Wall bound phenolics and total antioxidants in stored seeds of soybean (Glycine max) genotypes

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ABSTRACT

Quality seed is the most important input for sustainable agricultural productivity. Recent studies highlight the role of antioxidants in scavenging the reactive oxygen species, which is an indicator for quality seed. In natural ageing conditions, reactive oxygen species accumulates in the seeds that lead to seed deterioration. Wall bound phenolics in seed coat are potential antioxidants that encounter the reactive oxygen species. Four soybean genotypes with different seed coat wall bound phenolics were studied in 2018 at ICAR-Indian Institute of Seed Science and found that the black coat seed such as kalitur and JS76-205 showed highest phenolic content (117.00 and 128.50 µg/mL) and antioxidant activity of 51.38 and 46.07 µg eq. ascorbic acid, respectively. On contrary, the harasoya (green coated seed) resulted lowest phenolic content 14.30 µg/mL, while in RAUS-05 (white coated seed) the antioxidant activity is 2.58 µg eq. ascorbic acid, which is significantly lower than the other genotypes. Further, the genotypes were evaluated for the seed quality parameters and observed that kalitur and JS76-205 showed higher germination percentage (80%) and vigor indices I (900.33) and II (35.01) than the other genotypes. These results substantiate the hypothesis that the seed coat wall bound phenolics with antioxidant properties could alleviate the reactive oxygen species, protects from mechanical and chemical damage and helps in enhancement of seed quality parameters.

Key words: Antioxidants, Ferulic acid, Phenolics, Protocatechuic acid, Soybean

Recent observations on climatic changes and their influence on quality seed production have become a major challenge for sustainable agricultural productivity (Ali et al. 2017). Studies on seed deterioration substantiate that the fluctuations in weather conditions, i.e., biotic and abiotic factors contribute reactive oxygen species (ROS) production (Yadav et al. 2006, Sano et al. 2016). These radicals have high reactivity and damage lipids, DNA, RNA and proteins that ultimately lead to cell death (Kumar et al. 2015, 2016, Chandusingh et al. 2017). Phenolics that have the antioxidant capability because of reductox properties can neutralize or quench the highly reactive hydroxyl radical (OH·), singlet and triplet oxygen (Dona et al. 2013). Hence, mere possessing of quality phenolics is essential for antioxidant activity (Mhamdi et al. 2010). Talai and Sen-Mandi (2010) have proposed that the total antioxidant potential is mainly governed under genetic control in fresh harvested seeds; whereas in aged seeds, environmental factors determine the quality of the seed, which indicates that the aged seeds are prone to oxidative damage and need to be protected with antioxidants like seed coat phenolics. However, most of the hypothesis developed is based on the control deterioration or accelerated ageing experiments that need to be verified in naturally aged seeds. The problem of seed deterioration and field weathering are prominent in soybean genotypes. To determine the role of phenolics in seed longevity, 4 soybean genotypes such as kalitur (landrace having higher longevity) and JS76-205 (used in seed chain) with black seed coat phenolics, harasoya and RAUS-5 (defoliator) with green and white coat seeds (Agarwal et al. 2013) that differ in seed coat phenolics were chosen in the study. Further, evaluation of seed coat phenolics and their relation with quality seed parameters have been studied.

MATERIALS AND METHODS

Soybean genotypes: Soybean genotypes such as kalitur, JS75-206 (black coat seed), harasoya (green coat seed) and RAUS-05 (white coat) were supplied by ICAR-Indian Institute of Soybean Research, Indore, India. The seeds were naturally stored at 25°C for 1 year and used for experimental studies performed in 2018.

