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Development of Rapid Vigor Test Using Urine Sugar Analysis Paper for Soybean (*Glycine max* L.) Seeds

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Abstract

Seed vigor is an essential parameter of seed quality. It plays a critical value in the decision-making for the seeds to be used in agronomic and horticultural crops. The urinary sugar analysis paper (USAP) test determines seed vigor based on leakage concentration from the seed and corresponding color change of the USAP, which are obtained after soaking the seeds in water. This research was conducted from January 2021 to November 2021, intending to develop a rapid soybean vigor test using USAP, and it was composed of two experiments. Soybean seeds of the Biosoy1 variety were obtained from ICABIOGRAD (Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development). The seeds consisted of three different levels of seed lots (low, medium, and high viability with 35%, 64%, and 94% germination percentages, respectively). The first experiment was to determine moisture content and soaking period for the USAP seed vigor test. It was arranged in a completely randomized design with one factor as the combination of three seed lots, two levels of seed moisture content (10-12% and 13-14%), and four levels of the soaking period (0, 6, 8, and 10 hours). The second experiment was the optimization of the soaking period by seed number. It was arranged in a completely randomized design with one factor, which was combinations of three seed lots, three levels of seed numbers (50, 75, and 100 seeds), and the soaking period (four levels as 0, 6, 8, and 10 hours). The data were analyzed statistically by the Minitab package. The result showed that both combinations of 10-12% and 13-14% moisture content with the soaking period of 10 hours effectively differentiated the three seed lots into three vigor levels using USAP. Experiment 2 optimized the soaking period from 10 hours to 8 hours by using 100 seeds. The USAP color change was effectively significant to indicate seed lot vigor levels, mainly based on protein leakage concentration on USAP. The color changes from pale yellow-green for higher vigor seed lot to light greenish-blue for low vigor. The USAP was effective for the soybean vigor test for the seed lot with 10-14% moisture content by soaking 100 seeds into 50 ml of distilled water for 8 hours. The USAP is a promising rapid vigor method, but still needs to be developed further.

Keywords: seed leakage, seed lot, seed quality, seed vigor, soaking

Introduction

Soybean [(*Glycine max* (L.) Merrill] is a legume crop belonging to the family of Fabaceae. Soybean includes the essential oil crops of the world, which also has tremendous importance as a food legume (Pratap et al.,2012). Soybean is one of the food crops providing protein that favored the Indonesian population, resulting in high consumption of soybeans; this has increased their importation each year with an average percentage of 7.73%.

The import volume of soybeans reached 1,788, 962 tons in 2013, and it grew to 1,965,811 tons in 2014, and 2,256,932 tons in 2015 (Ningrum et al., 2016). The use of high-quality seeds with good vigor can potentially reduce the importation rate by increasing the domestic soybean production. Malik (2013) showed that seed aging was associated with different physio-biochemical changes at the cellular level, including membrane disruption, solute leakage, reduced energy metabolism, impairment of RNA, protein synthesis, and DNA degradation, and that resulted in the loss of quality. According to Senaratna et al. (1988) most soybeans are characterized by high lipid peroxidation, which contributes to the seed membrane degradation in storage and favors the measurement of seed quality loss based on the solute leakage after the seed water soaking period.

Sadjad et al. (1999) reported that the quality of seed can be determined by seed vigor by evaluation of the seed performance after being germinated under suboptimum conditions and that providing information about seed quality on the actual field.

Seed vigor tests help to determine the field performance of the seed lot. Different seed vigor testing methods have been developed in soybeans and validated by ISTA. According to Marin et al. (2018), the electric conductivity test may depend on the seed structure. It may be applied to a broader range of species for estimating both germination and vigor differences. Kaya et al. (2016) concluded that the accelerated aging test of soybean seeds at 41°C for 72 hours estimated the field emergence. Astuti et al. (2020) reported that the radicle emergence (RE) test for 42 hours ± 15 minutes at a temperature of 25 ± 2 ° C in soybean could be used as a vigor test. Soybean has short life storage as research shown that the seeds with high protein and lipids have limited longevity due to their specific chemical composition and lead to loss of germination ability and viability due to lipid peroxidation (Xie et al., 2003; Balesevic-Tubic et al., 2007). Therefore, the rapid vigor test method is very necessary to predict the seed lots' field performance.

