Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252019v32n317rc

SOLUBLE PROTEIN AS INDICATIVE OF PHYSIOLOGICAL QUALITY OF SOYBEAN SEEDS[1]

VANDERLÉIA MATHIAS[2], CILEIDE MARIA MEDEIROS COELHO[2]*, JAQUELINI GARCIA[2]

ABSTRACT - After physiological maturity, the seed is physiologically independent of the plant, and responds to climatic variations that can decrease its vigor, which is dependent on the cultivar. The objective of this work was to evaluate the vigor of soybean cultivars and identify the biochemical components that have the greatest contribution to the maintenance of the physiological quality of the seeds after physiological maturity. The experiment was conducted in Fraiburgo, SC, Brazil, during the 2015/2016 crop season, using four soybean cultivars NA 5909 RG, BMX Ativa RR, BMX VanguardaIPRO, and NS 5959 IPRO. The seed physiological quality and the biochemical composition were evaluated at the phenological stages R7, R7+5 days, R7+10 days (R8), and R7+20 days. The data were subjected to analysis of variance (F test) and the means were compared by the Tukey's test (p<0.01). The seed biochemical composition and physiological quality were correlated using multivariate statistics. The germination of the seeds decreased 6% after physiological maturity up to R7+20 days. This decrease in vigor was dependent on the cultivar; NA 5909 RG decreased 3%, and BMX Ativa RR and NS 5959 IPRO decreased 7%. The biochemical components soluble protein, phytate, soluble sugar, and lipids decreased as a function of the harvest times, indicating the beginning of the process of seed quality loss. The maintenance of seed vigor after physiological maturity was dependent on the cultivar. The soluble protein content can be used as an indicator of the maintenance of physiological quality of soybean seeds after R7.

Keywords: *Glycine max*. Vigor. Biochemistry composition.

PROTEÍNA SOLÚVEL COMO INDICATIVO DE MANUTENÇÃO DA QUALIDADE FISIOLÓGICA DE SEMENTES DE SOJA

RESUMO - Após a maturidade fisiológica a semente desliga-se fisiologicamente da planta, respondendo a variações climáticas que podem proporcionar decréscimo no seu vigor de forma dependente da cultivar. Desta forma, objetivou-se avaliar o comportamento de cultivares de soja quanto a variações no vigor e identificar quais os componentes bioquímicos que apresentam maior contribuição para a manutenção da qualidade fisiológica das sementes após a maturidade fisiológica. O experimento foi conduzido no município de Fraiburgo, SC, Brasil, na safra 2015/16 utilizando quatro cultivares de soja, NA 5909 RG, BMX Ativa RR, BMX Vanguarda IPRO, NS 5959 IPR. Foram avaliadas a qualidade fisiológica e a composição bioquímica nas épocas de colheita, R7, seguindo de R7 + 5 dias, R7 + 10 dias (R8), R7 + 20 dias. Os dados foram submetidos à análise de variância (teste F) e as médias comparadas pelo teste de Tukey (p<0,01). Para correlacionar a composição bioquímica e a qualidade fisiológica utilizou-se estatística multivariada. Verificou-se redução de 6% na germinação da maturidade fisiológica até R7 + 20 dias. Para o vigor, essa redução foi dependente da cultivar, enquanto a NA 5909 RG reduziu 3%, as BMX Ativa RR e NS 5959 IPRO tiveram redução de 7%. Os componentes bioquímicos, proteína solúvel, fitato, açúcar solúvel e lipídios reduziram em função das épocas de colheita, indicando o início do processo de perda de qualidade das sementes. A manutenção do vigor das sementes após a maturidade fisiológica foi dependente da cultivar. O teor de proteína solúvel pode ser utilizado como indicativo de manutenção da qualidade fisiológica de sementes de soja após R7.

Palavras-chave: *Glycine max*. Vigor. Composição bioquímica.

---

*Corresponding author
[1]Received for publication in 04/01/2019; accepted in 07/01/2019.
Paper extracted from the master degree of the first author.
[2]Department of Agronomy, Universidade do Estado de Santa Catarina, Lages, SC, Brazil; vanderleia.mathias@gmail.com – ORCID: 0000-0003-1640-7932, cileide.souza@udesc.br – ORCID: 0000-0001-9528-7371, jaquelini.garcia@hotmail.com – ORCID: 0000-0002-2073-2423.

INTRODUCTION

Vigor is the main physiological quality parameter of seeds, it establishes the potential of seedling emergence in the field (HENNING et al., 2010). The maximum physiological quality of seeds is reached at their physiological maturity, subsequently, they are subject to changes that reduce their quality (MARCOS-FILHO, 2015a). The natural quality loss process that occurs after their physiological maturity is one of the factors that reduce physiological quality of seeds (ROSA et al., 2017), which includes cytological, physiological, biochemical, and physical changes that can culminate in the death of the seed (JYOTI; MALIK, 2013). These changes include protein denaturation, free fatty acid increases, and decreases in sugar contents and storage reserves (MARCOS-FILHO, 2015b).

High temperatures and variations in relative air humidity increase the respiration process and reduce seed vigor, characterizing the pre-harvest quality loss process (WANG et al., 2012). Pre-harvest weather conditions have a direct effect on seed quality and may be the main cause of decreases in seed vigor (SMANIOTTO et al., 2014). This problem can be minimized using cultivars that present genetic characteristics to maintain seed vigor, even when the seed production coincides with adverse environmental conditions.

From a biochemical point of view, vigor is related to high soluble protein, starch, and soluble sugar contents (HENNING et al., 2010). Changes caused by the pre-harvest quality loss process in carbohydrate content lead to limitations in the availability of substrate for respiration, decreasing vigor and germination (MARCOS-FILHO, 2015a). Changes in lipids are due to enzymatic hydrolysis, peroxidation, and self-oxidation (MARCOS-FILHO, 2015a). The protein content is also affected during the seed quality loss; it decreases and undergoes denaturation due to high temperatures (MARCOS-FILHO, 2015a). Variations in protein profiles may provide efficient indicators for monitoring biochemical processes associated with seed vigor (HENNING et al., 2010; HAN et al., 2013; MARCOS FILHO, 2015a).

