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Development of Rapid Viability Test Using Urine Sugar Analysis Paper for Peanut (*Arachis hypogaea* L.) Seeds

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Abstract

Seed testing is important for determining seed quality. Seed testing must be easy, quick, and accurate. This study aimed to develop a rapid method for assessing peanut seed quality using Urine Sugar Analysis Paper (USAP). USAP is commonly used for detecting human urinary glucose and is currently being developed for evaluating seed quality. Three experiments were conducted. Experiment 1 investigated the most effective seed-cutting size (whole, halved, and seeds cut into six pieces) and soaking periods (0, 3, 6, and 9 hours) based on glucose and protein leakage, as well as specific gravity of the soaking water. The optimal method identified in Experiment 1 was then used to test all five seed lots in USAP Experiment 2, which aimed to evaluate the effectiveness of seed quality testing using the USAP method at various levels of seed viability. Experiment 3 investigated the correlation between USAP test results and other viability and vigor test parameters, including germination percentage, first count germination, speed of germination, and electrical conductivity. The results of experiments indicated that cutting seeds into six pieces and 6 6-hour soaking period was the most optimal. Experiment 3 showed that USAP testing correlated with viability and vigor testing across various peanut varieties. Protein and density testing using USAP were negatively correlated with germination percentage, first count germination, and germination speed parameters, but positively correlated with electrical conductivity. However, glucose leakage was not detectable using USAP.

Keywords: density, glucose, protein, storage, vigor

Introduction

Peanuts are a widely consumed nut in Indonesia, renowned for their high nutritional value, including 40% fat, 28% protein, 15% carbohydrates, vitamins, and minerals (Maurya et al., 2014). Increasing peanut consumption should be accompanied by a corresponding increase in peanut production. Seed quality is crucial for supporting plant productivity and ensuring high-quality harvests. Seeds with low viability can lead to non-uniform plant growth, decreased growth rates, and potential sources of seed-borne diseases (Ilyas, 2012). Peanuts deteriorate rapidly due to their high fat and protein content, resulting in a short shelf life (Liu et al., 2019). High fat content causes lipid auto-oxidation, which damages the phospholipid cell membrane (Groot et al., 2022). According to Gayathri et al. (2024), peanut seeds with an initial germination percentage of 90% showed a decline to 61% after 8 months of storage at room temperature.

Seed quality can be assessed based on viability and vigor. Seed quality testing aims to determine seed quality, evaluate the ability of seeds to germinate and grow, ensure the availability of high-quality seeds, and prepare seeds for planting activities (Widajati et al., 2013). Seed quality testing is crucial for assuring consumers that the seeds they acquire are of high quality (Pedrini and Dixon, 2020). Effective seed quality testing methods should be cost-effective, fast, easy to perform, objective, repeatable, and have a strong correlation with field performance (Copeland and McDonald, 2001). One of the rapid testing methods currently under development involves using Urine Sugar Analysis Paper (USAP) to detect cell metabolite leakage.

USAP or urine strips are commonly used in the medical field for urinalysis (Park and Ko, 2021). Urinalysis provides a rapid analysis of urine samples to obtain clinical information and assist in the early diagnosis of kidney and urinary tract conditions (Ourani et al., 2021). The seed viability test using USAP is conducted by measuring glucose, protein, pH, and specific gravity levels in the seed soaking water, based on color changes observed in the USAP reagent. When seeds are soaked, they release electrolytes and metabolites such as sugars, amino

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acids, fats, and other compounds. A higher level of metabolite leakage indicates lower integrity of the seed cell membrane. The use of USAP for seed testing was initially explored by (Takayanagi & Murakami, 1969) on Brassicaceae and wheat seeds. Their research showed that vigor could be detected in Brassica seeds after 5 hours of soaking, while wheat seeds required 40 hours for glucose leakage to be observed in the soaking water. (Rukundo et al., 2022) reported that USAP can effectively differentiate between low, medium, and high viability seed lots in soybeans. Similarly, Nursoleha et al. (2022) investigated the use of USAP for soybean seeds and found that this rapid test could accurately detect seed deterioration.