Rapid vigor test is essential for providing quick information related to seed lots performance (Zhao et al., 2008). According to McDonald et al. (1988) the development of the seed testing method should meet six criteria: cheap, fast, easy, objective, reproducible, and closely correlate with the plant growth in the field. Takayanagi and Murakami (1969) have developed a rapid viability test based on seed chemical leakage (glucose) using a urinary sugar analysis paper (USAP) to differentiate viable to non-viable seeds. Takayanagi and Murakami's method was successful in maize and rice after 40 hours of seed soaking do differentiate viable to non-viable seeds but this method was based only on glucose testing. The 1969 USAP method was not sensitive enough to indicate other seed leakages except glucose. Currently, there is other rapid vigor test method like extractive electrospray ionization mass spectrometry (ESMS) which is a new types of sub-chemical separation technology used to determine seed vigor; this method can be used for complex matrix samples without sample pretreatment (Jia et al., 2012). The ESMS method has good stability, high sensitivity and fast analysis speed, but it require specilized skills to analyse and also it is expensive. Urinary sugar analysis paper can now analyze ten different chemical substances from the solution, including glucose, pH, and protein (Henso Medical Co. Ltd). Our current study aimed to develop cheap, fast and easy soybean vigor testing method by analyzing glucose, protein, and pH from seed electrolite leakage by using USAP after seeds being soaked in water.

Material and Methods

Plant Materials

The experiment was done from January to November 2021 at the seed testing laboratory Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University. The research consisted of two experiments where soybean seeds cv. Biosoy1 used in both experiments. Three different seed lots (high, medium, and low viability with 35, 64, and 94 germination percentage, respectively) were obtained from ICABIOGRAD (Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development), Indonesia. Urinary sugar analysis paper (URS-10T type) was used to determine the seed leakage's protein, glucose, and pH. Royal Horticulture Society (RHS) color chart was used to identify the color names observed on USAP.

Moisture Content Calculation and Adjustment

The moisture content of the seeds was adjusted into two levels (10-12% and 13-15%). The determination of moisture content (MC) used a constant low temperature of 103^{o}C for 17 ± 1 hour, two replications of 5 g seed sample (ISTA 2018) based on the following formula:

$$\text{Sample weight (desired MC)} = \frac{(100 - \text{initial MC})}{(100 - \text{desired MC}} \text{x initial weight}$$

Experiment 1. Determination of the Moisture Content and Soaking Period on Soybean Seed Vigor Test by Using USAP

The experiment was arranged in a completely randomized design with combination treatments of three seed lots (low: 35%, medium: 64%, and high: 94% germination), two moisture content (10-12%, and 13-14%), and four soaking periods (0 h, 6 h, 8 h, and 10 h). These treatments were consisted of 24 combinations and replicated four times, resulting in 96 experimental units. All experimental tools were cleaned and sterilized by alcohol 70% and washed three times using distilled water according to Miguel and Filho (2002) method. Soybean seeds have various seed size and seed color and that may influence the results. Based on the objective of experiment to optimize the duration of soaking period by seed number (50, 75 and 100), differences in the seed weight was not included in this study. According Rodo et al. (1998) electric conductivity measurement in tomato was more effective by using 50 seeds compared to the 25 seeds. Similarly, Gaspar et al. (2002) reported that electric conductivity increased with the increase of seed number in millet with standardized water volume. Seeds from different treatment combination were soaked into 50 ml of distilled water, then kept at 25 to 30 ^{0}C (room temperature). Soaking was carried out without the replacement of the seeds and water.

