# Identification of protein markers and phylogenetic relationships in five potato cultivars*

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**Effat A. Badr*, A.A. Hemeida** O. M. Badawy*** and Hadia A. Hekal****

* Department Genetics, Faculty of Agriculture, Alexandria University

** Genetic Engineering and Biotechnology Research Institute, Menoufiya University, Sadat City

*** Department Breeding and Genetics, Agriculture Research, Station, Sabbahia, ARC, Alexandria

## ABSTRACT

*Molecular characterization of five potato cultivars, namely Alpha, Cara, Diamond, Draga and Spunta was performed using SDS-PAGE. Different developmental stages in each cultivar were also compared. Protein banding patterns showed differential gene expression in the different developmental stages in each cultivar. The dendrogram based on polymorphic protein bands showed the genetic distances between cultivars. The data showed that Spunta is closely related to Diamond, especially at the plant stage. Spunta is known to be drought-tolerant. Therefore, Diamond might also be planted under conditions of water stress.*

*The present research is the first to establish phylogenetic as well as ontogenetic relationships between important potato cultivars in Egypt. The method can be applied to other important crops, thus saving time, effort and expenses.*

***Key words:*** *Potato, protein markers, developmental stages, phylogenetic relationships.*

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## INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important and widely grown food crops in Egypt and in many other parts of the world. Molecular markers such as protein analysis, isozymes and randomly amplified polymorphic DNA (RAPD) have excellent potentiality to estimate genetic distances between cultivars, thus assisting breeding programs and gene-bank database (Badr and Mabrouk, 2000, Badr *et al*., 2001). They represent reliable tools for the identification of desired genotypes independent of environmental variations (Stuber, 1992).

Molecular characterization of different cultivars is of important economic value. Genetic fingerprinting and estimating genetic distances between cultivars can be of use in breeding programs, thus saving time, effort and expenses (El-Demerdash, 2000).

The objective of the present investigation is to characterize five potato cultivars, namely Alpha, Cara, Diamond, Draga and Spunta using protein analysis. Different developmental stages of each cultivar were also studied. Protein banding

patterns were analysed using SDS-PAGE. Dendrograms based on polymorphic protein bands were performed in order to estimate the genetic distances between the five cultivars.

## MATERIALS AND METHODS

### Plant material

Five potato cultivars (*Solanum tuberosum* L.), commonly grown in Egypt, namely; Alpha, Cara, Diamond, Draga and Spunta were used in the present study. The cultivars were obtained from the Ministry of Agriculture, Giza, Egypt.

### Callus, plantlet and microtuber formation

Young leaves of five potato cultivars were used as explants to initiate callus cultures (Figure 1A). The explants were plated on callus induction medium (Figure 1B) and the obtained calli were transferred on shoot induction medium (Figure 1C) (Visser, 1991). Microtubers (Figure 1D) were produced using specific protocols to maximize microtuberization according to Gopal *et al*. (1998).

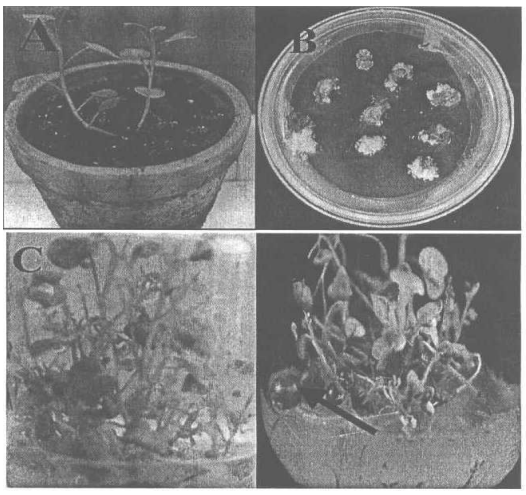


***Fig. (1): The stages of potato micro-propagation. A: plant, B: callus, C: plantlet, D: microtuber.***

### Protein analysis

One gram from each sample (plant, callus, plantlet and microtuber) was chopped into small pieces, frozen in liquid nitrogen and ground by mortar and pestle. The samples were extracted in 1 ml of extraction buffer consisting of 0.15 M Tris, 2% Triton X100 and 0.001 M EDTA. The extracts were centrifuged at 15000 rpm for 30 minutes and the supernatant was stored at -20ºC until used for electrophoresis.

