International Journal of Genetics
ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 10, Issue 4, 2018, pp.-390-393.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000226

Research Article

GENETIC VARIABILITY AND CHARACTER ASSOCIATION IN SOYBEAN GERMPLASM FOR PRE-HARVEST SPROUTING TOLERANCE AND ASSOCIATED TRAITS

SHARMA A.A.[1,2], SARMA M.K.*[1,2] AND RAMCHIARY N.[2,3]
[1]Advanced Level Biotech Hub, BN College of Agriculture, Biswanath, 784176, Assam Agricultural University, Jorhat, 785013, Assam, India
[2]Department of Bioengineering, Gauhati University Institute of Science and Technology, Gauhati University, Guwahati, 781014, Assam, India
[3]School of Life Sciences, Jawaharlal Nehru University, New Delhi, Delhi 110067
*Corresponding Author: Email- mksbnca@gmail.com

Received: April 10, 2018; Revised: April 23, 2018; Accepted: April 24, 2018; Published: April 30, 2018

Abstract- Evaluation of a set of 43 diverse genotypes of Soybean was carried out for their pre-harvest sprouting (PHS) tolerance and other important agroeconomic traits. Significant differences among the various characters of the genotypes under study were revealed through analysis of variance. Seed germination % in pods (SGP), was used as a measure of PHS tolerance. It ranged from 10.23 in DSb 30-2 to 74.4 in the cultivar JS 20-116. The character association studies revealed significant association of various plant, pod and seed traits with pre-harvest sprouting in soybean. The study also provided information on the performance of some of the popular soybean cultivars with respect to their PHS tolerance and identified some soybean genotypes with relatively low PHS. However, comprehensive field evaluation is required before these genotypes can be used in breeding programmes.

Key words- Soybean, Preharvest Sprouting, Resistance, Character association, Genetic Variability

Citation: Sharma A.A., *et al*., (2018) Genetic Variability and Character Association in Soybean Germplasm for Pre-Harvest Sprouting Tolerance and Associated Traits. International Journal of Genetics, ISSN: 0975- 2862 & E-ISSN: 0975-9158, Volume 10, Issue 4, pp.-390-393. DOI: http://dx.doi.org/10.9735/0975-2862.10.4.390-393

Academic Editor / Reviewer: Madhab Chandra Behera

Introduction

Soybean is potentially a high yielding crop playing an important role in production of vegetable oil and protein. It is the only oil seed which can be used as a provider of both protein (38-44%) and oil (18-22%). It has a wide range of geographical adaptation, unique chemical composition, very high nutritional value, functional health benefits and versatile end uses playing a major role in the world food trade. Being a leguminous plant, soybean naturally fixes nitrogen from the atmosphere [1]. However, Soybean production has been challenged by a number of Biotic and Abiotic stresses. Among various factors hampering realization of actual yield potential of soybean, pre-harvest sprouting (PHS) is an important one, causing damage not only in the standing crop but also in the harvested crop heaped in the fields and threshing-yards [2].

The phenomenon of germination of seeds in the pod, usually under wet conditions shortly before harvest, is termed pre-harvest sprouting (PHS). It is also termed as vivipary. It occurs in a wide range of cereal and legume crops like rice, wheat, barley, urdbean, mungbean, *etc.* Pre-harvest sprouting not only causes reduction of yield but also affects the quality of the crop [3]. The grain maturity time coincides with the rainy season of the region. Among grain legumes, the incidence of PHS is particularly high in urdbean and mungbean, where yield losses as high as 60-70% due to pre-harvest sprouting was reported [4]. Although pre-harvest sprouting is a major challenging factor in Soy production, very few reports in this context has been made so far. Therefore, information on PHS in Soybean is scarce. The present study was, therefore, undertaken to assess the genetic variation for PHS tolerance in soybean and to comprehend association of PHS with various morphological characters and their direct and indirect effects on PHS, and to identify potential sources of PHS tolerance in the germplasm.

Materials and Methods

The experiment was carried on during the kharif season of 2016-17 at Biswanath College of Agriculture, Biswanath Chariali, Assam with a set of 43 soybean genotypes comprising of released cultivars, local germplasm collections and wild variety of soybean. The field experiment was laid out in Randomised Complete Block Design in 4 blocks in 3 replications for each genotype.

