Advances in Environmental Biology, 5(7): 1554-1558, 2011
ISSN 1995-0756

American-Eurasian Network for Scientific Information
AENSI

This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLE

An Ultrastructural Study on Bolder and Smaller Grains Developing in the Same Spike or Spikelet of Wheat

Alireza Houshmandfar and Davood Eradatmand Asli

Department of Agronomy and Plant Breeding, Saveh Branch, Islamic Azad University, Saveh, Iran.

Alireza Houshmandfar and Davood Eradatmand Asli: An Ultrastructural Study on Bolder and Smaller Grains Developing in the Same Spike or Spikelet of Wheat

ABSTRACT

In order to have a further insight into the possible mechanism leading to differential growth of different grains, developing in the same spike or spikelet of wheat, the present investigation was conducted to examine the structure of different cell organelles and to point out correlations, if any, between their ultrastructural profiles and yielding abilities. The results divulged that the smaller grains, possessed bigger intracellular non-compact spaces in cell wall otherwise not prominent in the bolder grains. The bolder grains invariably showed Golgi structures in propinquity of cell wall, indicating its systematic role in cell wall construction. Further they were conspicuous in possessing a more prominent network of rough endoplasmic reticulum, profused ribosomes attached with the vesicles as compared to the relatively higher intensity of floating ribosomes on the endoplasmic reticulum matrix of smaller grains. In addition, data depicted the presence of more anastomosing mitochondrial cristae in the bolder grains as compared to the small grains. The differences within smaller grains in different segments of spike were significant in terms of number of chloroplasts as well as the number of thyllakoids.

Key words: Cell organelles; chloroplast; thyllakoids; cell wall; mitochondria; Triticum aestivum L.

Introduction

The position of grain within a spike to some extend determines its final grain weight which can range from 20 to 60 mg [2]. The grains from spikelets in the middle region of the spike and from the basal region within each spikelet are more towards the upper level of this range [1,4]. Various explanations such as assimilate availability and/or transport capacity [7] or the possible role of plant growth regulators [3,6,8,9] are offered to explain these differences. The objective of this present study was to have a further insight into the possible mechanism leading to differential growth of different grains, examine the structure of different cell organelles, and to point out correlations, if any, between their ultrastructural profiles and yielding abilities.

Materials and methods

Crop Management and Sampling:

The investigation was conducted with a common bread wheat (*Triticum aestivum* L. var. *PBW-343*), which was sown in circular earthenware pots (50x30x30 cm) containing 35 kg of soil mixed with farmyard manure (4:1). Eight seeds per pot were sown and after 15 days, seedlings were thinned to two. Hoagland's nutrient solution [5] was supplied to the pots. Main spikes were taken at 10th day after anthesis (DAA), divided into three grain positions included proximal (spikelet number 1 to 5), middle (spikelet number 6 to 15), and distal (spikelet number 16 to 20) regions, and further into two grain types included basal (bold) (grain No. 1 and 2) and apical (small) (grain No. 3 upward).

Corresponding Author

Alireza Houshmandfar, Department of Agronomy and Plant Breeding, Saveh Branch, Islamic Azad University, Saveh, Iran.
Email: houshmandfar@iau-saveh.ac.ir

The spikelets were numbered in ascending order with the most proximal one on peduncle side as number 1 with a sequential increase ending at number 20.

Pre and Post-fixation:

Grain material at 10th DAA were taken and dissected into half. This region was then cut into small cubes of 1 mm size. The material was washed in cacodylate buffer (pH of 7.3) and transferred to 6.5 percent gluteraldehyde in cacodylate buffer (pH of 7.3) and was kept for 6 hours at room temperature. Washing in several changes of buffer for 30 minutes was then followed by post fixation in 1 percent osmium tetraoxide in cacodylate buffer for 4 hours at room temperature. This was then followed by one change of cacodylate buffer for 5 minutes. The material was then pre-stained for 15 minutes (pre-stain 0.5 percent uranyl acetate in 10 percent alcoholic solution) after which it was hydrated in graded series of acetone (40, 50, 70 and 100 percents) for 5 minutes followed by 100 percent propylene oxide for 5 minutes. Infiltration by graded series of Epon resin in propylene oxide was followed by keeping for 30 minutes in 50 percent and 75 percent mixture and for one hour in 100 percent solution. At 100 percent a total vacuum 10^{-3} torr for 5 minutes was applied to facilitate infiltration of resin in the tissue. Polymerization of the resin was carried out after transferring the material into plastic capsules in same resin mixture at 60°C for 24 hours. Ultra thin sections were cut using Reichert Jung–ultramicrotome, model Ultra Cut–E. The sections were collected on formalin coated copper grids and stained with uranyl acetate for 20 minutes. To avoid precipitation of lead carbonates, lead citrate staining was carried out in wax sealed atmosphere in the presence of sodium hydroxide pellets. Washing of grids in 0.1 N sodium hydroxide was carried out after lead citrate staining to dissolve traces of lead carbonates formed during staining. The stain lead citrate was prepared fresh in warm double distilled water. Material was scanned on Zeiss transmission electron microscope, model EM-906 at 60 KV accelerating voltage with emission current of 40 micro-amperes (at PGIMER, Chandigarh). Micrographs were recorded on high contrast low speed 35 mm photographic films.