For loading with SDS, 50 µl of the extract were mixed with 25 µl of an SDS/mercaptoethanol (ME) solution (5% each), incubated at 100ºC for 3 min using 1 ml test tubes in boiling water bath. Ten-percent sucrose was added to the latter solution to increase the specific gravity of the supernatant. Eight molecular weight standards were used as markers (Sigma), consisting of Myosin, Rabbit Muscle (205,000 KDa), β-Galactosidase, *E. coli* (116,000 KDa), Phosphorylase b, Rabbit Muscle (97,000 KDa), Fructose-6-phosphate Kinase, Rabbit Muscle (84,000 KDa), Albumin, Bovine Serum (66,000 KDa), Glutamic Dehydrogenase, Bovine Liver (55,000 KDa), Ovalbumin, Chicken Egg (45,000 KDa) and Glyceraldehyde-3-phosphate Dehydrogenase, Rabbit Muscle (36,000 KDa).

SDS-PAGE was performed in a vertical slab gel electrophoresis unit (SE600). The separating (10%) and the stacking (4%) gels were made according to Laemmli (1970). The running buffer consisted of 0.25 M Tris, and 0.057 M citric acid, pH 8.3. Separation was allowed to proceed for 5 hrs at constant current (3 mA per cm) at 4ºC. SDS gels were kept overnight in the staining solution composed of 2.5 ml of 1% aqueous solution of Coomassie Blue R-250 added to 100 ml TCA / methanol / acetic acid aqueous solution. The latter solution was prepared by the addition of 6 ml TCA, 20 ml methanol, 2 ml acetic acid (96%) and 80 ml

distilled water. For destaining or removal of the background color, the gels were immersed overnight in a solution composed of 96% acetic acid, methanol, and distilled water (2:12:28, v:v:v, respectively) according to Stegemann *et al*. (1985).

Bands on the gels were recorded as present (1) or absent (0) and used to create a data matrix. The Phoretic 1D image analysis system (Phoretix International, London) was used to integrate the data of the protein bands. A similarity dendrogram was produced using NTSYS (Numerical Taxonomic and Multivariate Analysis System) software package, version 2.1, Applied Biostatistics Inc. (Rohlf, 2000).

## RESULTS AND DISCUSSION

The descriptive densitometric electrophoregrams and diagrams of total protein patterns of the four tested stages, namely plant (A), plantlet (B), callus (C) and microtuber (D) of the five potato cultivars; Alpha, Cara, Diamond, Draga and Spunta are illustrated in Figures 2, 3, 4, 5 and 6, respectively. It can be clearly observed that all of the five potato cultivars, at all tested developmental stages, exhibited fairly different norms of banding (Figs 2-6 and Table 1). The total number of detectable bands was similar in Alpha and Cara (56) and in Diamond and Spunta (60). Draga showed the highest number of total bands (67). Alpha cultivar showed a similar number of bands in plants and plantlets (13) as is the case with Diamond (11). The calli and microtubers, however, displayed additional bands in all cultivars. It is noteworthy that plants and plantlets each showed fairly similar numbers of protein bands in each cultivar.

***Table (1): Levels of polymorphic markers in the plants, calli, plantlets and microtubers of five potato cultivars.***

| | Stages | Cultivars | | | | | Total bands screened |
|---|---|---|---|---|---|---|---|
| | | Alpha | Cara | Diamond | Draga | Spunta | |
| **Plant** | Detectable bands | 13 | 12 | 11 | 16 | 13 | 65 |
| | Polymorphic markers | 2 | 1 | 1 | 4 | 2 | 10 |
| | Polymorphic markers% | 15.38 | 8.33 | 9.09 | 25.00 | 15.38 | 6.5 |
| **Callus** | Detectable bands | 16 | 17 | 21 | 18 | 19 | 91 |
| | Polymorphic markers | 4 | 5 | 7 | 5 | 6 | 27 |
| | Polymorphic markers% | 23.52 | 29.4 | 33.33 | 27.7 | 31.57 | 30 |
| **Plantlet** | Detectable bands | 13 | 14 | 11 | 15 | 12 | 65 |
| | Polymorphic markers | 8 | 8 | 6 | 10 | 7 | 39 |
| | Polymorphic markers% | 61.54 | 57.14 | 54.55 | 66.57 | 58.33 | 60 |
| **Microtuber** | Detectable bands | 17 | 13 | 17 | 18 | 16 | 81 |
| | Polymorphic markers | 4 | 1 | 4 | 5 | 3 | 17 |
| | Polymorphic markers% | 25.00 | 7.69 | 23.52 | 27.78 | 18.75 | 20 |
| | Total detectable bands | 56 | 56 | 60 | 67 | 60 | 302 |