Observation for days to 50% flowering was taken on plot basis from each replication. Plant height, number of clusters/plant, number of branches/plant, pods/cluster/plant, pods/plant and leaves/plant were recorded on five randomly selected plants in each genotype from each replication. Observations on pod length, pod diameter, pod wall thickness, water imbibition by pods (%) and seed germination in pods (%) were recorded on three sets of 15 pods each. Each set of 15 pods comprised of 3 pods from each of the five randomly taken plants. Seed length and seed diameter were recorded on 15 randomly taken seeds from the bulk seed of each genotype. 100-seed weight, seed density, water imbibition by seeds (%) and seed germination (%) were recorded on three sets of 100 seeds samples from the bulk harvest of each genotype. Pod wall thickness, seed length and seed diameter were recorded using a screw gauge [5]. The observations on other pod and seed related parameters were recorded as described below.

Water imbibition by pods: The dry weight of 15 pods was recorded using a digital balance. The pods were then soaked in water for 24 h. The soaked pods were surface-dried using a tissue paper and weight of the soaked pods was recorded. The procedure was repeated for the other two sets of 15 podseach. Water imbibition by pods (%) was calculated using the formula –

[(wt. of soaked pods- wt. of dry pods)/ wt. of drypods] x100

Seed germination in pods: The observation on seed germination in pods was recorded on the same set of pods used for recording water imbibition by pods. 15 soaked pods were placed in a petri-plate lined with blotting paper. The blotting paper was sufficiently wetted to retain moisture through the period of incubation. The petri-plate was sealed with paraffin film and incubated in a germination chamber maintained at 23-240C for 48 h. The number of germinated seeds and total number of seeds in the pods were counted.
Seed germination in pods (%) was calculated using the formula
- (number of germinated seeds/total number of seeds) x 100
and was used as a measure of PHS tolerance in the genotypes.

100-seed weight: The weight of 100-seed (g) was recorded using an electronic balance. The same set of seeds was used for recording seed density, water imbibition by seeds and seed germination.

Seed density: 100 seeds were immersed in a graduated test tube (100 ml) with a fixed volume of water. The rise in level of water following immersion of seed was recorded. The procedure was repeated for other two sets of 100 seeds each. Seed density was calculated using the formula –
wt. of 100 seeds (g)/volume of water displaced (ml)

Water imbibition by seeds: 100 seeds were soaked in water for 6 h. The soaked seeds were surface-dried using a tissue paper and weight of the soaked seeds was recorded. The procedure was repeated for the other two sets of 100 seeds each. Water imbibition by seeds (%) was calculated using the formula –
[(wt. of soaked seeds –wt. of dry seeds)/ wt. of dry seeds] x100

Seed germination: 100 soaked seeds were placed in a petri plate lined with blotting paper. The blotting paper was sufficiently wetted to retain moisture through the period of incubation. The petri-plate was sealed with paraffin film and incubated in a germination chamber maintained at 23-240C for 30 h. The number of germinated seeds out of a total 100 seeds was counted, which gave the seed germination (%).

Statistical analysis: The data were analyzed using INDOSTAT software.

Results and Discussion

Analysis of Variance indicated significant differences among genotypes. The parameters of variation for various characters are presented in [Table-1]. The mean seed germination in pods ranged from 10.23% in DSb 30-2to 74.4% in the variety JS 20-116 [Table-1]. Among the mentioned cultivated genotypes DSb 30-2 exhibited the lowest Seed Germination % in Pod (SGP) followed by RSC 10-30 and KDS 975 with 13.16 and 16.73, respectively. The afore mentioned varieties also exhibited low rate of seed germination % (SG) as well. The low SGP in these genotypes could be due to the hardness of the seeds. Study of association of various plant, pod and seed characters with SGP showed that among all characters, water imbibition (%) by pods (WIP) showed highly significant and highest positive correlation with SGP [Table-2]. Higher amount of water absorbed by the pod makes sufficient moisture available for the seeds present inside to initiate process of germination. Similar association of pre-harvest sprouting with rate of water imbibition through pod wall has also been reported [6] in greengram. Highly significant and positive correlation was observed between seed germination (%) in pods (SGP) and seed germination (%) (SG). Non–significant correlation was, however, observed between water imbibition by seeds (WIS) and SGP. The rate of water absorption and the amount of water absorbed determines the rate and percentage of germination of normal seed directly, some seeds that otherwise are normal, fail to imbibe water and do not germinate. This behaviour is known as hard seededness and results in failure of seeds to germinate even though sufficient moisture is available to the seed for initiation of germination process [5]. Lower germination percentage resulting from presence of hard seeds, therefore, lowers the incidence of seed germination inside the pod and indirectly reflects PHS resistance. The association of hard seededness and PHS resistance in soybean [7] and mungbean [8] has been reported in earlier studies. Reports have been made. No association was observed between certain morphological characters *viz*., plant height, number of clusters, pods per cluster, pods per plant, number of leaves, pod length with SGP.