The principle of seed viability testing using the USAP method involves measuring the increase in glucose, protein, pH, and specific gravity levels in seeds that are fully immersed in water during the soaking process. USAP changes color when the solution contains glucose, protein, or shows an increase in density (Abebayehu, 2023). High levels of metabolite in seed soaking water indicate damage to the seed cell membrane (Kumar & Mishra, 2014). Seeds with significant metabolite leakage exhibit compromised cell membrane integrity, which is strongly correlated with low seed viability (Panobianco et al., 2007). Decreased cell membrane integrity results in the release of various compounds from germ cells, including sugars, amino acids, fatty acids, enzymes, and organic ions, which can be observed through measurements of electrical conductivity and metabolite concentrations (Vieira et al., 2008).

Research on rapid seed testing methods using USAP can be further developed for other commodities and varieties, as each commodity has a distinct percentage of seed food reserves. This study specifically focused on peanut seeds (*Arachis hypogaea* L.) with varying levels of viability and several varieties. The primary objective was to develop a rapid test method for estimating the viability of peanut seeds using Urine Sugar Analysis Paper (USAP) by assessing the leakage of glucose, protein, and specific gravity values.

Materials and Methods

Location

This research was conducted from January to May 2023 at the Seed Storage and Quality Testing Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. The seeds used were peanut seeds of the “Katana 2”, “Domba”, “Kancil”, “Talam 1”, and “Talam 2” varieties from the Indonesian Agricultural Information Instrument Standard Testing Institute (ILESTI). The type of USAP used is the URS-10 T, which has 10 test parameters. The parameters assessed on USAP were glucose, protein, pH, and density. The research was conducted with three USAP experiments. Experiment 1 determines the best Method between seed cutting size and soaking duration. Experiment 2 tests seed viability using USAP on stored seed lots. Experiment 3 tests seed viability using USAP on different varieties.

Seed Physiological Quality Testing

The seeds were obtained from a two-month storage period to create several seed lots based on the viability levels. The experiment was designed using a completely randomized design with one factor: seed packaging. The packaging treatments included no packaging, aluminum foil, polypropylene plastic (PP), polyethylene plastic (PE), and paper. Seeds packaged in aluminum foil and no-packaging were stored at a cool temperature (18 °C and RH ± 50%), while seeds packaged in PP, PE, and paper were stored at room temperature (28 °C and RH ± 96%).

After storage, the seeds were tested for moisture content and viability. Viability was determined through germination percentage, first germination, and electrical conductivity tests, following the guidelines of ISTA (2018). Seeds with the highest and lowest viability were subsequently selected for USAP experiments to determine the optimal method for this purpose.

USAP Experiment 1: Evaluation of Seed Cutting Sizes and Soaking Durations

The method was optimized by evaluating the effects of two factors: seed cutting and soaking period. The variety used in this experiment was “Katana 2” peanut seeds. Seed-cutting treatments consisted of uncut/whole seeds (Figure 1A), halved seeds (Figure 1B), and seeds which are cut into six pieces (Figure 1C). The soaking period treatment consisted of a control (seeds not soaked in water) and 3, 6, and 9 hours in 50 mL of distilled water. This experiment was conducted using two seed lots: high-viability and low-viability seed lots. The high-viability seed lot exhibited a 95% germination percentage, a 27.5% first-count germination, and an electrical conductivity value of 2.64 $\mu S.cm^{-1}.g^{-1}$. The low-viability and vigor lot exhibited 86% germination percentage, 7% first count germination, and an electrical conductivity value of 4.74 $\mu S.cm^{-1}.g^{-1}$.

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The experiment was arranged in a completely randomized design with 12 treatment combinations replicated four times, resulting in 48 experimental units. Each treatment used 50 seeds. The experiment was conducted at room temperature (28°C) in a well-lit room. Fifty seeds were soaked in 50 mL of distilled water for each combination, varying the seed-cutting size and soaking period. Subsequently, the seed-soaking water was then moved to a test tube, and the urine strip (USAP) was dipped into the tube. The levels of glucose, protein, and specific gravity were then assessed. Color changes on the USAP strips were observed and compared with the color scale on the USAP bottle.