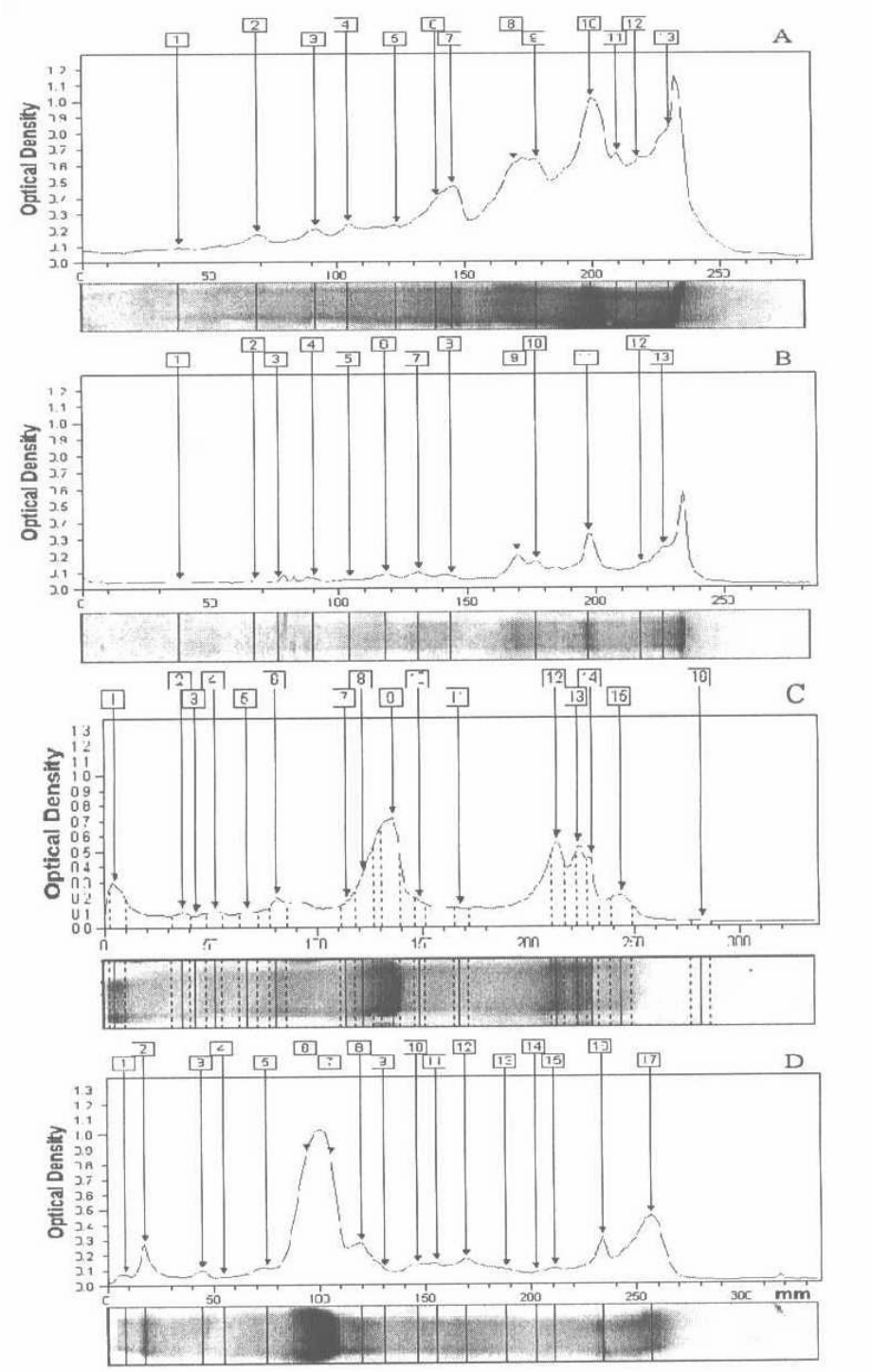


***Fig. (2): Total protein densitometric scans of Alpha cultivar of the tested stages. Plant stage (A), plantlet stage (B), callus stage (C) and microtuber stage (D).***