Table-1 *General statistical parameters for various characters in 43 soybean genotypes*

Character	Mean	Range	CV	CD	Sem
Days to 50% Flowering	27.4	23.5-32.5	7.69	1.4	0.81
Plant Height(PH)	41.2	29.62-52.2	10.65	3.4	1.99
No. of Branches(NB)	7.12	3.8-11.33	31.41	0.8	0.46
No. of Clusters(NC)	11.03	5.0-22.26	45.62	1.08	0.64
No. of Pods/Cluster(PC)	3.49	2.67-4.4	12.84	0.4	0.25
No. pods/plant	38.94	16.13-88.06	49.1	6.5	3.89
No. of Leaves(NL)	15.28	7.53-25.53	30.69	1.34	2.3
Pod Length(PL)	3.51	2.8-4.32	8.93	0.1	0.03
Pod Diameter(PD)	0.76	0.44-1.02	21.37	0	0.01
Pod Wall Thickness(PWT)	0.11	0.1-0.12		0	0.009
Seed Length (SL)	0.82	0.74-0.95	2.09	0.1	0.05
Seed Diameter (SD)	0.54	0.46-0.66	5.21	0	0.02
100 Seed Weight (SW)	11.67	8.31-23.44	21.26	0.1	0.03
Seed Density (SDT)	2.28	1.10-3.27	20.33	0.1	41.47
Water Imbibition (%) by pods(WIP)	49.98	40.7-55.93	8.64	1.4	0.84
Water Imbibition (%) by seeds (WIS)	22.81	18.3-29.7	11.67	1.6	0.92
Seed germination (%) of seeds (SG)	41.27	21.17-72.4	39.08	4.4	2.63
Seed germination (%) in pods (SGP)	38	10.23-74.4	44.6	3.4	2.04

Table-2 *Correlation coefficient of various characters with Seed Germination (%) in pods (SGP) and Water Imbibition by pods (%) (WIP)*

Character	Water Imbibition % in pods (WIP)	Seed Germination % in pods (SGP)
Plant Height cm	0.0050	-0.0175
Days to Flowering	0.0029	-0.3458**,*
No. of Branches	-0.0647	0.2956**
No. of Clusters	-0.0392	-0.1245
Pods Per Cluster	0.2254	-0.0647
Pods Per Plant	0.0391	-0.1430
No. of Leaves	-0.0507	0.0834
Pod Length	0.246**	0.3541**,*
pod diameter	-0.0159	-0.2527**
Pod Wall Thickness	-0.2719**	0.0714
Seed Length	-0.0298	-0.0448
Seed Diameter	0.2773**	0.0788
100 seed weight	-0.1660	-0.0801
Seed Density	0.1976	-0.4513**,*
Water Imbibition by Seeds	0.0658	-0.0621
Water Imbibition by Pod	1.0000	-0.2340
Seed Germination	-0.1821	0.9418**,*

Seed length also showed no correlation with SGP. Seed density showed highly negative correlation with SGP. This may indicate that denser the seed lower the imbibition of water and lower is the chance of SGP. Pod wall thickness showed significant negative correlation with water imbibitions (%). This negative association may be due to stronger barrier posed by a thicker pod wall to water leading to reduced incidence of PHS. Similar association was reported earlier by in Soybean [9] and in mungbean[10]. However, pod wall thickness alone may not account for lower imbibition of water by pods. The PHS resistant genotypes might exhibit higher wax content in pod wall of PHS tolerant genotypes which in turn might restrict water to come in contact with the seeds, causing failure of seed germination [11].