Ten ml water from each combination treatment (seed lots, moisture content and soaking period) was tested for electrolyte leakage (glucose and protein). After that, the urinary sugar analysis paper (USAP) was immersed in that 10 ml from each soaking combination treatment for 5 to 10 seconds. The USAP was left for 1-minute to see the color change related to leakage observations (glucose, protein, and pH) and compared the resulting USAP with the standard USAP color chart and the value corresponding to leakage concentration. The result indicated the optimum moisture content and soaking period to be used in Experiment 2.

Experiment 2. Optimization of Soaking Period and Seed Number

The best moisture content resulting in Experiment 1 was used for the second experiment. This experiment was arranged in a completely randomized design with one factor, i.e. the combination of three seed lots (low: 35%, medium: 64%, and high: 94% germination percentage), four soaking periods (0 h, 6 h, 8 h, and 10 h) and three different seed numbers (50, 75 and 100 seeds). Thirty-six combination treatments were replicated three times to get 108 total experimental units, indicating the number of urinary sugar paper used. The process for protein, glucose, and pH determination was the same as for moisture content determination in the first experiment. The qualitative data was obtained by observing the USAP color change (protein, pH, and glucose) compared with the USAP standard as each color has a correspondence concentration.

Statistical Analysis

The data were analyzed using simple linear regression analysis, Minitab, and Microsoft excel programs to determine a significant difference between combination treatments.

Result and Discussion

Experiment 1. Determination of the Moisture Content and Soaking Period on Soybean Seed

Quantitative result for protein, glucose, and pH from the leakage by USAP Test

The qualitative data (colors) were related to the color changes on USAP based on the observation (protein, glucose, and pH), and each color on USAP had a corresponding concentration quantity. The USAP results showed a significant difference for combination treatments based on protein, glucose, and pH leakage (Table 1). This result indicates that USAP is effective for the soybean vigor test. This result was confirmed by Walters et al. (2010) report on pea (*Pisum sativum*) and water melon that the seed with high protein and fat was characterized by different biochemical and physiological changes during storage, ending by alternation and transcription process. Senaratna et al. (1985) stated that most soybeans are characterized by high lipid peroxidation, which contributes to the seed membrane degradation in storage and favors the measurement of seed quality loss based on the solute leakage after a certain soaking period of seed.

The result (Table 1) showed that the combination of seed lots with moisture content (10-12% and 13-14%) at 10 hours of the soaking period was significantly effective for the USAP vigor test. This result was based on protein leakage, not on glucose and pH. The USAP was effective to differentiate the low vigor seed lot from medium and high but not effective enough to distinguish medium and high vigor (no significant difference). It means both moisture content 10-12 and 13-14% can be used for the USAP test. That combination was characterized by the performance of USAP color for each observation compared to the other combination treatments, which helped distinguish seed lots' vigor level. This result was confirmed by different studies on electric conductivity tests, which showed that the soaking period might range from 4 to 48 hours (Ramos et al., 2012). The moisture content result for USAP is confirmed by ISTA 2018 that the seed moisture content for the electrical conductivity test must be 10-14%. This combination treatment had high protein leakage of 3 g.L^{-1} for low vigor seed lot (35% germination), 0.475 g.L^{-1} for medium vigor seed lot (64%), and 0.15 g.L^{-1} for high vigor seed lot (94%) and agreed by Nacer et al. (2011) report that mostly soybean varieties contain at least 34% protein and 19% oil.

The USAP glucose observation resulted in 10 mmol.L^{-1} (low vigor), 7.5 mmol.L (medium vigor), and 5 mmol.L^{-1} (high vigor) for that combination of seed lots and 10-12% moisture content with 10 hours of the soaking period (Table 1) but not significantly different with those result on the moisture content of 13-14%. According to Ruth et al. (2018), the biochemical process can affect all seed composition and that including carbohydrates as the main glucose composition and soybeans contain around 16.31% of carbohydrates.