Phenolics extraction from seed coat: Seed coat
Seedling vigour index I = Germination (%) × Mean seedling length

Seedling vigour index II = Germination (%) × Mean seedling dry weight (mg)

**RESULTS AND DISCUSSION**

**Colour of different soybean genotypes:** Soybean cultivars with different colour are chosen to study the influence of seed coat phenolics in seed longevity. Cultivars such as kalitur and JS76-205 are having black seed coat, harasoya with green seed coat and RAUS-05 possess white seed coat. Seed coat colour of soybean is having a significant importance on the longevity and found the correlation between colour and germination. In the present study, kalitur and JS76-205 showed higher germination (80%), whereas harasoya and RAUS-05 resulted 60% each, respectively. The colour formation at seed coat is mainly attributed to the presence of phenolic compounds such as flavonoids. It is observed that in early seed development of Arabidopsis, the flavonoids accumulate as colorless compounds in the vacuoles (Gomes and Garcia 2013).

**Wall bound phenolics extraction from seed coat:** Seed coats have different phenolic compounds and to have optimum extraction, solvent systems that dissolve the desired products are essential. In the study, 07 solvent systems have been studied, of which acetone : water : acetic acid (70:29.8:0.2) solvent system resulted 129.4 µg/mL and 121.0 µg/mL of total phenolics from JS76-205 and kalitur (black seed coat) genotypes, respectively. Similarly, for harasoya (green coat) genotype, acetone: water: acetic acid (70:28:02) solvent system extracted 21.0 µg/mL of total phenolics; while for RAUS-05 (white seed coat) genotype 23.6 µg/mL of total phenolics has been obtained with ethanol:water in ratio of 7:3. Phenolic compounds have specific solubility in different solvent systems, as a result each genotype phenolics have been obtained in different solvent systems (Kumar et al. 2017, Singh et al. 2014).

**Estimation of wall bound seed coat phenolics from different soybean genotypes:** Total phenolic from seed coat have been standardized with different solvent systems. The black seed coated genotypes such as JS76-205 has showed highest phenolic content with the 128.50 µg/mL, while kalitur genotype resulted with 117.00 µg/mL phenolic contents. On the other hand, the white seed coated RAUS-05 genotype showed 19.40 µg/mL phenolic content; whereas, the green seed coat genotype harasoya has phenolic content of 14.30 µg/mL, which is lowest among all other genotypes (Fig 1).

Dona et al. (2013) have reported that the phenolics availability is one of the quality seed parameter. Seed coat phenolics have several functions in seed physiology. For
example, damaged seed coats are prone to chilling injury due to hydration stress in imbibition stage. Interestingly, Tully et al. (1981) have observed that black seed coat soybean imibe water slowly and are less prone to chilling injury. Another function of phenolics in seed coats possess chemical defense against microorganisms. Unlike in polyphenolic form, phenolics as monomers may serve as inhibitors to fungal growth that enhance this germination under humid storage conditions (Vinutha et al. 2014).

**Determination of antioxidant potential of soybean genotypes:** Phenolic compounds having antioxidant activity is highly desirable in the seed germination process. During seed germination, free radical synthesis occurs that can be neutralized by antioxidants. Seed coat of different soybean genotypes showed different antioxidant potential. The highest antioxidant potential was determined in kalitur 51.38 µg eq. ascorbic acid followed by JS76-205 with 46.07 µg eq. ascorbic acid and RAUS-05 resulted lower antioxidant potential with 2.58 µg eq. ascorbic acid (Fig 2). Phenolics with antioxidant potential are desirable because in germination process increase of ROS is widely reported (Tiwari et al. 2018, Sarangi et al. 2019).

For instance, flax seed coats are rich in polyphenolics (ligands), which act as antioxidants and may scavenge the ROS. This effect can protect the embryo, because the position of latter and its relation to seed coats and components could influence the seed storability (Chandusingh et al. 2018). Similar observations of viability lose in peanut and onion seeds due to radicle tip damage indicate the potential of phenolic antioxidant in the seed coats (Sinha et al. 2016, Singh et al. 2017b).