Figure 1. Peanut seed cuttings: (A) whole seed (uncut), (B) half-seeds, (C) seeds cut into six pieces

USAP Experiment 2: Seed Viability Testing using USAP on Stored Seed Lots

Based on the results of experiment 1, the optimal treatment combination for distinguishing seed vigor was identified. This combination was subsequently applied to evaluate seed viability and vigor in five seed lots with varying levels of viability and vigor. The variety used in this experiment was "Katana 2" peanut seeds. In experiment 2, all seed lots were tested using the optimal combination of the soaking period and seed-cutting size determined in Experiment 1. Seed lots were obtained based on storage packaging treatments, which included no packaging, aluminum foil, polypropylene (PP) plastic, polyethylene (PE) plastic, and paper. This experiment consisted of 20 experimental units, comprising five seed lots with four replications each. The experiment was analyzed using a completely randomized single-factor design, with seed lots as the treatment factor. Experiment 2, like Experiment 1, evaluated the color changes observed on the USAP strips resulting from dipping them into the seed-soaking solution. This experiment aimed to compare the changes in urine paper color on seeds with different viability.

USAP Experiment 3 (Seed Viability Testing using USAP on Different Varieties)

Experiment 3 utilized five varieties: "Domba", "Kancil", "Katana 2", "Talam 1", and "Talam 2". The seed moisture content was adjusted to 8-10% before the experiment. USAP tests were incubated at 35°C. The experiment was designed using a completely randomized design with four replicates. Data was analyzed using correlation analysis to assess the relationship between USAP test results and other viability and vigor test parameters, including germination percentage, first count germination, speed of germination, and electrical conductivity.

Statistical Analysis

The data were analyzed using the Analysis of Variance (ANOVA) test. Significant differences among treatments were determined using Duncan's Multiple Range Test (DMRT) at the 95% confidence level. Statistical analyses were performed using the Microsoft Excel and SAS Studio applications.

Results and Discussion

Experiment 1: Evaluation of Seed Cutting Sizes and Soaking Durations

The seed lots used for the first experiment were the highest and lowest viability lots obtained from the seed storage treatment. The experiment was conducted to investigate the effects of seed cutting size and soaking period. Seed cutting aims to increase the surface area of the seeds, which can accelerate membrane leakage processes.

USAP testing was performed by observing color changes on reagent paper for glucose, protein, and specific gravity parameters. Each parameter exhibited

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a distinct color change. Protein color changes related to albumin detection are based on the interaction between negatively charged and positively charged proteins. Albumin is the primary type of protein that can be detected using USAP. This interaction can result in ionization, altering the protein's charge and causing a color change that can be measured using specific indicators (Mahaffey, 2020).

The color change in the glucose parameter results from the reaction of glucose with the enzyme glucose oxidase, which catalyzes the formation of gluconate and hydrogen peroxide. The hydrogen peroxide is catalyzed by peroxidase to produce potassium iodide oxide, resulting in a brown color change. The specific gravity changes color due to the reaction of electrolytes, such as salts (M+X-), with ion-exchange bodies (methyl vinyl ether maleic copolymer), typically sodium ions (Na+), which then produce hydrogen ions. The hydrogen ions then react with the acid-base indicator on the strip, resulting in a color change (Fan and Bai, 2020).

The USAP test on protein and specific gravity parameters had a significant impact on seed viability and vigor levels, whereas glucose parameters showed no significant effect. USAP could not detect glucose content in the seed soaking water, and the pH value remained relatively constant across all treatments at approximately 6.5.

As shown in Table 1, the soaking period had no effect on the protein content in the soaking water. Seed soaking water containing high protein causes a color change on the USAP strip from yellow to green. Proteins within the seeds are broken down into amino acids, which are essential components for the synthesis of various molecules, including enzymes, hormones, structural proteins, pigments, and other compounds required for embryo development during germination. According to Rasheed et al. (2020), seeds contain several types of proteins, such as albumin, globulin, prolamin, and glutelin. Increased leakage from seeds, due to decreased cell membrane integrity, is also correlated with a loss of seed viability (Gu et al., 2024).

Protein leakage was not detected in the seed soaking water from whole seeds and halved seeds based on Table 1. However, protein was detected in the seed soaking water of six-cut seeds using USAP. The soaking significantly increased the protein content in the soaking water compared to the control, but there were no significant differences between the soaking periods of 3, 6, and 9 hours (Table 1).

