BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2016. Vol. **13**(4), 1959-1967

Characterization and Identification of Seed Storage Protein of Twelve Lettuce Cultivars

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http://dx.doi.org/10.13005/bbra/2350

(Received: 30 October 2016; accepted: 08 November 2016)

We used SDS-PAGE to evaluate and characterize the protein patterns of seed storage proteins in 12 lettuce cultivars. Total protein content of lettuce seeds in all cultivars did not show any significant difference. Results of SDS- PAGE pattern of a few protein bands were up regulated whereas some other bands showed down regulation. The identified protein patterns may be used protein marker for lettuce cultivars. The seed storage protein analyses helps in characterization and identification of diversity in lettuce crop varieties, cultivars and their wild varieties and also provides information on phylogenetic relationship of the accessions. It is also known that variation in protein bands provide information on the relationship among the used seeds. SDS-PAGE of seed protein using Tris-glycine buffer was performed to study the relationships within 12 cultivars of *Lactuca sativa* L. (lettuce). In the present study, SDS-PAGE was used to investigate and characterize the protein patterns of seed storage proteins in order to find protein bands as markers for cultivar characterization. Data were analyzed by clustering method and similarity coefficients using *NTSYSpc* version 2.02i. Thevalidity of cluster analysis of SDS-PAGE seed proteins profile data was discussed.

Keywords: SDS-PAGE; Seed Protein; Lettuce; (*Lactuca sativa*); Cultivars; Numerical Analysis; Cluster Method; Relationships.

The genus *Lactuca L.* is the one of the economically and medicinally important genera of *Asteraceae* family, subfamily *Cichorioideae*, tribe the *Lactuceae*, *lant* genetic resources comprise all agricultural crops and their wild relatives of valuable traits[1]. The genus *Lactuca* L. belonging to the Asteraceae family, is widely distributed in different geographical and ecological areas. Lettuce and most of the other species of the genus *Lactuca* L. have been cultivated for their economic and medicinal importance. This review summarizes recent knowledge of the application of biochemical (isozymes) and molecular technologies in *Lactuca germplasm* in order to better understand the genetic variation, interspecific relationships, taxonomy and breeding as a basis for further research studies. This is in order to better understand and improve breeding programmers and biology of crops for future use in agriculture and food security. Hence, advanced molecular genetic technologies including biochemical and molecular markers, have been developed to overcome those limitations of morphological and cytological traits[2,3,4,5,6]. Seed germination is a complex process that is influenced by many environmental factors, such as light, temperature, and moisture[7]. Recently,the genome of *Lactuca sativa* cv Salinas was sequenced[2]. Genetic variation studies are vital for providing information for propagation, taxonomy, disease resistance, and breeding programs as well as conservation and

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utilization of *Lactuca* genetic resources. Genetic diversity can be evaluated based on morphological, cytogenetic, biochemical and molecular markers[8-12]. However, the evaluation of genetic variation based on morphological and cytological traits has the disadvantages of being affected by both genetic and environmental factors and may not provide an accurate measure[13].

Lettuce (*Lactuca sativa*) is a temperate annual or biennial plant of the daisy family Asteraceae. It is most often grown as a leaf vegetable. In many countries, it is typically eaten cold, raw, in salads, sandwiches, hamburgers, tacos and in many other dishes. In some places, including China, lettuce is typically eaten cooked and use of the stem is as important as use of the leaf. Both the English name and the Latin name of the genus are ultimately derived from *lac*, the Latin word for "milk" referring to the plant's milky juice[14]. Mild in flavour, it has been described over the centuries as a cooling counterbalance to other ingredients in a salad. The earliest depiction of lettuce is in the carvings at the temple of Senusret at Karnak, where he offers milk to the god Min, to whom the lettuce was sacred. Lettuce was considered an aphrodisiac food in Ancient Egypt and appears as such in the contendings of Horus and Seth. Later, Ancient Greek physicians believed lettuce could act as a sleep-inducing agent. The Romans cultivated it, and it eventually made its way to the Papal Court at Avignon, France[15].

