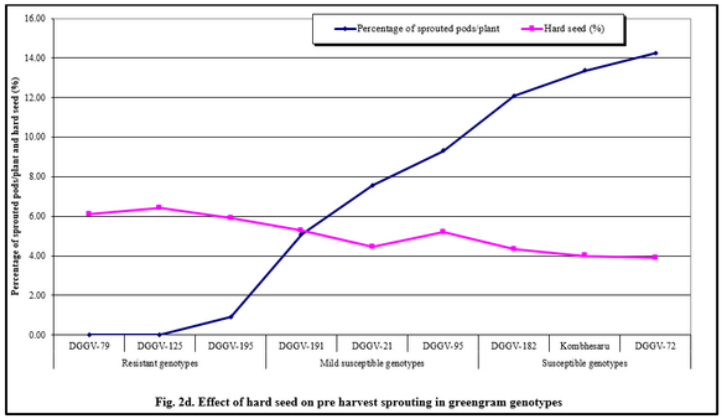

Fig. 2d. Effect of hard seed on pre harvest sprouting in greengram genotypes

Figure 2

Please See image above for figure legend.

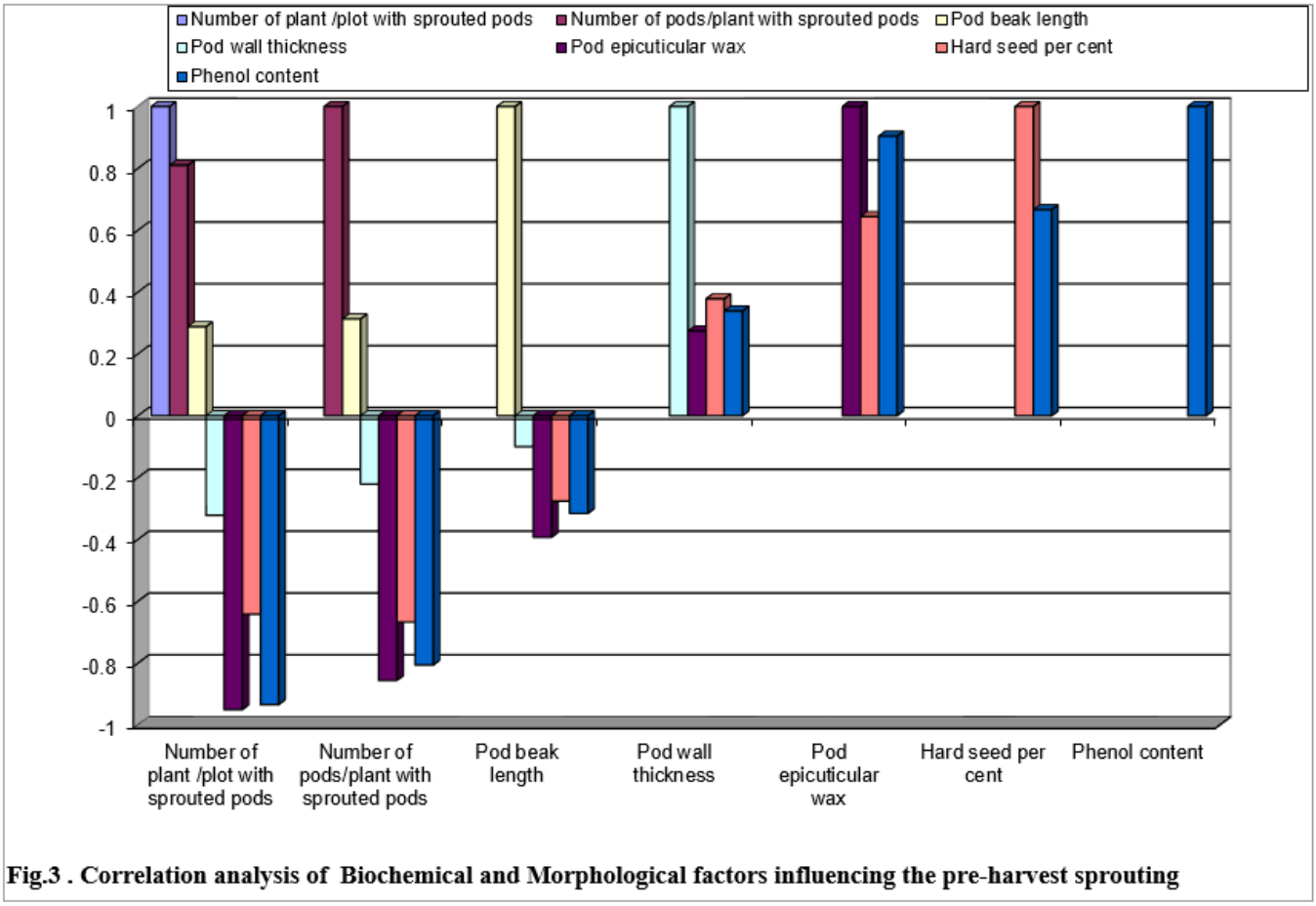

Fig.3 . Correlation analysis of Biochemical and Morphological factors influencing the pre-harvest sprouting

Figure 3

Please See image above for figure legend.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ESM29.04.22.docx