The biochemical components of soybean seeds that assist in the maintenance of physiological quality during the drying phase, when the pre-harvest quality loss process occurs, is not widely understood. Considering the climate changes, researches involving biochemical tools to differentiate cultivars regarding their tolerance to these changes have been conducted (HENNING et al., 2010; WANG et al., 2012; HAN et al., 2013; DELARMELINO-FERRARESI; VILLELA; AUMONDE, 2014). Thus, focused on explaining the physiological and biochemical changes involved with the pre-harvest quality loss process (R7), the objective of this work was to evaluate the seed vigor of soybean cultivars and identify the biochemical components that have the highest contribution to the maintenance of seed physiological quality after physiological maturity.

MATERIAL AND METHODS

The experiment and seed production were conducted in Fraiburgo, SC, Brazil (27°07'11"S, 50° 59'13"W, and 997 m altitude) during the 2015/2016 crop season. The experiment was conducted in a randomized block design with split plots and four replications. The plots (50 m²) consisted of the cultivars (NA 5909 RG, NS 5959 IPRO, BMX Ativa RR and BMX VanguardaIPRO), and the subplots (12.50 m²) consisted of harvest times (S1 = R7 phenological stage, corresponding to seed physiological maturity; and S2 = R7+5 days; S3 = R7+10 days, or R8; and S4 = R7+20 days). The evaluation area consisted of the central 4.05 m².

The seeds harvested at R7 (53% moisture) and R7+5 days (18% moisture) were kept in the pods and dried in an air-circulation oven at 35°C until reaching approximately 13% moisture. The seeds harvested at R7+10 days and R7+20 days did not need drying, since they had already moisture content of approximately 13%. The seed moistures were determined by drying them in an oven at 105ºC for 24 hours, with 2 replications of approximately 4.5 g, considering the difference between their wet and dry weights, times 100, and the arithmetic mean of the percentages of each replication as the final results (BRASIL, 2009).

After threshing, the seeds were stored in a dry chamber (10°C and relative humidity of 40%). The seed physiological quality was evaluated by germination and their vigor by accelerated aging and seedling length tests.

The germination test was conducted with four replications of 50 seeds on a germination test paper in a germinator set at 25 °C. The water volume for seed imbibition was 2.5 times the paper weight. Counts were performed at five days after sowing. The criteria for seedling evaluation are described in the Rules for Seed Analysis (RAS), considering the number of normal seedlings, abnormal seedlings, and dead seeds (BRASIL, 2009).

The accelerated aging test were performed in 200 seeds per treatment (four samples of 50 seeds). The seeds were distributed in a single layer on a steel mesh and placed inside plastic boxes (Gerbox®) containing 40 mL of distilled water. The distance between the water level and the seeds was approximately 2 cm. The boxes were closed and taken to an accelerated aging chamber set at 41ºC for 48 hours. After this period, the germination test was conducted, and their vigor was determined based on the number of normal seedlings, abnormal seedlings, and dead seeds on the fifth day after sowing (MARCOS-FILHO; KIKUTI; LIMA, 2009).

Seedling length was evaluated on germination test paper, using 20 seeds per replication, following the procedures used in the germination test; the total seedling, root, and shoot lengths were measured after five days (NAKAGAWA, 1999).

Total protein and total phosphorus contents were determined by sulfuric digestion. Total protein was calculated by multiplying the total nitrogen content of the sample by 6.25. Nitrogen content was determined using the Kjeldahl method. Total phosphorus content was read on a spectrophotometer, using a wavelength of 620 nm, and the results were expressed as mg g^{-1} of dry matter (TEDESCO; VOLKWEISS; BOHNEN, 1995).

Soluble protein was determined in 1 g samples of dried and ground seeds. It was extracted using 4 mL of KH_2PO_4 at pH 6.8, which was stirred and centrifuged at 3600 turns for 30 minutes. The supernatant was collected and a 20 µL aliquot of the extract was taken and mixed with 1 mL of the Bradford color reagent. The soluble proteins were determined in a spectrophotometer at 595 nm absorbance according to the method described by Bradford (1976) and the results were expressed in mg g^{-1} of dry matter.

Soluble sugars were quantified by homogenization with hot ethanol (85%), and subsequent agitation and centrifugation, according to the Antrona method (CLEGG, 1956); it was read in a spectrophotometer at 620 nm absorbance and the results were expressed as mg g^{-1} of dry matter.

The starch content was determined using the residue derived from the extraction of soluble sugars, following the methodology described by McCready, Guggoolz and Wens (1950). The reading was based on the Antrona method as in the quantification of soluble sugars and the results were expressed as mg g^{-1} of dry matter.

The phytate content was determined by the method described by Latta and Eskin (1980), based on the formation of a dark blue iron-sulfosalicylic acid compound called wade reagent. For the quantification, distilled water and treated resin were added to the sample, then added to each NaCl phase; in the last phase of the determination, 2 ml of the wade reagent was added, centrifuged and read in a spectrophotometer at 500 nm; the results were expressed as mg g^{-1} of dry matter.

Inorganic phosphorus was determined following the methodology of Raboy and Dickinson (1984); inorganic phosphorus was extracted and quantified by colorimetry, according to Chen et al. (1956). The reading was done at 820 nm wavelength and the results were expressed as mg g^{-1} of dry matter.

The lipid content was determined based on the Bligh-Dyer method. The sample was homogenized and- the layers were separated; the samples were placed in an oven at 80°C to evaporate the solvent (15-20 minutes). The sample was cooled in a dryer and weighed on an analytical balance; the result was expressed as percentage (BLIGH-DYER, 1959).

The data were subjected to analysis of variance by F test and means were compared by Tukey's test (p<0.01). Principal component analysis (PCA) and partial least squares regression (PLS-R) was used to correlate the seed biochemical response to physiological quality, using the R program (R CORE TEAM, 2016).

