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Micromorphology of palisade cuticle of bold seeded vegetable soybean and grain type soybean

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Abstract
Soybean and other legumes have hard seed (Rolston, 1978). Hard seed coat protect seeds from harsh environment and this trait gives special agronomic character for seeds. Hardness also protect from seed decay (Taylor, 1997). However, hardness is sometimes undesirable, Hence, breeding program need to be establish to maintain the lines to get good permeability and strong seed coat. Among the two soybean cultivars one is vegetable type Karune and another grain type William 82. In the study Karune has more permeability of water as compared to William 82. Crinkled formation in the seed coat showed of Karune is the indication of the primary site of hydration. The SEM study showed that the seed permeability of water due th small cuticular cracks present on the seed surface of vegetable type soybean, which was examined by the SEM. The size of micropyle, hilum, raphe, depression size near hilum and Hilar fissure in Karune mean was recorded 268.71µM, 1165.47µM, 120.34µM, 51.09 µM and 45.04 µM respectively.

Keywords: Imbibition, micropyle, hilum, raphe, Hilar fissure, Karune, William- 82

Introduction
Soybean and other legumes have hard seed (Rolston, 1978) [20]. Hard seed coat protect seeds from harsh environment and this trait gives special agronomic character for seeds. Hardness also protect from seed decay (Taylor, 1997) [23]. However, hardness is sometimes undesirable when it is used for food processing industry. For food industry seed should be ideal to take up water quickly. This particular trait is very important when whole seeds used for processing such as, production of soy-milk, soy-sauce, miso and tofu. Yet, permeable seed coat may be susceptible to damage when it undergoes pre-processing, ultimately leading to losses. Hence, breeding program need to be establish to maintain the lines to get good permeability and strong seed coat. The water imbibition property mainly depends upon the seed structure. A typical legume seed coat contains some of the special area such as, hilum, micropyle and raphe. Rest of the seed coat called extra- hilar region. Another property of seed coat of soybean seeds is that deposit is present of the seed coat most of the soybean genotypes. According to previous reports there are three classification of seed coat types such as, shiny, dull or coated with bloom depending on the amount of deposit present on the seed coat (Williams, 1950; Bernard and Weiss, 1973) [25, 2]. According to the literature some reports was claimed the deposits are the residues of pod endocarp which is adhere on the surface of seed coat (Newell and Hymowitz, 1978; Wolf *et al*., 1981; Yaklich *et al*., 1986) [18, 27, 29, 30]. In contrast, other reports suggested that deposits were cutin origin which has waxy nature and therefore had originated from the epidermal layer of the seed coat (Calero *et al.*, 1981; Ragus, 1987) [3, 4, 19]. Small pores are present in the seed coat of soybean seed is also well documented (Wolf and Baker, 1972; Calero *et al*., 1981; Wolf *et al*., 1981) [28, 3, 4, 27]. However, the role of deposits and pores of water uptake has not yet been fully identified. Calero *et al*. (1981) [3, 4], based on the SEM (Scanning Electron Microscopy) studies reported that, when deposit existed in the absence of pores, the seed is impermeable.
The seed coat and tissue components are greatly responsible for permeability. The origin of seed coat can be traced by the ovule primordial legumes have bi- tegmic ovules. Current seed identification criteria are based upon morphological characteristics including seed size, general shape, surface shape, color, pattern, hilum length and width. The outer integument of the seed coat forms a single layer of tightly packed palisade of radially elongated sclereids (called Malpighian cells, macrosclereids, or palisade cells) with heavily and unevenly thickened cell walls. The outer tangential cell walls are covered with cuticle and, because of specific cell wall thickening and modifications, they are commonly described as terminal caps. The cuticle forms a continuous layer covering the seed, except for the hilum, and is considered the outermost barrier to imbibition (White, 1908; Spurny, 1963; Ma *et al*., 2004; Shao *et al*. 2007) [24, 22, 14, 21].

In and around the hilum, the layer of osteosclereids merges with thick-walled, star-shaped parenchyma.
The micropyle and hilum, hilum is a distinctly oval or round abscission scar in the chalazal seed region, a relic of former connection of the seed to the maternal plant via the funiculus. There is another residual layer of palisade cells of funicular origin termed counter-palisade, which are part of the hilar scar (Lackey, 1981) [11]. There is a central fissure (hilar groove) in the hilum palisade layer overlaying the tracheid bar (from the micropyle to the ovular bundle on the other side. This strip of large, pitted and lignified tracheids is commonly found in legumes although there is some variability in structure and ovular bundle position (Lersten, 1982) [12]. A role for the hilar groove as a hygroscopically activated valve was suggested (Hyde, 1954 and Lush 1980) [9, 13]. The fissure in the hilum opens when relative humidity is low permitting the seed to dry out whereas high relative humidity causes the fissure to close preventing the absorption of moisture.

