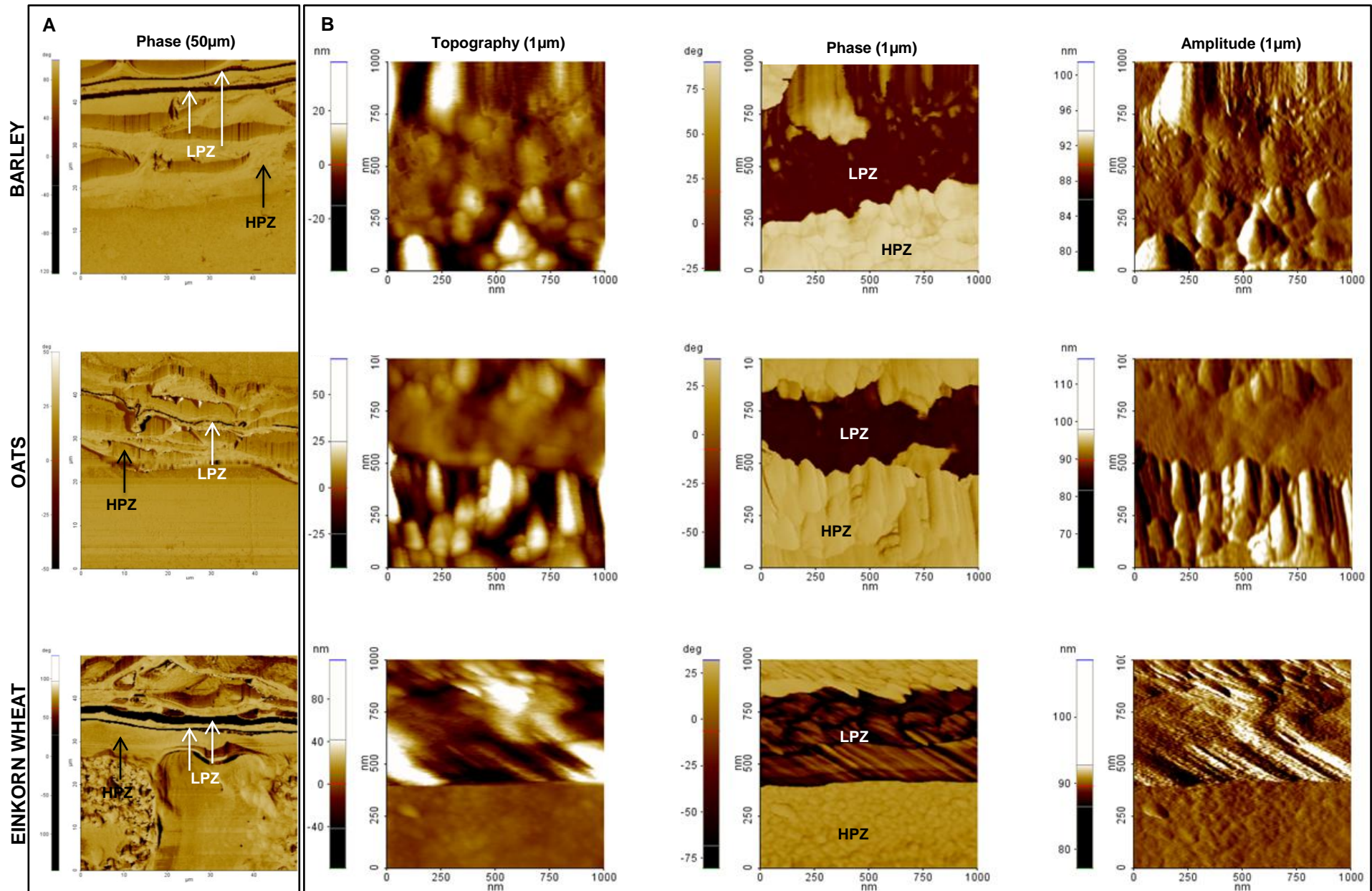


**Figure S1.** Further ESEM-EDS micrographs of starchy granules of barley (A), oats (B) and einkorn wheat (C). *c*, central analysis; *p*, peripheral analysis.