**Characterization of phenolics derived from different soybean genotypes:** Seed coat phenolics of soybean genotypes revealed presence of antioxidants such as protocatechuic acid, p-coumaric acid, t-ferulic acid, vanillin, 4-hydroxy benzoic acids and 4-hydroxy benzoic acid. These compounds quench the free radicals and help to maintain the homeostasis of ROS. Kalitur possessed highest amount of protocatechuic acid, 4-hydroxy benzoic acid, 4-hydroxy benzoic acid and vanillin with concentration of 3.41, 0.11, 1.39 and 0.06 µg/100 mg dry weight tissue. Similarly, in JS76-205 protocatechuic acid is 1.26 µg/100 mg dry weight tissue. On the contrary, RAUS-05 has showed 0.18 and 1.32 µg/100 mg of p-coumaric acid and t-ferulic acid, respectively. However, harsoya resulted with lower levels of phenolic content and antioxidant activity than the other genotypes (Table 1).

Several studies regarding the phenolic compounds have been performed and strong positive correlation between the phenolic compound contents and the antioxidant potential (Pang et al. 2018, Singh et al. 2019) has been observed. The seed antioxidants reduces the lipid oxidation (Kumar et al. 2015) and delay the ageing in seed tissues (Khurana et al. 2013). Besides, they protect the genetic material and essential biomolecules like proteins from the oxidative stress and enhance the nutraceuticals properties (Kumar et al. 2019).

**Quality seed parameters:** Quality seed parameters compromise of germination percentage, seedling length and vigour indices I and II. Among the soybean genotypes

![Fig 1](image1.png) **Fig 1** Correlation between seed coat phenolics, antioxidant and germination percentage of soybean genotypes.

![Fig 2](image2.png) **Fig 2** Electrical conductivity test from different soybean genotypes.

| Sample   | Protocatechuic acid | 4-Hydroxy benzoic acid | Vanillin | 4-Hydroxy benzoaldehyde | Vanillin | p-Coumaric acid | t-Ferulic acid |
|----------|---------------------|------------------------|----------|-------------------------|----------|----------------|----------------|
| JS-76205 | 1.2623 ± 0.0931      | 0.1076 ± 0.0105        | 0.0611 ± | 0.9048 ± 0.0836         | 0.0275 ± | 0.0335 ±       | 0.0977 ±       |
| Harasoya | 0.0059 ± 0.0004      | 0.0271 ± 0.0022        | 0.4581 ± | 0.9827 ± 0.0923         | 0.0229 ± | 0.0918 ±       | 0.7457 ±       |
| Kalitur  | 3.4114 ± 0.3112      | 0.1169 ± 0.0108        | 0.4343 ± | 1.3953 ± 0.1014         | 0.0645 ± | 0.1276 ±       | 0.8672 ±       |
| RAUS-05  | 0.0308 ± 0.0029      | 0.1059 ± 0.0108        | 0.5611 ± | 1.3711 ± 0.1132         | 0.0279 ± | 0.1810 ±       | 1.3259 ±       |

Table 1 Determination of seed coat phenolics in soybean genotypes using HPLC (±SD)
studied, the black coat genotypes kalitur and JS76-205 have germination percentage with 80 percent; whereas, white and green seed coat genotypes showed 60 percentage of germination. The vigour index-I of different soybean genotypes showed the descending order of kalitur (900.33 ± 1.76) > JS76-205 (818.67 ± 2.84) > harasoya (665.16 ± 2.48) > RAUS-05 (622.00 ± 2.16). Similarly, vigour index-II of soybean genotypes correspond to the trend that the kalitur (30.95 ± 1.76) > JS76-205 (35.01 ± 1.58) > harasoya (28.17 ± 1.00) > RAUS-05 (25.22 ± 0.75) as shown in Table 2.

**Electrical conductivity test:** Electrical conductivity (EC) is one of the important parameter that determined firmness of seed coat. Generally, in aged seed, the seed coat looses the firmness and allows leakage of electrolytes in the solvent. Higher the value of EC lower the quality of seed; while, lower the EC value signifies the quality of seed. In the present study, the highest EC among different soybean genotypes was in white coated seed of RAUS-05 with 213.00 μS/cm/g, lowest amount of EC was found in kalitur with the 168.37 μS/cm/g. The seed coats of harasoya and JS76-205 genotypes have 188.55 and 175.50μS/cm/g EC, respectively (Fig. 2). Thus, the current study exhibit correlation with Capeleti et al. (2005) that in soybean the phenolics polymerize to form lignin (polyphenol), which plays important role in seed permeability and resistance to mechanical damage.

