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Histological observations on grains of food crops and stems of vegetable and fiber crops applied by organic and inorganic (chemical) fertilizers

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Abstract

We investigated the effect of fertilizer type, organic (OF) and chemical (CF), on the histology of grains (rice, barley and soybean), vegetable (radish) and fiber crop (ramie). Each cross section was examined by optical, scanning electron and transmission electron microscopes. In both fresh (just after harvest) and samples stored long-term, some histological differences were observed between CF and OF products. Particularly with grains, gap (or separation) of cotyledon cells, obscure embryo sac liquid layer, thin seed coat or poor cell wall was observed in CF, but not in OF. Such a difference was more distinct in the grains stored long-term.

Key words: fertilizer type, chemical, organic, histology, grains, vegetable, fiber crop

INTRODUCTION

Organic farming has attracted attention throughout the world. Many people believe that organic foods are healthier than conventionally produced foods and that they are produced in a more environmentally compatible manner (Brandt and Mølgaard 2001).

Organic plant products are grown without the aid of chemical-synthetic pesticides and are grown largely without the use of readily soluble mineral fertilizers, a diverse range of crop rotation or extensive soil tillage (Woese *et al.* 1997). Accordingly, organic products inherently acquire minerals indirectly through ‘natural’ processes mediated by the soil biological activity, as suggested by Ryan *et al.* (2004). Analysis of δ15N revealed high values in organic products (Nakano *et al.* 2002; Fujita *et al.* 2003), indicating that organic products acquire nitrogen originating from organic materials. Recently, the possibility of direct acquisition of organic nitrogen by crops became clarified (Yamagata *et al.* 2001). It was indicated that fertilizer type was probably the cause of morphological differences of products (Yoshida *et al.* 1984, Nakamura 1994, Nakagawa *et al.* 2000, Brandt and Mølgaard 2001, Nakamura *et al.* 2007).

We observed the histological structure of several organic and conventional products to clarify the effect of fertilizer type (organic or chemical).

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MATERIALS AND METHODS

Materials

Except pot and field of fiber crops, agricultural practices were as follows: the fields of conventional products (CF) had been farmed conventionally (receiving chemical fertilizers and agricultural chemicals that were standard for the respective district). The fields of organic products (OF) had been managed for over 3 years without chemical fertilizers or agricultural chemicals and using no-till planting or weeding. The organic fertilizer was manure or chopped crop residue for field and vermicompost for pot. The agricultural practices and soil properties, including soil faunal makeup, were reported in detail by Nakamura (2003 for Fukushima pref. field) and Nakamura (1988 for Ibaraki pref. field). Soil properties of the Ehime pref. field were reported by Ueno and Suzuki (2005).

1: Grain (rice, *Oryza sativa* L., aquatic, non-glutinous)

1－1: variety Koshihikari, paddy fields

Sampled non-glutinous brown rice caryopsis was cultivated in paddy fields of Nagano pref. and had been harvested in 2003. After harvest, the hull of rice caryopsis was removed.

1－2: variety Matuyama-mii, paddy pots

Sampled non-glutinous brown rice caryopsis was cultivated in paddy pots under greenhouse conditions in Ehime pref. and had been harvested in 2003. After harvest, the hull of rice caryopsis was removed.

CF pots contained soil collected from a field where chemical fertilizers had been applied for many years, whereas the soil of the OF pots was taken from a field where chemical fertilizers had not been applied for over 3 years. OF pots employed vermicompost.

1－3: variety Nipponbare, paddy fields

Sampled non-glutinous polished rice caryopsis was cultivated in paddy fields of Ibaraki pref. and had been harvested in 1984. After harvest, the hull of rice caryopsis was removed. The brown rice caryopsis was polished and stored for 20 years under hermetic conditions at room temperature and shielded from light. Sampled rice caryopsis was in good condition with no evidence of mold, effects of harmful insects, or putrefaction. The color of OF rice caryopsis turned amber.

2: Grain (rice, *Oryza sativa* L., upland, glutinous, variety Hatakinumochi, upland fields)

Sampled glutinous polished rice caryopsis was cultivated in upland fields of Ibaraki pref. and had been harvested in 1986. After harvest, the hull of rice caryopsis was removed. The brown rice caryopsis was polished and stored for 18 years under hermetic conditions at room temperature and shielded from light. Sampled rice caryopsis was in good condition with no evidence of mold, effects of harmful insects, or putrefaction. The color of OF rice caryopsis turned amber.

3: Grain (barley, *Horduem vulgare* L., variety Benkei-oumugi, upland fields)

Sampled barley caryopsis was cultivated in upland fields of Fukushima pref. and had been harvested in 1994. After harvest, barley caryopses were stored for 10 years under hermetic conditions at room temperature and shielded from light. Sampled barley caryopsis was in good condition with no evidence of mold, effects of harmful insects, or putrefaction.

