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Analysis of Onion Seeds by Two Dimensional Electrophoresis

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ABSTRACT. Egyptian onion seeds (Giza 1 and Shandawel) were extracted with tris-borate buffer solution of pH 8.9. The protein buffer extract was examined by two dimensional electrophoresis (Mapping technique); with PAGIF in the first dimension, and Poro PAGE and SDS-PAGE in the second dimension. Differences between the cultivars, stored and fresh onion seeds could be detected.

The screening of enzyme patterns in onion seeds revealed the presence of different enzymes: alcohol dehydrogenase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, superoxide dismutase and alkaline phosphatase (Mallery 1972, Nakamura and Tahara 1977, Chupov and Kutyavina 1978, and Hadacova *et al.* 1981). Esterase isoenzyme of onion seed was studied by means of isoelectrofocusing which showed better resolution than polyacrylamide gel electrophoresis (Hadacova *et al.* 1983).

Compartive investigations of the protein content of onion seed are reported by Kubicz (1962), Brezhenv *et al.* (1971), and Klozova *et al.* (1981). Much more attention was paid to allicinase (Goryachenkova 1952) and alliinase (Selby *et al.* 1979).

In this work the protein of onion seeds was studied by two dimensional electrophoresis technique. The advantage of this method is that protein components are separated according to their isoelectric point in the first dimension and their MW by Poro PAGE and SDS-PAGE in the second dimension. The two cultivars of onion seeds (Giza 1 and Shandawel) were planted in Egypt and each cultivar differs markedly in its properties. Vakhtina *et al.* (1977) found morphological differences in DNA content between species within the genus Allium. Our

attempt was to verify the possibility of mapping technique as a tool for determining the differences between cultivars as well as between fresh and stored onion seeds, where the stored seeds lost part of their germination activity during the storage.

Materials and Methods

Materials

Egyptian onion seeds (cultivars Giza 1 season 1980 stored over calcium chloride, Giza 1 season 1985 and Shandawel season 1985) were supplied by Agriculture Research Center, Cairo, Egypt. The onion seeds were milled under cooling by using an electric mill (Jahke and Kunkel type A 10S) for one min.

Methods

Sample preparation and extraction

The meal was defatted by stirring with pure acetone (1:3 W/V) for 30 min., three time. The defatted meal was extracted with 0.125 M tris-borate buffer solution of pH 8.9 containing 0.02% sodium azide according to Afify and Ghali (1986).

Electrophoresis

Electrophoresis was performed in POOMA-PHOR apparatus 1 mm, (Labor-Müller, D-3510 Hann. Münden).

PAGIF was run in tube with 6% acrylamide and 0.16% N,N-methylene-bis-acrylamide, 1% Servalyt T-pH 4-9 in 6 M urea. The anode chamber was filled with 0.1% phosphoric acid and the cathode chamber with 0.15% 1-dimethyl-amino-propan-2-ol according to Stegemann *et al.* (1984).

Mapping: In the first dimension it was conducte by using PAGIF and in the second dimension PoroPAGE 5-25% PAA in 0.125 M tris-borate buffer solution of pH 8.9 (Margolis and Kenrick 1968) and SDS-PAGE 5 and 15% PAA in 0.1% SDS in 0.125 M tris-HCl buffer pH 6.8 and 0.375 M tris-HCl buffer -pH 8.8 respectively (Laemmli 1970) in 0.025 M tris-0.192 M glycin-pH 8.3 with 0.1% SDS were used.

The rods after PAGIF were treated with 2% SDS/ 1% ME for 5 min. at room temperature prior to the second dimension of SDS-PAGE (Stegemann *et al.* 1973).

SDS-Protein markers (Lysozyme, 14.3; Chymotrypsinogen A, 25.7; Alcohol dehydrogenase, 37.0; Bovin serum albumin, 67.0 and Phosphorylase b, 97.4 Kd) were used in SDS-PAGE.

Results and discussion

For the most detailed characterization and differentiation studies of the onion seed protein, two dimensional electrophoresis (Mapping technique) were used. Mapping technique was done with isoelectrofocusing in Servalyt T-pH 4-9 with 6 M urea in the first dimension; and PoroPAGE and SDS-PAGE in the second dimension. The present technique for instance, make it possible to compare protein component for differentiation between cultivars (Giza 1 and Shandawel) as well as between stored and fresh onion seed of Giza 1.