RESULTS AND DISCUSSION

The analysis of variance of the physiological quality showed significant differences for cultivars and harvest times in all analyzed variables, indicating genetic variability between cultivars (Table 1). The mean square of the interaction between cultivars and harvest times presented significance for seed vigor, and seedling, root, and shoot lengths, showing different performance of cultivars when subjected to different harvest times (Table 1). The coefficients of variation were low, showing the reliability of the results obtained (Table 1).

Table 1. Mean squares of analysis of variance for germination (G), vigor by accelerated aging (VI), seedling length (SL), root length (RL), and shoot length (SL) of four soybean cultivars subjected to four harvest times in the 2015/2016 crop season.

Source of variation	Degrees of freedom	Mean square G	VI	SL	RL	SL
Block	3	5.5	2.5	3.8	1.2	1.06
Cultivar (C)	3	64.7**	125.8*	45.3**	8.97**	20.5**
$Residue_1$	9	7.4	29.9	1.4	0.48	0.33
Harvest time (HT)	3	217.2**	147.5**	9.9**	4.34**	1.29**
C × HT	9	17.9	45.1*	4.9**	2.30**	0.479**
$Residue_2$	36	11.3	17.8	0.86	0.52	0.14
Mean	-	90.3	88	17.6	11.2	6.4
$CV (\%)_1$	-	3.0	6.2	6.8	6,2	9.0
$CV (\%)_2$	-	3.7	4.8	5.3	6.5	6.0

**Significant at the 1% probability level. *Significant at 5% probability level. CV = coefficient of variation.

The physiological quality evaluation showed no significant effect for the first three harvest times, thus, T3 (R7+10 days) and T4 (R7+20 days) were used to determine the contents of total protein, soluble protein, soluble sugar, starch, total phosphorus, phytate, inorganic phosphorus, and lipid (Figure 1a).

The germination percentages of the cultivars during the drying phase ranged, on average, from 92% (NA 5909 RG) to 87% (BMX Ativa RR) (Figure 1a), which is above the minimum required by the current legislation for soybean seed marketing (BRASIL, 2013). The period between physiological maturity and the last harvest time was approximately 20 days, and the main factors responsible for decreasing the germination from 91% (T1) to 85% (T4) were the climatic variations—average air temperature and rainfall - in this period (Figure 2).

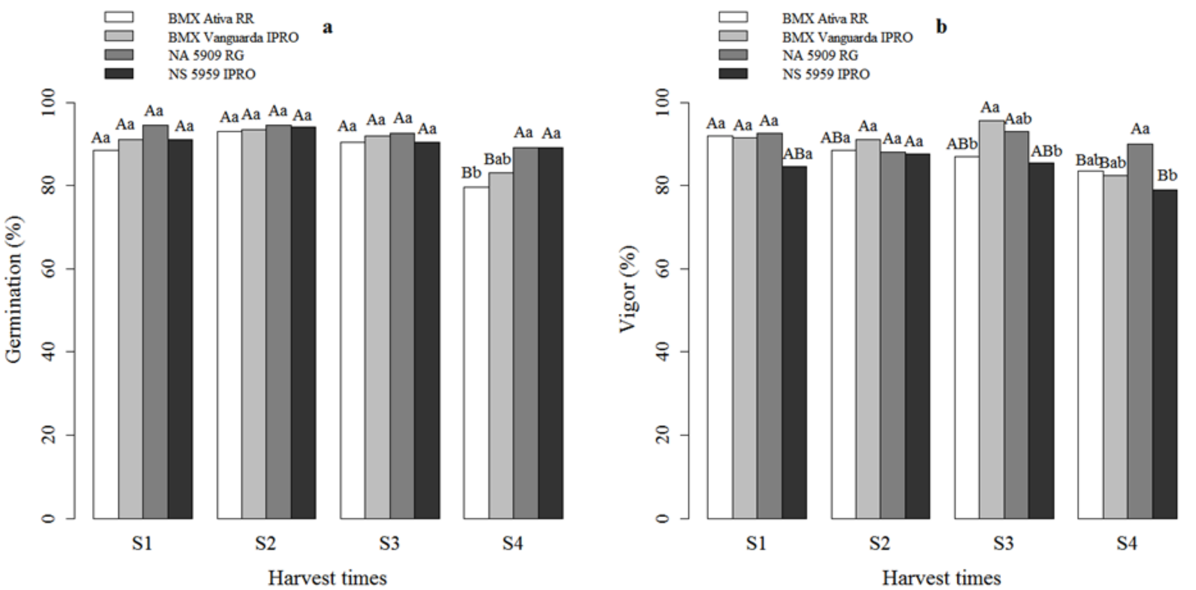

Figure 1. Germination (a) and vigor (b) of soybean seeds by accelerated aging test as a function of harvest times after physiological maturity. Means followed by the same uppercase letter between harvest times or lowercase letters between cultivars do not differ by the Tukey's test (*p*<0.01).

The period between T1 and T4 presented an average decrease of 6% in seed germination. Similar results were reported by Diniz et al. (2013) and Gris et al. (2010), who found a decrease in germination after the R8 stage, but this result was dependent on the evaluated cultivar. Differences between cultivars was also found in the present work; NA 5909RG and NS 5959IPRO maintained the germination between T1 and T4, whereas the others presented decreases in the last harvest time (Figure 1a).

