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Elemental Mapping and Quantities in Different Soybean Seed Colors Using Micro X-Ray Fluorescence and Their Correlations with Germination

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ABSTRACT

Micro X-ray fluorescence (μ-XRF) possesses a powerful analytical technique able to detect macro- and micro-elements. Each plant variety has a unique elemental composition and important role in the germination process. The aims of this study were to (1) map the elements and quantities in the soybean seed coat and endosperm, (2) investigate how the various elements might mediate the inter-relationship or correlation between elements within soybean seed genotypes with different seed coat colors, and (3) investigate that the targeted morphological characteristics especially in germination would be affected by seed elements. A μ-XRF technique was used for the elemental analysis and quantification. Three genotypes of Indonesian soybean were used in this study: greenish, black, and yellowish. In this study, we found that the silicon (Si) and magnesium (Mg) elements have a significant correlation. The high quantity of Si element in the embryo axis has a positive correlation with root length. The high quantity of Mg element which is evenly distributed on the endosperm has a positive correlation with normal germination. Si and Mg elements in the seeds have a negative correlation with imbibition water absorption. Based on the comparison between the three genotypes, the black genotype was superior in terms of germination and higher Si and Mg elements. Thus, the Si and Mg elements can be used as a reference in determining superiority of genotypes at the germination stage.

INTRODUCTION

Soybean (*Glycine max*) has been widespread throughout the world, and it offers a number of nutritional advantages [1]. Soybeans are the most important legume crop and the primary source of oil, protein, and mineral elements needed by human body such as iron (Fe), magnesium (Mg), and phosphorus (P) [2,3]. Soybean must obtain the following macro- and micro-elements from their growing base (soil): nitrogen (N), phosphorus (P), potassium (K); macro-elements (secondary and tertiary): calcium (Ca), sulfur (S), magnesium (Mg); and the micro-elements: iron (Fe), boron (B), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), molybdenum (Mo), sodium (Na), silicon (Si), selenium (Se), vanadium (V), aluminum (Al), nickel (Ni), titanium (Ti), and chlorine (Cl) [4].

Soybean is classified into several types based on the color of the seed coat [5]. In general, almost all of soybean seeds coat have 3 colors, i.e., yellowish, greenish, or black [6]. The colors of seed coat were produced by the accumulation of different pigments, such as anthocyanins, chlorophyll, and by combining these pigments with other types, such as flavonoids [7]. Dark seed coats in soybeans are caused by a high concentration of anthocyanins in the epidermal layer [8].

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Genetic and environmental factors influence variations in the elements accumulation in soybean seeds [9-11]. The diversity of soybean genotypes based on seed coat color, which is caused by genetic factors, have different uptake, transport, storage, and remobilization proceses in accumulating elements in seeds [12]. Increasing the concentration of seed elements can be accomplished by mapping the dominant element of the seed, so that it can be used as a specific strategy for plant breeding [13].

In order to measure physiological performance based on the quantity and mapping of seed elements, the use of micro X-ray fluorescence (μ-XRF) techniques may offer a potential option that can deliver information more quickly, practically, usefully, and consistently [14-16]. The μ-XRF is a nondestructive microscopic analytical technique used to investigate the distribution of the macro- and micro-elements in the seed structures and their concentration with 20 μ detailed resolutions [17]. As a result, μ-XRF is an ideal tool for qualitative and quantitative material composition analysis.

The primary concern in evaluating seed quality based on seed morphology is dependent on two key components, i.e., seed coat and endosperm (cotyledons and embryonic axis) [18]. But little is known about correlation between elements to seed coat, endosperm, and any morphological responses.

Each plant variety has a unique elemental composition [19]. The functions of elements in plants [20] were very important for plants, i.e., strengthening cell walls, cell elongation and division, second messengers in osmoregulation, chlorophyll molecular structure [21], photophosphorylation, and enzyme activation [22]. However, as far as we know, information about the elements that play an important role in the germination process are still lacking, especially in soybean plants.

Therefore, the aims of this study were to (1) map the elements and quantities in the soybean seed coat and endosperm, (2) investigate how the various elements might mediate the inter-relationship or correlation between elements within soybean seed genotypes with different seed coat colors, and (3) investigate that the targeted morphological characteristics especially in germination would be affected by seed elements differently. A μ-XRF technique was used in this study for elemental analysis and quantification.

MATERIALS AND METHODS

Plant material

Commercialized soybeans are used as the genetic material. Three genotypes of Indonesian soybean were used in this study: greenish seed coat genotype, black seed coat genotype, and yellowish seed coat genotype (Fig. 1). Of these, greenish genotype was harvested from Bogor Regency, West Java, Indonesia; black genotype was harvested from Indramayu Regency, West Java, Indonesia; and yellowish genotype was harvested from Grobogan Regency, Indonesia.

