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Spatial distribution of Pb and its correlation at different grain positions among wheat varieties for specific end-uses

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ABSTRACT

The relative content of Pb and its correlation were investigated in endosperm, aleurone layer and pericarp respectively among different wheat varieties for specific end-uses (WVFSE) using scanning electron microscope (SEM) equipped with the energy dispersive X-ray spectroscope (EDS). The results showed that Pb contents in grains at different positions followed the order of endosperm > pericarp > aleurone. The differences of Pb contents varied among the WVFSE, and wheat with strong gluten had a highest average content of Pb, while wheat with medium gluten had a lowest one. There were significant third order equation correlations between Pb content in endosperm and that in aleurone layer and that in pericarp, respectively. And good correlation coefficients were obtained. However, the correlation differed at different position among WVFSE, which indicated that Pb contents in endosperm, aleurone layer and pericarp were regulated by each other.

Keywords: Lead; Correlation; Wheat Varieties for Specific End-Uses; Grain Position

1. INTRODUCTION

The contamination of heavy metals into the environment is becoming a worldwide concern due to their transfer in food chain via the uptake of crops [1,2]. Pb has long been recognized as one of most toxic heavy minerals to human beings [3,4]. Pb absorbed by human body disturbs many body processes and is harmful to many organs and tissues such as the heart, bones, nervous systems and so on [5,6].

Wheat is one of most important food crops in the world, especially in China, due to the highest total yields and the most abundant types of food processing [7,8]. However, there are quite large differences in grain composition and processing quality among wheat cultivars. Based on the gluten contents and specific end uses, wheat cultivars are divided into three classes, *i.e.* wheat with strong gluten, wheat with medium gluten and wheat with weak gluten, respectively [7].

As Pb has strong wall penetrating ability and migration ability, its toxicity in wheat causes swelling of chloroplast in leaves cells [9], mitosis disturbances in root meristematic cells [10] and decreasing activities of antioxidant enzymes [11]. There were many factors affecting absorption of Pb in wheat including soil features [12,13], PH values [14], other mineral nutrients [15] and wheat varieties [16,17]. Study about Pb has been documented well, however, not much information is available about distribution of Pb and its correlation analysis at different position in wheat varieties for specific end-uses. With this background, in this report 15 genotypes of wheat grains were chosen to investigate the differences among the Pb contents at different positions and their relationships.

2. MATERIAL AND METHODS

2.1. Materials

The experiment was done at Yangzhou University, Yangzhou, China (32˚30'N, 119˚25'E) from October 2011 to July 2012. Based on wheat specific end-uses, 3 classes of wheat varieties, *i.e.*, wheat with strong gluten, wheat with medium gluten and wheat with weak gluten respectively, were used and grown on the same field (**Table 1**). The sowing date was 25 November. The field soil is sandy loam [Typic fluvaquents, Entisols (US taxonomy)] which contains organic materials at 2.45% and available nitrogen (N), phosphorus (P) and potassium (K) of 106, 33.8 and 66.4 mg·kg^{-1}, respectively. Available N as urea at 75 kg·hm^{-1} was applied into the soil on the day of sowing and at the jointing stage, respectively. All uniform agronomic practices were carried on for all varieties. When wheat ripe, the grains were collected for the analysis of elements

2.2. Observation of Wheat Grain Structure Using Scanning Electron Microscopy (SEM)

Representative grains were chosen and fractured in the middle region of grain by applying a slight pressure with a razor blade. During fracturing, the efforts were made to produce no contact between the razor blade and the fractured surface of grains. The thickness of slices was about 3 mm. Fractured grains were mounted on the specimen stub and sputtered with gold on the fractured region. The wheat grains were observed using SEM (XL30 ESEM, Philips, Holland) at 20 kV and the air pressure in the sample chamber was 4 Torr.

2.3. X-Ray Electron Probe Microanalysis

When the grains were being observed under the SEM, the energy spectrum was also analyzed with the energy dispersive X-ray spectroscope attached to the SEM. The relative content of Pb in pericarp, aleurone layer and endosperm, respectively, was determined, which was shown as atomic content among ten elements (Mg, Al, P, S, K, Ca, Cr, Cu, Cd, Pb) (**Figure 1**). Each kind of sample and the same position were scanned for 3 times respectively.