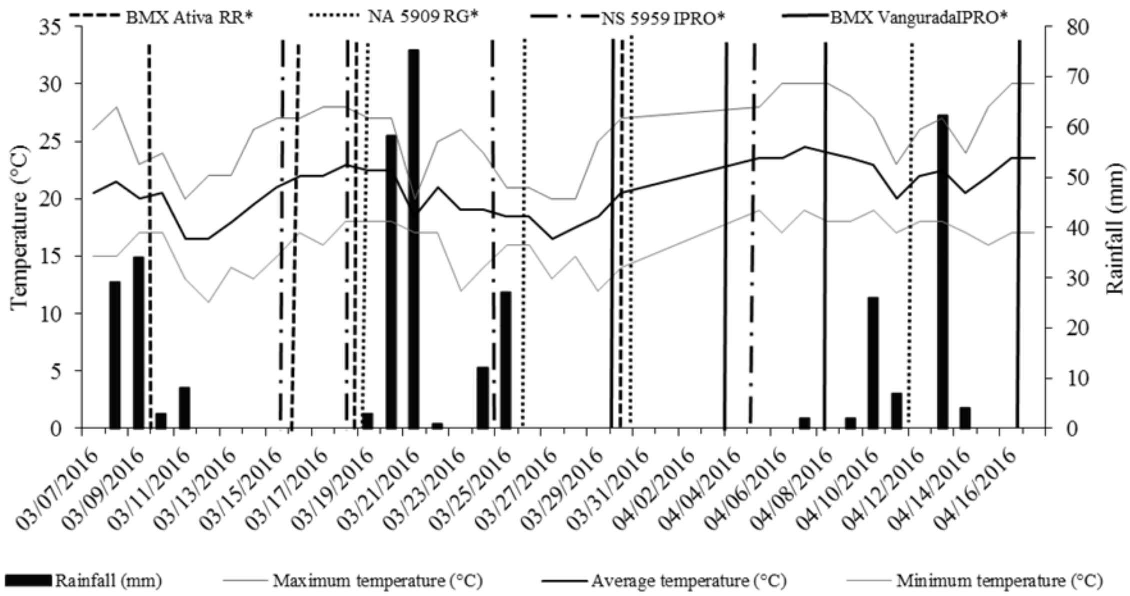

Figure 2. Rainfall, and maximum, average, and minimum air temperatures during the drying phase of soybean seeds in the 2015/2016 crop season. *Harvest times.

The vigor of the seeds was approximately 90% over the harvest times for the NA 5909 RG cultivar, but it decreased from 92% (T1) to 84% (T4) for the BMX Ativa RR; from 85% (T1) to 79% (T4) for the NS 5959 IPRO; and from 91% (T1) to 82% (T4) for the BMX VanguardaIPRO. The different vigor of the cultivars shows that it is dependent on genotype and its interaction with the environment, mainly by climatic conditions (temperature and relative humidity) after physiological maturity. Similar results of seed vigor were found by Pereira et al. (2015), with the cultivar NA 5909 RG, which maintained its seed physiological quality regardless of the harvest time in the 2011/2012 crop season.

The NA 5909 RG cultivar maintained the highest seedling length (Figure 3a) from T1, presenting mean of 19.6 cm, and the NS 5959 IPRO presented the lowest (15.2 cm). The first three harvest times showed no significant differences for the BMX Ativa RR cultivar (Figure 3a), indicating that the quality loss process had intensified after T3.

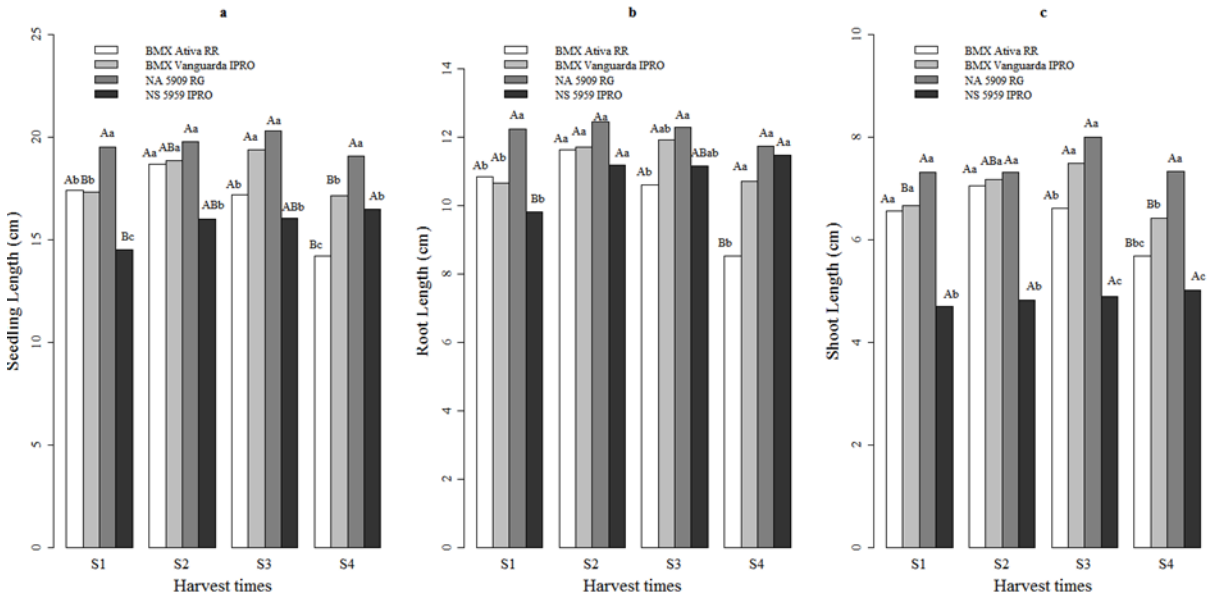

Figure 3. Seedling (a), root (b), and shoot (c) lengths (cm) of soybean plants as a function of harvest times after physiological maturity. Means followed by the same uppercase letter between harvest times or lowercase letters between cultivars do not differ by the Tukey's test ($p<0.01$).

The extension of the seed quality loss process directly affects seedling length, since the metabolic repair of damage caused by seed deterioration affects the germination process and consequently slows seedling growth (MARCOS-FILHO, 2015b). Considering that high-vigor seeds have higher metabolic repair efficiency, they will produce seedlings with higher emergence speed, resulting in larger and more vigorous plants (SILVA et al., 2016).

The NA 5909 RG cultivar showed no differences in root length between harvest times (Figure 3b). The BMX Ativa RR cultivar presented no differences between the first and third harvest times. A shorter root length is one of the first signs of seed deterioration after physiological maturity (MATTHEWS et al., 2012).