There are six commonly recognized cultivar groups of lettuce which are ordered here by head formation and leaf structure; there are hundreds of cultivars of lettuce selected for leaf shape and color, as well as extended field and shelf life, within each of these cultivar groups: (1) Butterhead (*L. sativa* var. *capitata* L.) forms loose heads. Its leaves have a buttery texture. Butterhead cultivars are most popular in Europe. Popular varieties include Boston, Bibb, Buttercrunch, and Tom Thumb. (2) Chinese lettuce (*L. sativa* var. *asparagina* Baily.) types generally have long, sword-shaped, non-head-forming leaves, with a bitter and robust flavour unlike Western types, for use in stir-fried dishes and stews. (3) Crisphead, also called Iceberg, forms tight, dense heads that resemble cabbage. They are generally the mildest of the lettuces, valued more for their crunchy texture than for flavour. Cultivars of iceberg lettuce are the most familiar lettuces in the USA. The name Iceberg refers to the crisp, cold, clean characteristics of the leaves. (4) Loose-leaf *(L. sativa* var.*crispa*L.) has tender, delicate and mildly flavored leaves. This group includes oak leaf and lollorosso lettuces. (5) Romaine (*L. sativa* var.*romana* Lam.), also called Cos, grows in a long head of sturdy leaves with a firm rib down the center. (6) Summer Crisp, also called Batavian, forms moderately dense heads with a crunchy texture. This type is intermediate between iceberg and loose leaf types.

Lettuces contain antioxidants and Vitamin K, romaine and loose leaf lettuce contain five to six times the Vitamin C and five to ten times the Vitamin A of iceberg. Romaine and butterhead lettuce are good sources of folate. Lettuce naturally absorbs and concentrates lithium[16]. The taxonomicstatus of cultivated lettuce (*Lactuca sativa*L.), the boundaries among *L. sativa* and close relatives and the boundaries of the genus *Lactuca* L., it self have been the subject of controversy among taxonomists for many decades.

Plant storage proteins can be classified into two classes; seed storage proteins (SSPs) and vegetative storage proteins (VSPs). SSPs are a set of proteins that accumulate at high levels in seeds during the late stages of seed development, whereas VSPs are proteins that accumulate in vegetative tissues such as leaves, stems and tubers, depending on the plant species. SSP genes were classic targets for work on plant molecular biology[17].

The abundant expression of plant storage proteins in seeds allowed for easy detection of the gene transcripts and cDNA cloning during research on plant molecular biology in late 70's to early 80's. Characterization of germplasm using biochemical fingerprinting has got special attention due to its increased used in crop improvement and the selection of desirable genotypes for breeding crops. The use of genetic markers and protein profiling has also been successfully used to resolve the taxonomic and evolutionary problems of several crop plants[18,19,20,21,22].

Seed protein is highly stable, being unaffected by environmental conditions[23]. Thus electrophoretic banding patterns of total seed protein as revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl

sulfate (SDS-PAGE) have provided a valid source of taxonomic evidences and were used to address taxonomic relationships at the generic and specific levels, for example *Trifolium*[24]**,** *Sesbania*[25]**,** the genus *Vicia*[26], the genus *Lathyrus*[27],*Lens esculenta*[28], *Hordeum vulgare*[29], and *Psidium*[30], *Rosaceae*[31], *Cruciferae*[32], *Raphanus* L.[33], *Campanulaceae*[33], *Gentiana* L. and *Gentianella* Moench,[34], canola[35],Onobrychis[36]and *Hordeum Vulgare* L. [37].

The seed storage protein analyses help in identification and characterization of diversity in crop varieties, cultivars and their wild varieties and provides information on phylogenetic relationship of the accessions. It is also known that variation in protein bands provide information on the relationship among the used seeds collected from various geographical regions[38,22,39]. There are different amounts of storage proteins in all plant seeds. They play two main roles including nitrogen and energy source and defense against insects and pathogens such as bacteria and fungi.

The aim of this study is to summarize recent knowledge of the seed storage protein analysis can be a useful tool for identification of species, varieties and cultivars, in the present study, SDS-PAGE was used to investigate and characterize the protein patterns of seed storage proteins in 12 cultivars of *Lactuca sativa* L. (lettuce) to find protein bands as markers for cultivar characterization. Data were analyzed by clustering method and similarity coefficients using NTSYS-pc version 2.02i. The validity of cluster analysis of SDS-PAGE seed proteins profile data was discussed.

MATERIALS AND METHODS

Plant materials

In the present study, sample of 12 cultivars representing *Lactuca sativa* L. collected from different botanic garden and from the ministry of agriculture in Egypt were used Table (1).