Reagents:

1. Cacodylate-HCL buffer (0.2 M): (i) Sodium cacodylate (42.8 g), Distilled water (1000 ml); (ii) 0.2 M HCl – Concentrated HCl 36-38% (10 ml), Distilled Water (603 ml).

   Adding 4.2 ml of solution B to 50 ml of solution A and diluting it to a total volume of 200 ml could give a desired pH of 7.2.
2. Embedding mixture: (i) Mixture A-Epon 812 (5 ml), DDSA (8 ml); (ii) Mixture B-Epon 812 (8 ml), NMA (7 ml). Final embedding mixture -(i) Mixture A (13 ml); (ii) Mixture B (15 ml) and DMP 30 (16 drops).

   Each of the above mixtures was mixed thoroughly. A further increase in the proportion of mixture B will invariably result in harder blocks.

Pre and Post Staining:

1. Stain A: Lead citrate – (i) Distilled water (100 ml); (ii) Lead citrate (0.4 g) and 10 N NaOH fresh (1.0 ml).
2. Stain B: Uranyl acetate (post stain) – (i) Uranyl acetate (0.2 g) and Distilled water (40 ml).

The above reagents were vigorously shaken for 10 minutes to get a clear solution with a pH of 7.0.

Results and discussion

In order to have a further discernment into the possible mechanism leading to differential growth of different grains, developing in the same spike or spikelet, it was considered worthwhile to examine the structure of different cell organelles and to point out correlations, if any, between their ultrastructural profiles and yielding abilities.

As is well known through the innumerable reports and findings, middle region of spike as compare with proximal and distal regions, and bolder the grain as compared the smaller one, produces the maximum level of grain dry matter accumulation [1,2,4]. The investigations were carried out on 10 days old grains, developing within the same spikelet at three different segments of spike (proximal, middle and distal). Electron micrographs of bold and small grains in the same spike or spikelet are given in Figures 1, 2, 3 and 4. As evident from the following sections there were salient demarcations which separated the two types of grains (bold and small) and the same are discussed under the following sub heads:

Cell Wall and its Orientation:

The pattern of cell wall and its constituents could be distinguished in two types of grains (bold and small) at three different segments of spike (proximal, middle and distal) and is reproduced at Figure 1 at X28000, X36000 and X48000 magnifications. Cell wall in the bolder grains (left) reveals an elaborate presence of macro-fibrils which were oriented centripetally around a longitudinal axis, had relatively more thickness (average of 1.10 micrometer) with tandem interweaving of macro-fibrils within the matrix. On the other hand, smaller grains (right), revealed bigger interacellular non-compact spaces in cell wall which otherwise were not so prominent in the bolder grains. Bolder grains invariably showed the Golgi structure in propinquity of cell wall, indicating its systematic role in cell wall construction.

The dictyosome bodies were not detected in close proximity to the cell wall of smaller grains in almost the entire length of the spike. There was also a considerable difference in the cell wall thickness of smaller grains in middle segment as compared to proximal and distal segments of spike respectively (the thickness was 0.82, 0.90 and 0.79 micrometers in small grains as compared to 1.10, 1.12 and 1.09 micrometers of the bolder grains in proximal, middle and distal segments of spike respectively).

Endoplasmic Reticulum (ER) and its Distribution:

A look in distribution of endoplasmic reticulum in the 10 days old grains revealed that (Figure 2) the bolder grains were conspicuous in possessing a more prominent network of rough endoplasmic reticulum, profused ribosomes attached with the vesicles as compared to the relatively higher intensity of floating ribosomes on the endoplasmic reticulum matrix of smaller grains. There was an increase in the endoplasmic reticulum network in both bolder and smaller grains in middle segment of spike as compared to proximal and distal segments respectively.

Amongst different segments of a spike, bolder grains revealed a better distribution of endoplasmic reticulum in the cell cytoplasm. The difference between bolder and smaller grains in endoplasmic reticulum network was more pronounced in distal segment of spike as compared to proximal and distal segments respectively.