Table 1. Characterization of wheat varieties for specific end-uses.

Classes	Representative varieties	Protein content (%)	Wet gluten content (%)	Food types used
Strong Gluten	Qing 11,Waimai 33, Xumai30, Yannong 19, Zheng 9023	≥15%	≥32%	Bread, dumpling
Medium Gluten	E21, Yangfu 4, Yangmai 11, Yangmai 16, Yangmai 158	14% ± 1%	30% ± 3%	steamed bread, noodles
Weak Gluten	Yangfu2, Yangmai 13, Yangmai15, Yangmai 18, Yangmai 19	<13%	<20%	Cookies, cakes, pastries

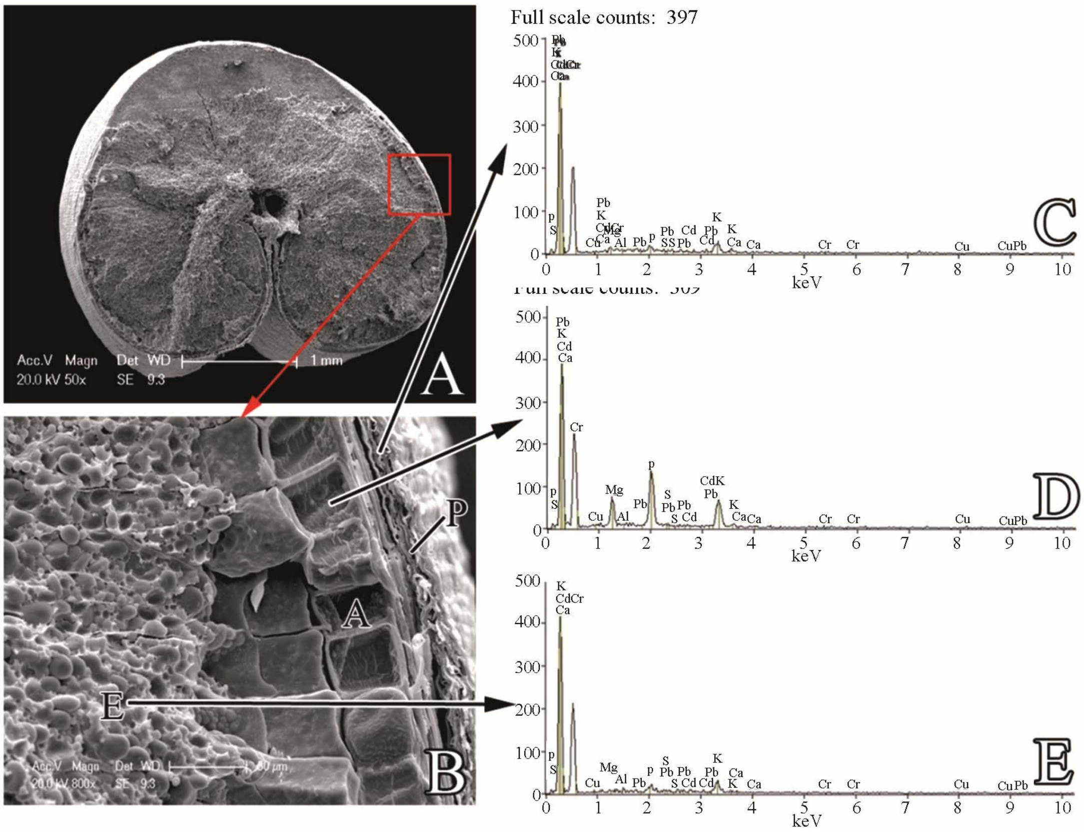

Figure 1. SEM-EDS images of mature wheat grain; (A) SEM images of transversely section of wheat grain; (B) Magnification of A showing details of pericarp, aleurone and endosperm, respectively; (C) EDS image of pericarp; (D) EDS image of aleurone; (E) EDS image of endosperm.

Statistical analysis

SPSS 19.0 software was carried out to analyze variance test and significance, and the relative analysis and the graph were done with Sigmaplot 12.0 software.