Methods of Analysis

Protein Extraction and Electrophoresis

Seed proteins of the studied samples were analyzed using cont-SDS PAGE based on the method of Weber & Osbon[40]. The electrophoretic analysis was carried out using Tris-glycine (pH 8.2) as an extraction buffer. 0.1 g of seeds was mixed with an equal weight of pure, clean, sterile fine sand and powdered using a mortar and pestle. Extraction was performed overnight. After centrifugation at 15.000 rpm for 10 min, the supernatant was firstly mixed with equal volume of Tris-HCl pH 6.8 (as a digestion buffer) and boiled for 5 min in water path[41], then the supernatant was taken for loading on 12% polyacrylamide gels. Electrophoresis was carried out in a Tris-glycine buffer pH 8.3 at 150 V for 2 - 2.5 h, using a low molecular weight protein mixture as a marker in each run. Gels were then stained in Comassie brilliant blue R-250 for 2 h, distained, photographed and the number of bands revealed in each gel lane were counted and compared with each other. Gel Pro-Analyzer software version 2.0 was used to determine the molecular mass (MM) of each protein band.

Numerical Analysis

The data of SDS-PAGE seed protein banding patterns were scored as present (1) and absence (0). The data was analyzed to compare various similarity coefficients and clustering methods and to study the relationships among the cultivars detail. Clustering methods and similarity coefficients were tested using the procedures SIMQUAL, SAHN and TREE from the program NTSYS-pc version 2.02i[42].

RESULTS AND DISCUSSION

SDS-PAGE Electropherogram of the Storage Seed Proteins

Seed protein analysis was carried out on 12 cultivars of lettuce represented the four type of lettuce and the electropherograms produced from seed protein analysis using Tris-glycine buffer revealed great polymorphism among these cultivars as illustrated in Table (2) and Fig. (1). A total number of 73 protein bands were observed within the studied cultivars (57 unique bands and 16 polymorphic bands). Each cultivar was characterized by the presence of unique bands (ranged from 3 bands, recorded in cv. no; 2&3 to 7 bands observed in cv. no; 1). Cultivar no; 10 was found to have six bands and all of them were unique for this cultivar. Cv. no; (11) was found to have the highest number of bands (10), while the lowest number of bands (6) was observed in the sample of cv. no; (10).

Table 1. The Names of Lettuce Plant (*Lactuca sativa*) Cultivar and The Sources in This Study

S. No.	Scientific Name	CV.	Type	Source
1	*Lactuca sativa* var. *capitata*L.	Great Lakes	Cabbage, Butter Head or Headed	659 (WS) U S A
2	*Lactuca sativa* var. *capitata* L.	All The Year Round	Cabbage, Butter Head or Headed	Italy
3	*Lactuca sativa* var. *longifolia* Lam.	Local	Cos or Romaine	Egypt
4	*Lactuca sativa* var. *longifolia* Lam.	Romaine	Cos or Romaine	Egypt
5	*Lactuca sativa* var. *crispa* L.	Salad Bowl	Leafy or Curled	U S A
6	*Lactuca sativa* var. *crispa* L.	Simpson's Curled	Leafy or Curled	U S A
7	*Lactuca sativa* var. *capitata* L.	Butter crunch	Cabbage, Butter Head or Headed	U S A
8	*Lactuca sativa* var. *capitata* L.	Lattuga	Cabbage, Butter Head or Headed	Egypt
9	*Lactuca sativa* var. *capitata* L.	Great Lakes	Cabbage, Butter Head or Headed	Egypt
10	*Lactuca sativa* var. *capitata* L.	Great Lakes	Cabbage, Butter Head or Headed	Bonn - Germany
11	*Lactuca sativa* var. *longifolia* Lam.	Romaine	Cos or Romaine	206 Marseille - France
12	*Lactuca sativa* var. *longifolia*Lam.	Romaine	Laitue Romaine verteMaraichère	France

Table 2. The molecular weights of protein bands extracted in Tris-glycine Buffer of the Studied different lettuce Plants for 12 cultivars