The NA 5909 RG cultivar had no differences in shoot length between harvest times (Figure 3c), and the highest mean (7.5 cm). Contrastingly, the NS 5959 IPRO cultivar had the lowest shoot length (4.9 cm), regardless of the harvest time. The cultivar BMX Ativa RR had no significant difference in shoot length between the first three harvest times (Figure 3c).

Decreases in vigor caused by harvest after physiological maturity are due to the exposure of seeds to adverse climatic conditions (MARCOS-FILHO, 2015b). The harvest at 20 days after physiological maturity resulted in a decreased in vigor of 7%. Excessive rainfall combined with variations in air temperature during the drying phase is one of the main problems that decrease seed quality (Figure 2). This decrease in vigor (Figure 1b) was dependent on the cultivar; the cultivars showed differences for harvest times after physiological maturity, even when exposed to similar climatic conditions (Figure 2).

The analysis of variance of biochemical components showed significant differences between cultivars, except for soluble protein content (Table 2), confirming their genetic variability. Significant effect of the harvest times was found for all variables, except for protein and total phosphorus contents (Table 2). The mean square of the interaction between cultivars and harvest times was significant for soluble sugar, inorganic phosphorus, phytate, and lipid contents (Table 2).

Table 2. Mean squares of analysis of variance for contents of total protein (TP), soluble protein (SP), starch (S), soluble sugar (SS), phosphorus (P), inorganic phosphorus (IP), phytate (PT) and lipid (LI) of four soybean cultivars subjected to 2 harvest times in the 2015/2016 crop season.

		Mean square							
Source of variation	Degrees of freedom	TP	SP	S	SS	P	IP	PT	LI
Block	2	2.8	0.37	0.009	2.17	0.06	0.002	0.002	0.22
Cultivar (C)	3	92.2**	0.98	4.4**	139.38**	0.98*	0.044**	0.21**	3.77*
$Residue_1$	6	2.67	0.43	0.12	12.94	0.15	0.002	0.017	0.66
Harvest times (HT)	1	1.43	9.13**	0.32*	702.9**	0.20	0.052**	0.81**	6.00**
C × HT	3	17.29	0.49	0.09	315.48**	0.26	0.022**	0.30**	10.0**
$Residue_2$	8	4.73	0.32	0.03	5.43	0.10	0.0010	0.02	0.33
Mean	-	38.9	29.5	7.9	90.9	4.0	0.67	5.0	20.5
$CV (\%)_1$	-	4.2	2.2	4.3	4.0	9.8	7.1	2.6	4
$CV (\%)_2$	-	5.6	1.9	2.4	2.6	8.1	4.8	3.0	2.8

**Significant at the 1% probability level. *Significant at 5% probability level. CV = coefficient of variation.

The BMX Ativa RR cultivar had the highest the total protein content (Figure 4a) (42.5%), differing only from the NS 5959 IPRO, which had the lowest (35.3%). The NS 5959 IPRO cultivar had the lowest vigor. According to Henning et al. (2010), protein contents are directly related to the physiological quality of seeds, more vigorous seeds presented higher protein contents. In addition to genetic factors, seed protein contents are affected by environmental conditions, especially during maturation (RAO et al., 1993). Therefore, considering that all cultivars were produced under the same conditions, the differences found in total protein contents are due to genetic factors.

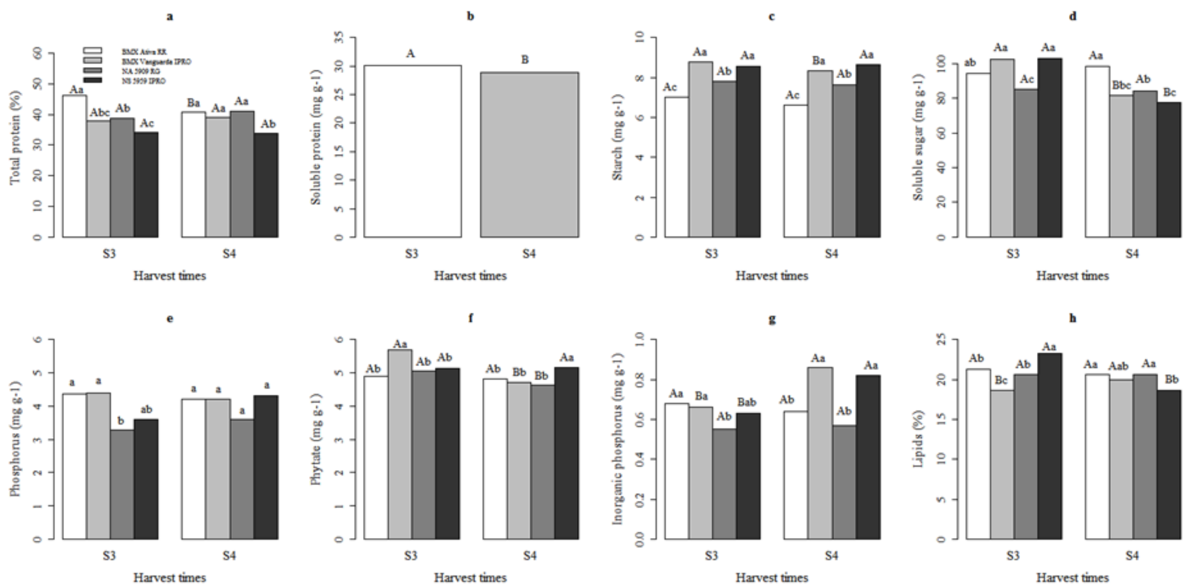

Figure 4. Total protein (a), soluble protein (b), starch (c), soluble sugar (d), phosphorus (e), phytate (f), inorganic phosphorus (g), and lipids (h) contents in soybean seeds as a function of harvest times after physiological maturity. Means followed by the same uppercase letter between harvest times or lowercase letters between cultivars do not differ by the Tukey's test (p<0.01).

No genetic effect on soluble protein content was found, considering the significant decreases over the harvest times, from 30.5 mg g^{-1} to 28.9 mg g^{-1} (Figure 4b). This decrease is explained by the intense seed respiration process caused by high temperatures, decreases in protein content, and increases in amino acids in the seeds, which directly affect the seed physiological quality (HAN et al., 2013).