It was proved that the best method for differentiation between stored and fresh onion seed was by extraction with tris-borate buffer solution of pH 8.9 (Afify and Ghali 1986). Therefore the protein patterns of the buffer extract of onion seeds were examined by mapping technique.

Mapping with PoroPAGE of different cultivars (Giza 1 and Shadawel) proved that Shandawel contained more specific protein bands of 80 and 140 Kd, where protein bands of 210, 270 and 300 (I, II and III) appeared as strong spots compared to Giza 1 (Fig. 1-A). Mapping with SDS-PAGE in the second dimension ensured that Shandawel seeds contain lower MW proteins of 12 and 14 Kd, which could not be detected in Giza 1. Also, the higher MW protein subunits of 60 Kd appeared as strong band in Shandawel corresponding to the same protein in Giza 1 (Fig. 2-A). Stored onion seeds by mapping with Poro PAGE in the second dimension proved increasing in the intensity of three protein bands with MW of 210, 220 and 240; beside the lower protein of 67 Kd in the stored onion seed as compared to the fresh seed. The rest of protein bands appeared to be identical in the stored and fresh onion seeds (Fig. 1-B).

We focus our attension to the physical and chemical properties of the protein band (30 Kd) detected in the stored onion seed after SDS-PAGE (Afify and Ghali 1986). Physical properties of this band were studied especially by mapping with SDS-PAGE in the second dimension. The results showed that this protein was detected as three subunits with the same MW beside the original subunit detected in the fresh and stored onion seed. This fact indicate the protein (30 Kd) consisted of four homogeneous protein subunits with different charges, separated in the basic region of isoeletrofocusing in the first dimension (Fig. 2 B). This point needs further investigation to insure that the three subunits come out from the protein band of 30 Kd as a results of onion seed storage. The chemical properties of this protein as enzyme, enzyme inhibitor or the nature of it as glycoprotein etc....., are in progress in our laboratory to show its role during the storage, which may be related to enzyme inhibitor (Ryan 1973).

Isoelectrofocusing in Servalyt T-pH 4-9 proved that most of the onion proteins were separated in the acidic region with Ip of pH 4 to 7 (Fig. 3) which is in agreement with the finding of Afify and Ghali (1986).