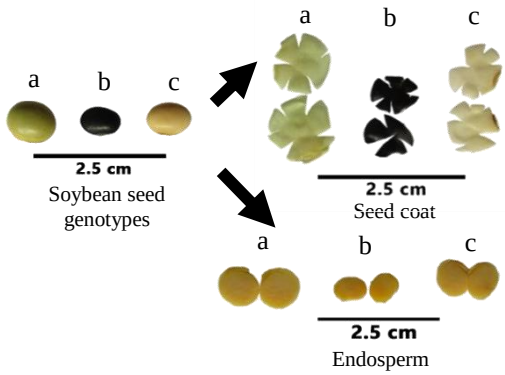

Fig. 1. Soybean seeds genotypes: seed coat and endosperm. a) greenish genotype 2; b) black genotype; c) yellowish genotype.

Sample preparation

Four soybean seeds from each genotype were analyzed by using a μ-XRF technique. First, the soybeans were longitudinally split into 2 equal parts, then the seed coat and endosperm were separated. After that, the seed coat was cut on the sides, placed between 2 object glasses (the outside of the seed coat was faced upwards). The seed coat was pressed gently and slowly, until the surface of the seed coat becomes flat. The endosperm was placed on a glass object that has been given double-sided tape (approximately 1 mm^2/endosperm), the longitudinal section of the endosperm was faced upwards. For morphological analysis, i.e., seed total area (in cm^2), seed coat area (in cm^2), endosperm area (in cm^2), seed color (used image processing analysis), and germination (used germination test analysis), a total of 100 samples of seeds were used randomly from each genotype for the observe of seed total area, seed coat area, endosperm longitudinal section area, germination test, and imbibition water absorption. For 25 g seeds-weight, 1000 seeds were used for each genotype. A total of 40 seeds for each genotype were used for confirmation of seed coat color through red, green, and blue color value.

μ-XRF analysis

The elemental analysis was conducted at Nuclear Minerals Technology Laboratory, National

Research and Innovation Agency (NRIA), South Jakarta, Indonesia. The μ-XRF type M4 Tornado155 plus (Bruker nano GmBH, Berlin, Germany) was used for analyzing the distribution of elements. In particular, the micro-XRF scanning analyses were performed at 13.5 keV incident beam energy with mapping parameters of 35 μm pixel size and 10 × 10 mm of total area. The acquisition parameters were pixel time of 20 ms/pixel, measurement time of 20 min, and stage speed of 1.8 mm/s. The tube parameters were high voltage of 50kV, anode current of 600 μA, and air chamber pressure of 19.8 mbar. The μ-XRF scanning analysis employed selected detectors 1.2, a detector that is sensitive to a specific range of X-ray energies, and was chosen based on the desired analysis conditions. The number "1.2" likely corresponds to the specific channel or energy range that the detector is optimized to detect. The maximum pulse throughput of 275,000 cps. Elemental mapping and quantities were recorded as data.

Image processing

Images of the seed were acquired with a Canon PowerShot SX410 IS camera with a 20 megapixels resolution. The camera was mounted overhead 0.32 m above the object. Images were taken on a plain black background with natural light. White balance of the camera was adjusted according to the manufacturer's instructions before images were captured for the experiments. The camera was set to auto mode with flash disabled to automatically adjust shutter speed, aperture, and ISO speed. For each sample of seed placed on the black background. Each image is renamed according to the sample that shows the treatment. Images were saved in JPEG format for analysis.

The image analysis was developed in ImageJ software (version 1.52e, NIH, https://imagej.nih.gov/ij/). The first step was to set the scale of the image (Fig. 2a). Then, object of interests were extracted from the background, and the extracted seed images were converted into black and white (Fig. 2b) using color thresholding [23]. Then, the total seed area, seed coat area, and endosperm longitudinal section area were observed using particles analyzer (Fig. 2b). Polygon selection following the shape of the seed coat for color analysis (Fig. 2c). For confirmation of seed coat color through red, green, and blue (RGB) value was done using histogram analyzer (Figs. 2d-f). The visualization converted from RGB values using color picker adobe photoshop version CS3 (Fig. 2h). Based on the RGB values (Fig. 2g), it is known that the seed coat color of genotypes were significantly different ($p<0.05$). Remarks (Fig. 2): values marked with different letters in the same single color space show significant differences based on Duncan Multiple Range Test at $p<0.05$; G = greenish genotype; B =black genotype; Y = yellowish genotype.