4: Grain (soybean, *Glycine max* (L.) Merr.)

4－1: variety Hoseki, upland fields

Sampled green soybean seeds were sown in June and harvested in September 2003 from upland fields in Nagano pref. After harvest, the soybean seeds were analyzed immediately while still in fresh condition.

4－2: variety Horei, upland fields

Sampled soybean seeds were cultivated in upland fields of Fukushima pref. and had been harvested in 1995. After harvest, soybean seeds were stored for 9 years under hermetic conditions at room temperature and shielded from light. Sampled seeds were in good condition with no evidence of mold, effects of harmful insects, or putrefaction.

5: Vegetable (radish, *Rhanus sativus* var. *hortensis* Baskier, variety Mino-Wase, pot)

Sampled stems of radish were cultivated in pots in the Shikoku District, Ehime pref., and had been harvested in 2005. After harvest, the radish was immediately analyzed while still in fresh condition.

CF pots contained soil collected from a field where chemical fertilizers had been applied for many years, whereas soil for OF pots was taken from a field where chemical fertilizers had not been applied for over 3 years. OF pots employed vermicompost.

6: Fiber crop (ramie, *Boehmeria nivea* Hooken, upland field)

The seedlings used were cultivated using only organic fertilizer over a long period of many years before transplantation in Showa village, Fukushima pref. The seedlings were transplanted to an upland field in Ehime pref. in 2005. This field had not had any fertilizer applied for over 3 years. Before transplantation, the fields were tilled. The OF field employed vermicompost.

Stems of ramie had been harvested once reaching a height of about 1 m and were cut in the middle part for the samples.

Optical microscopy (OM)

Sampled grains were randomly selected from storage bottles of each product group (CF and OF), and they were fixed in 5% glutaraldehyde (0.1M phosphate buffer, pH 7.4) for 2 weeks at 4℃. Sampled vegetable and fiber crops were randomly obtained from either field or pot and fixed in TAF liquid for 2 weeks at 4℃. All samples were cut in two longitudinally and stored in the fixative at 4℃. After dehydration through a 50-to-100% ethnol series, samples were embedded in Technovit (Technovit 7100; Ohken Co., Tokyo, Japan), and 3μm thick longitudinal sections were sliced by microtome (Nippon Optical Works, Tokyo, Japan). Sections were stained with 0.1% toluidine blue and observed under optical microscope (BX-50; Olympus, Tokyo, Japan).

Scanning Electron Microscopy (SEM)

Sampled grains were randomly selected from storage bottles of each product group (CF and OF), and were cut longitudinally by microtome (Nippon Optical Works, Tokyo, Japan). The specimen was then mounted on an aluminum holder and coated with platinum-palladium (Eiko IB3, Japan). The structure of the section was examined under scanning electron microscope (Super Scan SS-550, Shimadzu, Japan) at an accelerating voltage of 15 kV.

Transmission Electron Microscopy (TEM)

All fixed samples were further fixed with a 1% osmic acid medium (0.1M phosphate buffer, pH 7.4). Dehydration using a 50-to-100% ethanol series was performed before saturation with propylene oxide. After embedding samples in epoxy resin (Epon 812; Ohken Co., Tokyo, Japan), ultra-thin sections (90 nm thick) were prepared using an ultra-microtome (MT 6000 Sorvall Instruments; Du Pont Co., Delaware, USA). They were stained with 1% uranyl acetate and lead citrate, and observed under a transmission electron microscope (H-800, Hitachi, Tokyo, Japan) with an accelerating voltage of 100kV.

RESULTS AND DISCUSSION

1: Grain (rice, *Oryza sativa* L., aquatic, non-glutinous)

1−1: variety Koshihikari, field, fresh sample, Fig. 1 (a-h).

The albumen cells in the central and external parts of caryopsis were of various forms (round, square and elongated (Fig. 1a-f), and their cell walls were clearly in OF (Fig. 1b, d, f). The cell walls of CF were less distinct and had some collapsed structures (Fig. 1a, c, e). Numerous globular starch grains were observed in both fertilizers (Fig. 1g and h), and sticky materials were abundant in the central part of OF rice caryopsis (Fig. 1h). In the internal part of CF rice caryopsis, many gaps, indistinct cell walls and square starch grains that did not have sticky granules between them were observed. In contrast, the internal part of OF rice caryopsis had a clear cell wall, lacked gaps, and had globular starch grains with clear gaps containing many sticky granules between them. The external part of CF rice caryopsis had many cracks which were similar to

