BIOLOGIA PLANTARUM 44 (4): 623-625, 2001

BRIEF COMMUNICATION

Characterization and localization of a novel protein (HFN 40) in maize genotypes without husk leaf blades

H.A. EL-SHEMY*[1], T. NISHIMURA** and K. FUJITA*

*Department of Environmental Dynamic and Management, Faculty of Applied Biological Science, Hiroshima University, 1-4-4 Kagamiyama, Higashihiroshima, 739-8528 Japan**
*Department of Food Science, Faculty of Applied Biological Science, Hiroshima University, 1-4-4 Kagamiyama, Higashihiroshima, 739-8528 Japan***

Abstract

Some maize (*Zea mays* L.) genotypes produced husk leaves without leaf blades. However, the physiological implication of this leaf deformity is unclear. Difference in protein pattern was observed between maize with and without husk leaf blades. A clear band around 38~40 kDa in seeds of maize genotypes without husk leaf blades appeared, while it was not detected in ones with husk leaf blades. These protein might be involved in leaf blade intiation.

Additional key words: polypeptide patterns, seed proteins, *Zea mays*.

Some specific maize cultivars expand husk leaf blades from husk (leaf sheath of husk leaf) enclosing the developing grains. Several reports have shown that the husk leaves contribute to grain production with higher efficiency than culm leaves (Fujita *et al.* 1994), which is mainly due to a higher efficiency of carbon translocation (Sawada *et al.* 1995). No significant difference in net photosynthetic rate between husk leaves and culm leaves was observed, though some characteristics suggested an C_3 and C_4 intermediate type of carbon metabolism in husk leaves (Langdale *et al.* 1988). Therefore, we are interested in leaf area expansion of husk leaves (Sato *et al.* 1997).

During seed development, the specialized proteins are synthesized and used as a source of nitrogen, amino acids, and energy during seed germination and subsequent seedling establishment. There are many families of storage proteins that can be synthesized and stored in the same seed. Many proteins are synthesized in response to stress. For example, a thaumatin-like protein was isolated and characterized from mercuric chloride-treated maize (Frendo *et al.* 1992). Mistrík *et al* (2000) reported the quantitative changes in maize membrane proteins induced by aluminium treatment. However, the proteins involved in husk leaf formation have not yet been identified. Therefore, the current study was undertaken to examine the differences between maize with and without husk leaf blades in terms of seed protein composition.

Zea mays L. three lines (B: N-19, C: CM-80 and D: CE-78) with husk leaves blades and three lines (F: F_1 derived from crossing the parents N-19 and X-15, G: W-41 and E: W-79) without husk leaf blades were grown in the experimental field of Hiroshima University, located in Higashihiroshima city, Japan. We define here the leaf initiating from shank of an ear as the husk leaf. The husk leaf is composed of leaf sheath and leaf blades (Fig. 1).

Ten plants from respective maize line were harvested in each of the three replications after physiological maturity, and were separated into various plant parts. Seeds were fixed in liquid nitrogen and preserved at the deep freezer (-80 °C) until analysis. After lyophilization, the harvested seeds were ground into fine powder with a vibrating mill (*Model T1-100, Heiko Co.*, Fukushima, Japan). Proteins were extracted from 0.5 g defatted seed powder plus 4 cm^3 extraction buffer essentially as described by the method of Ohtake *et al.* (1994), with some modification (El-Shemy *et al.* 2000). The

Received 27 December 2000, *accepted* 25 March 2001.
[1]Corresponding author's permanent address: Cairo University, Biochemical Department, Faculty of Agriculture, Giza, Egypt.
Fax: (+81) 824 247963, e-mail: helshemy@hiroshima-u.ac.jp

supernatant fraction (0.15 cm^3) was heated at 90 °C for 5 min with 0.30 cm^3 of 0.0625 M Tris-HCl buffer at pH 8.8 containing 20 g dm^{-3} sodium dodecyl sulfate, 1.0 cm^3 2-mercaptoethanol and 2.0 cm^3 glycerol. Then the 0.040 cm^3 of the solution mixture was separated by SDS-PAGE according to the procedure described by Laemmli (1970), and the peptide bands were stained by incubation in Coomassie Brilliant Blue (CBB) R-250 for 10 min and destined by methanol, acetic acid and water (5:7.5:87.5, v/v/v) for 8 h. Identification of the components of the major storage proteins in the total extract was done by using protein markers.