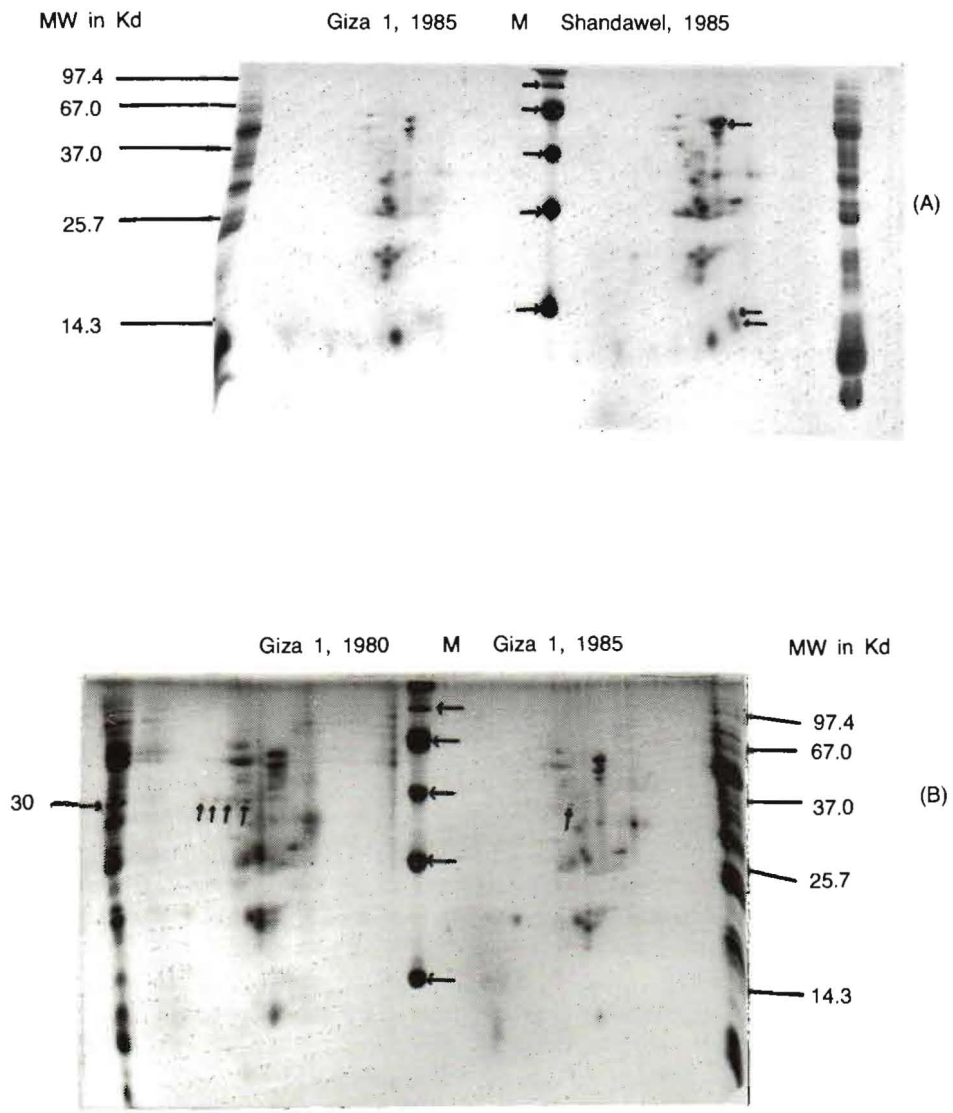

Fig. 1. Mapping (2-D)
First dimension: PAGIF in Servalyt T-pH 4-9 with 6 M urea.
Second dimension: PoroPAGE 5-25% PAA in tris-borate buffer pH 8.9.
S = Bovin serum albumin.