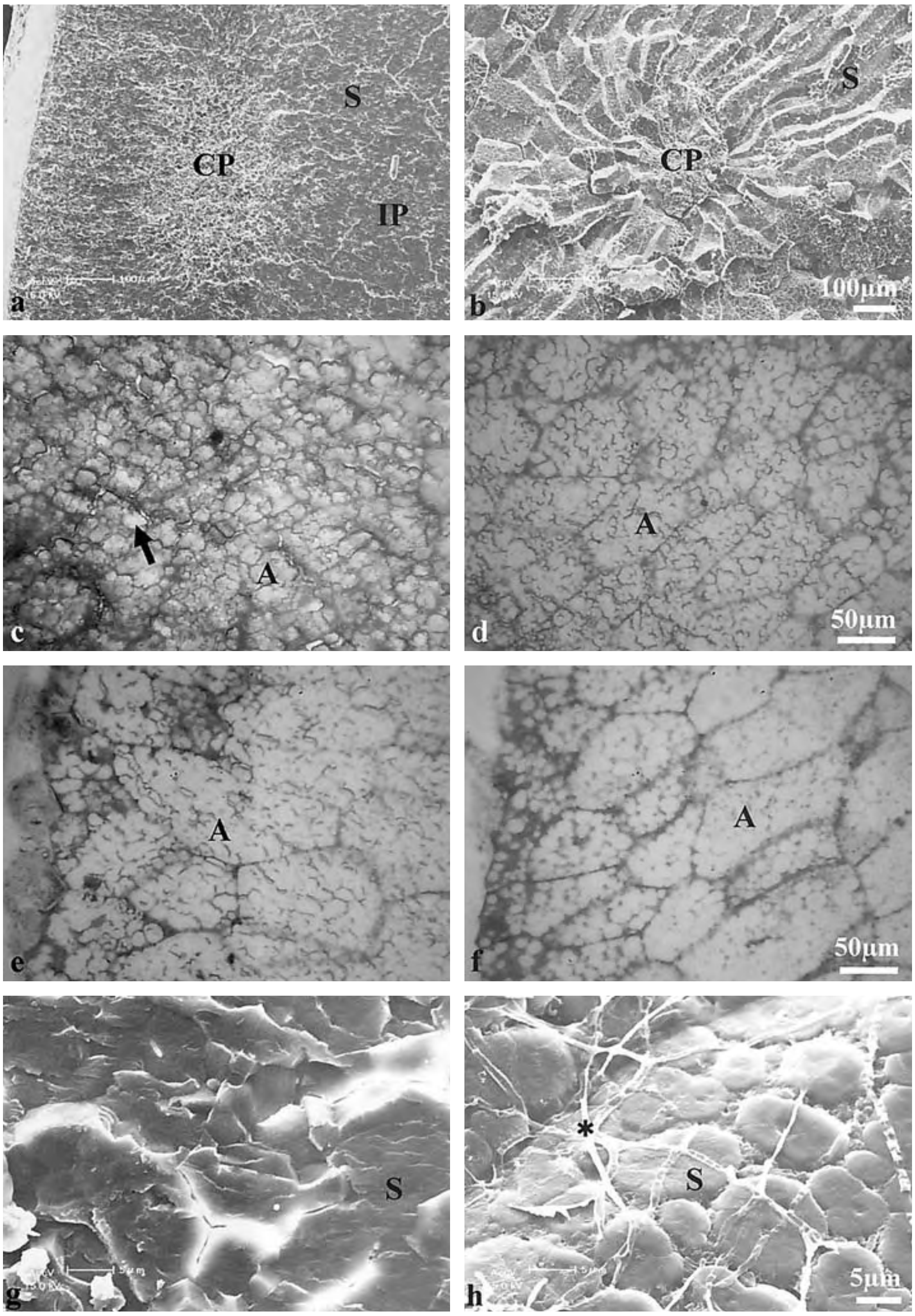

Figure 1 (a-h). SEM (a, b, g and h) and OM (c-f) photographs of grain 1-1 (rice, *Oryza sativa* L., aquatic, non-glutinous, variety Koshihikari, field, fresh sample).
a, c, e, g: CF (chemical fertilizer); b, d, f, h: OF (organic fertilizer). CP: central part; IP: internal part; A: albumin cell; S: starch grain; black arrows: gaps between albumin cells; asterisks: sticky material.

the internal part, but that of OF rice caryopsis did not have a collapsed structure.

1−2: variety Matuyama-mii, pot, fresh sample, Fig. 2 (a-d)

Both types of rice caryopsis (CF and OF) cultivated in pots had albumen cells with various structures (Fig. 2a-d). Numerous cracks were observed in the external part of CF brown rice caryopsis, but not in OF (Fig. 2b). In the internal part, many gaps similar to those of the central part of albumen cell were observed in CF, but not in OF. In the external part of the albumen cell, the cell walls were indistinct in CF, but were distinct in OF (Fig. 2c, d).

1−3: variety Nipponbare, field, 20 years stored sample, Fig. 3 (a-b)

CF polished rice caryopsis had distinct areas of severe structural damage, especially in the external part, which was not evident in OF (Fig. 3).