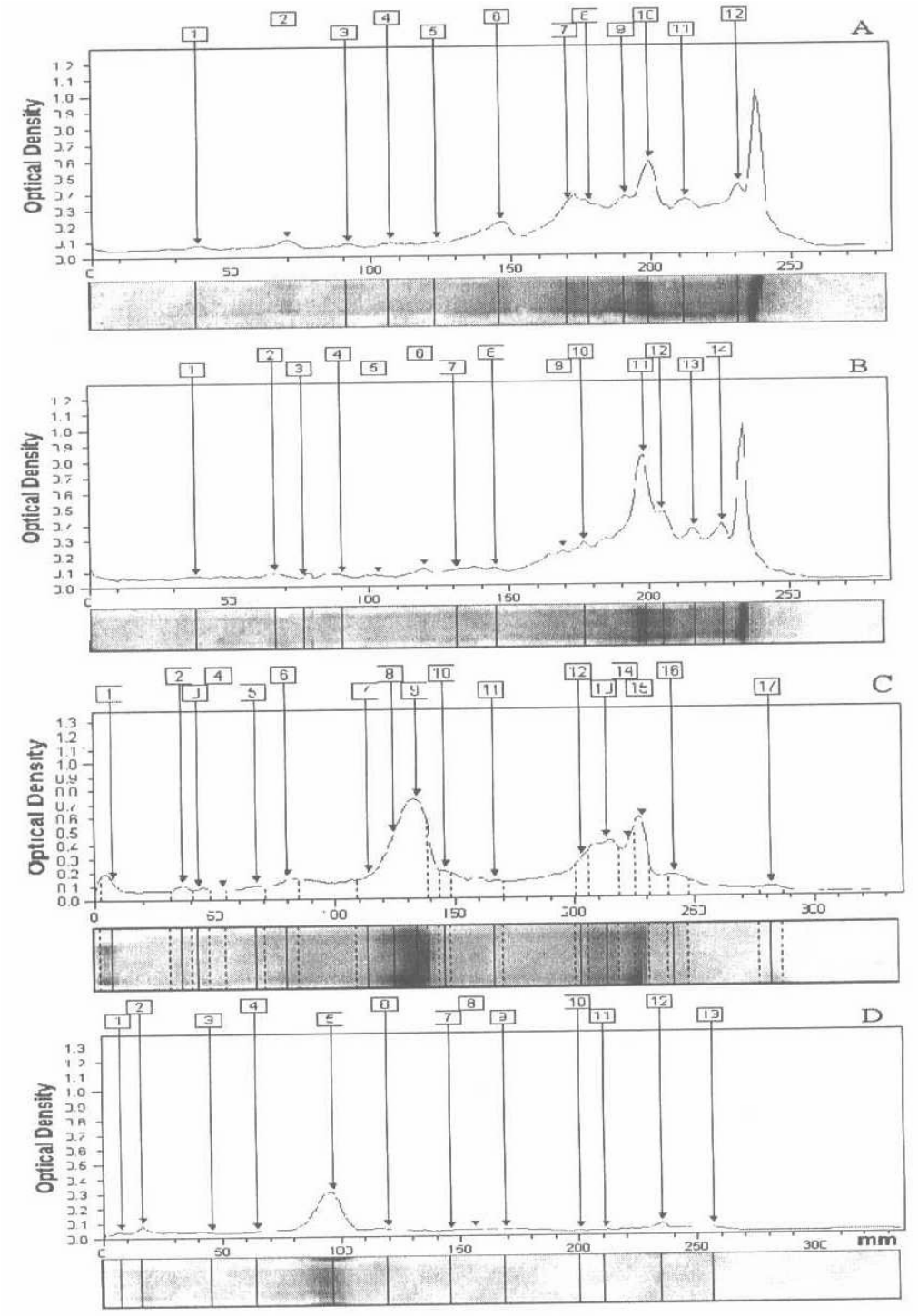


***Fig. (3): Total protein densitometric scans of Cara cultivar of the four tested stages. Plant stage (A), plantlet stage (B), callus stage (C) and microtuber stage (D).***