Materials and Methods

Soybean cultivars

Two distinct featured soybean *Glycine max* L. Merr. cultivar were included in the study. One cultivar Karune, is popular vegetable soybean cultivated in Karnataka state India, released from University of Agricultural sciences Bangalore. It is bold seeded, green seed coat. Other cultivar used is William-82, is popular grain type soybean medium seeded with creamy yellowish seed coat widely grown in United States of America. The plants were grown on the experimental farms of University of Horticultural Sciences Bagalkot, Karnataka during 2019-20 year. Seeds were hand harvested and stored at 4 ^{0}C.

Examination of seed morphology and water uptake

All the seeds were examined with 10x compound microscope, only intact seeds without any damage on seed coat were taken and used for examination. From both the cultivar eight to ten seeds were used for the study. Single seeds were weighed immersed in water, blotted with tissue paper, weighed it and restored to the water. Seeds were weighed at 5 min interval for the first 30 min. and 10 min. for next 30 min and 30 min. till 3 hour. Penultimate weight was taken at 3 hour, final measurement was taken at 24 hour. The rate of water uptake was expressed as weight increase (g) per gram of seed weight. Both the cultivar seeds were taken and made impermeable to water by coating them with paraffin wax and considered as control.

Scanning electron microscopy (SEM)

Soybean seed coat surface were studied using SEM. Approximately 1-5 mm^2 sample from abaxial and dorsal region were dissected. Samples from the ventral region were also excised and continued the hilum, micropyle and raphe (Fig. 1). For each cultivar 8-10 seeds were examined. All samples were coated with colloidal nano gold particles (Sigma Pvt. Ltd.). All sample were examined at Indian institute of Sciences, Bengaluru using Hitachi scanning electron microscope at 15 kV.

Results

Seed permeability of soybean seed coat as indicated by imbibition rate

Among the two contrast cultivar used for the study vegetable soybean (Karune) imbibed water faster indicating their coat were more permeable to the water than grain type soybean (William-82). (Fig. 1). Both the cultivar seeds normally took 12-24 hour to achieve full hydration. In the case of Karune seeds took 30-60 per cent of water during the first 12 hour and William-82 took was less permeable than vegetable soybean the data is taken for initial 3 hour as these time is more crucial for water imbibition and germination. The impermeable (I) seed (coated with paraffin wax) did not imbibe the water.

