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Seed Coat Permeability Studies in Wild and Cultivated Species of Soybean

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A B S T R A C T

Soybean [*Glycine max* (L.) Merr.] Seeds loose viability at a rapid pace under tropical and subtropical conditions. Poor seed longevity in cultivated soybean genotypes may associates with seed size, testa colour and seed coat permeability. Present study aims to understand kinetics of imbibation and evaluate the seed coat permeability in *Glycine tomentella*, *Glycine soja* and *Glycine max.* Out of all 125 genotypes of three species, 20 genotypes of wild annual and perennial species were small and hard seeded. All black seeded genotypes of three species including cultivated soybean were small in size, suggesting positive correlation of testa colour with seed size. There was strong positive correlation between 100 seed weight and seed coat permeability percentage based on 6 hours rapid imbibation test (0.83) and Seed coat permeability assessment in germination paper after 7 days (0.81). Kinetics of Imbibation suggest that *G. max* starts uptake water after some minutes, but seeds of *G.tomentella* imbibes water after 78 hours. Seed weight of *G. soja* genotype remained constant even after 4 days. Genotype of *G. max* (DS 9712) imbibes water up to 24 hours with steady linear curve, after that it becomes constant up to germination. Variability in seed coat permeability (44%) after 7 days slow imbibation in *G. soja* (DC 2008-1) suggests its use in introduction of optimum permeability behavior in *G. max* to slow down the extra leaching for improvement in seed longevity.

Introduction

Soybean is a major component in the diet of million people throughout the world. It plays vital role in nutritional security as it is a rich source of protein and oil. Although soybean is grown across the world, yet United States of America, Brazil, Argentina, China and India are the major producers. Taxonomically, the genus *Glycine* is divided into two sub-genera: *Glycine* (with perennial species) and *Soja* (with annual species). Sub-genus *Soja* includes the diploid species *Glycine max* (cultivated soybean) and *Glycine soja* (wild progenitor). Subgenus Glycine is currently consisting of 30 wild perennial species including *Glycine tomentella, Glycine tabacina* etc. (Singh *et al.,* 2014). The typical cultivated soybean displays a bush-type growth habit with a stout primary stem and sparse branches, bearing large seeds with variable seed coat colors, whereas the wild type is a procumbent or climbing vine with a slender, many-branched stem bearing small, coarse black seeds.

Soybean seeds are the important regenerative unit which cannot retain their viability indefinitely. Seed coat, the outer covering of every mature seed, is an important part of the seed and is associated with preservation of integrity of internal seed parts, protection of embryo against mechanical injuries and regulation of gaseous exchange between embryo and environment. It also offers tolerance to fungal activity, flooding, and protection to microbial attacks. The seed coat is also related to seed viability under delayed-harvest field condition, seed longevity under humid environments and inhibition against rapid imbibation of water, which often deteriorates the germination (Kebede *et al.,* 2014). Seeds with impermeable seed coat often results in adverse quality, and cost factors in processing seeds for vegetable oil and soy foods, and they affect the texture and consistency of products such as fermented soy food (Jang *et al.*, 2015).

Soybean seed reaches its maximum potential for germination and vigour at physiological maturity (Crookston *et.al.,* 1978), which then gradually declines till harvest, followed by a more rapid decline thereafter. Loss of germination potential or viability is more acute in tropical and sub-tropical regions compared to temperate environments.

However, hard seed coat (Tiwari *et al.*, 1995), small seed size (Tiwari *et al.*, 1989), black seed coat (Dassou *et al.*, 1984) and tight attachment of the seed coat to the cotyledons (Kuchlan *et al.*, 2010) are useful parameters for viability in tropical environment. Seeds of the wild type soybeans with hard seed coat exhibit better germination under tropical environment than the cultivated type. However, limited information is available on seed coat permeability and pattern of imbibitions in wild and cultivated germplasm of soybean.

Therefore, the current study was conducted to evaluate seed coat permeability in the seeds of cultivated (*G max*) and two wild type soybean species (*G soja* and *G tomentola*) under ambient storage and to see their effect on seed viability.

Materials and Methods

Plant material

The experimental material (125 genotypes) consisted of an accession of *G tomentella*, 19 accession of *G soja* and 105 genotypes of *G max*. The cultivated genotypes included high yielding popular cultivars, advanced breeding lines and exotic stocks (Table 1). The seeds, which were obtained from the Division of Genetics, IARI, New Delhi, varied in testa colour, seed size and seed weight.