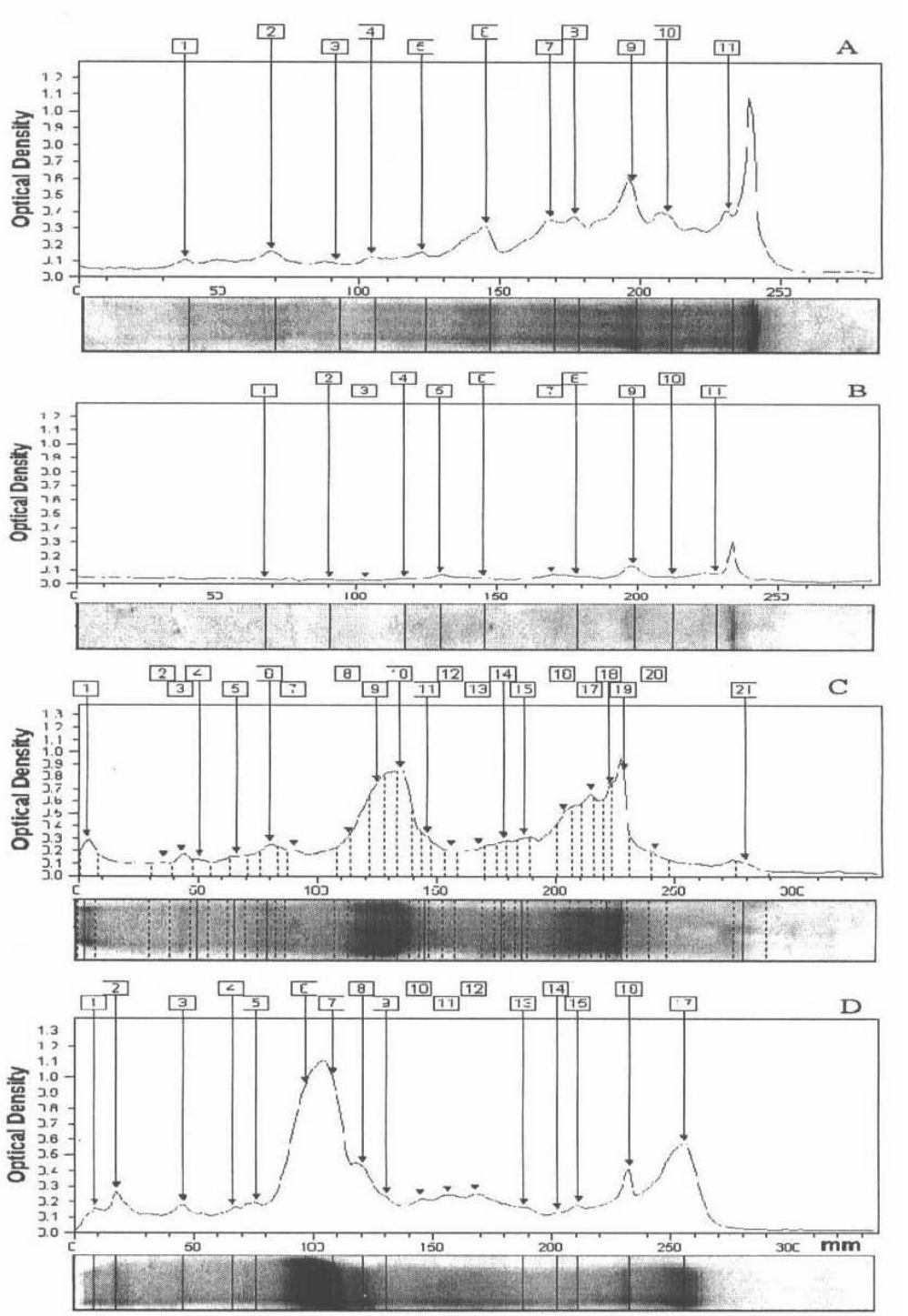


***Fig. (4): Total protein densitometric scans of Diamond cultivar of the four tested stages. Plant stage (A), plantlet stage (B), callus stage (C) and microtuber stage (D).***