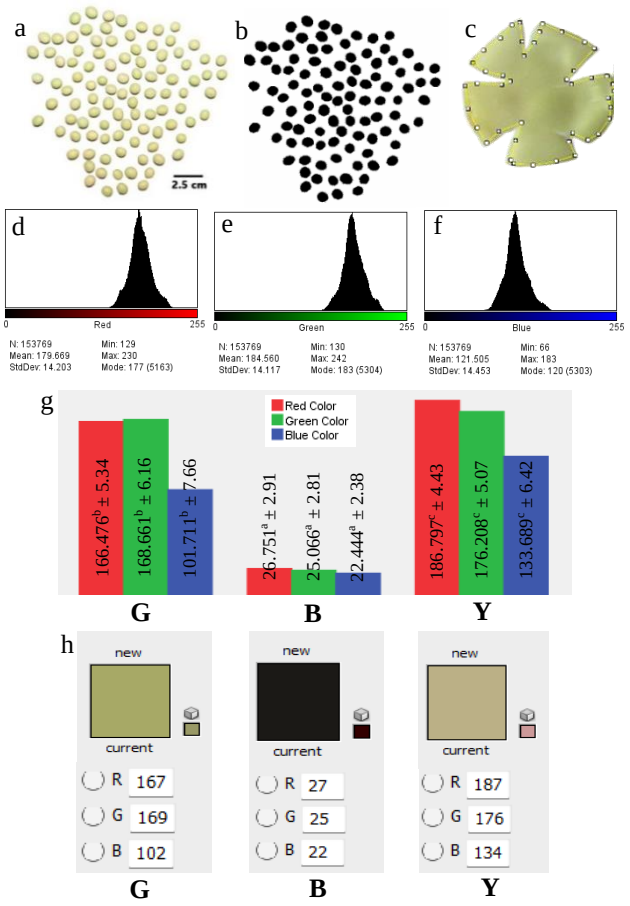

Fig. 2. Image processing of soybean genotype using ImageJ and adobe photoshop version CS3. a) set the scale of image and extract the object of interest from the background; b) binary image from color thresholding for area analysis; c) polygon selection following the shape of the seed coat for color analysis; d-f) red, green, and blue (RGB) value using analyze histogram; g) red, green, and blue color value (mean ± standard deviation) of soybean seed coat; h) visualization of soybean seed coat color using color picker adobe photoshop version CS3.

Germination test

The germination test standard was applied in this study. Germination paper with 100 seeds each genotype (25 seeds × 4 replications) was folded and placed in a germination chamber for 5 days at 25 °C and 90 % relative humidity. The seeds were classified as normal viable when the root size was > 15 mm, abnormal viable when the root size was 5 mm ≤ x ≤ 15 mm, and nonviable seed when the root size was < 5 mm. The data recorded were the number of normal, abnormal viable seeds, root length, and root area. The analysis steps using ImageJ (version 1.52e) consist of setting scale of

image (Fig. 3a), then measuring the root area using the polygon selection (Fig. 3b), and measuring root length using the segmented line (Fig. 3c).

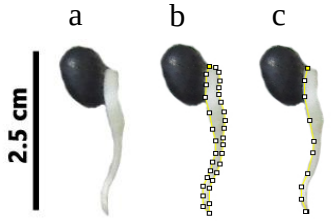

Fig. 3. Germination test of soybean genotype 3 days after planting. a) set scale of image; b) measure the root area using polygon selection; c) measure the root length using segmented line.

Data analysis

Soybean seed coat colors value were analyzed of variance and continued with Duncan Multiple Range Test at $p<0.05$[24]. Elementals mapping were described based on figure and compared between the 3 genotypes. Analysis of variance was carried out to determine the difference quantities of elements and continued with Duncan Multiple Range Test at $p<0.05$. Elements in each part of the endosperm or seed coat correlations were analyzed using Pearson correlation. Morphological characters variance were analyzed and continued with Duncan Multiple Range Test at $p<0.05$ through boxplot . Elemental quantities in each endosperm and seed coat correlations were analyzed with morphological characters using Pearson correlation. All analysis were performed using SPSS Statistics version 22 at $p<0.05$.

RESULTS

Figure 4 shows the element mapping of macro-elements (P, S, K, Ca, and Mg) and micro-elements (Al, Si, and Fe) in the endosperm and seed coat for three different genotypes of soybeans. In general, none of the elements of the three seed coat genotypes were concentrated in one area. The three genotypes are distinguished by the concentration of elements in the seed coat as shown in Table 1.

The macro-elements of P, S, K, and Mg appear to be evenly distributed throughout the endosperm in the three genotypes. However, P element appears to be more concentrated in the embryo axis and plumule in three genotypes. Meanwhile, the Ca element was seen to be more concentrated in the cotyledons compared to the embryo axis and plumule. In the three genotypes, micro-elements Al and Fe appears to be distributed equally among the part of endosperm. In the black genotype, the Si element appears to be concentrated in the embryo axis, while the cotyledon tends to contain less Si element. In the same way, the Si element in the endosperm of the yellowish genotype appear to be more concentrated in the embryo axis than the cotyledon.