Seed coat color of all the 125 genotypes was recorded by visual comparison with the ‘color chart’ of Royal Horticultural Society, London under natural day light. Three replicates of 100 randomly selected seeds were taken for determination of seed weight. The mean value of the 3 replications was expressed in grams. Based on 100-seed weight, the genotypes were categorized as bold (>10 g), medium (8 - 10 g) and small (< 8 g) seeded type on basis of size diversity.

For understanding the kinetics of imbibitions, the water uptake pattern was measured as per Hahlis and Smith (1997). Ten seeds of five genotypes *viz.*, *G. tomentella*, DC 2008-1, PI 424079, DS9712 and JS 335 were soaked in 25 ml of distilled water at room temperature and their weight was recorded at every two hours interval up to 24 h. Three genotypes, DC 2008-1 (*G. soja*) and DS 9712 (*G. max*) and *G. tomentella* were used to understanding pattern of imbibation up to 96 hours with interval of 4 hours in another experiment. The rate of water uptake was depicted as percentage increase over the initial weight of the seeds with time. Seed coat permeability (SCP) was tested through Rapid and Slow imbibitions tests. For rapid imbibitions test, 20 seeds of each genotype in 3 replications were soaked in 100 ml of distilled water for 6 hrs at room temperature. For slow imbibitions test, 50 seeds of each genotype in three replications were placed in water-moistened germination paper towels, which were then rolled up and placed vertically in plastic containers on a shelf in a germinator. The towels were maintained at near 100% humidity for 7 days. SCP (%) was recorded as percent of seeds imbibed water in respective tests (Kebede *et al.*, 2014). As per Sun *et al.*, (2015), the genotypes with SCP < 20% were categorized as hard-seeded and genotypes with SCP > 80% were grouped as soft-seeded. Data were analyzed for ANOVA, MSD, correlation coefficient using SAS software package version 9.4. All the replicated data were subjected to Tukey's Studentized Range (HSD) Test.

Results and Discussion

Seed color

Seeds of all but one *G. soja* accessions were dull black; PI366120 had shiny black seeds. Seeds of the single *G. tomentella* accessions were also dull black. However, enough variability for color was observed in the seeds of cultivated type i.e. *G max*. Out of 105 *G max* genotypes, 69 had yellow seeds and 25 had black seeds. Besides, 6, 3 and 2 genotypes had yellow-green, brown and green seeds, respectively. During domestication, people preferred yellow colored seeds over black and hence most of the cultivated genotypes have yellow seeds. However, black colored genotypes like Kalitur are also popular locally.

Seed weight

Weight of the seeds is an important trait. It influences consumers' preferences and market price, as well. In general, wild type genotype possesses smaller seeds and *vice versa*. In this study, wide variation was observed among the genotypes for the seed weight. *G. tomentella* accession had the smallest seeds (100-seed wt. =0.5312g) and the *G. max* genotype Dsb19 had the largest seeds (100-seed wt. =13.52g). The 100-seed weight of the *G. soja* accessions ranged from 0.561g (DC 2008-1) to 2.072g (PI 406684) with an average of 1.26g, while that in *G. max* ranged from 5.06g (UPSL-34) to 13.52g (Dsb 19) and had an average weight of 8.43g (Table 2). Among the cultivated genotypes, yellow seeded genotypes had highest mean seed weight (9.27g/100 seeds), followed by brown (8.16g/ 100 seeds), yellow-green (7.46g/100seeds) and green (7.01g/100 seeds). Accordingly, all the 20 accessions of wild type soybean were categorized as small seeded, while 48, 42 and 20 genotypes of the cultivated type were classified as small, medium and large seeded, respectively.

Dynamics of imbibition

Imbibition pattern of seeds is primarily governed by permeability of the seed coat (Kuchlan *et al.*, 2010). More permeable the seed coat more is the imbibitions and *vice-versa*. It was observed that the cultivated genotypes viz., DS9712 and JS335 started imbibing quickly after soaking, and there was an increase in fresh weight of the seeds up to 24 hrs. However, no imbibitions were observed in the seeds of *G. soja* (DC 2008-1 and PI 424079) and *G. tomentella* accessions (Fig. 1). Therefore, the seeds of the *G soja* and *G tomentella* were classified as hard-seeded while that of *G max* was rated as soft-seeded. Among the soft seeds, the rate of imbibitions was not uniform. It was more in JS335 (0.196 g/h) than DS9712 (0.032 g/h) in the first two hours, which was later reversed.