Table-3 *Mean Seed Germination % in pods (SGP) and Seed Germination in 43 Soybean Genotypes*

Genotype	SGP	SG		Genotype	SGP	SG
PS 1569	17.6	27.63		PS 1570	43.16	53.83
RVS 2010-1	43.46	53.8		DS 3104	24.56	27.97
DSb 30-2	10.23	21.16		SL 1074	44.13	54.13
RSC 10-30	13.16	23.5		NRC 120	47.26	54.3
MACS 1491	48.6	55.6		TS 69	65.63	65.63
JS 20-94	46.4	56.46		BAUS 72	25.1	25.13
NRC 117	34.6	37.96		MAUS 1488	24.46	24.8
AMS 115	33.13	33.46		VLB 202	70.43	70.5
KDS 975	16.73	26.76		NRC 121	26.6	26.63
VLS 91	21.53	21.86		KDS 754	40.86	47.56
MAUS 710	22.4	22.8		RVS 2010-2	28.53	28.56
TS 72	27.53	27.53		RSC 10-29	20.16	34.5
DS 3103	26.86	26.9		MAUS 740	26.43	26.76
NRC 118	41.23	48.23		NRC 122	24.3	24.66
PS 1572	30.13	30.46		KBS 24-2014	43.46	53.46
MACS 1480	31.7	32.36		JS 20-116	74.46	72.4
AMS 100-1	55.06	55.4		AMS 1001	66.4	66.4
Himso 1686	26.9	26.9		NRC 123	62.66	62.73
KDS 775	53.26	56.6		JS 9752	36.33	33.06
VLS 90	24.4	24.76		JS 335	65.83	62.53
NRC 119	49.6	56.63		Bragg	67.43	60.46
DSb 29	31.4	31.73				

Table-4 *Direct and Indirect of various characters on SGP*

Character	Plant Height cm	No. of Clusters	Pods Per Cluster	Pods Per Plant	No. of Leaves	Pod effect Length	Seed Length	Seed Diameter	100 seed weight	Seed Density	Water Imbibition by Seeds	Water Imbibition by Pod	Seed Germination
Plant Height cm	**-0.1825**	0.0079	-0.0664	-0.0039	-0.0232	0.0489	-0.0444	0.0115	0.0815	0.0168	0.0287	-0.0137	-0.0192
No. of Clusters	-0.0491	**1.1386**	0.2785	1.1105	-0.3852	-0.2066	-0.5121	-0.3989	-0.1299	0.1217	-0.1502	-0.0338	-0.1421
Pods Per Cluster	0.1527	0.1026	**0.4196**	0.1885	-0.0877	-0.0555	0.0321	-0.1143	-0.1123	-0.0188	0.0044	0.1167	-0.0221
Pods Per Plant	-0.0275	-1.2658	-0.5831	**-1.2978**	0.4759	0.2462	0.5225	0.5468	0.1965	-0.1010	0.1011	-0.0573	0.1870
No. of Leaves	0.0120	-0.0320	-0.0198	-0.0347	**0.0945**	0.0093	-0.0107	0.0184	0.0075	-0.0145	0.0386	-0.0046	0.0061
Pod Length	0.0285	0.0193	0.0141	0.0202	-0.0105	**-0.1066**	-0.0122	-0.0123	0.0024	-0.0176	0.0088	-0.0032	0.0377
Seed Length	-0.0147	0.0273	-0.0046	0.0244	0.0068	-0.0070	**-0.0606**	0.0004	0.0187	0.0108	0.0171	0.0040	-0.0002
Seed Diameter	0.0006	0.0035	0.0027	0.0042	-0.0020	-0.0012	0.0001	**-0.0101**	-0.0015	-0.0020	0.0010	0.0040	-0.0011
100 seed weight	-0.0096	-0.0025	-0.0057	-0.0033	0.0017	-0.0005	-0.0066	0.0031	**0.0215**	0.0045	0.0083	-0.0037	-0.0041
Seed Density	-0.0021	0.0024	-0.0010	0.0018	-0.0035	0.0037	-0.0041	0.0044	0.0047	**0.0227**	-0.0017	0.0047	-0.0115
Water Imbibition By Seeds	0.0084	0.0071	-0.0006	0.0042	-0.0219	0.0045	0.0152	0.0051	-0.0209	0.0040	**-0.0538**	-0.0037	0.0030
Water Imbibition By Pod	-0.0069	0.0027	-0.0254	-0.0040	0.0044	-0.0027	0.0061	0.0361	0.0156	-0.0190	-0.0062	**-0.0914**	0.0175
Seed Germination	0.0945	-0.1123	-0.0474	-0.1296	0.0582	-0.3178	0.0028	0.1019	-0.1699	-0.4578	-0.0494	-0.1725	**0.8996**