2: Grain (rice, *Oryza sativa* L., upland, glutinous, variety Hatakinumochi, 18 years stored sample), Fig. 4 (a and b)

Multiple and extensive gaps were observed and the cell structure had collapsed uniformly in CF polished rice caryopsis. Collapsed layers and gaps were not detected in OF (Fig. 4).

3: Grain (barley, *Horduem vulgare* L., variety Benkei-oumugi, field, 10 years stored sample), Fig. 5 (a-d)

In CF barley caryopsis, many collapsed areas and gaps were extensively observed, whereas only a few were observed in OF (Fig. 5). Pericarp near crease was clearer in OF than in CF. Aleurone layer was thicker in CF than in OF (Fig. 5c, d).

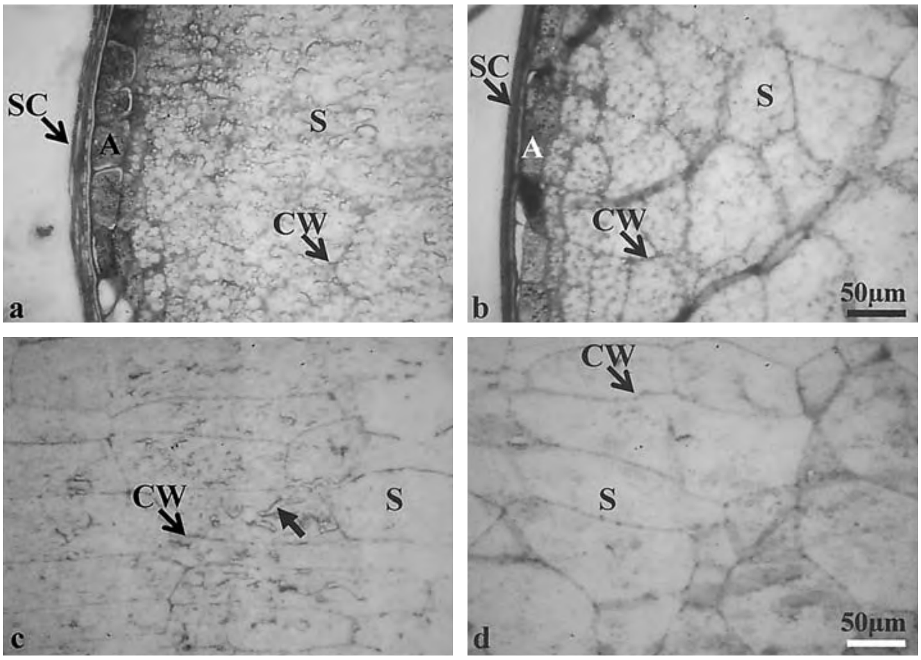

Figure 2 (a-d). OM photographs of grain 1-2 (rice, *Oryza sativa* L., aquatic, non-glutinous, variety Matuyama-mii, pot, fresh sample).
a, c: CF; b, d: OF. SC: seed coat; A: albumin cell; S: starch grain; CW: cell wall; black arrows: gaps between albumin cells.

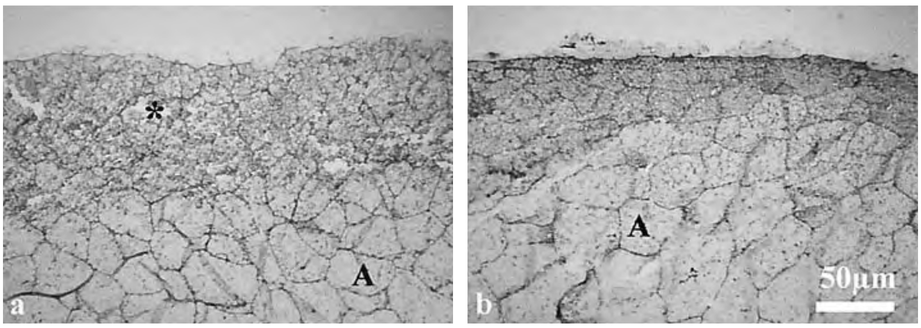

Figure 3 (a and b). OM photographs of grain 1-3 (rice, *Oryza sativa* L., aquatic, non-glutinous, variety Nipponbare, field, 20 years stored sample).
a: CF, b: OF. A: albumin cell; asterisks: collapsed cells.

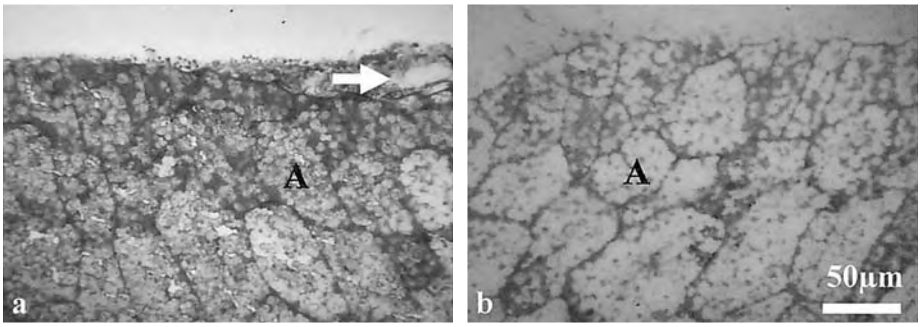

Figure 4 (a and b). OM photographs of grain 2 (rice, *Oryza sativa* L., upland, glutinous, variety Hatakinumochi, 18 years stored sample).
a: CF; b: OF. A: albumin cell; white arrows: gaps with cell wall.