Table 2 shows that the soaking period and the seed cutting size had a significant effect on color change in the density parameter. Density is defined as the mass of a substance per unit volume. According to Poon (2022), the density value represents the concentration of solutes, specifically the seed soaking water. Seed-soaking water with a higher solute concentration causes the USAP strip to change color from yellow to pale yellow.

The density value of 1.017 was observed in the low-viability seed lot after 9 hours of soaking with six-cut seeds, and it was 1.002 in the high-viability seed lot with a 3-hour soaking period of the uncut seeds (Table

Table 1. Protein content of the peanut soaking water measured by USAP

Seed cutting	Soaking period (hours)	Protein content ($mg.L^{-1}$)	
		Low viability (A)	High viability (B)
Whole seeds	0	0 b	0 b
	3	0 b	0 b
	6	0 b	0 b
	9	0 b	0 b
Halved seeds	0	0 b	0 b
	3	0 b	0 b
	6	0 b	0 b
	9	0 b	0 b
Seeds cut into six pieces	0	75.0 b	37.5 b
	3	187.5 a	150.0 a
	6	225.0 a	150.0 a
	9	225.0 a	150.0 a

Notes: The mean values followed by the same letter in one column are not significantly different based on the Duncan Multiple Range Test at α=0.05.

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2). These density values correspond to urine density values, which typically range from 1.00 to 1.03. The best method was determined based on data from the protein and specific gravity parameters. The glucose parameter was excluded because no color changes were observed in any of the treatments.

Cutting peanut seeds into six pieces was optimal for testing, as the detectable protein leakage was observed only in these seeds. Cutting seeds into six pieces increases the surface area exposed to water, which accelerates cell membrane leakage. The soaking period showed no significant differences; hence, a soaking period of 6 hours was chosen.

USAP Experiment 2: Seed Viability Testing using USAP on Stored Seed Lots

Experiment 2 was conducted based on the best method determined from the results of the first experiment. Seeds of the five lots were cut into six pieces and soaked for six hours.

Table 3 showed that the seed lots significantly affected the protein leakage and specific gravity of the seed soaking water. The protein leakage of the two seed lots was 0.0–75.0 mg.L^{-1} and 187.5 mg.L^{-1}. Among the seed lots, the fifth seed lot exhibited the highest protein leakage compared to the other lots.

Seeds with high metabolite leakage have damaged cell membrane structures (Lin et al., 2022), which can be associated with reduced viability and vigor. Protein leakage is primarily influenced by alterations in the membrane structure and phospholipid composition, serving as a crucial indicator of cell membrane function and permeability (Ratajczak et al., 2015).

Based on specific gravity parameters, seed lots were categorized into two groups: 1.01 - 1.011 (seed lots 1-4) and 1.017 (seed lot 5). The fifth lot had a significantly different effect from the other lots. This observation aligns with the results of other viability tests, such as germination percentage, first count germination, and electrical conductivity, as the fifth lot also showed lower viability compared to the other lots. According to Demir et al. (2019), seeds with high membrane leakage indicate low viability. One of the primary causes of cell membrane leakage is the appearance of reactive oxygen species (ROS), which are highly reactive oxygen-containing compounds. ROS can trigger a chemical reaction known as lipid peroxidation, leading to increased membrane permeability (Wang et al., 2024).

Correlation between Seed Viability and Vigor with Protein Content and Specific Gravity Using USAP

Germination percentage, first count germination, and germination speed were negatively correlated with protein leakage as measured by the USAP method (Table 4). Correlation analysis aims to determine the direction and strength of the relationship between two or more variables (Schober et al., 2018). A positive correlation coefficient (r) indicates a direct relationship between variables, while a negative coefficient signifies an inverse relationship. In contrast, the electrical conductivity parameter was positively correlated with protein leakage. Hayat and Ahmad (1996) reported that protein leakage is negatively

Table 2. Specific gravity values of peanut seed soaking water measured by USAP

Seed	Soaking period (hours)	Specific gravity values	
		Low viability	High viability
Whole Seed	0	1.006 bc	1.006 bc
	3	1.004 c	1.002 c
	6	1.005 bc	1.005 bc
	9	1.007 bc	1.006 bc
Half seed	0	1.007 bc	1.006 bc
	3	1.005 bc	1.004 bc
	6	1.009 bc	1.007 abc
	9	1.011 abc	1.009 abc
Seeds cut into six pieces	0	1.010 abc	1.007 abc
	3	1.009 bc	1.007 abc
	6	1.012 ab	1.010 ab
	9	1.017 a	1.014 a

Note: The mean values followed by the same letter in one column are not significantly different based on the Duncan Multiple Range Test (DMRT) at α=0.05.