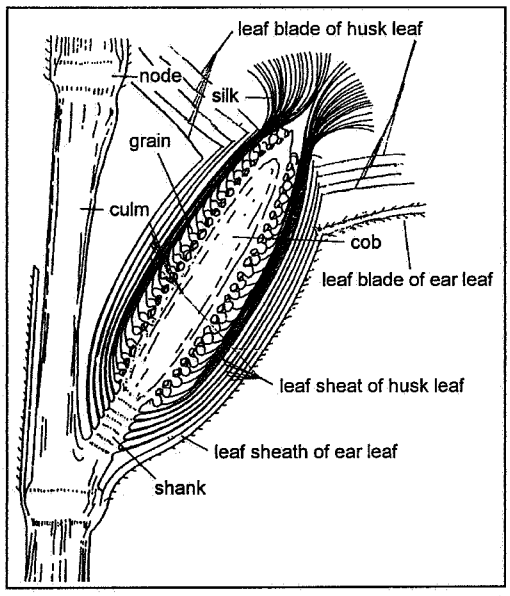

Fig. 1. Transversal illustration of structure of an ear with leaf blades in maize.

Digestion was carried out as described by Kawasaki *et al.* (1990) with lysylendopeptidase in 100 mM Tris-HCl (pH 9.0) in the presence or absence of 0.1 % SDS at 37 °C. When digestion was carried out in the gel, the polyacrylamide gel stained with R-250, CBB was transferred from the destaining solution to a large volume of water and soaked about 1 h to remove acetic acid and methanol. After removal of the destaining solution, the CBB stained protein band (38 ~ 40 kDa) were cut out with a razor blade or a microspatula, and transferred to polypropylene tubes. The gel pieces were incubated for 1 h at 37 °C, and then lysylendopeptidase was added at a concentration greater than 2 μg cm^{-3}. Protease digestion was carried out 24 h at 37 °C. Solution after digestion was aspirated with a pipet or microsyringe and applied to SDS-PAGE and electroblotted onto a polyvinylidene difluoride (PVDF) membrane.

For N-terminal amino acid sequencing proteins band of maize seeds separated by SDS-PAGE were digested by lysylendopeptidase and peptides were separated by SDS-PAGE and electroblotted onto a polyvinylidene difluoride (PVDF) membrane. After Coomassie Brilliant Blue R-250 staining, 38~40 kDa protein band was excised from the membrane sheet. The N-terminal amino acid sequence was determined using a *Hewlett-Packard G1005A* (Wilmington, USA) automatic protein sequencing system calibrated with phenylthiohydantoin (PTH)-amino acid standards prior to each sequencing run by the Edman degradation method. The PVDF-blotted sample was loaded directly into the sequencer. Searches for sequence similarity were performed with the *SWISS-PROT* and *Blastp* databases.

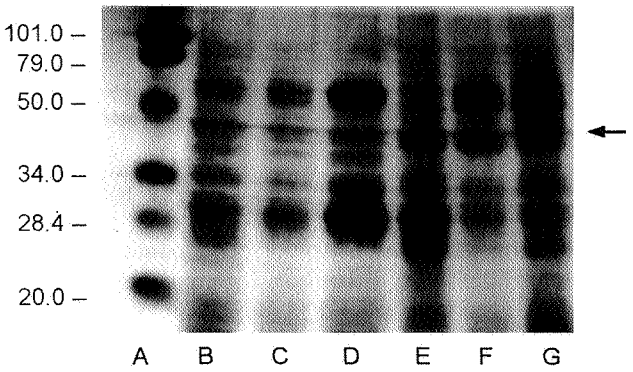

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of seed proteins from maize. Gel was stained with Coomassie Brilliant Blue G-250. *Lane A* - a protein size marker, *B* - N19, *C* - CM80, *D* - CE78, *E* - W79, *F* - F1, *G* - W41.