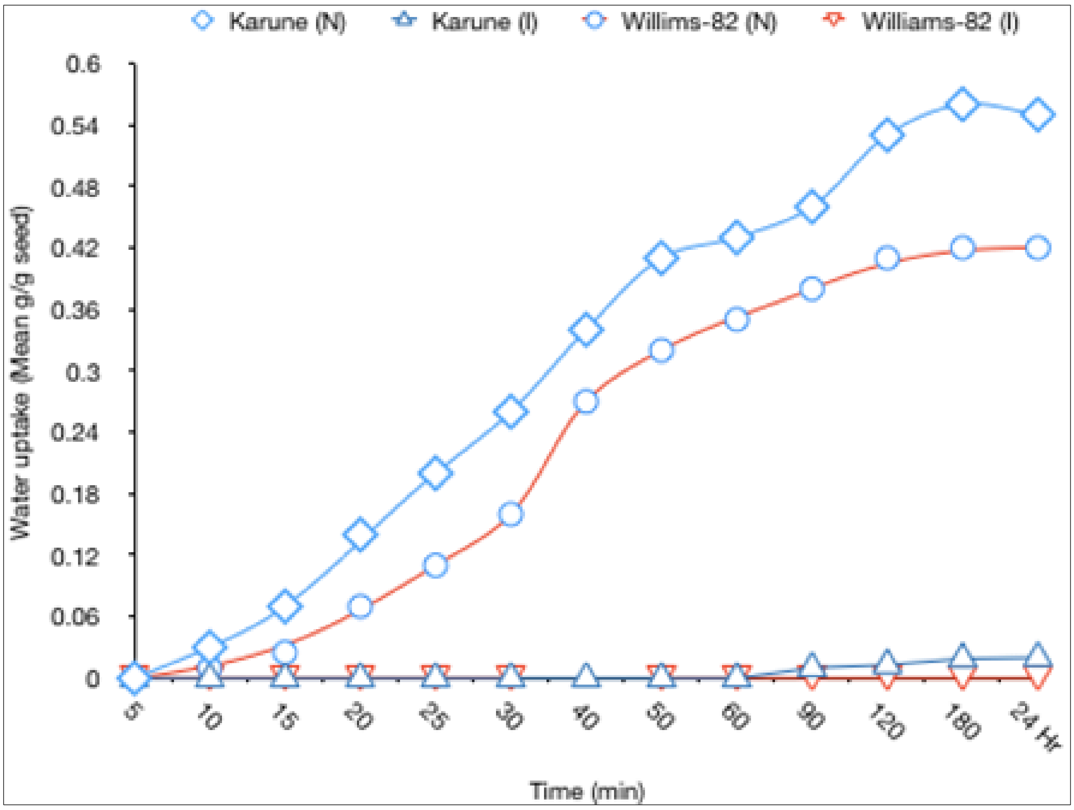

Fig 1: Permeabilities to water of vegetable soybean (Karune cv.) and grain soybean (William-82). Seeds were harvested in 2020 and measured in the same year (Only three hour data is indicated.

The crinkle formed in the seed coat of Karune first appearing within 1-6 min of incubation and usually on the dorsal side of the seeds. These crinkles spread towards the ventral side as the imbibition proceeded. Later, they all disappeared when the seed was completely hydrated (increased the weight of 2.2-2.5 fold of its original weight). Concern was particularly the location of primary sites of hydration in the seed coat.

Micromorphology of soybean seed

The soybean seed morphology is shown in Fig. 2. is made up of four basic parts: a seed coat or hull (8%), the plumule and hypocotyl-axis (2%) and two large cotyledons (90%).The cotyledons are of primary interest since they contain the storage tissue of the seed. Developing seeds are attached by the funiculus to the two large vascular bundles in the pod placenta. The hilum region of the seed coat is composed of several distinct tissues, few of which are involved in photosynthate import. At the hilum, the seed coat is considerably thicker and exhibits marked cellular differences from the remainder of the seed coat.

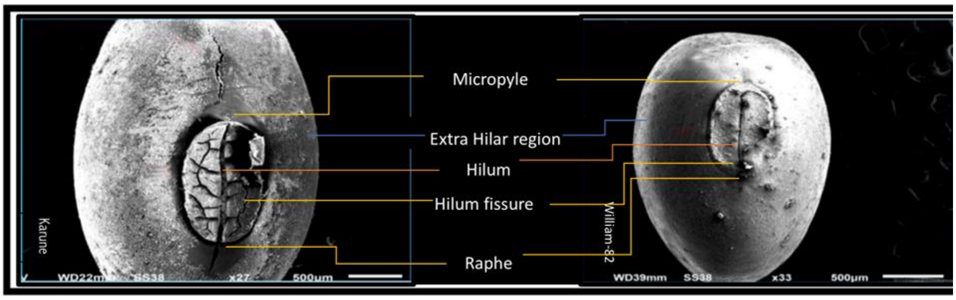

Fig 2: Micro-morphology two contrast different cultivar of soybean

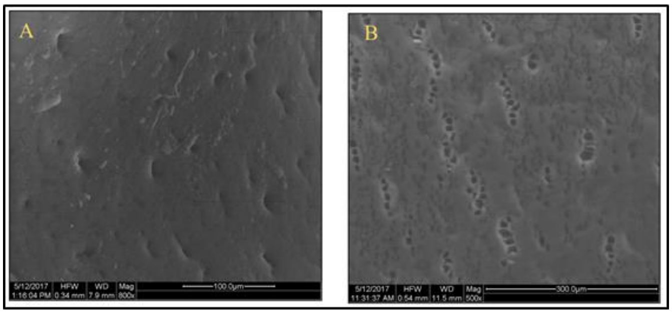

Fig 3: Porousness of seed surface; Left to right A) Karune, more irregular pore spread throughout the surface; B)William-82,

In the Fig.3. here it described the permeability of the seed coat depends upon the uptake of water which shows the permeability character of particular seed which has ultimately striking of the character of hard and softness of the seeds. Permeable seeds have larger pore size compare to less permeable seed as shown in Fig. 3. Some examples are tightly bound palisade cells (Corner, 1951; Ballard, 1973) [6, 1], thickened coat (Wyatt, 1977; Miao *et al*., 2001) [28, 16], lack of pits (Yaklich *et al*., 1986; Harris, 1987; Hahalis *et al*., 1996) [29, 30, 8, 7]. The SEM study showed the seed permeability of water due to small cuticular cracks present on the seed surface, visible with SEM.