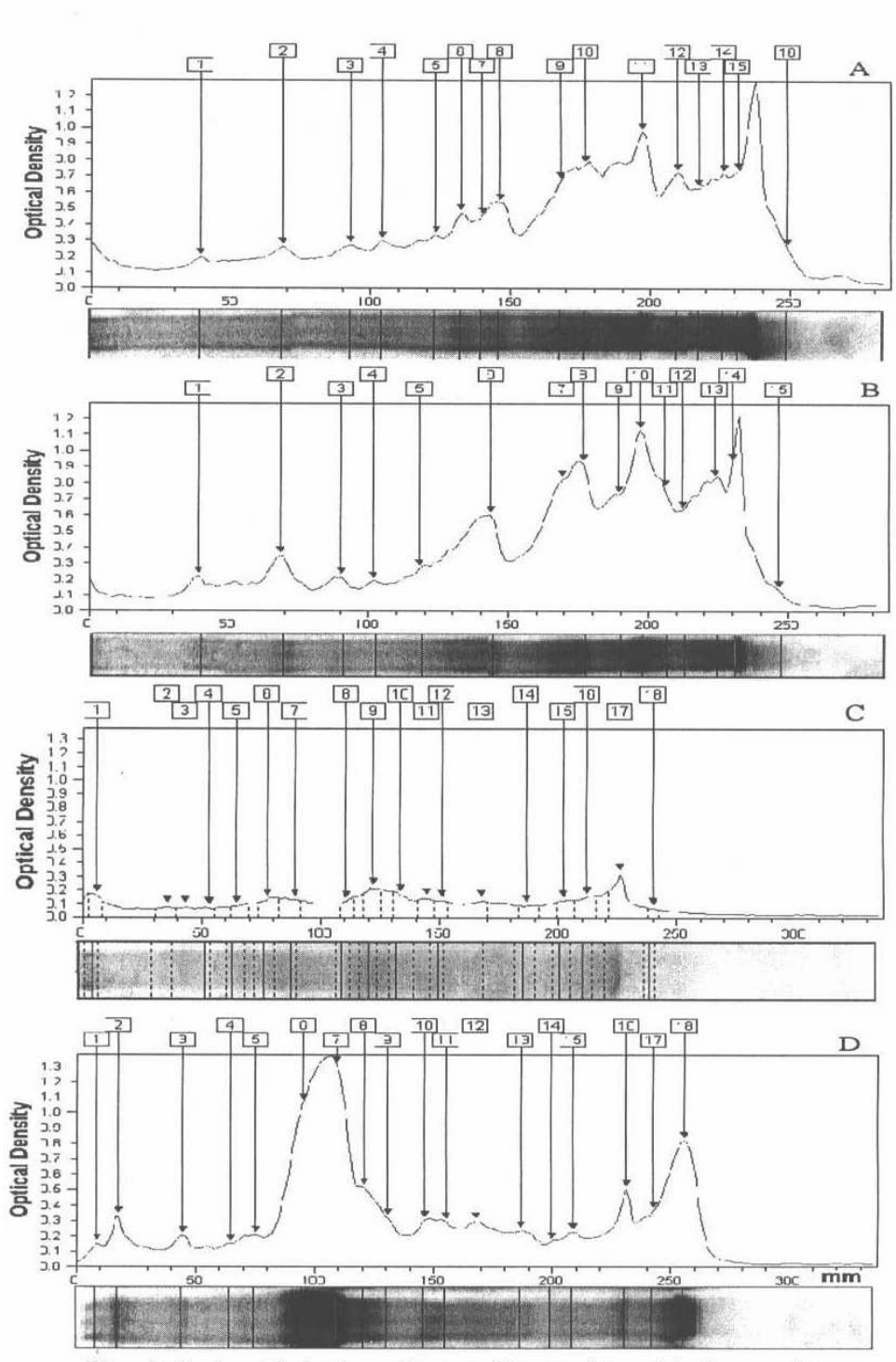


***Fig. (5): Total protein densitometric scans of Draga cultivar of the four tested stages. Plant stage (A), plantlet stage (B), callus stage (C) and microtuber stage (D).***