3. RESULTS

3.1. Distribution of Pb Content at Different Position in Wheat Varieties for Specific End-Uses (WVFSE)

The spatial distribution of Pb in different positions differed and the order was endopsperm > pericarp > aleurone (**Table 2**). Pb contents were different among three classes of wheat varieties. Wheat with strong gluten had a highest average content of Pb in whole grain, while wheat with medium gluten had a lowest. Moreover, in the same position the relative content of Pb distributed differently among three classes of wheat varieties. In endosperm and aleurone layer, Pb content changed as wheat with strong gluten > wheat with medium gluten > wheat with weak gluten, but in pericarp, it followed the order of wheat with strong gluten > wheat with weak

Table 2. Relative content of Pb at different positions of WVFSE.

Classes	Representative varieties	Endosperm	Aleurone layer	Pericarp	Total
Strong gluten	Qing 11,Waimai 33, Xumai30, Yannong 19, Zheng 9023	8.84a	1.00a	3.47a	13.3a
Medium gluten	E21, Yangfu 4, Yangmai 11, Yangmai 16, Yangmai 158	4.07b	0.85a	2.13b	7.05b
Weak gluten	Yangfu2, Yangmai 13, Yangmai15, Yangmai 18, Yangmai 19	3.89b	0.35b	3.22a	7.46a

Different letters means difference significant at $P < 0.05$ and the same letters means difference no significant.