The average seed vigor decreased in 6.5% from T3 and T4, which is related to the decrease in soluble protein content. Thus, the vigor of plants of cultivars that present maintenance of protein contents possibly will not change. Proteins are related to metabolism efficiency and seedling vigor (BORTOLOTTO et al. 2008), which denotes the correlation between chemical composition and physiological quality of soybean seeds (DELARMELINO-FERRARESI; VILLELA; AUMONDE, 2014). The correlation between soluble protein and seed vigor can be due to the functions of proteins in the seeds-formation of new tissues at embryonic axis growth points during germination, chemical reactions, and regulation of physiological processes (MARCOS-FILHO, 2015a).

The BMX VanguardaIPRO cultivar had the highest mean starch content (8.83 mg g^{-1}) in the two evaluated harvest times, and BMX Ativa RR had the lowest (6.04 mg g^{-1}). Considering the harvest times, significant differences were found for the BMX VanguardaIPRO; the other cultivars maintained the starch content with a harvest delay of 20 days after R7. The starch in the seed is metabolically inactive and needs to be hydrolyzed to become available for mobilization and to serve as an energy source during germination (HAGER; MÄKINEN; ARENDT, 2014). Therefore, the efficiency in starch hydrolysis and mobilization during germination characterizes a cultivar of high vigor, not its initial content.

The NA 5909 RG and BMX Ativa RR cultivars maintained their soluble sugar contents over the harvest times, which may be associated with the maintenance of physiological quality. NS 5959 IPRO presented the largest decrease in soluble sugar content; it presented its highest content (103 mg g^{-1}) in T3, which decreased 25% (77 mg g^{-1}) due to variations in air humidity and temperature (Figure 4d). Decreases in soluble sugar contents are related to the process of seed quality loss. Soluble sugar and total sugar contents - sugars such as sucrose, which are directly related to membrane integrity - decrease during this process and cause limitations for respiration, leading to decreases in seed vigor and germination (MARCOS-FILHO, 2015a).

The cultivars presented significant different phosphorus content in T3 (Figure 4e); the NA 5909 RG cultivar had the lowest content (3.4 mg g^{-1}). Approximately 80% of the total phosphorus in the seed is in phytate form, which provides phosphorus during germination (RABOY, 2009), and is related to vital seed functions (SILVA et al. 2011). The cultivars BMX VanguardaIPRO presented higher phytate content in T3 (Figure 4f); however, it was significantly lower in T4. This decrease was also found for NA-5909RG cultivar. These results indicate a great genetic effect on the phosphorus composition in the seeds.

The NA 5909 RG and BMX Ativa RR cultivars maintained their inorganic phosphorus contents, whereas the other cultivars had increases of

0.62 mg g^{-1} to 0.81 mg g^{-1} (NS 5959 IPRO) to 0.65 mg g^{-1} to 0.86 mg g^{-1} (BMX VanguardaIPRO) (Figure 4g). This increase is connected to the increase in seed respiration rate, since inorganic phosphorus is consumed during the respiration process, resulting in a higher energy demand. Inorganic phosphorus content is an important parameter to identify differences in performance between samples, since it directly affects the seed physiological quality (NERLING; COELHO; BRÜMMER, 2018).

The lipid content presented significant decreases between harvest times for the cultivar NS 5959 IPRO cultivar (23% to 18%) (Figure 4h), presenting negative effect of the seed deterioration process on the lipid content of this cultivar. Lipid deterioration are involved with several reactions that produce toxic products, characterizing lipid peroxidation, which consists in the oxidation of fatty acid chains, producing free radicals and hydroperoxides (MARCOS-FILHO, 2015a).

Principal component analysis (PCA) was used to determine the biochemical components that explain the maintenance of physiological seed quality after physiological maturity (Figure 5). The first two components explained 63.81% of the variance, 40.27% by the first component (PC1), and 23.54% by the second component (PC2) (Figure 5).

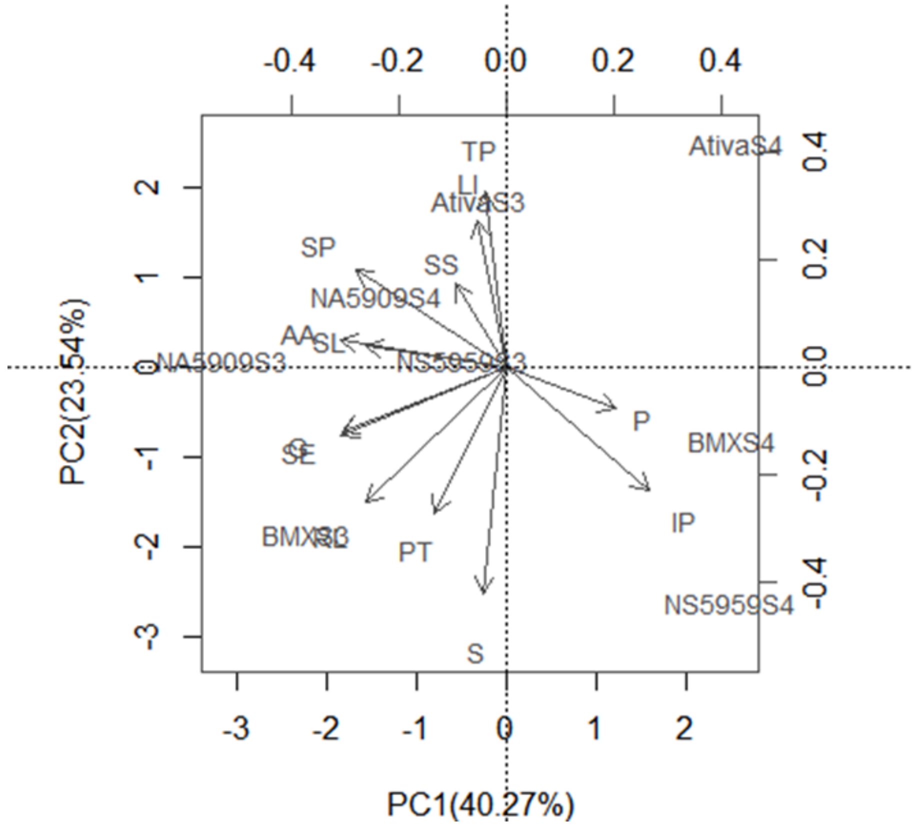

Figure 5. Principal component analysis (PCA) for biochemical components and physiological quality of soybean seeds. G: germination; AA: vigor by accelerated aging test; SE: seedling length; RL: root length; SL: shoot length; TP: total protein; SP: soluble protein; S: starch; SS: soluble sugar; P: phosphorus; PT: phytate; IP: inorganic phosphorus; LI: lipids.