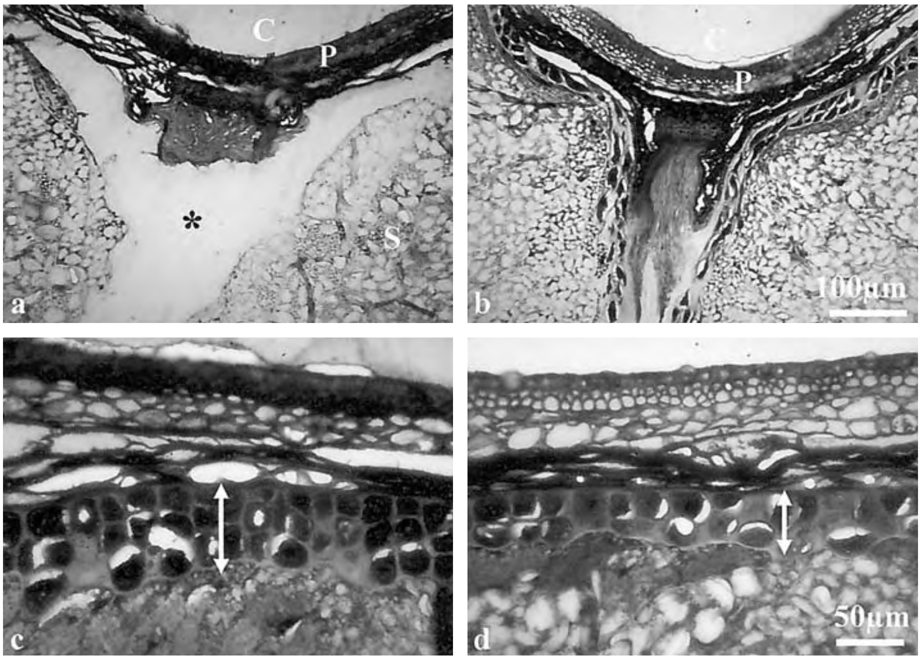

Figure 5 (a-d). OM photographs of grain 3 (barley, *Horduem vulgare* L., variety Benkei-oumugi, field, 10 years stored sample).
a, c: CF; b, d: OF. C: crease; P: pericarp; S: starchy endosperm; asterisks: gaps and collapsed layers; arrows: aleurone layer.

4: Grain (soybean, *Glycine max* (L.) Merr.)

4−1: variety Hoseki, field, fresh sample, Fig. 6 (a-d)

The seed coat of CF soybean was thinner than that of the OF seeds (Fig. 6a and b). An obscure embryo sac liquid layer was observed in CF seeds (Fig. 6a and b). Gaps were extensively observed in the cotyledon cells of CF seeds (Fig. 6c and d).

4−2: variety Horei, field, 9 years stored sample, Fig. 7 (a-d)

There was no difference between CF and OF seeds regarding the morphological structure of the surface of the seed coat and surface of seeds which had stripped off seed coats. CF seeds contained many collapsed areas, whereas only a few such areas were found in OF seeds (Fig. 7a, b). Cotyledon cells of CF seeds were transformed (Fig. 7 a, b). In seeds of CF soybean, collapsed cell walls were observed (Fig. 7c). OF seeds had clear protein bodies, lipid granules and thick cell walls (Fig. 7d).

5: Vegetable (radish, *Rhanus sativus* var. *hortensis* Baskier, variety Mino-Wase, pot, fresh sample), Fig. 8 (a-d)

Collapsed cells were observed in the stems of CF radish. In OF radish, cells were larger than those of CF and organic granules or something similar was observed near the cell wall (Fig. 8).