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correlated with viability and vigor in chickpea (*Cicer arietinum)* seeds.

In this study, seed protein content was found to have a negative correlation with protein leakage in the "Domba", "Kancil", "Talam 1", and "Talam 2" varieties. Still, no significant correlation was observed in the Katana 2 variety. Demir et al. (2019) found that cell membrane leakage was negatively correlated with germination percentage in cress (*Lepidium sativum*) seeds. Proteins are crucial for maintaining cellular structure and function, including the integrity of cell membranes. Increased protein content within the membrane can enhance its structural stability and minimize the risk of leakage (Dhaliwal and Angeles-Shim, 2022).

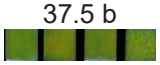
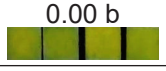
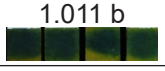
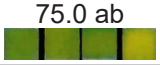
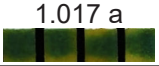
The electrical conductivity showed a positive correlation with the results of the protein leakage test using USAP. Both parameters indicate damage to seed cell membranes. Ozden (2022) stated that damage to the seed cell membrane leads to the leakage of ions and metabolites in the seed.

Based on Table 5, the parameters of germination percentage, first count germination, and germination speed were negatively correlated with the density value of USAP, indicating that higher density values are associated with lower germination values. The differences in correlation values among varieties may be influenced by the content of seed food reserves, cell membrane structure, seed coat thickness, and seed size.

According to Adetunji et al. (2020), during the soaking process, seeds release various substances, including organic compounds and ions, into the surrounding water, which can increase the density of the solution. The concentration of solutes in the seed-soaking water reflects the extent of metabolite and electrolyte leakage from the cells, indicating damage to the cell membrane (Dauwe et al., 2021). Damaged cell membranes are often associated with seed deterioration and decreased viability.

The fat content of the seeds showed a positive correlation with seed viability in the "Domba" and "Talam 1" varieties but no significant correlation in the "Kancil", "Katana 2", and "Talam 2" varieties. Fat is a substantial component of peanut seeds, serving as

Table 3. Value and color change in protein and specific gravity parameters, and the values of vigor and viability test

Seed lots	Protein ($mg.L^{-1}$) and color change on paper	Specific gravity and color change on paper	Moisture content (%)	Germination percentage (%)	First count germination (%)	Electrical conductivity ($\mu S.cm^{-1} g^{-1}$)
1	37.5 b 	1.011 b 	7.05 b	95 a	27.5 a	2.64 b
2	0.00 b 	1.011 b 	7.05 b	88 b	25 ab	3.14 b
3	75.0 ab 	1.010 b 	7.15 b	87 b	23 ab	2.73 b
4	75.0 ab 	1.012 b 	7.26 b	92 ab	19 b	2.83 b
5	187.5 a 	1.017 a 	9.52 a	86 b	7 c	4.74 a

Note: The mean values followed by the same letter in one column are not significantly different based on the Duncan Multiple Range Test (DMRT) at α=0.05.

Table 4. Correlation of seed viability and vigor with the protein leakage using USAP

Correlations "Domba"		Varieties				
		"Kancil"	"Katana 2"	"Talam 1"	"Talam 2"	"Talam 2"
Protein leakages	Germination percentage	-0.874	-0.807	-0.977**	-0.724	-0.932*
	First count germination	-0.832	-0.334	-0.967**	-0.676	-0.846
	Germination speed	-0.905*	-0.642	-0.828	-0.914	-0.933*
	Electrical conductivity	0.866	0.673	0.949*	0.925*	0.828
	Seed fat content	-0.906	-0.276	0.047	-0.762	-0.617

Notes: ρ (r) = Pearson's correlation coefficient, * = significant at 0.05 level, ** = significant at 0.01 level.