**Table S1.** Further elemental composition of starchy granules revealed by ESEM-EDS analysis.

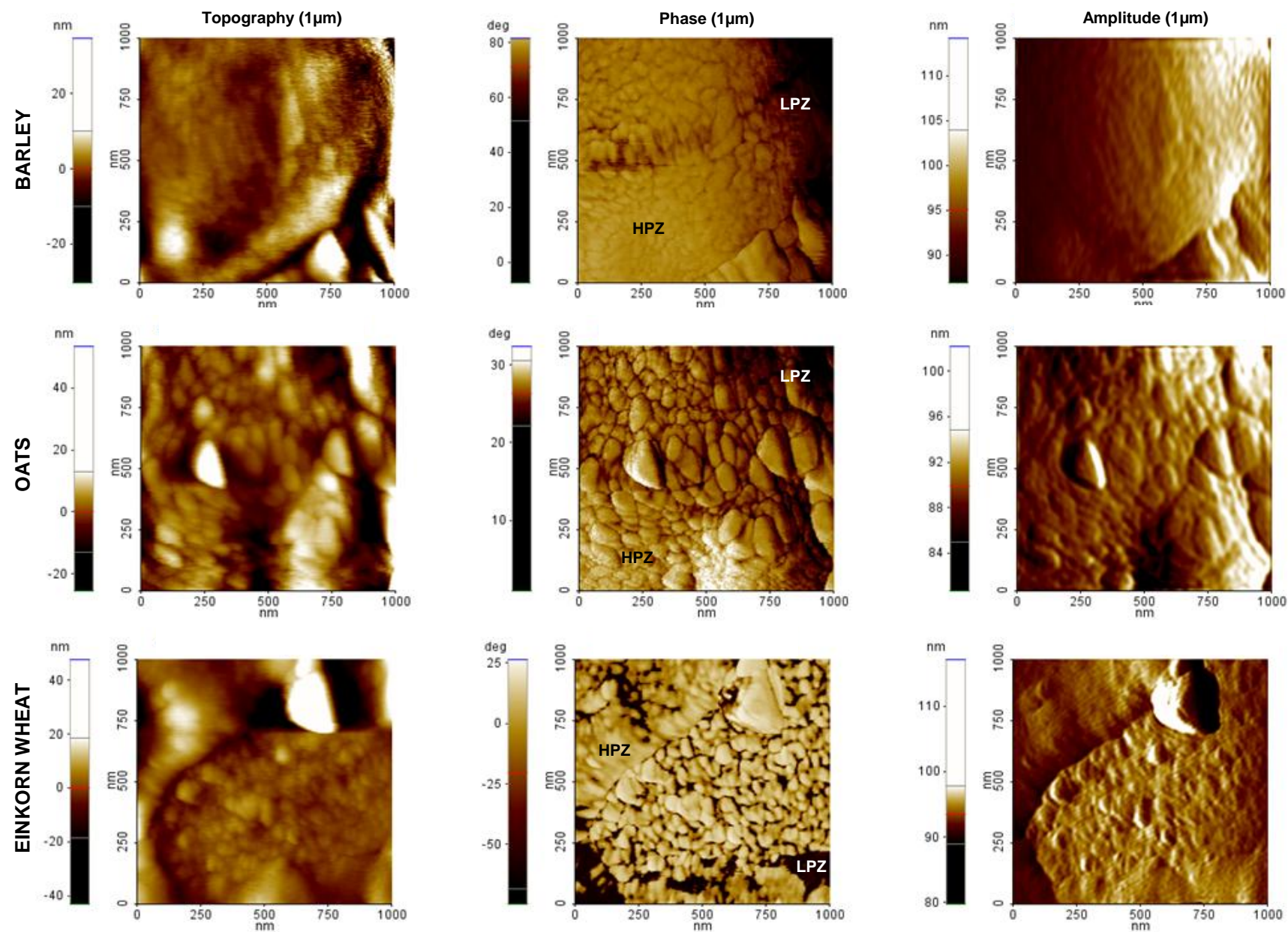
Elements	BARLEY		OATS		EINKORN WHEAT	
	Center <sup>a</sup>	Periphery <sup>b</sup>	Center	Periphery	Center	Periphery
<b>C</b>	70.57±0.52	75.64±1.94 (*)	74.33±0.61	80.04±2.96 (*)	71.86±0.71	77.23±3.24 (*)
<b>O</b>	29.18±0.53	23.95±1.96(*)	25.16±0.56	19.43±2.92 (*)	27.92±0.71	22.35±3.13 (*)
<b>P</b>	0.08±0.01	0.11±0.02 (*)	0.19±0.01	0.22±0.02 (*)	0.05±0.02	0.10±0.01 (*)
<b>S</b>	N.D.	0.06±0.02 (*)	0.15±0.01	0.12±0.02 (ns)	0.06±0.01	0.10±0.04 (ns)
<b>Cl</b>	0.02±0.01	0.06±0.01 (*)	N.D.	N.D.	0.04±0.03	0.07±0.02 (ns)
<b>K</b>	0.17±0.03	0.18±0.04 (ns)	0.19±0.03	0.20±0.01 (ns)	0.09±0.02	0.15±0.05 (ns)