It was further observed that seeds of *G soja* accession DC2008-1 did not imbibe even up to 96 hrs. It indicated that the seed coat of this accession is highly impermeable. Similarly, *G. tomentella* seeds started imbibing only after 78 hrs, of course at a very slow rate. On the other hand, seeds of DS9712 (*G max*) started imbibing minutes after soaking. There was a linear increase in rate of imbibitions up to 24 hrs after which it remained stagnant up to 72 hrs beyond which weight gain was observed due to germination.

Table.1 Genotypes used in experiment for testing seed coat permeability

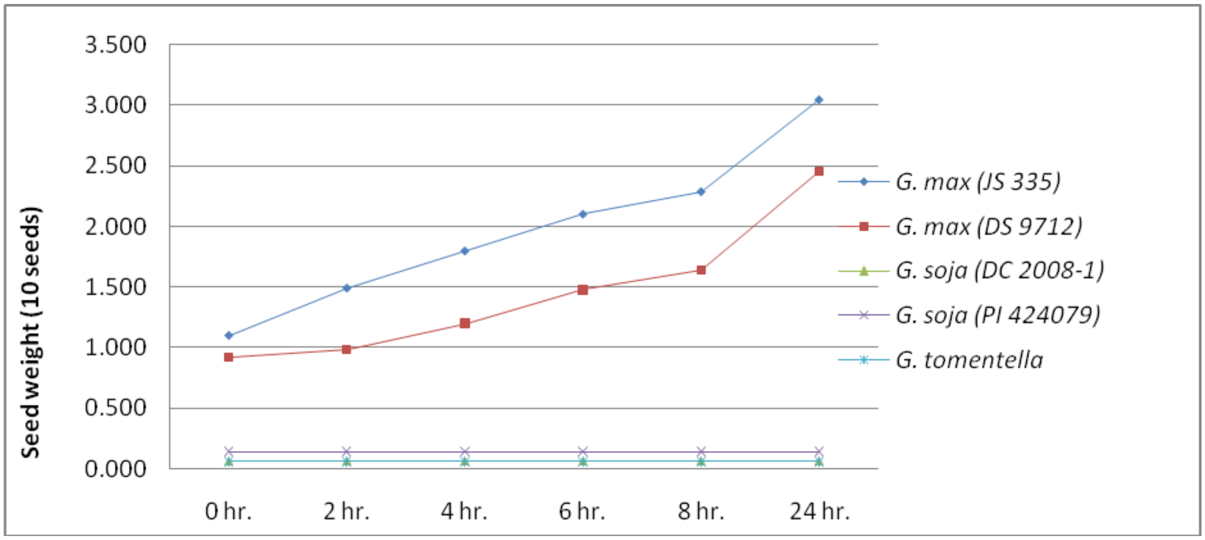
S.No.	Genotype	S.No.	Genotype	S.No.	Genotype	S.No.	Genotype
1	*G. tomentella*	33	UPSL-117-B	65	PS-1477	97	JAVA-16
2	PI-464925-B	34	USSL-291	66	PS-1480	98	J-231
3	PI-464925-A	35	EC-456615	67	MACS-1336	99	JS-76-257
4	PI-561355	36	EC-457442	68	SL-900	100	M253
5	PI-464869-A	37	EC-457472	69	DSB-19	101	MACS565
6	PI-522183-B	38	EC-471635	70	PS-1503	102	MAUS-2
7	PI-464889-A	39	EC-457189	71	DSB-21	103	NRC-21
8	PI-407294	40	DS-9909	72	PS-1505	104	PK-1225A
9	PI-407292	41	MAUS-164	73	NRC-89	105	UPSL-19
10	PI-406684	42	PK-1347	74	AMS-56	106	UPSL-29
11	PI-366120	43	TS-148-22	75	DS-2708	107	UPSL-34
12	PI-507805	44	MACS-869	76	PS-1518	108	UPSL-736
13	PI-423991	45	DS-22005	77	DS 61	109	EC-458342
14	PI-522229	46	DS-9817	78	DS 178	110	EC 1023
15	PI-507830-B	47	DS-9819	79	DS 76-37-2	111	DS MM 64
16	PI-326582-B	48	His-01	80	DS 93-3	112	G 2603
17	PI-424051 B	49	JS-94-67	81	EC13969	113	G 2253
18	PI-424079	50	KB-222	82	EC343361A	114	G 2601
19	PI-424032	51	MACS-450	83	EC1021	115	MACS 694
20	DC-2008-1	52	PK-1024	84	EC36961	116	EC 471919
21	DS 9712	53	PK-1041	85	EC39873	117	EC 472134
22	DS-76-1-2-1	54	VLS-61	86	EC97351	118	G 395
23	G-2215	55	SL-679	87	EC105790	119	UPSL 163
24	G-2132	56	SL-710	88	G2265	120	UPSL 332
25	G-2144	57	E-20	89	G2344	121	DS 74
26	PK-1243	58	SL688	90	G2608	122	AMSS 34
27	PK-1135	59	PKS-34	91	JS 335	123	NRC 7
28	Bragg	60	NRC-71	92	G2651	124	PK 416
29	TGX1855-530	61	NRC-78	93	G3023	125	PK 472
30	TGX1864-15F	62	DS-2614	94	GUJ-SOY-1		
31	TAMS-38	63	JS-20-05	95	IC-144409		
32	UGM-34	64	DSb-15	96	IC-141446		