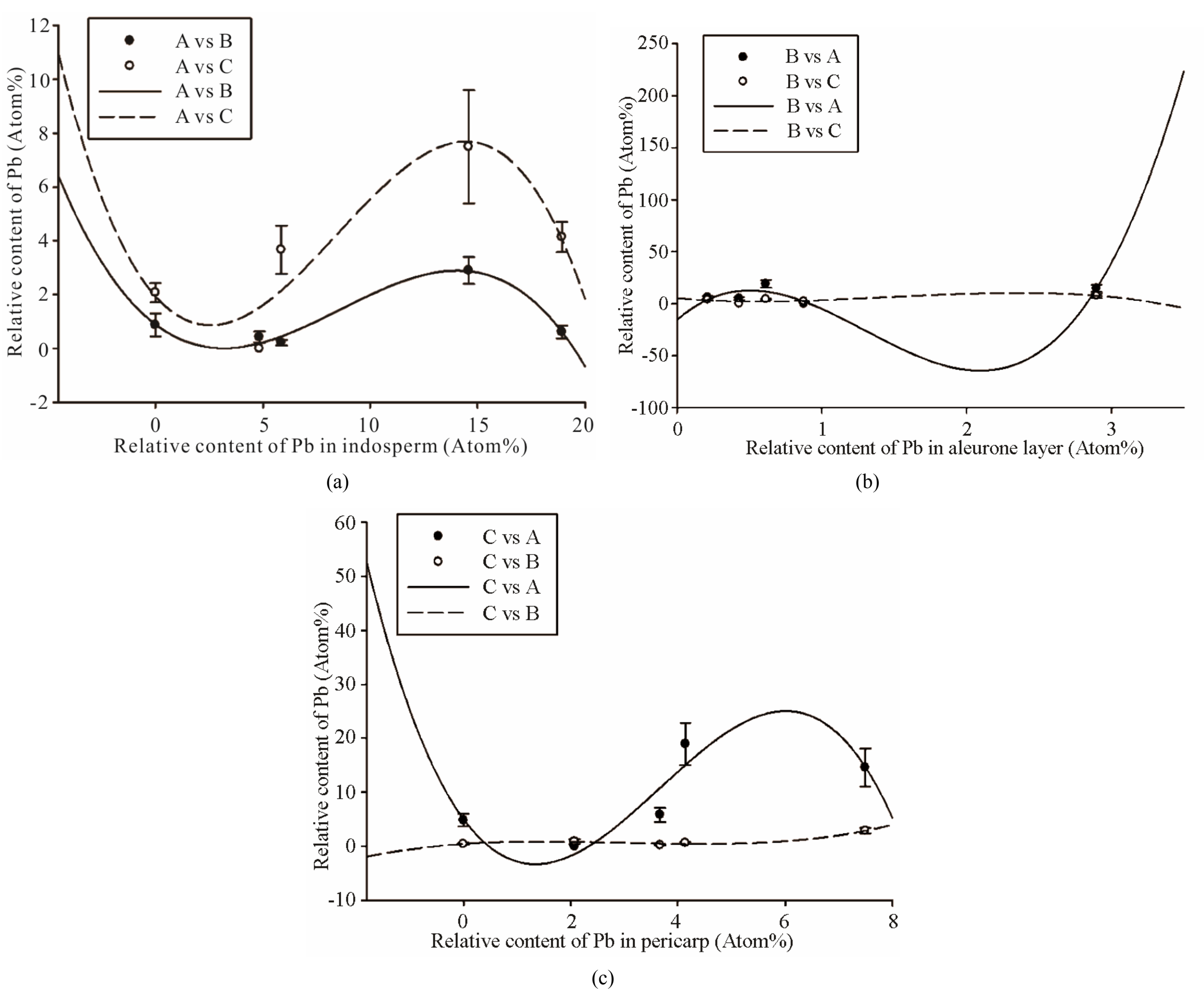

Figure 2. Relationship between the relative content of Pb in the endosperm (A) and that in aleurone layer (B) and that in pericarp (C) among wheat varieties with strong gluten.

gluten > wheat with medium gluten. This indicated Pb contents in wheat with strong gluten was the highest at any position, probably due to genetic differences.

3.2. The Relations between Pb Contents at Different Position among WVFSE

The relations of Pb contents at different position of WVFSE were performed with curve fitting and the correlations were fitted well with three order equation (**Figures 2-4** and **Tables 3-5**), which showed the Pb contents at different position were influenced by each other.

Among wheat varieties with strong gluten, the relations of Pb content at different position and the relevant equations were shown in **Figure 2** and **Table 3**, respectively. The correlation between Pb content in endosperm, that in aleurone layer and that in pericarp were highly significant at P < 0.01 and significant at P < 0.05, respectively, which indicated Pb distribution in endosperm could affect that of the two positions; Correlation coeffi-cient showed degree of influence followed the order of aleurone layer > pericarp. The correlation between Pb content in aleurone layer, that in pericarp and that in endosperm were not significant, which indicated Pb content in aleurone layer had no effect on that of other two positions. The correlation between Pb content in pericarp, that of endosperm and that of aleurone were significant at P < 0.05 and highly significant at P < 0.01, respectively, correlation coefficient showed degree of influence changed as aleurone layer > endosperm.

Among medium gluten wheat, the relations of Pb content at different position and the relevant equations were shown in **Figure 3** and **Table 4**, respectively. The correlation between Pb content in endosperm and that in pericarp were highly significant at P < 0.001 but not significant for that in aleurone layer, which showed Pb content in endosperm affected greatly that in pericarp; The correlation between Pb content in aleurone, that of pericarp and that of endosperm were significant at P < 0.05 and