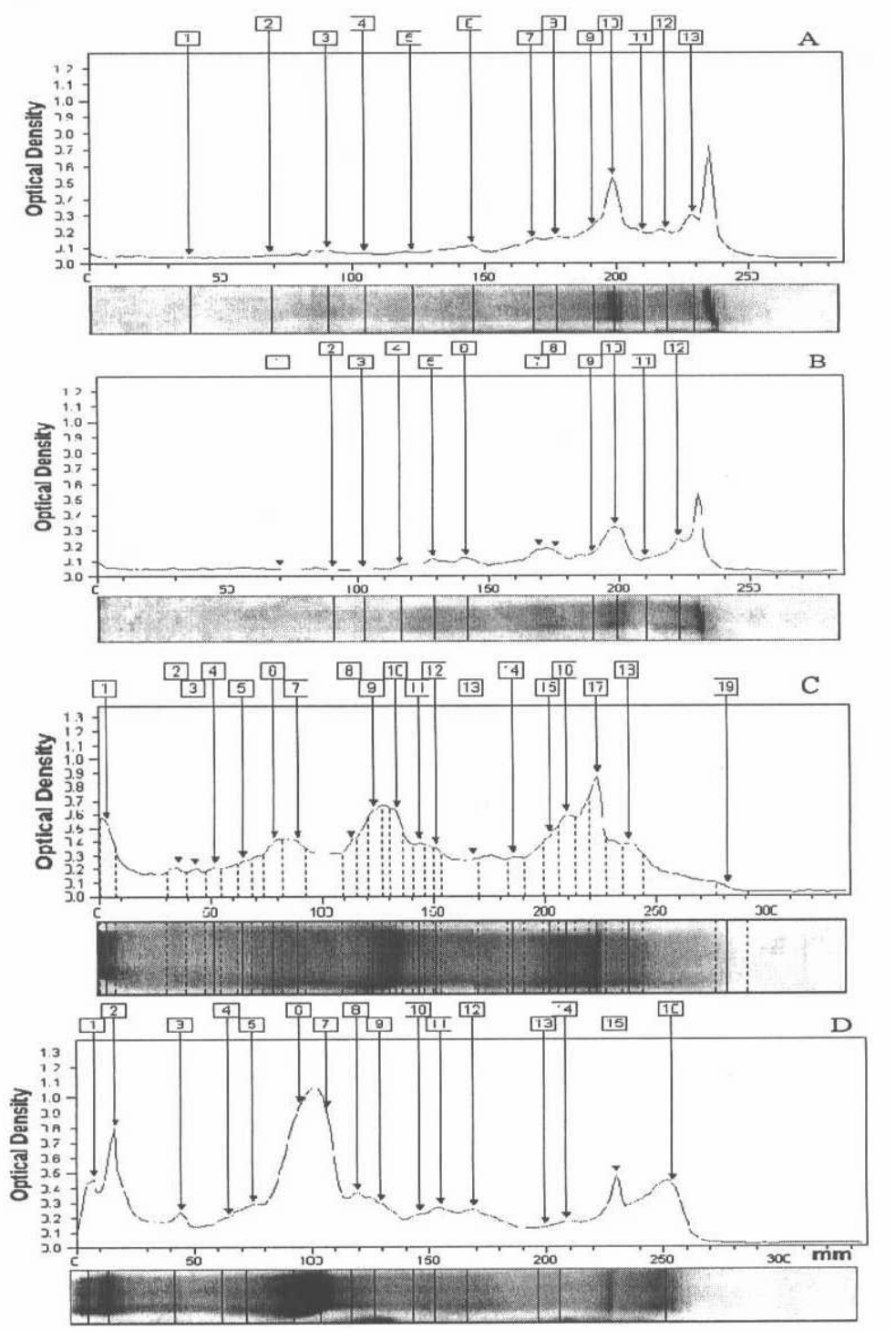


***Fig. (6): Total protein densitometric scans of Spunta cultivar of the four tested stages. Plant stage (A), plantlet stage (B), callus stage (C) and microtuber stage (D).***

The results of screening the polymorphic markers in the different stages are given in Table (1). Only clearly scorable bands were used. The chosen electrophoretic protein patterns revealed a total of 302 bands. The number of detectable bands obtained for each stage ranged from 11 to 21, with molecular weights ranging from 212,480 - 12,620 Daltons. The number of polymorphic bands ranged from 1 to 10. In total, 93 clearly scorable polymorphic bands (30.8% of total bands) identified the different stages and were analyzed. Furthermore, the obtained results showed that in the plantlet stage of all cultivars, the percentages of polymorphic markers were significantly higher compared with the other developmental stages (about 60%, in average).

The dendrograms obtained by the cluster software NTSYS method, showing the phylogenetic positions based on the electrophoretic protein patterns of plants, calli, plantlets and microtubers of the five potato cultivars are shown in Figure (7). In each case two major clusters appeared. Calli of Alpha, Diamond, Draga and Spunta were closely related, being in the same cluster. However, in the plantlet stage, Spunta appeared in a different cluster from all of the other cultivars. Cara, Diamond and Spunta plants were very closely related, belonging to the same group. In microtubers, Diamond, Draga and Spunta appeared in the same cluster. Thus, different developmental stages, in all cultivars, have different dendrogram patterns. This can be attributed to differential gene expression at different developmental stages (Abdel-Tawab *et al*., 1998a).

The present study is a preliminary step to determine and locate molecular markers in economic plants (Stegemann *et al*., 1985; Fahmy and Okasha, 1992; Abdel-Tawab *et al*., 1998b; and DaSilva *et al*., 1993). Our results represent a method for characterization of genetic distances using protein markers. Different protein bands, shown in different cultivars indicate differential gene expression (Singh *et al*., 1985; El-Farash *et al*., 1998 and Abdel-Tawab *et al*., 1998a). It is of practical value to note that Spunta cultivar, which is drought-tolerant (El-Demerdash, 2000) is closely related to Diamond cultivar; plants of both cultivars belong to the same group. The latter cultivar can thus be cultivated under conditions of water stress. These methods can be applied to other economic plants and can be of great value for rapid and accurate characterization of different cultivars, contributing to gene-bank database as well as breeding strategies.

It may be emphasized that the present research is the first to identify and establish phylogenetic as well as ontogenetic relationships between important potato cultivars in Egypt.