6: Fiber crop (ramie, *Boehmeria nivea* Hooken, field, fresh sample), Fig. 9 (a and b)

The stem cells of OF ramie were irregular in shape and size, and were fairly large (Fig. 9b). In CF ramie, the cells were almost the same shape and size (Fig. 9a). In the growing district of the seedlings, people believed that inorganic fertilizer reduced the quality of fiber. However,

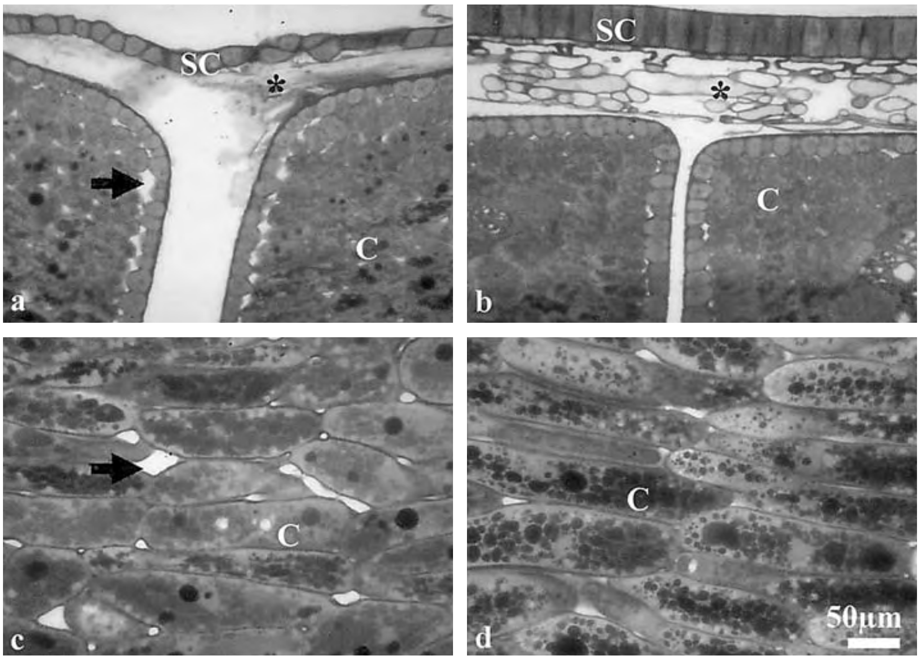

Figure 6 (a-d). OM photographs of grain 4-1 (soybean, *Glycine max* (L.) Merr., variety Hoseki, field, fresh sample).
a, c: CF; b, d: OF. C: cotyledon cell; SC: seed coat; arrows: gaps; asterisks: embryo sac liquid layer.

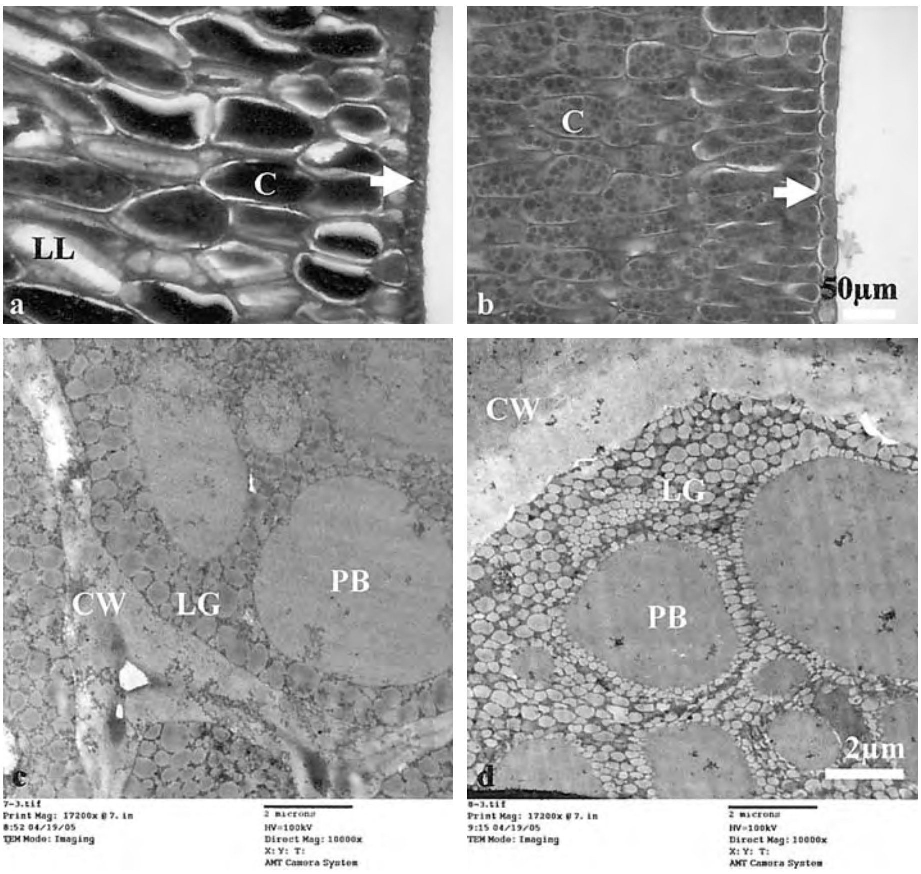

Figure 7 (a-d). OM (a and b) and TEM (c and d) photographs of grain 4-2 (soybean, *Glycine max* (L.) Merr., variety Horei, field, 9 years stored sample).
a, c: CF; b, d: OF. C: cotyledon cell; CW: cell wall; LG: liquid granule; PB: protein body; LL: embryo sac liquid layer; white arrows: seed coat.