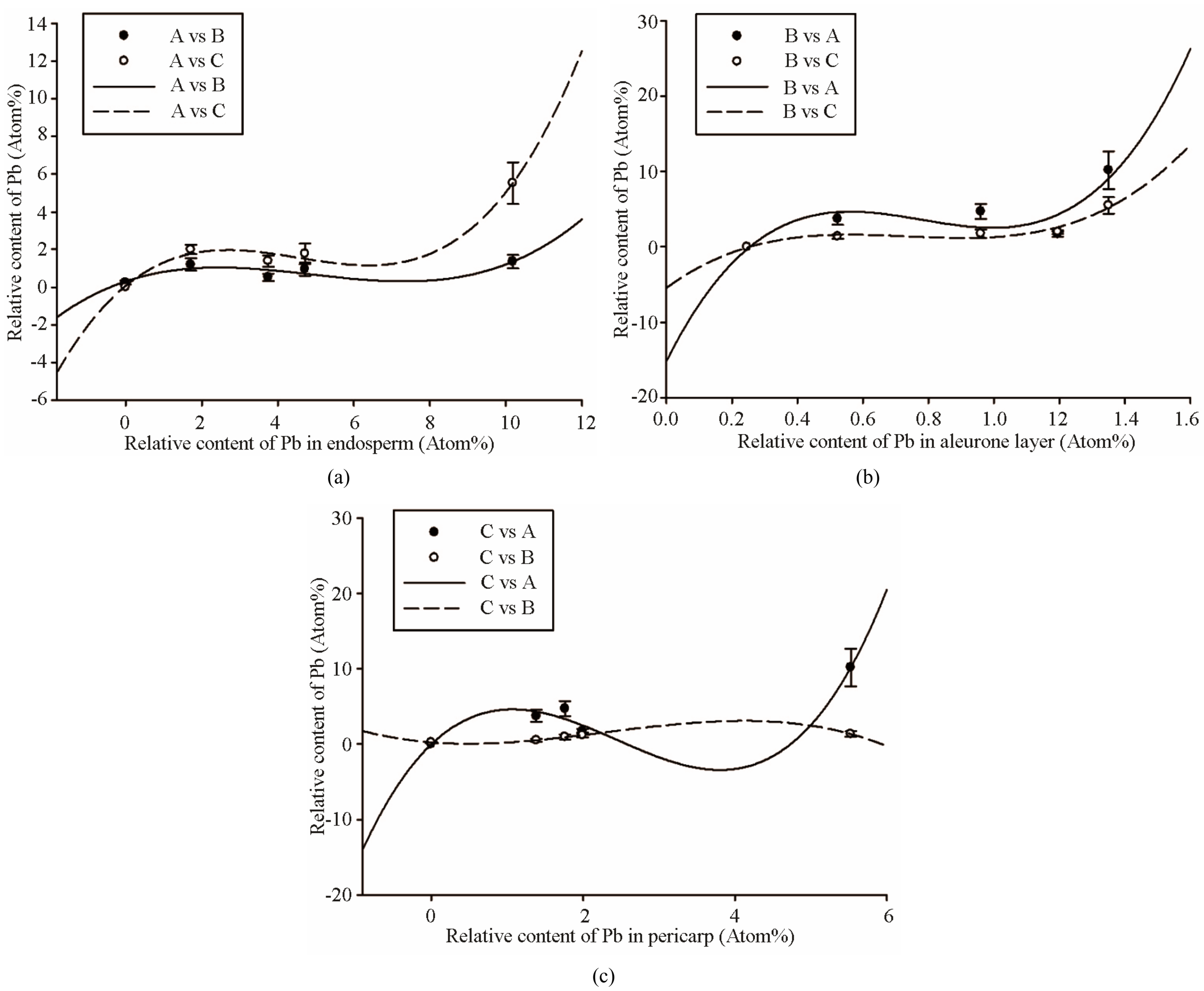

Figure 3. Relationship between the relative content of Pb in the endosperm (A) and that in aleurone layer (B) and that in pericarp (C) among wheat varieties with medium gluten.