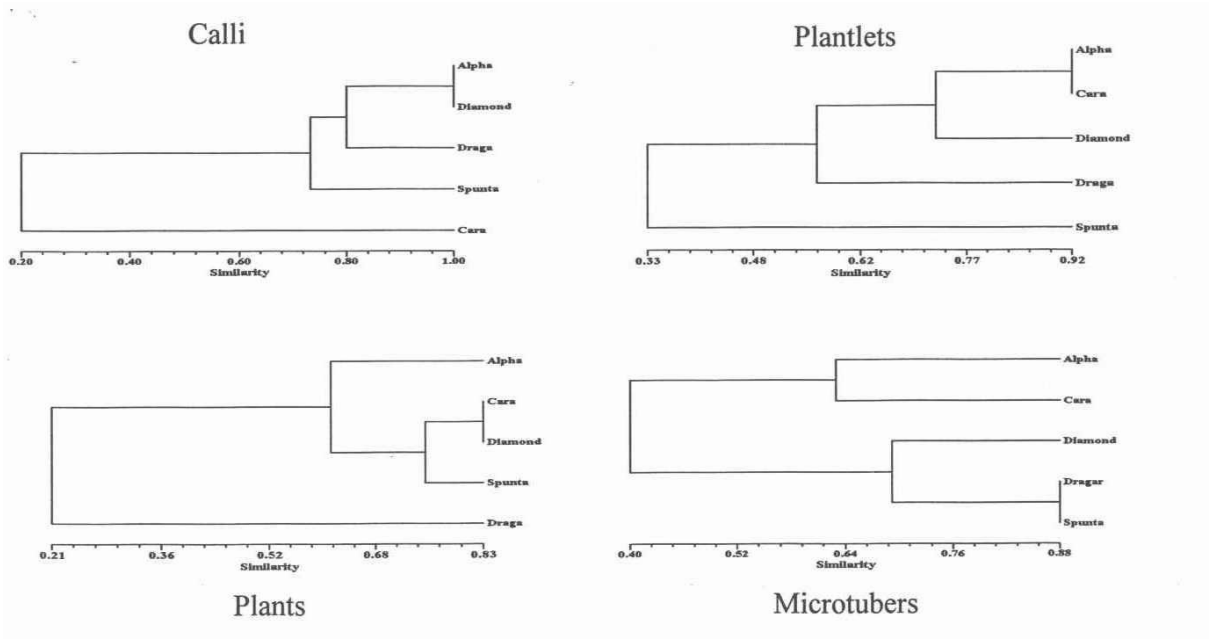


***Fig. (7): Dendrograms showing the relationships among the electrophoretic protein patterns of plants, plantlets, calli and microtubers of five potato cultivars.***

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## الملخص العربي

### تحديد واسمات بروتينية وعلاقات القرابة الوراثية لخمسة أصناف من البطاطس

**عفت عبداللطيف بدر* ، علاء الدين عبدالله حميدة** ، أسامة مصطفى بدوى*** وهادية أحمد هيكل****

* قسم الوراثة – كلية الزراعة – جامعة الأسكندرية – الأسكندرية – مصر

** معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية –جامعة المنوفية – مدينة السادات – مصر

*** قسم الوراثة والتربية – محطة البحوث الزراعية – الصبحية – مركز البحوث الزراعية – الأسكندرية

أستخدمت فى هذه الدراسة تقنية التفريد الكهربى للبروتينات (SDS-PAGE) للتعرف والتمييز الجزيئى بين خمسة أصناف من البطاطس بمراحلها التطورية المختلفة والمنزرعة فى مصر ، حيث تم تحليل النتائج المتحصل عليها إعتماداً على نظام حزم البروتين ، وحسبت على أساسه المسافة الوراثية بين هذه الأصناف . وقد أظهرت النتائج أن الصنف سبونتا قريب وراثياً من الصنف دياموند خاصة فى مرحلة النبات الكامل . وحيث أن الصنف سبونتا معروف بأنه يتحمل الجفاف فيمكن التنبؤ بأن الصنف دياموند ربما يمكن زراعته تحت ظروف قلة مياه الري . ويمكن تطبيق مثل هذه الدراسة على نباتات إقتصادية أخرى حيث يمكن التعرف بسرعة وسهولة على الأصناف القريبة وراثياً من بعضها مما يساعد كثيراً في برامج التربية وبنوك الجينات .

والجدير بالذكر أن هذه الدراسة هي الأولى من نوعها لتحديد العلاقات الوراثية بين أصناف من البطاطس الهامة فى مصر باستخدام الواسمات الجزيئية .