Table 1. Mass element concentrations (mean ± standard deviation) in different soybean seed color.

Elements		G (wt.%)	B (wt.%)	Y (wt.%)
P	e	$0.8221^{a} \pm 0.009$	$0.7927^{a} \pm 0.029$	$0.7916^{a} \pm 0.032$
	sc	$0.0788^{a} \pm 0.002$	$0.1153^{b} \pm 0.002$	$0.1228^{c} \pm 0.004$
S	e	$0.9400^{a} \pm 0.006$	$0.9403^{a} \pm 0.045$	$0.9413^{a} \pm 0.031$
	sc	$0.1614^{a} \pm 0.003$	$0.2198^{b} \pm 0.013$	$0.1446^{a} \pm 0.019$
K	e	$12.9203^{c} \pm 0.011$	$10.7072^{a} \pm 0.130$	$11.9943^{b} \pm 0.698$
	sc	$5.4391^{b} \pm 0.255$	$4.9305^{b} \pm 0.182$	$4.1115^{a} \pm 0.578$
Ca	e	$2.1786^{a} \pm 0.015$	$2.1317^{a} \pm 0.279$	$2.6404^{b} \pm 0.054$
	sc	$3.0042^{a} \pm 0.158$	$2.9069^{a} \pm 0.134$	$2.9574^{a} \pm 0.249$
Mg	e	$0.2593^{a} \pm 0.024$	$0.3811^{b} \pm 0.048$	$0.3619^{b} \pm 0.032$
	sc	$0.5225^{a} \pm 0.035$	$0.9196^{c} \pm 0.023$	$0.7900^{b} \pm 0.124$
Al	e	$0.0340^{a} \pm 0.004$	$0.0696^{c} \pm 0.007$	$0.0569^{b} \pm 0.004$
	sc	$0.3970^{b} \pm 0.033$	$0.5194^{c} \pm 0.005$	$0.2504^{a} \pm 0.047$
Si	e	$0.1146^{a} \pm 0.009$	$0.2009^{c} \pm 0.012$	$0.1690^{b} \pm 0.015$
	sc	$0.5913^{a} \pm 0.039$	$0.9683^{b} \pm 0.009$	$0.5362^{a} \pm 0.086$
Fe	e	$0.1697^{b} \pm 0.003$	$0.1803^{b} \pm 0.011$	$0.1289^{a} \pm 0.006$
	sc	$0.4251^{a} \pm 0.009$	$0.4705^{a} \pm 0.008$	$0.4564^{a} \pm 0.067$

Remarks: G = greenish genotype; B = black genotype; Y = yellowish genotype; e = endosperm; sc = seed coat; wt.% = weight percent; Mg = magnesium; Al = aluminum; Si = silicon; P = phosphorus; S = sulfur; K = potassium; Ca = calcium; Fe = iron.

Figure 5 shows the quantity of elements in the endosperm and seed coat areas. In general, the pattern of macro-elements in the endosperm does not differ between genotypes, i.e., K > Ca > S > P > Mg. However, there was a pattern difference in the micro-elements, i.e., Fe > Si > Al in greenish genotype and Si > Fe > Al in black and yellowish genotypes.

The quantity of P and S elements in the endosperm area was not significantly different ($p>0.05$) between genotypes (Table 1). K element was higher in the greenish than in the black and yellowish genotype ($p<0.05$). Ca element was higher in the yellowish than in the black and greenish genotypes ($p<0.05$). Mg element was higher in the black than in the greenish genotype ($p<0.05$), but not significantly different with the yellowish genotype ($p>0.05$). The black genotype had the highest quantity of micro-elements of Al and Si in the endosperm area compared to greenish and genotypes ($p<0.05$). Interestingly, based on the elemental mapping of the black genotype, Si element were concentrated in the embryo axis (Fig. 4), which means that Si element may have an important role on the embryo axis. Meanwhile, Fe element in the black genotype was not

significantly different with the greenish genotype ($p>0.05$), but significantly different with the yellowish genotype ($p<0.05$). Thus, in simple terms, the black genotype has a higher quantity of micro-elements than the greenish and the yellowish genotypes in the endosperm area.