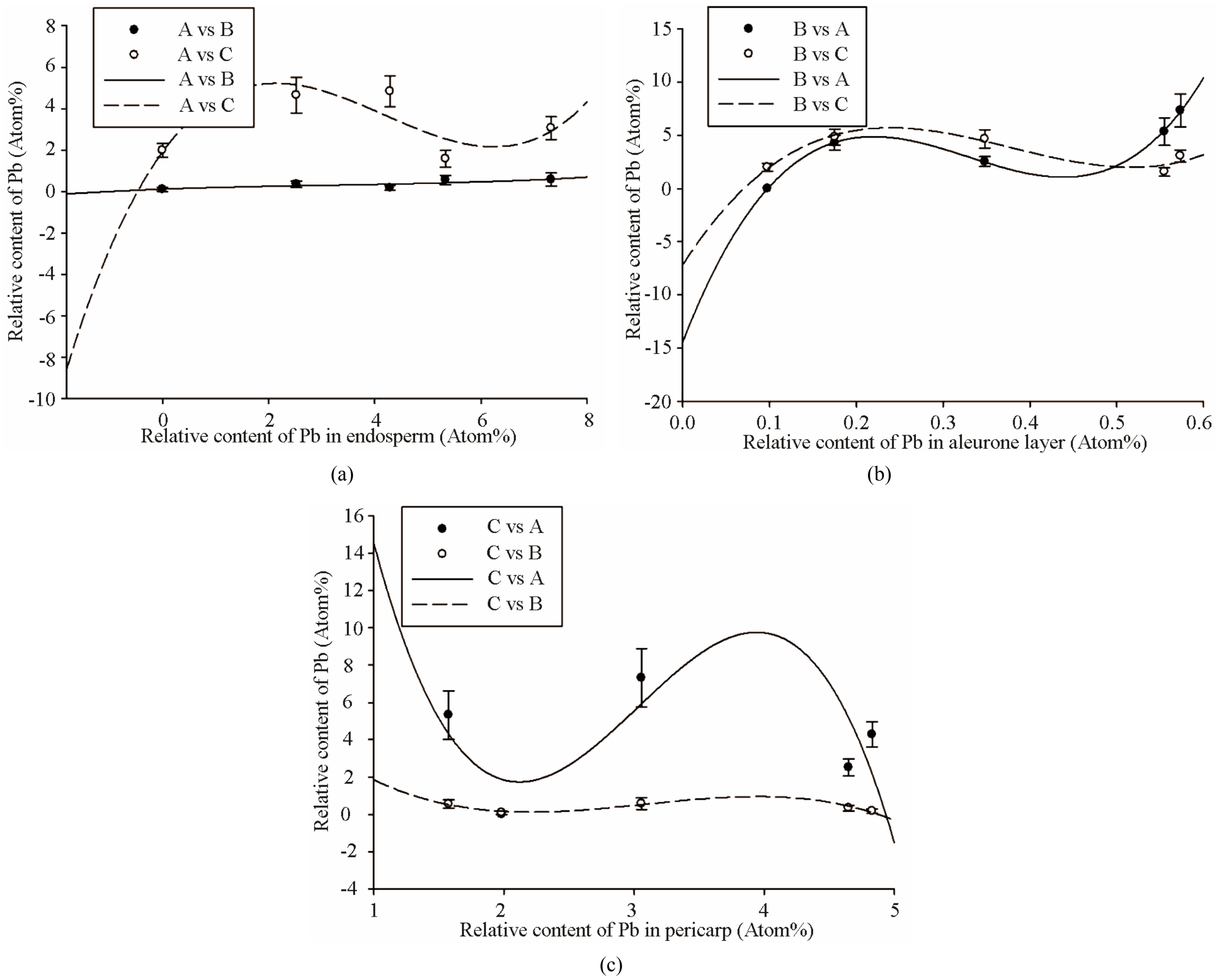

Figure 4. Relationship between the relative content of Pb in the endosperm (A) and that in aleurone (B) and that in pericarp (C) among wheat varieties with weak gluten.

Table 3. The correlation of relative content of Pb at different position of wheat varieties with strong gluten.

	Endosperm	Aleurone layer	Pericarp
Endosperm	1	$y = 0.8958 - 0.6032x + 0.1163x^2 - 0.0045x^3 R^2 = 0.9756^{**}$	$y = 1.9375 - 0.8956x + 0.2066x^2 - 0.0081x^3 R^2 = 0.8479^{*}$
Aleurone layer	$y = -15.4986 + 121.5540x - 149.5574x^2 + 38.3808x^3 R^2 = 0.5185 n.s$	1	$y = 4.9202 - 11.3663x + 12.5710x^2 - 2.8791x^3 R^2 = 0.68106 n.s.$
Pericarp	$y = 5.0571 - 13.4312x + 6.1301x^2 - 0.5560x^3 R^2 = 0.8245^{*}$	$y = 0.4391 + 0.6544x - 0.3034x^2 + 0.0347x^3 R^2 = 0.9758^{**}$	1

Note: n.s. indicates not significant, *indicates the correlation is significant at p < 0.05, **: indicates the correlation is significant at p < 0.01, ***: indicates the correlation is significant at p < 0.001.

highly significant at P < 0.01, respectively, which indicated that degree of influence followed the order of pericarp > endosperm. The correlation between Pb content in pericarp, that of endosperm and that of aleurone layer were highly significant at P < 0.01 and at P < 0.001, respectively, which showed Pb distribution in pericarp affected greatly that of the two positions.

Among weak gluten wheat, the relations of Pb content at different position and the relevant equations were shown in **Figure 4** and **Table 5**, respectively. The correlation between Pb content in endosperm, that of aleurone and that of pericarp were not significant, this indicated Pb content in endosperm did not affect that of other both positions; The correlation between Pb content in aleu-

Table 4. The correlation of relative content of Pb at different position of wheat varieties with medium gluten.