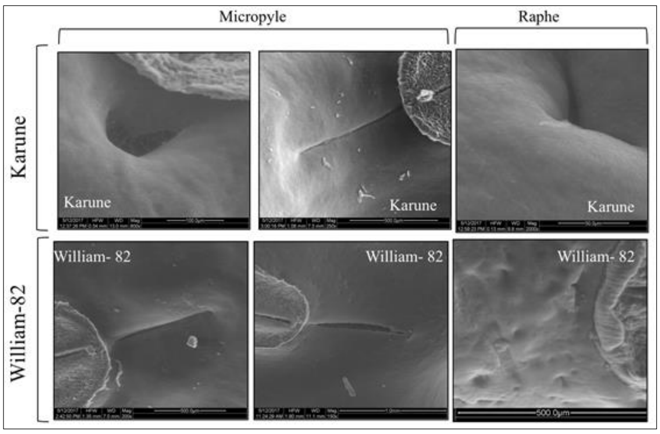

Fig 4: Scanning electron micromorphology of micropyle, raphe of Karun e cv, William-82 respectively

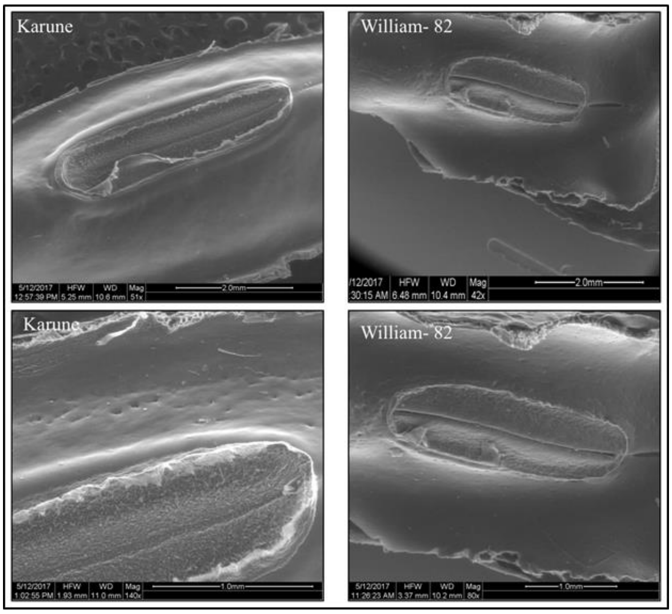

Fig 5: Scanning electron Micor-morphology of hydrated seeds of vegetable and grain type seeds Hilum and Hilar fissure structure.

Micro-morphology of seed permeability factors between W-82 and Karune

Common developmental patterns in the different types of seed coats were observed with the scanning electron microscope. SEM showed that generally pores begin to form first around the hilum (Fig. 3, 4 and 5). The hilum, which is the scar resulting from the ovule attachment to the funiculus, was considered the ventral surface. Pores also formed in a straight line with the axis of the hilum and micropyle encircling the seed to the area opposite the hilum on the dorsal surface. and completing the encirclement back around to the raphe. As the seed coat developed, the pores formed outward across the abaxial surface (round face of the cotyledon). Initially, pores varied in shape from elongated (Fig 3) to circular (Fig 4) and were either shallow or deep (Fig 5). Pore development continued throughout the depressed ovate stage until most of the seed coat surface was covered (Table 1 and 2). The pattern of pore development, except for William-82, changed during the transversely broadly elliptic stage. Seeds entering this stage had numerous pores (Fig. 3, 4 and 5). The size of micropyle, hilum, raphe, depression size near hilum and hilar fissure in Karune mean was recorded 268.71µM, 1165.47µM, 120.34µM, 51.09 µM and 45.04 µM respectively shown in Table 1

Table 1: Micro-morphology of seed permeability factor (µM) determining germination in Karune