In the seed coat area, the pattern of macro-elements quantity in the three genotypes were not different, i.e., K > Ca > Mg > S > P. The pattern of micro-elements quantity in black genotype i.e., Si > Al > Fe, while in greenish and yellowish, i.e., Si > Fe > Al (Fig. 5). The quantity of P element in the yellowish genotype was significantly higher ($p<0.05$) than the black and the greenish genotypes (Table 1). The quantity of S and Mg elements in the black genotype was significantly higher than the greenish and the yellowish genotypes ($p<0.05$). The quantity of K elements in the greenish and the black genotypes was not significantly different ($p>0.05$), but significantly higher than the yellowish genotype ($p<0.05$). Meanwhile, Ca element was not significantly different in the three genotypes ($p>0.05$). The quantities of Ca and Mg were higher in the seed coat than endosperm area for the three genotypes, while the quantities of P, S, and K elements were higher in endosperm than seed coat.

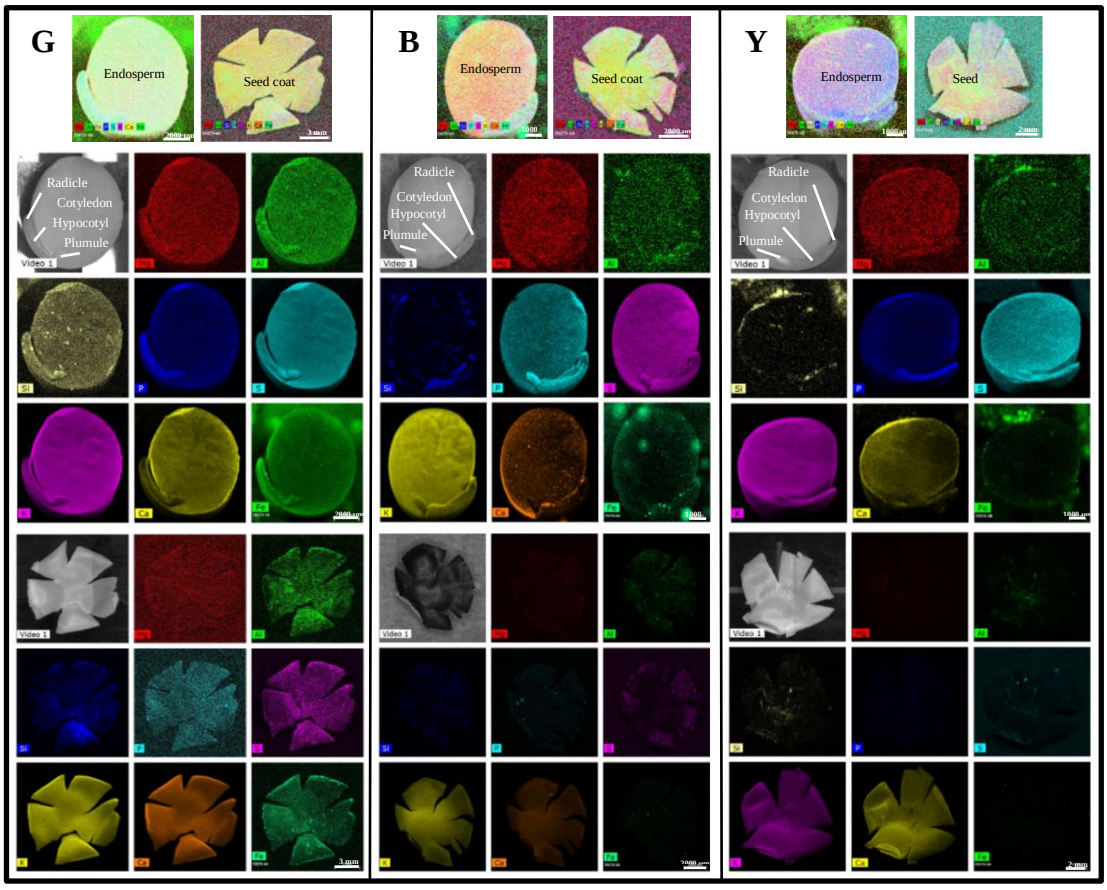

Fig. 4. Elemental mapping in different endosperm and seed coat of soybean genotypes. Remarks: G = greenish genotype; B = black genotype; Y = yellowish genotype; Mg = magnesium; Al = aluminum; Si = silicon; P = phosphorus; S = sulfur; K = potassium; Ca = calcium; Fe = iron.

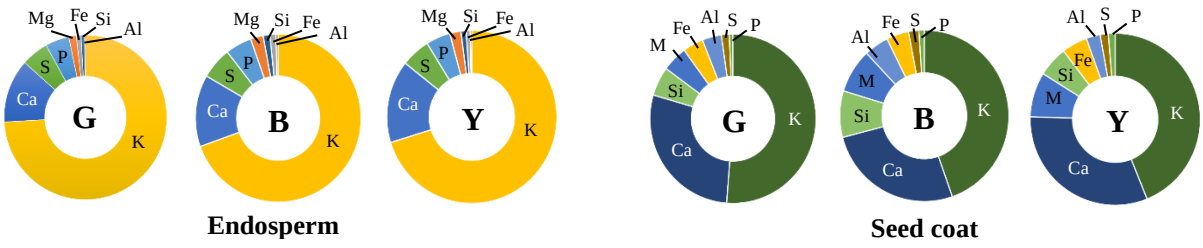

Fig. 5. List of elements based on the highest quantity in different soybean colors. Remarks: G = greenish genotype; B = black genotype; Y = yellowish genotype; Mg = magnesium; Al = aluminum; Si = silicon; P = phosphorus; S = sulfur; K = potassium; Ca = calcium; Fe = iron.