	Endosperm	Aleurone layer	Pericarp
Endosperm	1	$y = 0.3057 + 0.6767x - 0.1846x^2 + 0.0126x^3$ $R^2 = 0.6958n.s.$	$y = 0.0638 + 1.6501x - 0.4372x^2 + 0.3222x^3$ $R^2 = 0.9823^{***}$
Aleurone layer	$y = -15.1377 + 87.0232x - 121.1866x^2 + 51.8678x^3$ $R^2 = 0.7794^{*}$	1	$y = -5.4213 + 31.8126x - 46.1262x^2 + 21.0439x^3$ $R^2 = 0.9499^{**}$
Pericarp	$y = -0.0291 + 9.6281x - 5.7626x^2 + 0.7882x^3$ $R^2 = 0.9527^{**}$	$y = 0.2454 - 0.7774x + 0.8905x^2 - 0.1292x^3$ $R^2 = 0.9988^{***}$	1

Note: n.s. indicates not significant, *: indicates the correlation is significant at P < 0.05, **: indicates the correlation is significant at P < 0.01, ***: indicates the correlation is significant at P < 0.001.

Table 5. The correlation of relative content of Pb at different position of wheat varieties with weak gluten.

	Endosperm	Aleurone layer	Pericarp
Endosperm	1	$y = 0.1079 + 0.0953x - 0.0154x^2 + 0.0016x^3$ $R^2 = 0.6871n.s.$	$y = 1.9111 + 3.5222x - 1.1106x^2 + 0.0885x^3$ $R^2 = 0.6886n.s.$
Aleurone layer	$y = -14.3736 + 210.3609x - 718.7944x^2 + 728.2636x^3$ $R^2 = 0.9988^{***}$	1	$y = -7.1491 + 127.3948x - 390.2065x^2 + 344.3020x^3$ $R^2 = 0.9086^{**}$
Pericarp	$y = 59.4457 - 66.3821x + 24.0915x^2 - 2.6507x^3$ $R^2 = 0.4190n.s.$	$y = 7.4239 - 8.1499x + 2.8833x^2 - 0.3126x^3$ $R^2 = 0.8813^{**}$	1

Note: n.s. indicates not significant, *: dicates the correlation is significant at P < 0.05, **: indicates the correlation is significant at P < 0.01, ***: indicates the correlation is significant at P < 0.001.

rone, that of pericarp and that of endosperm were highly significant at P < 0.001 and at P < 0.01, respectively, which showed Pb distribution significantly influenced that of the two positions. The correlation between Pb in pericarp and that in aleurone were significant at P < 0.01 but not significant for that in endosperm, which showed Pb content in pericarp was regulated by endosperm.

The results also indicated Pb correlation varied among different WVSE. Pb contents in aleurone layer and pericarp were regulated by endosperm and degree of influence followed the order of wheat with strong gluten > wheat with medium gluten > wheat with weak gluten; Pb contents in endosperm and pericarp were affected by that in aleurone and the order changed as wheat with weak gluten > wheat with medium gluten > wheat with strong gluten; Pb contents in endosperm and aleurone layer were adjusted by pericarp and the order changed as medium gluten > strong gluten > weak gluten.

4. DISCUSSION

Generally speaking, there were two approaches to reduce Pb accumulation in grain, one is to breed new genotypes with low Pb concentrations in grain [15,17], the other is to use some agricultural practices [16,18]. Dietary fibers in wheat bran can also obviously bind Pb to prevent the body from toxicity [19].

Distribution of Pb varied with different organs and followed the order of root > shoots > shells > grains. Moreover, Pb contents in different parts of wheat plants were mainly relative to the bound-to-carbonate metal fractions [20]. In the present study, there were genetic differences in Pb enrichment, which reflected the differences of Pb contents in different WVFSE. Because the wheat cultivars were grown in the same fields and environmental conditions, we can confirm that the Pb distribution was controlled by polygene quantitative characters, which agrees with the findings of previous experiment studies [21,22].

We also found that there were significant third order equation correlations between Pb content in endosperm and in aleurone layer and in pericarp, which indicated that Pb accumulation in different position of WVFSE was influenced by each other. Furthermore, there were differences in the regulation of Pb distribution among the different positions. When wheat grains were ground into flour, the aleurone, pericarp and germ should be removed from the endosperm. Thus, the Pb accumulation in endosperm was closely linked with human's health, which was regulated by other positions of wheat grain, particularly in aleurone and pericarp.

Therefore, in order to reduce the risk of Pb toxicity from the daily consumption of wheat, we should choose the genotypes with low Pb content as the main wheat cultivars. At the same time, some suitable measures should be also taken to remove potential hazards of Pb [16,18].

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