The pH result (Table 1) indicates that pH decreased according to the increase of seed deterioration where low seed vigor had 5.75 pH, medium seed vigor had 6 pH, and high seed lot vigor with 6.15 pH. For all combination treatments, USAP has resulted in high pH levels but is not effective to differentiate seed vigor levels, they were not significantly different.The USAP was not adequate for all control combination treatments (seed lots,10-12% and 13-14% moisture content at 0 hour of soaking). The pH has been observed to be decreased contrary to the protein and glucose leakage result due to leakage exudate, which increased ions (H^+ and HO^-) in imbibed water. This result is confirmed by electric conductivity as USAP also depends on seed leakage. According to Loomis and Smith (1980), when the cell membrane deteriorates, the contents of carbohydrates, amino acids, organic acids, and ions increase in the leakage leading to an increase in electric conductivity when the seeds are imbibed. Also, Rastegar and Mehdi (2013) indicate that high-quality seeds have low electrical conductivity, and an increase in electrical leakage can result from membrane permeability and seed deterioration during aging.

Table 1. Determination of protein (g.L^{-1}), glucose (mmol.L^{-1}) leakage, and pH from the combination of seed lots at the different moisture content and soaking period by using USAP

Moisture content (%)	Soaking period (h)	Seed lots	Protein g (g.L^{-1})	Glucose (mmol.L^{-1})	pH
10-12	0	Low	0.00c	0.00c	6.5a
		Medium	0.00c	0.00c	6.5a
		High	0.00c	0.00c	6.5a
	6	Low	0.30c	5.00abc	6.0ab
		Medium	0.75c	3.75abc	6.2a
		High	0.00c	1.25bc	6.3a
	8	Low	1.00bc	7.50ab	6.0ab
		Medium	0.22c	5.00abc	6.1a
		High	0.11c	3.75abc	6.3a
	10	Low	3.00a	10.00a	5.5b
		Medium	0.47c	7.50ab	6.0ab
		High	0.15c	5.00abc	6.1a
13-14	0	Low	0.00c	0.00c	6.5a
		Medium	0.00c	0.00c	6.5a
		High	0.00c	0.00c	6.5a
	6	Low	0.86c	3.75abc	6.1a
		Medium	0.47c	2.50bc	6.2a
		High	0.00c	0.00c	6.3a
	8	Low	1.00bc	5.00abc	6.0ab
		Medium	0.30c	2.50bc	6.1a
		High	0.03c	2.50bc	6.2a
	10	Low	2.00ab	7.50ab	6.0b
		Medium	0.47c	2.50bc	6.1a
		High	0.07c	1.25bc	6.2a
F-Test			*	*	*
CV			1.88	1.21	0.4

Note: 1.The means in the same observation that sharing the same letters are not significantly different according to the Tukey test at α = 0.05
2.*Means significant different P<0.05 for combination treatments based on observations
3. CV: Coefficient of Variation

The USAP is a very rapid soybean vigor test method compared to the existing methods such as electrical conductivity test which needs 24 hours of seed soaking (ISTA ,2018). Astuti et al. (2020) reported that the radicle emergence test for soybean required 42 hours after seeds radicle emerged whereas Kaya et al. (2016) concluded that the accelerated aging test of soybean seeds must be done at 41°C for 72 hours. The USAP method is easy as a result based on observation of the color (qualitative). The USAP can be considered a cheap method for seed vigor as Fang et al. (2005) reported that the "Dip-and-read" type of test strips does not require electricity and electronic device that made to be more convenient and less costly for the end-users. The USAP test is not familiarized in seed science but is known as an easy and more beneficially testing method in other science. Shah et al. (2013) reported that paper-based analytical devices had been widely used for biomedical, environmental, and food-quality testing with good results.