Karune	Micropyle	Hilum	Raphe	Depression size near hilum	Hilar Fissure
1	273.214	426.885	116.311	48.652	48.53
2	268.354	4150.24	120.365	51.248	42.53
3	271.658	421.02	116.546	53.214	43.214
4	259.641	398.21	118.254	50.103	46.57
5	270.682	431.012	130.246	52.254	44.357
Mean	268.71	1165.47	120.344	51.094	45.040
Std. Dev	5.37	1668.58	5.769	1.789	2.481
Min	259.64	398.21	116.311	48.652	42.530
Max	273.21	4150.24	130.246	53.214	48.530

Table 2: Micro-morphology of seed permeability factor (µM) determining germination in Williaam-82

William-82	Micropyle	Hilum	Raphe	Depression size near hilum	Hilar Fissure
1	151.816	26.43	83.34	34.924	44.8
2	148.314	152.021	72.63	40.256	36.56
3	153.45	315.24	78.261	38.654	32.64
4	160.24	67.24	60.384	36.54	35.25
5	148.615	30.256	91.45	34.25	30.25
Mean	152.49	118.24	77.213	36.9248	35.9
SD	4.845	121.17	11.68	2.52	5.54
Min	148.314	26.43	60.384	34.25	30.25
Max	160.24	315.24	91.45	40.256	44.8

Note: Std. Dev= Standard deviation, Min= Minimum value, Max= Maximum value

The size of the micropyle, hilum, raphe, depression size near hilum and hilar fissure was recorded in W-82 *i.e* 151.81 µm, 26.43µM, 83.34µM, 34.92µM and 44.80µM respectively, presented in Table 2.

Discussion

Proper seed germination is vital for better crop yield (Munawar *et al.*, 2012) [17]. Seed coat plays an important role in the process of water imbibition (Ma *et al.*, 2004; Shao *et al.*, 2007; Meyer 2007; Koizumi *et al.*, 2008) [14, 15, 21]. The major change observed during the depressed ovate stage was the differentiation of the seed coat to form characteristics of the mature seed coat surface These characteristics are i) a surface morphology common to all soybean seed coats (Fig 3, 4 and 5) ii) pores, and iii) an impermeable seed coat. The development of pores and an impermeable seed coat were not characteristics of all genotypes. The mature surface was possibly caused by the epidermis collapsing into the lumen of the palisade cells (Fig 3, 4 and 5), resulting a folded surface that becomes compressed and compact with further development (Plate 3). This ability for change in the surface of the developing seed may provide protection for the embryo when severe climatic conditions prematurely terminate seed growth. Calero *et al*., (1981) [3, 4] observed that two seeds from the same F6 plant had different seed coat surfaces; one was an impermeable seed, without pores and the other was a normal seed with waxy material embedded on the epidermis and occasional pores on the surface. They reasoned that the difference between these two seed coats was not genetic because the seed coat was maternal tissue. We observed similar differences during the development of the seed coat in W-82 and Karune in that the quantity of pores, their location, depth, and structure was dependent on the individual seed and stage of development at which they were allowed to desiccate during maturation (Calero *et al*., 1981) [3, 4].

Wrinkling of the seed coat was the first visible sign of imbibition by the seed findings supported by Yaklich *et al.,* 1986 [29, 30]. Water imbibition in seeds is a two-phase process, the first dominated by hydration of the seed coat and the second by hydration of the cotyledons, which is rate-limited by the coat. When hydrated, coats of seeds is permeable to water but their hydraulic conductivity, as measured with a pressure probe, is smaller than that of coats from permeable seeds by a factor of five. Hydrated seed coats permeable and the seeds showed osmometer properties. Wrinkling of the seed coat can occur within a few minutes for a cultivar with a permeable seed coat such as Williams-82 (Chris *et al.,* 2007) [5].

Conclusion

The genotypes of vegetable type soybean are soft seeded. However, soft seeded genotypes are having short viability. The information generated in this study would pave the only for developing of soybean genotypes with high viability permeability.

In order to retain seed viability and life span of seed there is a urgent need to set up the breeding programme to imrove the seed micro- morphological tarits with out compromising the seed permeability.