In the PC1-/PC2+ component, the cultivars NA 5909 RG (T3 and T4), BMX Ativa RR (T3), and NS 5959 IPRO (T3) were grouped according to vigor by accelerated aging, shoot length, and soluble protein, total protein, lipid, and soluble sugar contents. The sample of the component PC1-/PC2- was composed by the cultivar BMX VanguardaIPRO (T3), which was grouped according to germination, total seedling length, root length, and starch and phytate contents. The cultivars BMX VanguardaIPRO (T4) and NS-5959IPRO (T4) were allocated in the quadrant PC1+/PC2-, grouped according to inorganic phosphorus and total phosphorus contents. The cultivar BMX Ativa RR (T4) was allocated in the PC1+/PC2+ component. According to the PCA, the NA 5909 RG cultivar was affected by the soluble protein content. Moreover, only the cultivar NA 5909 RG was grouped in PC1-/PC2+, regardless of the harvest time, showing the cultivar potential in maintain seed physiological quality and a correlation of this quality to the soluble protein content. Seed vigor was affected by soluble protein content, and germination was affected by starch content. More detailed analyses were applied to establish reliable parameters and confirm the effect of soluble protein on seed vigor.

Partial least squares regression analysis (PLS-R) was used to confirm the association of soluble protein content with vigor. In addition, it was assessed the existence of other causes and effects involving biochemical components and physiological quality of soybean seeds (Figure 6).

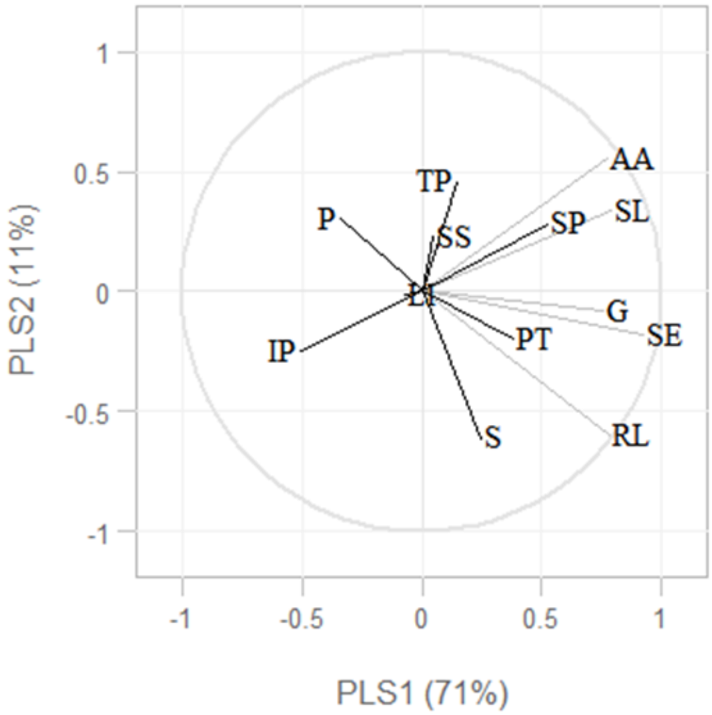

Figure 6. Partial least squares regression analysis (PLS-R) for association of biochemical components with physiological quality of soybean seeds G: germination; AA: vigor by accelerated aging test; SE: seedling length; RL: root length; SL: shoot length; TP: total protein; SP: soluble protein; S: starch; SS: soluble sugar; P: phosphorus; PT: phytate; IP: inorganic phosphorus; LI: lipids.

According to the PLS-R model, besides soluble protein content, soluble sugar is among the biochemical components that affect vigor (Figure 8), confirming the results obtained with PCA. The phytate content had effect on root length. Inverse effect was found for starch and total protein contents. Overall, there was a positive effect of soluble protein content on vigor, according to the accelerated aging and shoot length tests. The other physiological attributes showed significant correlations with starch and phytate contents.

When the biochemical components were evaluated separately, soluble protein, starch, soluble sugar, phytate, and lipid contents decreased; and inorganic phosphorus content increased in the 10-day period in which the seeds remained in the field (T3 to T4), indicating the pre-harvest quality loss process.

The results showed the importance of soluble protein in the maintenance of the physiological quality of pre-harvest seeds. Thus, this research offers perspectives for studies on the effect of biochemical components on the seed germination process, focused on identifying the mechanisms of the hydrolysis and mobilization processes in these components that are important for the formation of vigorous plants.

CONCLUSION

The maintenance of seed vigor after physiological maturity is dependent on the cultivar. The cultivar NA 5909 RG maintained the vigor percentage up to 20 days after R7.

Soluble protein content can be used as an indicator of the maintenance of physiological quality of pre-harvest soybean seeds.

ACKNOWLEDGEMENTS

The financial support of CAPES-BRAZIL. The second author thanks FAPESC-UDESC-PAP for article processing charges and thanks CNPq for Research Productivity Fellowships.

REFERENCES

BORTOLOTTO, R. P. et al. Teor de proteína e qualidade fisiológica de sementes de arroz. **Revista Bragantia**, v. 67, n. 2, p. 513-520, 2008.