Surprisingly, the quantity of micro-elements in the seed coat was higher than in the macro-elements in the three genotypes, i.e., Si and Fe. In the greenish and the black genotypes, Si and Fe were ranked third and fifth, respectively, whereas in yellowish genotype they were ranked fourth and fifth, respectively. The quantity of Si and Al elements in the seed coat area of the black genotype was higher than that of greenish and yellowish genotypes ($p<0.05$). Thus, the black genotype has an advantage in terms of high micro-elements content, both in the seed coat and the endosperm.

Meanwhile, Fe element was not significantly different between the three genotypes ($p>0.05$). Another interesting fact, the quantities of all micro-elements were higher in the seed coat than in the endosperm.

Table 2 shows that in each area of the seed, i.e., seed coat or endosperm, there were dependencies between elements. We observed that there were significantly correlated ($p<0.05$) elements in both areas of the seed, i.e., P-K ($r>0.65$), Fe-Ca ($r>0.61$), Mg-Si ($r>0.68$), K-Al ($r>0.63$), and Si-Al ($r>0.89$).

Table 2. Pearson correlation analysis of elements in the seed coat (above the diagonal line) and endosperm (below the diagonal line) soybean genotypes.

Elements	P	Mg	Fe	K	Ca	S	Si	Al
P	1	0.843**	0.498	**-0.716****	-0.141	0.163	0.251	-0.193
Mg	-0.090	1	0.754**	-0.265	0.11	0.603*	**0.683***	0.301
Fe	0.397	-0.022	1	0.172	**0.613***	0.484	0.499	0.281
K	**0.651***	-.609*	-0.19	1	0.528	0.408	0.32	**0.639***
Ca	-0.434	0.037	**-0.919****	0.142	1	-0.007	0.005	0.065
S	0.781**	0.465	0.261	0.252	-0.336	1	0.974**	0.906**
Si	-0.639*	**0.723****	-0.008	-0.922**	0.117	-0.21	1	**0.892****
Al	-0.633*	0.711**	-0.022	**-0.906****	0.148	-0.21	**0.982****	1

Remarks: * = correlation was significant at the 0.05 level (2-tailed); ** = correlation was significant at the 0.01 level (2-tailed); P = phosphorus; Mg = magnesium; Fe = iron; K = potassium; Ca = calcium; S = sulfur; Si = silicon; Al = aluminum.

Table 3. Pearson correlation analysis of elements in the seed coat and endosperm with morphological characters before and after germination.

Char.		STA	SCA	ELSA	25W	IWA3	GN5	RL5	RA5
P	**e**	0.342	0.462	0.498	0.484	0.538	-0.473	-0.204	-0.115
	sc	-0.773**	-0.800**	-0.777**	-0.825**	-0.898**	0.940**	0.448	0.336
Mg	**e**	**-0.811****	**-0.836****	**-0.794****	**-0.838****	**-0.865****	**0.884****	0.655*	0.555
	sc	**-0.949****	**-0.910****	**-0.910****	**-0.903****	**-0.906****	**0.955****	0.559	0.432
Fe	**e**	-0.161	-0.129	-0.114	-0.088	0.069	-0.149	0.321	0.305
	sc	-0.610*	-0.501	-0.463	-0.458	-0.441	0.588*	0.219	0.181
K	**e**	0.804**	0.897**	0.899**	0.905**	0.887**	-0.787**	-0.670*	-0.497
	sc	0.242	0.380	0.373	0.431	0.541	-0.506	-0.203	-0.137
Ca	**e**	-0.020	0.016	0.004	-0.010	-0.140	0.264	-0.320	-0.320
	sc	0.068	0.246	0.203	0.285	0.266	-0.090	-0.409	-0.346
S	**e**	-0.046	-0.005	0.066	0.010	0.008	0.059	0.171	0.224
	sc	-0.684*	-0.647*	-0.621*	-0.605*	-0.497	0.455	0.649*	0.560
Si	**e**	**-0.925****	**-0.950****	**-0.966****	**-0.969****	**-0.950****	0.911**	**0.626***	0.474
	sc	**-0.760****	**-0.729****	**-0.724****	**-0.695***	**-0.588***	0.547	**0.601***	0.470
Al	**e**	-0.914**	-0.937**	-0.944**	-0.957**	-0.941**	0.911**	0.526	0.357
	sc	-0.395	-0.374	-0.360	-0.324	-0.194	0.133	0.385	0.305