(S.No. 1: *G. tomentella*; 2 to 20: *G. soja*; 21 to 125: *G. max*)

Table.2 Descriptive statistics for 100 seed weight and Seed coat permeability among the three species of soybean

Species	100 seed Weight			SCP% (7 days basis)		
	Range	Mean	SD	Range	Mean	SD
G. max	5.06-13.52	8.48	1.91	92.0-100	99.69	0.971
G. soja	0.56- 2.07	1.26	0.42	3.1 – 44.0	3.37	10.5
G. tomentella	0.53	0.53	-	66.0	66.0	-

Fig.1 Pattern of water imbibation in wild and cultivated soybean



Seed coat permeability

Rapid permeability test: Permeability of the seed coat of all the 125 genotypes was checked after 6 hrs of soaking (rapid imbibitions) and 7 days of soaking (slow imbibitions). Those genotypes whose <20% seeds imbibed within 6 hrs were classified as hard seeded. Similarly, genotypes whose >80% seeds imbibed within 6 hrs were classified as soft-seeded. Accordingly, all the accessions of *G. soja* and *G. tomentella* were rated as hard-seeded as it did not imbibe any water (SCP=0%) within 6 hrs. Similarly, 81 genotypes of *G. max* out of 105 had 90-100% SCP and were classified as soft-seeded.

Slow permeability test: Slow imbibitions test indicated 66% permeability in seeds of *G. tomentella*. Similarly, 14 out of 19 *G. soja* genotypes showed 0% permeability in the slow imbibitions test. *G. soja* accession DC2008-1 had 44% SCP and PI507830B, PI366120, PI464889A and PI424032 had 3%, 4%, 5% and 6% permeability, respectively. Therefore, all the accessions of *G. soja* were rated as hard-seeded. Genotypes of the cultivated soybean found to have soft seeds as all the genotypes had SCP ranging from 92-100%. Mean value of SCP % in the slow permeability test were recorded as 3.37%, 66% and 99.69% in *G. soja*, *G. tomentella* and *G. max*, respectively.

A very strong correlation (0.98) was observed between the result of SCP under rapid and slow permeability test. It indicated that rapid permeability test i.e. imbibitions of 6 hours can be effectively used for testing permeability of seeds. It will save time and energy. Similarly, seed weight found to have strong correlation with the seed coat permeability. So, large seeds would have high seed coat permeability.

The morphological and physiological changes associated with domestication can be delimitated into adaptation syndromes resulting from natural or deliberate human selection (Harlan *et al.*, 1992). Hard seed is an adaptive trait which ascertained in wild relatives of soybean and maintained longer viability in them. In the present study, all the accessions of *G. soja* except DC 2008-1 had permeability less than 7%. The seeds of DC2008-1 are highly viable (data not shown). Therefore, transferring partial permeable trait of DC 2008-1 to cultivated type might increase seed viability in soybean. Seed size found to have strong correlation with seed coat color and viability. Black genotypes of all the species including *G.max* had small seeds (<8 g) and in general were highly viable. Seed coat permeability was strongly associated with process of domestication (Liu *et al.,* 2007). All genotypes of domesticated soybean are soft seeded. However, soft seeded genotypes are having short viability. Therefore, breeding efforts need to be oriented to increase viability of soybean seeds without compromising seed coat permeability. The information generated in this study would pave the way for developing soybean genotype with high viability and permeability.

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