References

1. Ballard L, *et al* Physical barriers to germination. Seed Sci. and Technol 1973;1:285.
2. Bernard R, Weiss MU, Qualitative genetics. In: Caldwell BE (ed) Soybeans: improvement, product, and uses. American Society of Agronomy, Madison 1973, 117-164.
3. Calero E, West SH, Hinson K. Water absorption of soybean seed quality. Agron. J 1981;65:440-441.
4. Calero E, West SH, Hinson K. Water absorption of soybean seed and associated causal factors. Crop Science 1981;21:926-933.
5. Chris J, Meyer E, Steudle Carol A. Peterson, Patterns and kinetics of water uptake by soybean seed Journal of Experimental Botany 2007;58(3):717-732.
6. Corner EJH. The Leguminous Seed. Phytomorphology 1951;(1):117–121.
7. Hahalis D, Cochrane MP, Smith ML. Water penetration sites in the testa of soybeans (*Glycine max* L. Merril) during seed imbibition Science of Legumes 1996;(3):218-226.
8. Harris WM. Comparative ultrastructure of developing seed coats of ‘hard-seeded’ and ‘soft-seeded’ varieties of soybean, *Glycine max* (L.) Merr Botantical Gazette 1987;148:324-331.
9. Hyde EOC. The function of the hilum in some Papilionaceae in relation to the ripening of the seed and the permeability of the testa Annals of Botany 1954;18:241-256.
10. Koizumi M, Kikuchi K, Isobe S, Ishida N, Naito S, Kano H. Role of seed coat in imbibing soybean seeds observed by micro-magnetic resonance imaging Ann. Bot 2008;102:343-352.
11. Lackey JA. Systematic significance of the epihilum in Phaseoleae (Fabaceae, Faboideae). Bot. Gaz 1981;142:160–164.

12. Lersten NR. Tracheid bar and vestured pits in legume seeds (Leguminosae: Papilionoideae). Am. J Bot 1982;69:98-107.
13. Lush AD, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR 1949;60;2859–2876.
14. Ma FE, Cholewa T, Mohamed CA, Peterson M Gijzen. Cracks in the palisade cuticle of soybean seed coats correlate with their permeability to water. Ann. Bot 2004;94:213-228.
15. Meyer CJ, Steudle E, Peterson CA. Patterns and kinetics of water uptake by soybean seeds. J Exp. Bot 2007;58:717-732.
16. Miao ZH, Fortune JA, Gallagher J. Anatomical structure and nutritive value of lupin seed coats. Australian Journal of Agricultural Research 2001;52:985–993.
17. Munawar IM, Dil UH, Zia J, Yasir MR, Ahmad. Effect of pre-sowing magnetic field treatment to garden pea (*Pisum sativum* l.) seed on germination and seedling growth. Pak. J Bot 2012;44(6):1851-1856.
18. Newell CA, Hymowitz T. A reappraisal of the subgenus Glycine. Am. J Bot 1978;65(2):168-179.
19. Ragus L, Role of water absorbing capacity in soybean germination and seedling vigour. Seed Science and Technology 1987;15:285–296.
20. Rolston MP. Water impermeable seed dormancy Bot. Rev 1978;44:365.
21. Shao SC, Meyer J, Ma F, Peterson CA. Bernards MA. The outermost cuticle of soybean seeds: chemical composition and function during imbibition. J Exp. Bot 2007;58(5):1071-1082.
22. Spurny M. Cell wall structure of epidermal cells of the pea seed coat (*Pisum sativum* L.) studied by micro cinematography. Mikroskopie 1963;18:272–279.
23. Taylor GB, Revell CK, Seed softening, imbibition time, and seedling establishment in yellow serradella. Aust. J Agric. Res 2002;53:1011–1018.
24. White J. The occurrence of an impermeable cuticle on the exterior of certain seeds. Proc. Royal Soc. Victoria 1908;21:203–210.
25. Williams LF. Structure and genetic characteristics of the soybean. In: Markley KS, ed. Soybeans and soybean products: New York: Inter-science Publishers 1950;1:111-134.
26. Wolf WJ, Baker FL. Scanning electron microscopy of soybeans. Cereal Sci. Today 1972;17:125-132.
27. Wolf WJ, Baker FL, Bernard RL. Soy bean seed-coat structural features: Pits, deposits and cracks. Scanning Electron Microscopy 1981;3:531-544.
28. Wyatt JE. Seed coat and water absorption properties of seed of near-isogenic snap bean lines differing in seed coat colour. Journal of the American Society of Horticultural Science 1977;102:478–480.
29. Yaklich RW, Vigil EL, Wergin WP. Pore Development and Seed Coat Permeability in Soybean. Crop science 1986;26(3):980-1001.
30. Yaklich RW, Vigil EL, Wergin WP. Pore development and seed coat permeability in soybean. Crop Science 1986;26:616−62.