Values are reported as atomic percent (%) of the elements and are the mean  $\pm$  SD of 7 independent analysis performed at the center of type-A starchy granules <sup>(a)</sup> and the mean  $\pm$  SD of 6 independent analysis performed at the periphery <sup>(b)</sup>. See Fig. S1 for correspondence between central and peripheral analysis. N.D. = non detected element. (\*) statistically significant difference between the central and peripheral elemental composition, for each cereal ( $p \leq 0.05$ , Student's *t* test); (ns) not statistically significant difference.

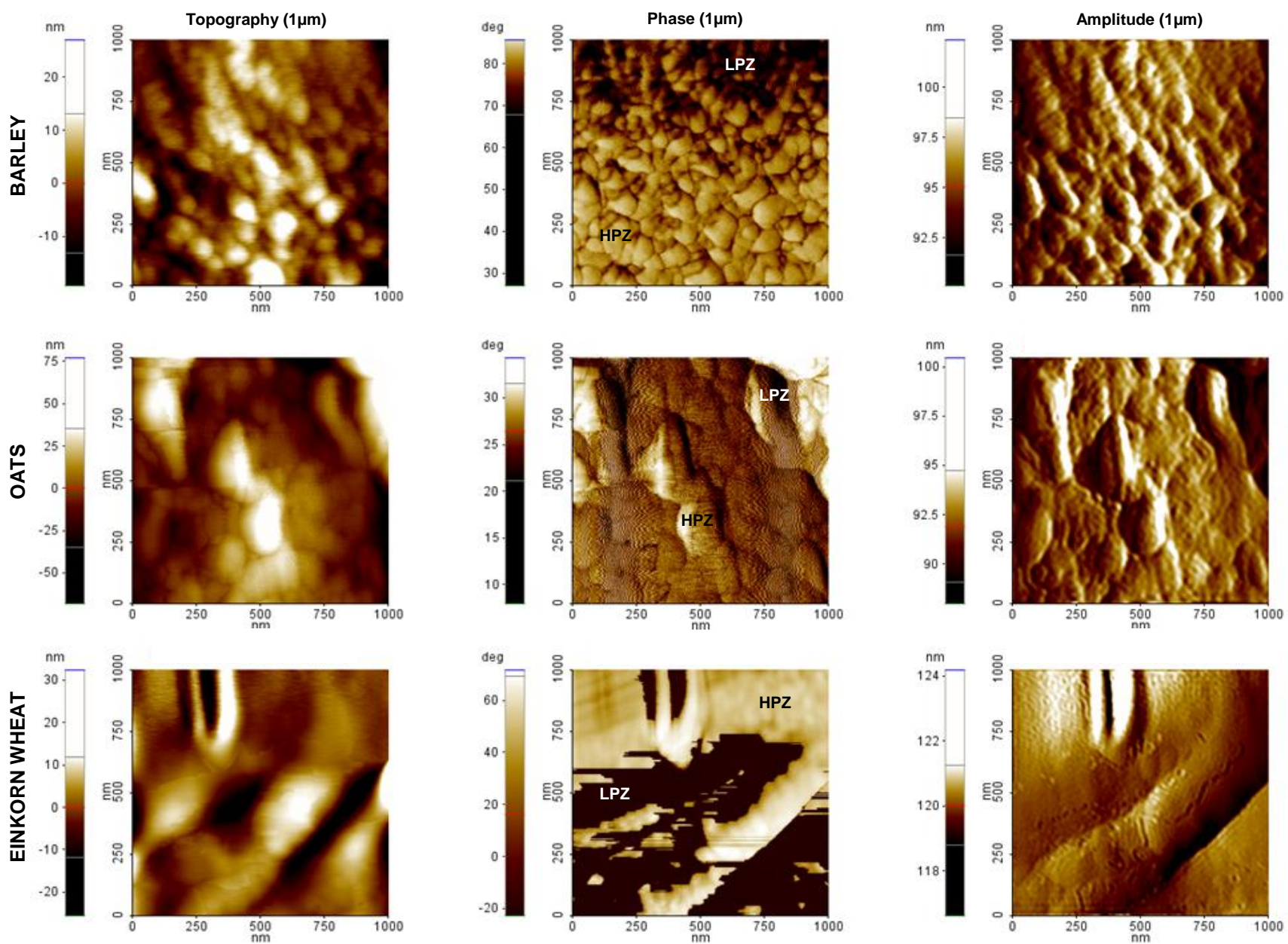


**Figure S2.** AFM analysis of the pericarp of barley, oats and einkorn wheat.

(A) Phase (50 $\mu$ m): 50x50 $\mu$ m AFM phase images of the outer layers. (B) Topography (1 $\mu$ m), Phase (1 $\mu$ m), Amplitude (1 $\mu$ m): 1x1 $\mu$ m AFM topographic, phase, amplitude images of the outer layers. LPZ, low-phase zone; HPZ, high-phase zone.



**Figure S3.** Sub-micrometric AFM analysis of protein body globoids of the aleurone layer of barley, oats and einkorn wheat. Topography (1 $\mu$ m), Phase (1 $\mu$ m), Amplitude (1 $\mu$ m): 1x1 $\mu$ m AFM topographic, phase, amplitude images of globoids of aleurone. LPZ, low-phase zone within the 1x1 $\mu$ m area; HPZ, high-phase zone within the 1x1 $\mu$ m area.



**Figure S4.** Sub-micrometric AFM analysis of starchy granules of barley, oats and einkorn wheat. Topography (1 $\mu\text{m}$ ), Phase (1 $\mu\text{m}$ ), Amplitude (1 $\mu\text{m}$ ): 1x1 $\mu\text{m}$  AFM topographic, phase, amplitude images of endosperm starchy granules. LPZ, low-phase zone within the 1x1 $\mu\text{m}$  area; HPZ, high-phase zone within the 1x1 $\mu\text{m}$  area.

**Table S2.** Phase signal roughness parameters revealed by AFM analysis.

		BARLEY					OATS						EINKORN WHEAT						