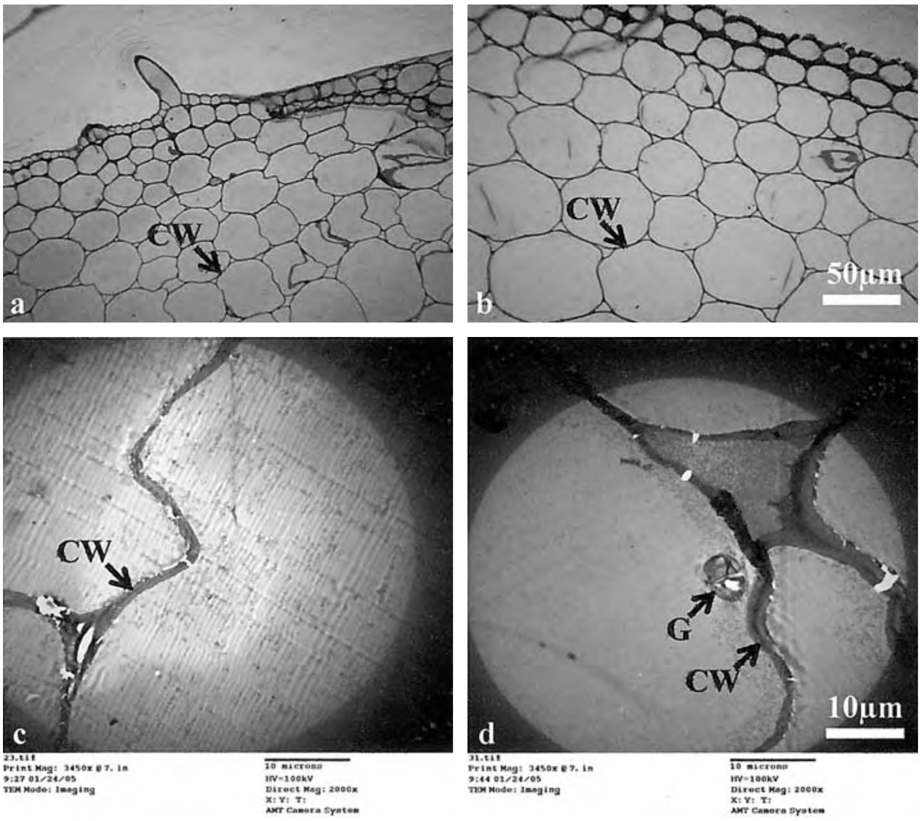

Figure 8 (a-d). OM (a and b) and TEM (c and d) photographs of vegetable (radish, *Rhanus sativus* var. *hortensis* Baskier, variety Mino-Wase, pot, fresh sample). a, c: CF; b, d: OF. CW: cell wall; G: granule.

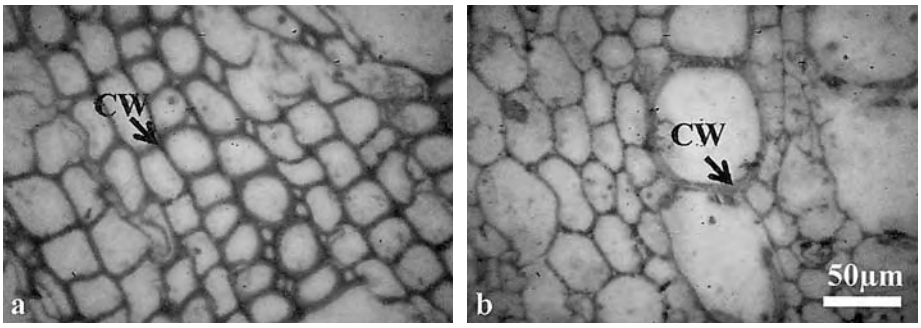

Figure 9 (a and b). OM photographs of fiber crop: ramie, *Boehmeria nivea* Hooken, field, fresh sample). a: CF; b: OF. C: cell wall.

Table 1. Condition of materials for analysis

	Material	Crop	Variety	Place of culture	Harvest (year)	Analysis (year)	Locality (prefecture)	Fertilizer of organic (OF) product
grain 1-1	non-glutinous brown rice	*Oryza sativa* L.	Koshihikari	paddy field	2003	2003	Nagano	manure
grain 1-2	non-glutinous brown rice	*Oryza sativa* L.	Matuyama-mii	paddy pot	2003	2003	Ehime	vermicompost
grain 1-3	non-glutinous polished rice	*Oryza sativa* L.	Nipponbare	paddy field	1984	2005	Ibaraki	chopped crop residue
grain 2	glutinous polished rice	*Oryza sativa* L.	Hatakinumochi	upland field	1986	2005	Ibaraki	chopped crop residue
grain 3	barley caryopsis	*Horduem vulgare* L.	Benkei-oumugi	upland field	1994	2005	Fukushima	chopped crop residue
grain 4-1	soybean seed	*Glycine max* (L.) Merr.	Hoseki	upland field	2003	2003	Nagano	manure
grain 4-2	soybean seed	*Glycine max* (L.) Merr.	Horei	upland field	1995	2005	Fukushima	manure
vegetable	stem of radish	*Rhanus sativus* var. *hortensis* Baskier	Mino-Wase	pot	2005	2005	Ehime	vermicompost
fiber crop	stem of ramie	*Boehmeria nivea* Hooken		upland field	2005	2005	Ehime	vermicompost

the relationship between the quality, shape and size of cells was not ascertained.