**Correlation of wall bound phenolics, antioxidant activity and seed quality parameters:** Evidences on correlation of antioxidant potential, phenolics and seed quality parameters substantiate that the phenolics could increase the quality seed parameters by protecting from mechanical and chemical damage in maize (Vinutha et al. 2014). In the present study, soybean black seed coat showed highest germination, vigour indices – I and II. Presence of phenolics and antioxidant activity is higher in these genotypes and showed positive correlation. However, with the harasoya (green black coat) and RAUS-05 genotypes the phenolics is low concomitantly resulted with poor seed quality parameters (Fig 2). These results indicate that the presence of phenolics coupled with antioxidant potential could enhance the quality of seed parameters.

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### Table 2 Seed quality parameters of soybean genotype (±SD)

| Genotype | Germination (%) | Vigor Index-I | Vigor Index-II |
|----------|----------------|---------------|---------------|
| Kalitur  | 80.00 ± 1.52    | 900.33 ± 1.76 | 35.01 ± 1.58  |
| JS-76205 | 80.00 ± 0.90    | 818.67 ± 2.84 | 30.95 ± 1.17  |
| Harasoya | 60.00 ± 0.76    | 665.16 ± 2.48 | 28.17 ± 1.00  |
| RAUS-05  | 60.00 ± 1.61    | 622.00 ± 2.16 | 25.22 ± 0.75  |

### REFERENCES

Agarwal D K, Billore S D, Sharma A N, Dupare B U and Srivastava S K. 2013. Soybean: introduction, improvement, and utilization in India-problems and prospects. *Agricultural Research* 2: 293–300.

Ali S, Liu Y, Ishaq M, Shah T, Abdullah Ilyas A and Din I U. 2017. Climate change and its impact on the yield of major food crops: Evidence from Pakistan. *Foods Basel, Switzerland* 6: 39.

Campbell M M and Ellis B E. 1992. Fungal elicitor-mediated responses in pine cell cultures; cell wall-bound phenolics. *Phytochemistry* 31:737–42.

Capeleti I, Ferrarese M L L, Krzyzanowski F C and Ferrarese-Filho O. 2005. A new procedure for quantification of lignin in soybean (*Glycine max* (L.) Merrill) seed coat and their relationship with the resistance to mechanical damage. *Seed Science and Technology* 33: 511–5.

Chandusimg, Sripathy K V, Kumar S P J, Naik B K, Pal G, Udayabahaskar K, Ramesh K V, Somasundaram G. 2017. Delineation of inheritance pattern of aleurone layer colour through chemical tests in rice. *Rice* 10: 48–55.

Chandusimg, Boraih M K, Singh R K, Kumar S P J, Singh G, Chand R. 2018. Comparative study of floral biology using detergent and confirm self-incompatibility system in protogynous lines of Indian mustard (*Brassica juncea*). *Indian Journal of Agricultural Sciences* 88: 1190–7.

De Ascensao A R and Dubery I A. 2003. Soluble and wall-bound phenolics and phenolic polymers in *Musa acuminata* roots exposed to elicitors from *Fusarium oxysporum* f. sp. *cubense*. *Phytochemistry* 63: 679–86.

Don M, Balextrazzia A, Mondoni A, Rossi G, Ventura L, Buttafava A, Macovei A, Sabatini ME, Valassi A, Carbonera D. 2013. DNA profiling, telomere analysis and antioxidant properties as tools for monitoring ex situ seed longevity. *Annals of botany* 111:987–98.

Gomes M P and Garcia Q S. 2013. Reactive oxygen species and seed germination. *Biologia* 68: 351–7.

ISTA. 2008. International Rules for Seed Testing. Zurich. 31. pp. 288 (suppl.).

Khrana S, Venkataraman K, Hollingsworth A, Piche M and Tai T. 2013. Polyphenols: benefits to the cardiovascular system in health and in aging. *Nutrients* 5: 3779–827.