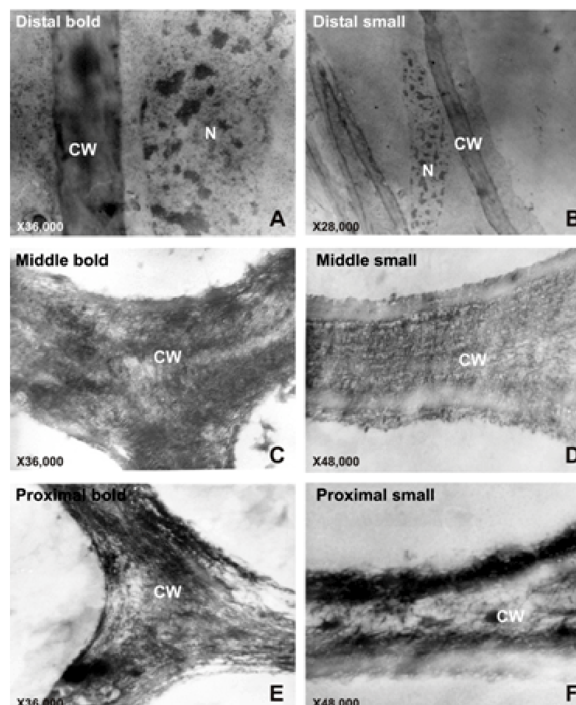

Fig. 1: A-F: Electron micrographs showing orientation of cell wall (CW) in the bold and small grains of wheat in three different segments of spike (proximal, middle and distal) at 10 days after anthesis (N, nucleus).

Mitochondrial Complex:

Studies on the distribution of mitochondria in the two types of grains in different segments of spike i.e., proximal, middle and distal at 10th DAA depicted the presence of more anastomosing mitochondrial cristae in the bold grains as compared to the small grains (Figure 3). The number of mitochondria per unit cellular area could not be ascertained as these were more or less randomly distributed or pushed aside by the expanding vacuoles. On an average of 10 photographs each with 50 to 100 mitochondria, 45 ± 6.2 cristae per mitochondrion were present in the cells of bolder grains (X36000) as compared to the mitochondrion of smaller grains which had a significantly lesser number of cristae (28 ± 5.3) per mitochondrion in almost equal number of samples. These mitochondria were detected in close association of chloroplasts. Smaller grains, in middle segment of spike showed presence of circular mitochondrial structure with more number of cristae per mitochondrion as compared to proximal and distal segments of spike. Surprisingly bolder grains in three different segments of spike did not exhibit much difference in the structure or number of mitochondria per cell in them.

Chloroplasts:

The studies on the distribution of chloroplasts in two types of grains in three different segments of spike (Figure 4) at X20000 and X28000 show that bolder grains were conspicuous in possessing higher number of chloroplast per cell (25 to 35), higher number of starch granules (4-5) and number of grana per chloroplast (20 to 28) as well as higher number of thyllakoids per grana (6-7) in all the three segments of spike.

Smaller grains, on the other hand, possessed only 12-15 chloroplasts per cell, 2-3 starch bodies, 17-23 grana per chloroplast and 3-4 thyllakoids per grana. Bolder grains in different segments of spike did not reveal a conspicuous variation in the chloroplast structure. The differences within smaller grains in different segments of spike was significant in terms of two parameters (i.e., number of chloroplasts as well as the number of thyllakoids) which were significantly more in middle segment of spike as compared to proximal and distal segments respectively.

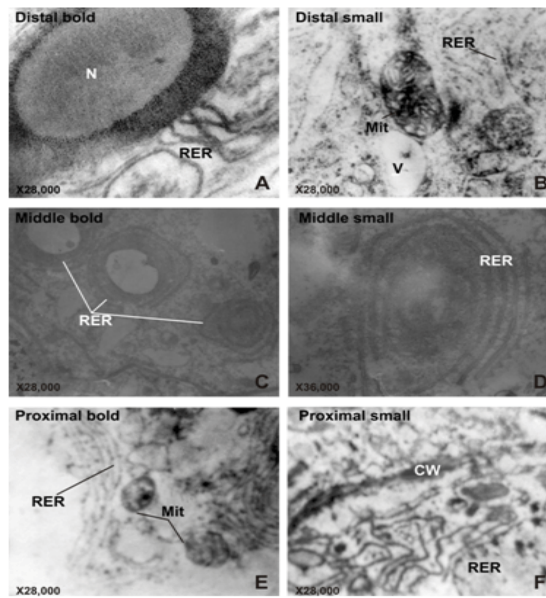

Fig. 2: A-F: Electron micrographs showing the distribution of extensive arrays of endoplasmic reticulum (ER) in bold and small grains of wheat in three different segments of spike (proximal, middle and distal) at 10 days after anthesis. (RER, Rough endoplasmic reticulum; Mit, Mitochondrion; N, Nucleus; CW, Cell wall; V, Voculus).