The present differences in the histological characteristics between CF and OF products coincide to some extent with several studies which have been done on the quality of products. Yoshida *et al.* (1984) reported that tomato fruits cultured with chemical fertilizers had many loosened adhesive cell walls as observed by scanning microscopy. Nakamura (1994) showed that organic upland glutinous-rice had clear cell walls and eight amino acids even after eight years of storage, though inorganic rice had almost no cell walls and three (one: trace) different amino acids. Further studies need to be conducted on whether the present differences were inextricably induced from the applied fertilizer type, or not.

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REFERENCES

Brandt, K. and J. P. Mølgaard (2001) Organic agriculture: does it enhance or reduce the nutritional value of plant foods?. J. Sci. Food Agr., **81**: 924—931.

Fujita, M., S. Iwaishi, T. Minami, Y. Matsuda and S. Fujiyama (2003) Quality evaluation of the organic certified rice using δ15N values and taste qualities. Jpn. J. Soil Sci. Plant Nutr., **74**: 805—808. (In Japanese)

Nakagawa, S., Y. Tamura and Y. Ogata (2000) Comparison of rice grain qualities as influenced by organic and conventional farming systems. Jpn. J. Crop Sci., **69**: 31—37. (In Japanese)

Nakamura, Y. (1988) The effect of soil management on the soil faunal makeup of a cropped andosol in central Japan. Soil Tillage Res., **12**: 177—186.

Nakamura, Y. (1994) Organic products are fit for long-term storage. News Tohoku Agr. Experi. Station, **72**: 6. (In Japanese)

Nakamura, Y. (2003) Earthworm and enchytraeid numbers in soybean-barley fields under till and no-till cropping systems in Japan during nine years. Mem. Fac. Agr., Ehime Univ., **48**: 19—29.

Nakamura, Y-N., M. Fujita, Y. Nakamura and T. Gotoh (2007) Comparison of nutritional composition and histological changes of the soybean seeds cultivated by conventional and organic farming systems after long-term storage. -Preliminary study. Mem. Fac. Agr., Kyushu Univ., **52**: 1—10.

Nakano, A., Y. Uehara and I. Watanabe (2002) Qualities and δ^{15}N values of the organic certified fruit vegetables. Jpn. J. Soil Sci. Plant Nutr., **73**: 307—309. (In Japanese)

Ryan, M. H., J.W. Derrick and P. R. Dann (2004) Grain mineral concentrations and yield of wheat grown under organic and conventional management. J. Sci. Food Agr., **84**: 207—216.

Ueno, H. and T. Suzuki (2005) Herbicidal effects and nutrients supply by applying of distilled spirit waste and rice bran in organic rice cultivation. Japanese J. Farm Work Research, **40**: 191—198. (In Japanese with English summary)

Woese, K., D. Lange, C. Boess and K.W. Bögl (1997) A comparison of organically and conventionally grown foods-Results of a review of the relevant literature. J. Sci. Food Agr., **74**: 281—293.

Yamagata, M., S. Matsumoto and N. Ae (2001) Possibility of direct acquisition of organic nitrogen by crops. (Ae *et al.* eds.), Plant Nutrient Acquisition, Springer, 399—420.

Yoshida, K., S. Mori, K. Hasegawa, N. Nishizawa and K. Kumazawa (1984) Texture of tomato fruits cultured with organic fertilizers in comparison with inorganic fertilizers. J. Jpn. Soc. Nutr. Food Sci., **37**: 267—272. (In Japanese)

摘　　要

中村好男・加藤奈都実・中村好德：有機肥料または化成肥料農作物の電子顕微鏡による微細構造観察

有機および無機（化成）肥料で栽培した穀粒や茎を，収穫直後あるいは長期保存し，その形態を光学顕微鏡および電子顕微鏡（SEM および TEM）で比較したところ，有機および無機肥料栽培間に差異が観察された．とくに穀粒においては，有機肥料栽培に比して無機肥料栽培で細胞間の隙間が多く，細胞膜が薄かった．この差異は長期保存した水稲（日本晴，20年間常温にて精米で保存）と陸稲（はたきぬもち，18年間常温にて精米で保存）で顕著であった．