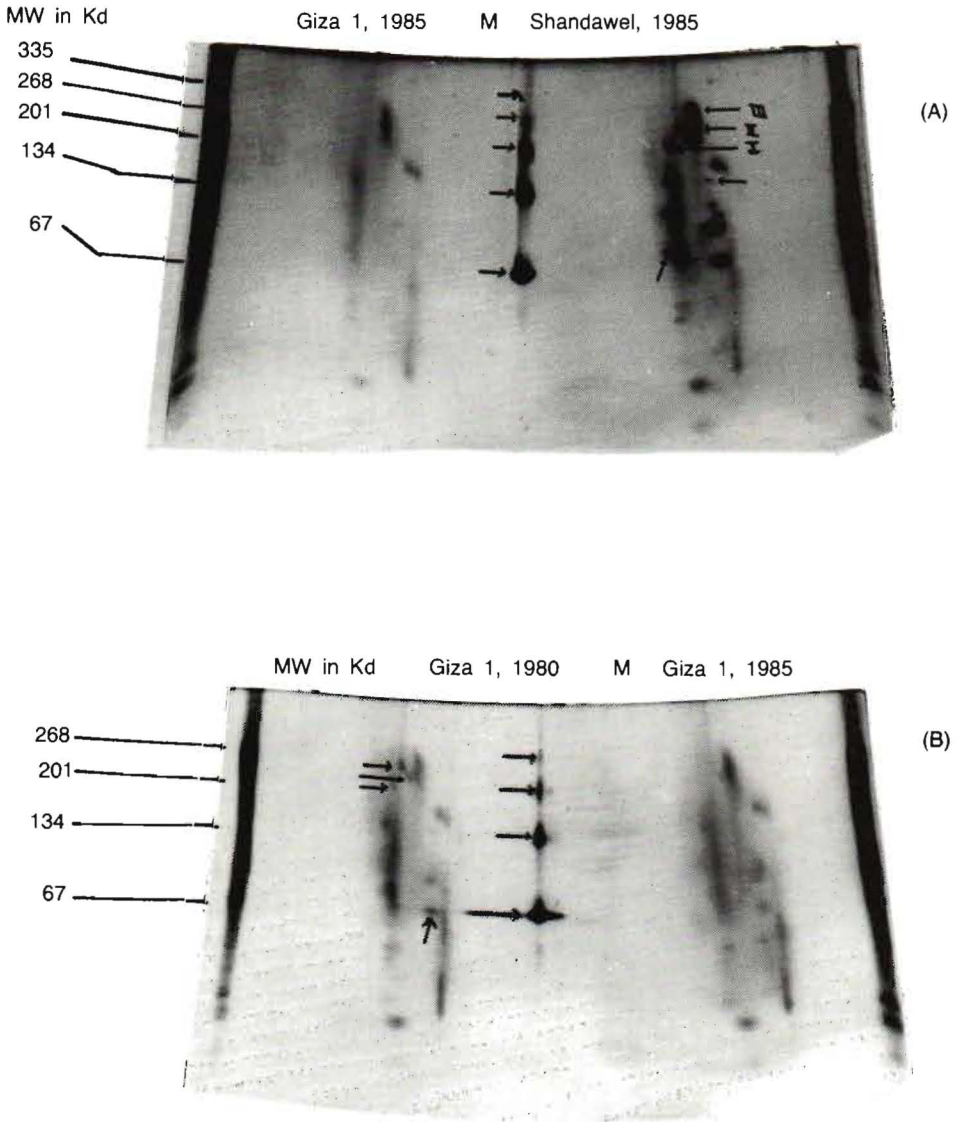

Fig. 2. Mapping (2-D)
First dimension: PAGIF in Servalyt T-pH 4-9 with 6 M urea.
Second dimension: SDS-PAGE (Laemmli) 5 and 15% PAA in tris-glycin buffer-pH 8.3 with 0.1% SDS.
M = SDS-Molecular weight markers.

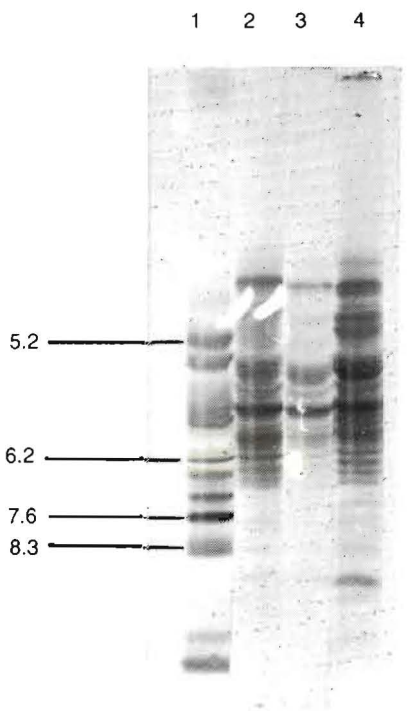

Fig. 3. PAGIF in tubes with Servalyt T-pH 4-9 with 6 M urea.
1 = Ip protein markers
2 = Giza 1, 1980
3 = Giza 1, 1985
4 = Shandawel, 1985

The results indicated that the protein map obtained from different onion seeds cultivars by combined techniques is even more specific for a given variety than the electrophoresis or electrofocusing patterns alone. Some other experiments should be done in the future to characterize the onion seed protein by fingerprinting of the peptides after tryptic digestion.

Finally mapping gel electrophoresis with SDS-PAGE and PoroPAGE in the second dimension has the potenial to provide precise detailed to differentiate between cultivars as well as between stored and fresh onion seeds.

Abbreviation:

Ip	= Isoelectric point
Kd	= Kilo dalton
MW	= Molecular weight
PAA	= polyacrylamide
APGIF	= Polyacrylamide gel isoelectric focusing
PoroPAGE	= Porosity gradient polyacrylamide gel electrophoresis
SDS	= Sodium dodecyl sulphate
SDS-PAGE	=SDS-polyacrylamide gel electrophoresis
Tris	= Hydroxymethyl-aminoethan
ME	= Mercaptoethanol.

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تحليل بذور البصل بالتفريد الكهربي في اتجاهين

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تم استخلاص بذور البصل المصرية أصناف جيزه ١، وشندويل بمحلول منظم مكون من الترس ـ حامض البوريك درجة حموضته ٨,٩. فحصت هذه البروتينات المستخلصة بالتفريد الكهربي (الالكتروفوريسيس) في اتجاهين، باستخدام جيل البولى اكربلاميد.

أجرى الفصل في الاتجاه الأول على حسب نقطة التعادل الكهربي للبروتينات باستخدام جيل يحتوي على محاليل مختلفة في درجة حموضتها. بينما أجرى الفصل في الإتجاه الثاني على حسب الوزن الجزئي للبروتينات تحت الدراسة بطريقتين مختلفتين. أثبت استخدام هذه الطريقة إمكانية التفرقة ما بين الأصناف المختلفة من بذور البصل بالإضافة إلى التمييز بين البذور المخزنة والبذور حديثة الإنتاج.