We have found differences in the composition of proteins isolated from seeds (Fig. 2) between maize genotypes with and without husk leaf blades. This means that there may be some mechanisms in plants regulating initiation of husk leaf development. All the genotypes of maize without husk leave blades enhanced the synthesis of a 38~40 kDa polypeptide localized in the protein pattern of all genotypes without husk leaf blades (Fig. 2). Presumably, it assumes that such a specific polypeptide is involved in impairment of husk leaf initiation in genotypes without husk leaf (Fig. 2). Searches in the *SWISS-PROT* and *Blastp* databases revealed that the purified HFN40 protein shows no significant similarity to any protein sequence in the database (Table 1). In our initial study, it is intended to isolate a protein involved in husk leaf development from maize seeds.

Grain proteins from all three genotypes displayed a similar protein pattern. After digestion by lysylendo-

Table 1. The sequences of peptides derived from maize seed proteins after digestion with lysylenendopeptidase. The sequences were determined from PVDF membrane.

Peptide	Amino acid sequence
P1	DAQEXK
P2	RVLGQLHGGPFSASAK
P3	YFGQAHGGXEK

peptidase enzyme the peptides were separated by SDS-PAGE and electroblotted onto a polyvinylidene difluoride (PVDF) membrane, the sequence of the N-terminal amino acids of the HFN40 protein was determined (Table 1). HFN40 is a new type of maize protein and shows no homology to other known maize proteins.

Further studies on isolation and characterization of cDNA clones coding for HFN40 protein and other maize proteins may help us to better understand the interaction between with and without husk leaf blades.

References

El-Shemy, H., Yamana, H., Saneoka, H., Fujita, K.: Phylogenetic comparative analysis of storage proteins structure in some legume seeds. - Amer. Biotech. Lab. **18**: 60-62, 2000.

Frendo, P., Didierjean, L., Passelegue, E., Burkard, G.: Abiotic stresses induce a thaumatin-like protein in maize: cDNA isolation and sequence analysis. - Plant Sci. **15**: 61-69, 1992.

Fujita, K., Furuse, F., Sawada, O., Bandara, D.: Effect of defoliation and pod removal on dry matter production and inorganic elements absorption in sweet corn. - Soil Sci. Plant Nutr. **40**: 581-591, 1994.

Kawasaki, H., Emori, Y., Suzuki, K.: Production and separation of peptides from proteins stained with CBB R-250 after separation by SDS-PAGE. - Anal. Biochem. **191**: 332-336, 1990.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. - Nature **227**: 680-685, 1970.

Langdale, J.A., Zelitch, I., Miller, E., Nelson, T.: Cell position and light influence C_4 versus C_3 patterns of photosynthetic gene expression in maize. - EMBO J. **7**: 3643-3651, 1988.

Mistrík, I., Tamás, L., Huttová, J.: Quantitative changes in maize membrane proteins induced by aluminium. - Biol. Plant. **43**: 85-91, 2000.

Ohtake, N., Nishiwaki, T., Mizukoshi, K., Chinushi, T., Takahashi, Y., Ohyama, T.: Lack of β-subunit of β-conglycinis in non-nodulating isolines of soybean. - Soil Sci. Plant Nutr. **40**: 345-349, 1994.

Sato, H., Sakurai, N., Sendo, S., Oka, T., Saneoka, H., Nobuyasu, H., Fujita, K.: Factors affecting leaf area development in husk leaf of flint corn. - Crop Sci. **37**: 1826-1831, 1997.

Sawada, O., Ito, J., Fujita, K.: Characterization of photosynthesis and translocation of ^{13}C-labelled photosynthate in husk leaves of sweet corn. - Crop Sci. **35**: 480-485, 1995.