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Table 5. Correlations of seed viability and vigor test with the specific gravity test using USAP

Correlations "Domba"		Varieties				
		"Kancil"	"Katana 2"	"Talam 1"	"Talam 2"	"Talam 2"
Specific Gravity	Germination percentage	-0.729	-0.960**	-0.756	-0.988**	-0.984**
	First count germination	-0.04	-0.696	-0.917*	-0.958*	-0.917*
	Germination speed	-0.775	-0.910*	-0.561	-0.965**	-0.99**
	Electrical conductivity	0.766	0.979**	0.629	0.920*	-0.974**
	Seed fat content	0.645	-0.141	0.252	0.715	-0.016

Notes: ρ (r) = Pearson's correlation coefficient, * = significant at 0.05 level, ** = significant at 0.01 level.

an energy reserve for embryo development during germination and as a structural component of the cell membrane in the form of phospholipids. Fat content testing is conducted using the proximate analysis method, which is a more comprehensive process compared to the USAP method.

Fats, particularly unsaturated fatty acids, are susceptible to lipid peroxidation, a process that involves the oxidative degradation of lipids. This process is initiated by reactive oxygen species (ROS), which can react with unsaturated fatty acids, damaging the cell membrane structure (Oenel et al., 2017). This oxidative process generates free radicals that can disrupt chemical bonds within fats, proteins, and other membrane components, ultimately increasing membrane permeability. High fat content in seeds can potentially increase the susceptibility of cell membranes to oxidative damage and subsequent leakage.

Relationship between Seed Viability and Vigor with Glucose Testing Using USAP

The experimental results of glucose content testing using USAP revealed no detectable glucose leakage, as no color change was observed on the USAP strips in any of the treatments (Table 3). This finding aligns with Rukundo et al. (2022), who reported that USAP tests on soybean seeds were practical primarily for detecting protein content. In contrast, Nursoleha et al., (2022) observed that USAP could detect glucose leakage in soybean seeds and found a negative correlation with germination parameters, including germination percentage, first count germination, and germination speed. The lack of detectable glucose leakage in peanut seeds can be attributed to their relatively low carbohydrate content. Peanuts primarily utilize fat as an energy source rather than carbohydrates. According to Nayak et al. (2020) the carbohydrate content of peanuts is approximately 11-17%.

The USAP method determines seed viability based on color changes observed on the test strip. Regarding the protein parameter, high viability in seeds is indicated by a color change to pale brilliant yellow-green (HEX #E6F57E), medium viability by a change to light lime green (HEX #CEEA86), and low viability by a change to bright sea green (HEX #8CC690).

For the density parameter, color changes on the strip are also used to assess the viability of peanut seeds. High viability is indicated by a dark green color (forest green, HEX #045956), medium viability is marked by a yellowish green color (dark olive green, HEX #677B39), and low viability is indicated by a greenish yellow color (mustard green, HEX #9A9433).

Urine strips used in seed viability testing can rapidly and conveniently detect metabolic compounds, such as protein, through color changes. However, further method development is required to improve their effectiveness.

Conclusions

Urine sugar analysis paper (USAP) can effectively assess peanut seed viability and vigor by analyzing protein and density parameters through the color change on the paper strip. Color changes from yellow to green for protein parameters and from dark green to yellowish green for density parameters can be used to assess seed viability. The optimal USAP method, determined through experimentation, involves cutting the peanut seeds into six pieces and a six-hour soaking period because it can distinguish between peanut seeds with high and low viability. Across all five peanut varieties tested, both protein and density parameters exhibited significant negative correlations with germination percentage, first count germination, and germination speed, while showing significant positive correlations with electrical conductivity. Glucose parameters were ineffective in predicting seed viability and vigor using USAP, as no color change was observed on the USAP strips. However, USAP effectively differentiated between seed lots with varying levels of viability and varying varieties based on the measured protein and specific gravity

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levels. The USAP method can be further developed by exploring different USAP types, correlating its test results with the metabolite content of seed soaking water, and expanding its application to seeds of other species.

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