Kumar A, Agarwal D K, Kumar S, Reddy Y M, Chintagunta A D, Sarita KV, Pal G and Kumar S J. 2019. Nutraceuticals derived from seed storage proteins: Implications for health wellness. *Biocatalysis and Agricultural Biotechnology* 17: 710–9.

Kumar S P J, Rajendra Prasad S, Kumar M, Singh C, Sinha A K and Pathak A. 2016. Seed quality markers: a review. *Research & Reviews: Journal of Botanical Sciences* 3: 13–17.

Kumar S P, Rajendra Prasad S, Banerjee R and Thammmineni C. 2015. Seed birth to death: dual functions of reactive oxygen species in seed physiology. *Annals of Botany* 116: 663–8.

Kumar S P J, Prasad S R, Banerjee R, Agarwal D K, Kulkami K S, Ramesh K V. 2017. Green solvents and technologies for oil extraction from oilseeds. *Chemistry Central Journal* 11: 1–9.

Mhamdi B, Wannes W A, Sriti J, Jellali I, Ksouri R and Marzouk M. 2010. Effect of harvesting time on phenolic compounds and antiradical scavenging activity of *Borago officinalis* seed extracts. *Industrial Crops and Products* 31: 1–4.

Pang Y, Ahmed S, Xu Y, Beta T, Zhu Z, Shao Y and Bao J. 2018. Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. *Food Chemistry* 240: 212–21.
Panobianco M and Vieira R D. 1996. Electrical conductivity of soybean soaked seeds. I. Effect of genotype. Pesquisa Agropecuária Brasileira 31:621–7.

Sano N, Rajjou L, North H M, Debeaujon I, Marion-Poll A and Seo M. 2015. Staying alive: molecular aspects of seed longevity. Plant and Cell Physiology 57: 660–74.

Sarangi S, Mandal C, Dutta S, Mukherjee P, Mondal R, Kumar S P J, Choudhury P R, Singh V P, Tripathi D K, Mandal A B. 2019. Microparticle based particle bombardment in development of transgenic indica rice involving AmSOD gene to impart tolerance to salinity. Plant Gene 19: 100183.

Singh A, Karmakar S, Jacob B S, Bhattacharya P, Kumar S P J, Banerjee R. 2015. Enzymatic polishing of cereal grains for improved nutrient retention. Journal of Food Science and Technology 52: 3147–57.

Singh R P, Chintagunta A J, Agarwal D K, Kureel R S, Kumar S P J. 2019. Varietal replacement rate: Prospects and challenges for global food security. Global Food Security, 100324.

Sinha A K, Agarwal D K, Kumar S J, Chaturvedi A and Tiwari T N. 2016. Novel technique for precluding hybrid necrosis in bread wheat. International Journal of Tropical Agriculture 34: 761–5.

Talai S and Sen-Mandi S. 2010. Seed vigour-related DNA marker in rice shows homology with acetyl CoA carboxylase gene. Acta Physiologiae Plantarum 32:153–67.

Tiwari T N, Kumar S P J, Tiwari A K, Agarwal D K. 2018. Seed coating in relation to minimizing the effects of seed ageing in rice (Oryza sativa L.). Journal of Rice Research 10 (2): 27–32.

Tully R E, Musgrave M E and Leopold A C. 1981. The seed coat as a control of imbibitional chilling injury. Crop Science 21: 312–7.

Vinutha K S, Prasad S R, Murthy P, Kumar S P J and Rame Gowda, R P. 2014. Influence of staggered sowing, planting ratio and subtending cob leaf clipping on seed quality parameters of maize. Seed Research 42: 91–97.

Yadav S, Bhatia V S and Guruprasad K N. 2006. Oxyradical accumulation and rapid deterioration of soybean seeds due to field weathering. Indian Journal of Biochemistry and Biophysics 43: 41–47.

Zlotek U, Mikulska S, Nagajek M and Świeca M. 2016. The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (Ocimum basilicum L.) extracts. Saudi Journal of Biological Sciences 23: 628–33.