BLIGH, E. G.; DYER, W. J. A rapid method of total lipid extraction and purification. **Canadian Journal**

of Biochemistry and Physiology, v. 37, n. 8, p. 911-917, 1959.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 37, n. 8, p. 248-254, 1976.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ ACS, 2009. 395 p.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento**. Instrução Normativa nº 45, de 17 de setembro de 2013**. Brasília: MAPA: 2013. Disponível em: <http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-agricolas/sementes-e-mudas/publicacoes-sementes-e-mudas/copy_of_INN45de17desetembrode2013.pdf>. Acesso em: 05 set. 2018.

CLEGG, K. M. The application of the anthrone reagent to the estimation of starch in cereals. **Journal of the Science of Food and Agricultural**, v. 7, n. 3, p. 40-44, 1956.

CHEN, P. S. et al. Microdetermination of Phosphorus. **Analytical Chemistry**, v. 28, n. 11, p. 1756–1758, 1956.

DINIZ, F. O. et al. Physiological quality of soybean seeds of cultivars submitted to harvesting delay and its association with seedling emergence in the field. **Journal of Seed Science**, v. 35, n. 2, p. 147-152, 2013.

DELARMELINO-FERRARESI, L. M.; VILLELA, F. A.; AUMONDE, T. Z. Desempenho fisiológico e composição química de sementes de soja. **Revista Brasileira de Ciências Agrárias**, v. 9, n. 1, p. 14-18, 2014.

GRIS, C. F. et al. Qualidade fisiológica e teor de lignina no tegumento de sementes de soja convencional e transgênica RR submetidas a diferentes épocas de colheita. **Revista Ciência Agrotecnica**, v. 34, n. 2, p. 374-381, 2010.

HAGER, A. S.; MÄKINEN, O. E.; ARENDT, E. L. Amylolytic activities and starch reserve mobilization during the germination of quinoa. **European Food Research and Technology**, v. 239, n. 4, p. 621-627, 2014.

HAN, C. et al. Analysis of proteome profile in germinating soybean seed, and its comparison with rice showing the styles of reserves mobilization in different crops. **Plos One**, v. 8, n. 2, p. 1-9, 2013.

HENNING, F. A. et al. Composição química e mobilização de reservas em sementes de soja de alto e baixo vigor. **Bragantia**, v. 69, n. 3, p727-734, 2010.

JYOTI; MALIK, C. P. Seed deterioration: a review. **International Journal of Life Sciences Biotchenology and Pharma Reserch**, v. 2. n. 3, p. 374-385 ,2013.

LATTA, M.; ESKIN, M. A simple and rapid method for phytate determination. **Journal Agricultural Food Chemistry**, v. 28, n. 6, p. 313-315, 1980.

MARCOS-FILHO, J.; KIKUTI, A. L. P.; LIMA, L. B. Métodos para avaliação do vigor de sementes de soja, incluindo a análise computadorizada de imagens. **Revista Brasileira de Sementes**, v. 31, n. 1, p. 102-112, 2009.

MARCOS-FILHO, J. Seed vigor testing: an overview of the past, present and future perspective. **Scientia Agricola**, v. 72, n. 4, p. 363-374, 2015a.

MARCOS-FILHO, J. **Fisiologia de sementes de plantas cultivadas**. 2. ed. Londrina, PR: ABRATES 2015b. 660 p.

MATTHEWS, S. et al. Evaluation of seed quality: from physiology to international standardization. **Seed Science Research**, v. 22, n. 1, p. S69–S73, 2012.

McCREADY, R. M.; GUGGOOLZ, J.; WENS, H. S. Determination of starch and amylase in vegetables. **Analytical Chemistry**, v. 22, n. 9, p. 1156-1158, 1950.

NAKAGAWA, J. Testes de vigor baseados no desempenho das plântulas. In: KRZYZANOSKI, F.C.; VIEIRA, R.D.; FRANÇA NETO, J.B. (Ed.). **Vigor de sementes**: conceitos e testes. Londrina: ABRATES, 1999. v. 1, cap. 2, p. 1-24.

NERLING, D.; COELHO, C. M. M.; BRÜMMER, A. Biochemical profiling and its role in physiological quality of maize seeds. **Journal of Seed Science**, v. 40, n. 1, p. 007-015, 2018.

PEREIRA, T. et al. Physiological quality of soybean seeds depending on the preharvest desiccation. **Planta Daninha**, v. 33, n. 3, p. 441-450, 2015.

RABOY, V.; DICKINSON, D. B. Effect of phosphorus and zinc nutrition na soybean seed phytie acid and zine. **Plant Physiology**, v. 75. n. 4, p. 1094-1098, 1984.

RABOY, V. Approaches and challenges to engineering seed phytate and total phosphorus. **Plant Science**, v. 177, n. 4, p. 281-296, 2009.

RAO, A.C.S. et al. Cultivar and climatic effects on the protein content of soft white winter wheat. **Agronomy Journal**, v. 85, n. 5, p. 123-128, 1993.

ROSA, D. P. et al. Genetic diversity in soybean seed quality under different storage conditions. **Semina: Ciências Agrárias**, v. 38, n. 1, p. 57-72, 2017.

R CORE TEAM. **R:** A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2016. Disponível em: <https://www.R-project.org/>. Acesso em: 06 jun. 2016.

SILVA, H. P. et al. Qualidade de sementes de *Helianthus annuus* L. em função da adubação fosfatada e da localização na inflorescência. **Ciência Rural**, v. 41, n. 7, p. 1160-1165, 2011.

SILVA, T. A. et al. Condicionamento fisiológico de sementes de soja, componentes de produção e produtividade. **Ciência Rural**, v. 46, n. 2, p. 227-232, 2016.

SMANIOTTO, T. A. S. et al. Qualidade fisiológica de sementes de soja armazenadas em diferentes condições. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 18, n. 4, p. 446-453, 2014.

TEDESCO, M. J.; VOLKWEISS, S. J.; BOHNEN, H. **Análises de solo, plantas e outros materiais**. 1. ed. Porto Alegre, RS: UFRGS, 1995. 174 p.

WANG, L. et al. Comparative proteomics analysis reveals the mechanism of pre-harvest seed deterioration of soybean under high temperature and humidity stress. **Journal of Proteomics**, v. 75, n. 7, p. 2109–2127, 2012.