Qualitative results for protein, glucose, and pH tested from the seed leakage

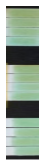
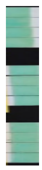
Qualitative results based on color observations and shown that USAP was more effective for the combination treatments of seed lots with both 10-12% and 13-14% moisture content for 10 hours of the soaking period as compared to the one for 0 hour-soaking periods. The protein color on USAP changed from pale yellow-green to light greenish-blue, while glucose color has changed from light greenish blue to strong yellowish-green from high, medium, and low seed lot vigor, respectively. Contrary to pH, the color changed from deep orange-yellow to moderated yellow from low, medium, and high vigor shown in Table below:

Experiment 2. Optimization of the Soaking Period for USAP for Soybean Vigor Test by Seed Number

Quantitative result for optimization of soaking period by soybean seed number

USAP results showed a significant difference between combinations of seed lots, seed number, and the soaking period. This result was based on protein leakage, not on glucose leakage detection and pH (Table 3). The deteriorated seed has high leakage, and those leakages substances (protein and glucose) affect soaked water pH. This result was confirmed by Senaratna et al. (1985) that most soybeans are

Table 2. The USAP qualitative (color change) result from combinations of seed lots with 10-12% and 13-14 % moisture content at 0 and 10 hours of soaking period

Moisture content (%)	Soaking period (h)	Seed lots	Protein	pH	Glucose
10-12	0	Low Medium High			
	10	Low Medium High			
13-14	0	Low Medium High			
	10	Low Medium High			

Note: Low, medium, and high vigor seed lots had 35%, 64%, and 94% of germination respectively

Table 3. Determination of protein (g.L^{-1}), glucose (mmol. L^{-1}) leakage, and pH from the combination of seed lots with the combination of seed number and soaking period by using USAP

Seed number	Soaking period (h)	Seed lots	Protein (g.L^{-1})	Glucose (mmol. L^{-1})	pH
50	0	Low	0.00c	0.00c	6.50a
		Medium	0.00c	0.00c	6.50a
		High	0.00c	0.00c	6.50a
	6	Low	0.25bc	5.00abc	6.30ab
		Medium	0.15c	1.66bc	6.30ab
		High	0.00c	0.00c	6.50a
	8	Low	0.76bc	5.00abc	6.00ab
		Medium	0.20bc	8.33ab	6.10ab
		High	0.10bc	3.33bc	6.30ab
	10	Low	1.00bc	11.66ab	5.60ab
		Medium	0.76bc	8.33ab	6.00ab
		High	0.15bc	5.00abc	6.10ab
75	0	Low	0.00c	0.00c	6.50a
		Medium	0.00c	0.00c	6.50a
		High	0.00c	0.00c	6.50a
	6	Low	0.48bc	3.33bc	6.10ab
		Medium	0.25bc	3.33bc	6.00ab
		High	0.10bc	0.00c	6.30ab
	8	Low	1.43b	11.66ab	5.60ab
		Medium	0.53bc	8.33abc	6.00ab
		High	0.15bc	3.33bc	6.10ab
	10	Low	3.00a	15.00a	5.30b
		Medium	1.00b	11.66ab	5.60ab
		High	0.25bc	5.00abc	6.10ab
100	0	Low	0.00c	0.00c	6.50a
		Medium	0.00c	0.00c	6.50a
		High	0.00c	0.00c	6.50a
	6	Low	0.53bc	5.00abc	6.10a
		Medium	0.30bc	3.33bc	6.10a
		High	0.15bc	3.33bc	6.10a
	8	Low	3.00a	11.66ab	5.30b
		Medium	1.43b	11.66ab	5.60ab
		High	0.30bc	5.00abc	6.00ab
	10	Low	3.00a	15.00a	5.30b
		Medium	1.43b	11.66ab	5.60ab
		High	0.25bc	5.00abc	6.00ab
F-Test			*	*	*
CV			1.57	1.07	0.007

Note:

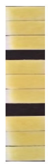
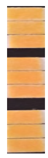
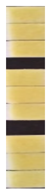
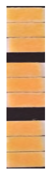
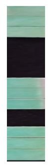
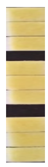
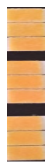
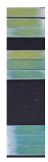
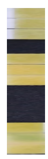
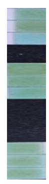
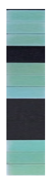
1. The means in the same observation that sharing the same letters are not significantly different according to the Tukey test at α = 0.05
2. *Means significant different P<0.05 for combination treatments based on observations
3. CV: Coefficient of Variation

characterized by high lipid peroxidation, which contributes to the seed membrane degradation in storage and favors the measurement of seed quality loss based on the leakage after a certain soaking period of seed.