Remarks: * = correlation was significant at the 0.05 level (2-tailed); ** = correlation was significant at the 0.01 level (2-tailed); phosphorus (P); magnesium (Mg); iron (Fe); potassium (K); calcium (Ca); sulfur (S); silicon (Si); and aluminum (Al); e = endosperm; sc = seed coat; STA = seed total area (cm^2); SCA = seed coat area (cm^2); ELSA = endosperm longitudinal section area (cm^2); 25W = 25 seeds-weight (g); IWA3 = imbibition water absorption 3 days (g); GN5 = normal germination 5 days (%); RL% = root length 5 days (cm); RA5 = root area 5 days (cm^2).

There is an interesting finding in the correlation of P-K, Fe-Ca, and K-Al, where the seed coat and endosperm show the opposite correlation. In P-K elements, the seed coat has a negative correlation, while the endosperm has a positive correlation ($p<0.05$), or conversely in Fe-Ca and K-Al elements ($p<0.05$). Mg-Si and Al-Si elements have a positive correlation ($p<0.05$) on both areas of the seed.

Table 3 shows the morphological characteristics of three genotypes before and after germination. From the characteristics of the three genotypes, it can be seen that the seed total area (STA) of greenish genotype was the widest and significantly different ($p<0.05$) compared to yellowish and black genotypes. Likewise, for seed coat area (SCA), endosperm longitudinal section area (ELSA), and 25 seeds-weight (25W), the greenish genotype was shown to be larger, heavier, and significantly different ($p<0.05$) than other genotypes.

Interestingly, during the germination process, the black genotype was generally superior compared to the yellowish and the greenish genotypes. In contrast, the greenish genotype, which looked bigger and heavier ($p<0.05$), was not superior compared to the black genotype. The black genotype could reach 88 % in normal germination 5 days (GN5) and was significantly different from the other genotypes ($p<0.05$). Likewise, for root length 5 days (RL5) and root area 5 days (RA5), the black genotype has longer, wider, and significantly different ($p<0.05$) compared to other genotypes. Black genotype needed less water for imbibition 3 days (IWA3) compared to other genotypes ($p<0.05$). The black genotype, which has the smallest and lightest seed weight, was better than the other genotypes in terms of germination. This may be related to the elemental content of the seed.

Table 3 shows that Mg element in the seed coat and endosperm have a negative and significant correlation ($p<0.01$) to the character of the seed total area ($r>0.81$), seed coat area ($r>0.83$), endosperm longitudinal section area ($r>0.79$), and 25 seeds-weight ($r>0.83$), which are characters before germination. Mg element had a positive and significant correlation ($p<0.01$) to normal germination 5 days characters ($r>0.88$), negative and significant correlation ($p<0.01$) to imbibition water absorption 3 days ($r>0.86$), which are characters after germination.

Si element in the seed coat and endosperm was also like Mg, there was a negative and significant correlation ($p<0.01$) to seed total area ($r>0.76$), seed coat area ($r>0.73$), endosperm longitudinal section area ($r> 0.72$), and 25 seeds-weight ($r>0.69$). Si element had a positive and significant correlation ($p<0.05$) to root length 5 days ($r>0.60$), negative and significant correlation ($p<0.01$) to imbibition water absorption 3 days ($r>0.86$).

Based on Table 1, the black genotype has the highest Mg and Si elements compared to other genotypes. Thus, it makes sense if the black genotype has the most superior germination capacity, with smaller seed characters. These results may be used as a reference in plant breeding to increase the quantity of elements in soybean seeds.

DISCUSSION

Over the past two decades, the μ-XRF technique has advanced significantly, faster than many other techniques [25]. In the future, it is expected that μ-XRF technique will be able to offer new capabilities with huge benefits in various sectors including macro-, and micro-elements profiling, including concentration, nondestructive, and utility for plant breeding programs. The μ-XRF technique is a promising powerful tool in food and agriculture sciences.

Elemental mapping in this study has many similarities with the work of Romeu et al. [26], where P and S elements were detected throughout the seeds, but most of them are accumulated in the hypocotyl along the embryo axis and plumule indicated by thicker colors. K elements were distributed homogeneously at several points between the embryo axis and cotyledon. Ca element was more concentrated in the seed coat and cotyledon areas. Fe element was found in the embryo axis, especially in root. We hypothesize that the genotypes have different uptake, transport, storage, and remobilization proceses in accumulating elements based on the color of the soybean seeds, especially the endosperm and seed coat area, which means it is influenced by genetic factors.