Pod diameter also showed highly significant and negative correlation with water imbibition by pods. The correlation of this character, however with SGP was non-significant. Pod length showed highly significant positive correlation with SGP, while non-significant positive correlation was observed with WIP. The positive association of PL with SGP may be explained by the fact that a longer pod on account of its higher surface area is likely to absorb more water compared to the smaller pod, thereby causing higher percent of seed germination in the pod. Seed density showed negative and significant correlation with seed germination (%) in pods (SGP), suggesting that genotypes with seeds of higher density may be more tolerant to PHS. Path coefficient analysis revealed highest positive direct effect for number of clusters followed by seed germination (%) and pods per cluster. The characters *viz*., number of leaves, 100 seed weight, and seed density also showed positive direct effect but the values were very low. The characters plant height, pods per plant, pod length, seed length, seed diameter, water imbibition (%) by seeds and water imbibition (%) by pods showed negative direct effect. Indirect effect of seed germination (%) was considerably low via other traits. However, the traits number of clusters and pods per cluster showed considerable negative indirect effect via pods per plant. Pods per plant, number of leaves, pod length, seed length and seed diameter were observed showing considerable negative indirect effect via number of clusters. Similar effect was observed in number of clusters and pods per cluster via pods per plant. Number of leaves, pod length, seed length and seed diameter were observed showing considerable positive effect via pods per plant. The residual effect was calculated to be 0.2039 which suggests the parameters under study considerably explained the effect of various traits on the dependent variable.

Conclusion

Pre-harvest sprouting is a complex trait and is controlled by many genes showing significant interaction with the environment. For the development of PHS tolerant cultivars, availability of suitable donors is a prerequisite. In this study, soybean genotypes with relatively lower incidence of PHS have been identified. The genotypes *viz*., MAUS 710, DS3104, MACS 1480, NRC 121 *etc*. were identified as

the possible donor for Preharvest Sprouting Resistance in soybean which may be used in breeding for development of varieties with PHS resistance particularly in Noon conventional regions like North East India with high rainfall during the growing season. Comprehensive field evaluation of these genotypes, however, is required before these genotypes can be used in breeding programmes. However, detailed studies at both morphological and physiological level are required to elucidate the mechanisms and the underlying genetics of this complex trait.

Application of research: The study provides information about the performance of some of the popular soybean cultivars with respect to their PHS tolerance. The character associations revealed in the study give a preliminary idea about various plant, pod and seed traits that play role in the governance of pre-harvest sprouting in mungbean.

Research Category: Genetic and plant breeding

Abbreviations:

DF : Days to 50% Flowering
PH : Plant height
NB : No. of Branches
NC : No. of Clusters
PC : No. of Pods/Cluster
PP : No. pods/plant
NL : No. of Leaves
PL : Pod Length
PD : Pod Diameter
PWT : Pod Wall Thickness
SL : Seed Length
SD : Seed Diameter
SW : 100 Seed Weight
SDT : Seed Density
WIP : Water Imbibition (%) by pods
WIS : Water Imbibition (%) by seeds
SG : Seed germination (%) of seeds
SGP : Seed germination (%) in pods
PHS : Pre Harvest Sprouting

Acknowledgement / Funding: Author thankful to Advanced Level Biotech Hub, BN College of Agriculture, Biswanath, 784176, Assam Agricultural University, Jorhat, 785013, Assam, India

***Research Guide or Chairperson of research: Dr M.K. Sarma**
University: Assam Agricultural University, Jorhat, 785013, Assam, India
Research project name or number: Nil

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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