The USAP (Table 5) has resulted in three combination treatments, which were not significantly different between them but significantly distinguished seed lot vigor level based on protein leakage. That USAP effectiveness was not efficient to distinguish medium to high vigor as their results were not significantly different which means USAP was able to distinguish only low vigor. Those are the combination of seed lots with 100 seeds for 8 hours, 100 seeds for 10 hours, and 75 seeds for 10 hours of the soaking period. This experiment aimed at optimization of USAP by seed number so that the soaking period can be optimized by combination treatment of seed lots for 100 seeds with 8 hours of the soaking period. Generally, increasing seed number with constant water volume during this experiment resulted in increased leakage in the imbibed water. Other research on electric conductivity (EC) has confirmed this obtained result as EC and USAP depend on seed leakage. Gaspar et al. (2002) reported that electric conductivity increased with the increase of seed number in millet with standardized water volume.

The USAP (Table 3) has resulted in no glucose leakage in all combinations treatments with 0 hours of the soaking period because distilled water was tested as a control. The highest glucose leakage (with 15 mmol/L) was observed in the combination of seed

Table 4. The USAP qualitative for optimization of soaking period by different seed numbers.

Soaking Period (hour)	Seed lots	Seed number	Protein ($g.L^{-1}$)	pH	Glucose ($mmol. L^{-1}$)
0	Low	50 75 100			
	Medium	50 75 100			
	High	50 75 100			
8	Low	50 75 100			
	Medium	50 75 100			
	High	50 75 100			

Note: Low, medium, and high seed lot has 35%, 64%, and 94% of germination, respectively lots with 75 and 100 seeds for 10 hours. The USAP (Table 3) has resulted with the highest pH (6.5) in all combination treatments with 0 hour of the soaking period, while the lowest was pH (5.3) observed in the combination of 75 and 100 seeds with 10 hours of the soaking period. Still, those are not significantly different from the other combinations.

Qualitative result for optimization of soaking period by seed number for soybean vigor test by USAP

The quantitative result confirms this qualitative result (Table 6). The USAP was effectively differentiating seed vigor levels. The protein color change on USAP for the combination of seed lots with 100 seeds and 8 hours of the soaking period resulted in pale brilliant yellow-green (high vigor), very pale blue (medium vigor), and light greenish-blue (low seed vigor). The glucose color had changed from very light bluish-green to strong yellowish-green from high to low seed lot vigor. The pH color changed from deep orange-yellow for low vigor to moderate yellow in high vigor soybean seed lot. Table 6 shows qualitative results from two extremes combination treatments such as control test where distilled water used as control at 0 hour of soaking period and combination at 8 hours of soaking period as this has successfully optimized USAP from 10 hours to 8 hours of soaking period.

Conclusion

Urinary sugar analysis paper (USAP) is effective for soybean seed vigor test by using 100 soybean seeds with the moisture content of 10-14% and soaked in 50 ml of distilled water for 8 hours. The USAP method was effectively based on protein leakage, not glucose and pH. In addition, the USAP method was only successful to differentiate the low vigor seed lot from both medium and high vigor ones, because the medium and high vigor seed lot were not significantly different. The USAP qualitative protein result indicated that the soybean seed lot with USAP color ranging from yellow to pale yellow-green can be used in agriculture as indicators of seeds with high performance in the field as that color was provided by seed lot with low protein leakage, which mean those seed lots still has high capacity to produce crop with good vigor. In contrast, the seed lot with light greenish-blue USAP color cannot be used in agriculture because they can provide crops with low vigor due to high nutrient leakage. USAP is a promising rapid vigor test method but still needs to be developed further using different types of USAP paper, using different varieties with different color and size as this method has shown a effectiveness depending on seed composition. The result interpretation is simple and it can be based on observation and measurement of the USAP color change and the measurement of color corresponding concentration on USAP by comparing with USAP standard chart.

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