Regions		Middle	Mean	Rpv	Rq	Ra	Rz	Middle	Mean	Rpv	Rq	Ra	Rz	Middle	Mean	Rpv	Rq	Ra	Rz
<i>Out</i>	<b>Whole</b>	<b>31.91</b>		116.55	46.85	44.91	116.15	<b>-14.58</b>		107.23	34.71	31.54	104.87	<b>-24.20</b>		112.56	31.65	27.65	111.44
	<b>LPZ</b>		-19.01	55.88	4.60	2.07	50.50		-49.86	41.42	4.51	2.70	38.09		-36.32	98.78	25.00	21.92	97.37
	<b>HPZ</b>		83.81	22.74	3.83	3.09	21.11		27.03	32.22	3.81	2.56	29.67		17.17	18.50	2.61	2.02	17.39
<i>Al</i>	<b>Whole</b>	<b>53.81</b>		55.00	5.50	4.24	52.99	<b>16.66</b>		31.45	2.15	1.61	26.02	<b>-24.16</b>		101.05	24.56	18.85	95.80
	<b>LPZ</b>		71.13	8.04	1.23	1.00	7.46		26.03	12.78	1.54	1.22	11.91		-59.40	55.22	4.03	2.49	44.67
	<b>HPZ</b>		79.48	5.09	0.76	0.61	4.50		27.85	10.49	1.22	0.92	9.59		7.62	87.55	8.39	6.39	77.95
<i>Se</i>	<b>Whole</b>	<b>65.83</b>		48.44	5.23	4.12	46.52	<b>22.64</b>		20.83	2.17	1.55	20.63	<b>-40.89</b>		190.34	25.80	21.55	150.53
	<b>LPZ</b>		73.05	44.07	5.64	4.31	42.51		25.35	16.30	1.36	0.92	16.05		-22.48	76.98	22.24	19.71	72.89
	<b>HPZ</b>		84.70	18.91	2.35	1.80	16.61		30.93	5.78	0.85	0.69	4.97		15.37	80.12	6.06	4.03	46.33

Regions: *Out*, pericarp; *Al*, protein body globoids of the aleurone layer; *Se*, starchy granules of endosperm; Rpv, peak-to-valley roughness; Rq, root mean square roughness; Ra, average roughness; Rz, Ten point average roughness. Phase signal roughness parameters were calculated from AFM phase images (1x1µm) and are expressed in degree. Whole = entire 1x1µm area; HPZ, high-phase zone within the whole area; LPZ, low-phase zone within the whole area.