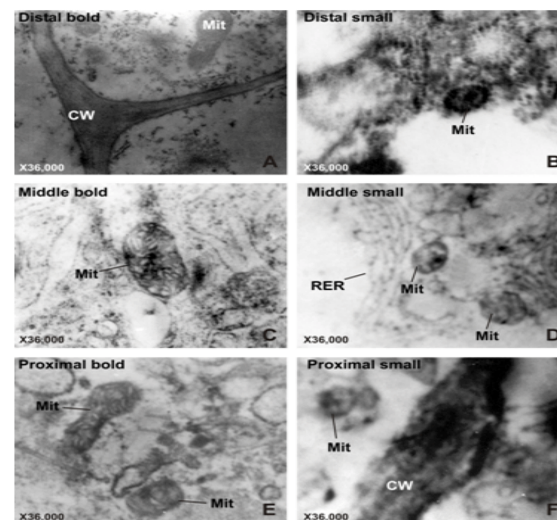

Fig. 3: A-F: Electron micrographs showing mitochondrial complex in the bold and small grains of wheat in three different segments of spike (proximal, middle and distal) at 10 days after anthesis. (RER, Rough endoplasmic reticulum; Mit, Mitochondrion; CW, Cell wall).

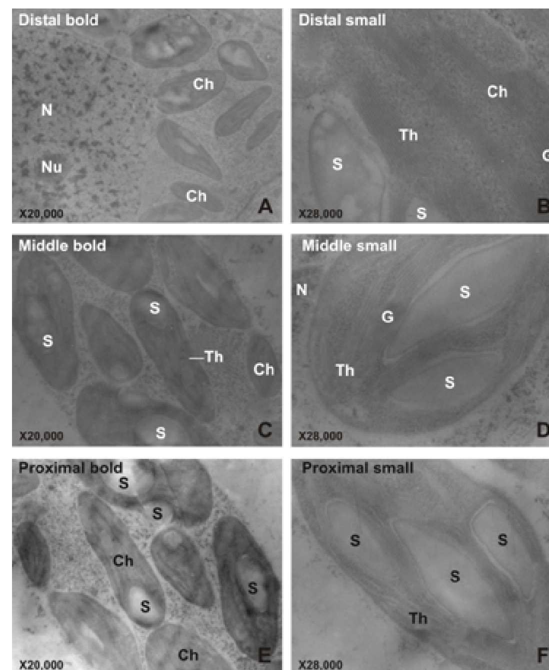

Fig. 4: A-F: Electron micrographs showing chloroplast in the bold and small grains of wheat in three different segments of spike (proximal, middle and distal) at 10 days after anthesis. (Ch, Chloroplast; S, Starch granules; Th, Thyllakoid; G, Grana; N, Nucleus; Nu, Nucleolus).

References

1. Aufhammer, W., P. Zinsmaier, F. Bangerth, 1986. Variation of dry matter accumulation at definite positions within wheat ears and levels of indole-3yl acetic acid (IAA). Plant Growth Regulation 4: 305-310.
2. Bangerth, F., W. Aufhammer, O. Baum, 1985. IAA level and dry matter accumulation at different positions within a wheat ear. Physiol Plant, 63: 121-125.
3. Eradatmand Asli, D., A. Houshmandfar, 2011. Dry matter accumulation and auxin levels within developing grains of different durum wheat genotypes. Adv. Environ. Biol., 5(4): 678-682.
4. Eradatmand Asli, D., A. Houshmandfar, 2011. Dry matter accumulation within developing grains of different wheat genotypes (Triticum aestivum L.). Adv. Environ. Biol., 5(5): 908-911.
5. Hoagland, D.R., D.I. Arnon, 1939. The water culture method for growing plants without soil. Calif. Agri. Expt. Stn. Cir., 12: 347-352.
6. Michael, G., H. Beringer, 1980. The role of hormones in formation. In, Physiological aspects of crop production. International Potash Institute, Bern. Proc. 15h Coll. Int. Potash Inst. Held in Wageningen.
7. Simmons, S.R., D.N. Moss, 1978. Nitrogen and dry matter accumulation by kernels formed at specific florets in spikelets of spring wheat. Crop Sci., 18: 139-143.
8. Yang, J., S. Peng, R.M. Visperas, A.L. Sanico, Q. Zhu, S. Gu, 2000. Grain filling pattern and cytokinin content in the grains and roots of rice plants. Plant Growth Regulation, 30: 261-270.
9. Yang, J., J. Zhang, Z. Wang, Q. Zhu, 2003. Hormones in the grains in relation to sink strength and post-anthesis development of spikelets in rice. Plant Growth Regulation, 41: 185-195.