We also found that the quantity of micro-elements in the three genotypes was higher in the seed coat compared to the endosperm (Table 1). As reported by Moriyasu et al. [27] and Karatas et al. [28], that the seed coat on soybean and Faba bean (*Vicia faba* L.) has a great potential as a functional food. This information is very useful for the purposes of public consumption in processing soybeans, especially when a higher micro-element is expected, it is necessary to include a seed coat in the processing. The most important micro-elements in human diets were zinc, iron, iodine, selenium, and cobalt [4].

In this study, several elements seem to have a strong correlation in the seed coat and endosperm, namely Mg-Si and Al-Si (Table 2). In line with this information, it was also reported by Malle et al. [29] that the element content in soybean seeds is proven to correlate with each other, i.e., K-S and K-P ($r>0.6$; $p<0.001$). In addition, there is information which states that some elements are only influenced by genetic factors in accumulating elements in seeds, such as Ca.

According to Malle et al. [29], phenotypic variation for Ca element in soybean was influenced by genetic factors. The elements of K, P, and S were influenced not only by genetic factors but also by environmental factors. On the other hand, the interaction between genetics and environment was reported to be not significant in influencing K, P, S, and Ca elements in soybean seeds. These results may be used as a reference by plant breeders in strategies for developing varieties to increase the content of elements in seeds. However, further research is still needed to determine the factors that affect an element in the seed, especially the Mg-Si and Al-Si elements. From Table 3, it is known that the Mg and Si elements have strong correlation with the characters before and after germination.

Si and Mg elements have a negative correlation with the characters before germination (Table 3). Smaller seeds were more likely to be produced in plants with higher quantity of Si and Mg elements. This should be a concern for plant breeders, because the advantages of increasing Si and Mg will increase the probability of normal germinate and root length, but on the other hand it will affect the smaller seed size (Table 3). Nevertheless, more research on the effect of Si and Mg on seed size and weight are still required.

Si element has a negative correlation with the imbibition process (Table 3), which is related to the component of the seed coat structure on the seed (Table 1). The higher quantity of Si element in the genotype has a negative correlation with water requirement during imbibition (Table 1).

Furthermore, it was found in the previous study that the more water a seed needs to absorb, the higher potential of elemental leakage and the lower ability to germinate [30]. It is also proven that the greenish genotype, which requires more water, has a lower germination ability compared to the black genotype.

Silicon is the second most prevalent element on Earth after oxygen, and inorganic silica, $SiO_2 \bullet nH_2O$, was a key component of plants, especially in seed coat and husk [31]. Phytoliths, silica bodies that were found in plants, help them to have more rigid and stronger structures [32]. The seed coat was crucial in controlling the amount of water that the embryo receives [33]. Embryos enveloped by seed coats absorb water slower than embryos alone [34,35]. When seed coats are removed, there is more leakage from the embryos during absorption [30,36]. Pathogens have a higher chance of killing seeds that leak water a lot during imbibition than leak less water [37].

Previous studies have reported that depending on the its levels, Si enhanced the growth of the root and the nitrogen fixed from the nodules [38]. This is consistent with the results of this study that Si concentrations have a positive correlation with root length (Table 3). It increases the number and size of nodules in legumes by increasing primary root and lateral root (secondary roots) [39], because of the accumulation of abscisic acid in the roots [40-42]. In the future, it may be useful to measure abscisic acid in the roots so that Si can be regarded as a crucial element to increase the effectiveness of nitrogen fixation in soybean [43].

Table 3 shows that the Mg element has a high positive correlation against abnormal germination. Research conducted by Varnagiris et al. [44] and Ceylan et al. [45] supports the results of our study that Mg is an essential element of plants that controlled biochemical processes and supported in seed germination. An investigation has been carried out on the *Zea mays* to determine the effect of Mg on germination, with the result shows that Mg was able to increase germination performance [46]. Another study also reported that Mg in the seeds can improved germination performance on *Vigna radiata* [47].

CONCLUSIONS

In this study we found that the Si and Mg elements have a significant correlation. The high quantity of Si element in the embryo axis has a positive correlation with root length. The high quantity of Mg element which is evenly distributed on the endosperm has a positive correlation with normal germination. Si and Mg elements in the seeds has a negative correlation with imbibitions water absorption. Based on the comparison between the three genotypes, the black genotype was superior in terms of germination and has higher Si and Mg elements. Thus, the Si and Mg elements can be used as a reference in determining superiority of genotypes at the germination stage.

AUTHOR CONTRIBUTION

K. Wibisono is the main contributor and W. Nurcholis is the supporting contributor of this paper. All authors read and approved the final version of the paper.

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