No;	Mol. Weights	OTU's												Polymorphism
		1	2	3	4	5	6	7	8	9	10	11	12	
1	71.579	0	0	0	0	0	0	0	0	0	0	0	1	Unique
2	62.255	0	0	1	0	0	0	0	0	0	0	0	0	Unique
3	56.846	0	0	0	0	0	0	1	0	0	0	0	1	Polymorphic
4	56.662	0	0	0	0	0	0	0	0	1	0	0	0	Unique
0	56.479	0	1	0	0	0	0	0	0	0	0	1	0	Polymorphic
6	55.931	0	0	0	0	0	0	0	1	0	0	0	0	Unique
7	55.75	1	0	1	0	0	0	0	0	0	0	0	0	Polymorphic
8	55.21	0	0	0	1	0	0	0	0	0	0	0	0	Unique
9	54.321	0	0	0	0	0	1	0	0	0	0	0	0	Unique
10	53.795	0	0	0	0	1	0	0	0	0	0	0	0	Unique
11	49.925	0	0	0	0	0	0	0	0	0	0	1	0	Unique
12	46.184	1	0	0	0	0	0	0	0	0	0	0	0	Unique
13	45.736	0	0	0	0	0	0	0	0	1	0	0	1	Polymorphic
14	45.44	0	0	0	0	1	1	0	1	0	0	0	0	Polymorphic
15	45.293	0	1	0	0	0	0	1	0	0	0	1	0	Polymorphic
16	44.357	0	0	0	1	0	0	0	0	0	0	0	0	Unique
17	41.276	0	0	0	0	0	0	0	0	0	0	1	0	Unique
18	40.249	1	0	1	0	0	0	0	0	0	0	0	0	Polymorphic
19	40.105	0	0	0	1	1	0	0	0	1	0	0	1	Polymorphic
20	39.961	0	1	0	0	0	0	1	1	0	0	0	0	Polymorphic
21	39.817	0	0	0	0	0	1	0	0	0	0	0	0	Unique
22	36.918	1	0	0	0	0	0	0	0	0	0	0	0	Unique
23	36.522	0	0	1	0	0	0	0	0	0	0	1	0	Polymorphic
24	36.26	0	0	0	0	0	0	0	0	0	0	0	1	Unique
25	36	0	0	0	0	0	0	0	0	1	0	0	0	Unique
26	35.545	0	0	0	0	0	0	1	0	0	0	0	0	Unique

27	35.245	0	1	0	0	0	0	0	1	0	0	0	0	Polymorphic
28	34.947	0	0	0	0	1	1	0	0	0	0	0	0	Polymorphic
29	34.799	0	0	0	1	0	0	0	0	0	0	0	0	Unique
30	34.652	0	0	0	0	0	0	0	0	0	1	0	0	Unique
31	32.655	1	0	0	0	0	0	0	0	0	0	0	0	Unique
32	32.38	0	0	0	0	0	0	0	0	0	0	1	0	Unique
33	32.106	0	0	0	0	0	0	0	0	1	0	0	0	Unique
34	31.835	0	0	0	0	0	0	0	0	0	1	0	0	Unique
35	31.566	0	0	1	0	0	0	0	0	0	0	0	0	Unique
36	31.433	0	1	0	1	1	0	1	0	0	0	0	1	Polymorphic
37	31.3	0	0	0	0	0	0	0	1	0	0	0	0	Unique
38	31.167	0	0	0	0	0	1	0	0	0	0	0	0	Unique
39	26.785	0	0	0	0	0	1	0	0	0	0	0	0	Unique
40	26.66	0	0	0	0	0	0	1	0	0	0	0	0	Unique
41	25.984	0	0	0	1	0	0	0	0	0	0	0	0	Unique
42	24.111	0	0	0	0	0	0	0	0	1	0	0	0	Unique
43	23.554	1	0	0	0	0	0	0	0	0	0	0	0	Unique
44	22.796	0	1	0	0	0	0	0	0	0	0	0	0	Unique
45	22.322	0	0	0	0	0	0	0	0	0	1	0	0	Unique
46	22.063	0	0	0	1	0	1	0	0	0	0	1	0	Polymorphic
47	21.96	0	0	1	0	0	0	1	0	1	0	0	1	Polymorphic
48	21.908	0	0	0	0	1	0	0	0	0	0	0	0	Unique
49	21.857	0	0	0	0	0	0	0	1	0	0	0	0	Unique
50	20.141	1	0	0	0	0	0	0	0	0	0	0	0	Unique
51	16.883	1	0	0	0	0	0	0	0	0	0	0	0	Unique
52	16.833	0	0	0	0	0	0	0	0	0	0	1	0	Unique
53	16.783	0	0	0	0	0	0	1	0	0	0	0	0	Unique
54	16.733	0	0	0	0	0	1	0	0	0	0	0	0	Unique
55	16.634	0	0	0	0	0	0	0	0	0	1	0	0	Unique
56	16.486	0	0	0	0	1	0	0	0	0	0	0	0	Unique
57	16.437	0	0	1	0	0	0	0	0	0	0	0	0	Unique
58	16.389	0	0	0	1	0	0	0	1	0	0	0	0	Polymorphic
59	16.34	0	0	0	0	0	0	0	0	0	0	0	1	Unique
60	16.051	0	0	0	0	0	0	0	0	1	0	0	0	Unique
61	15.581	0	0	0	0	0	0	0	0	0	1	0	0	Unique
62	15.535	0	1	0	0	0	0	0	0	0	0	0	0	Unique
63	14.902	0	0	0	0	0	0	0	0	0	0	1	0	Unique
64	13.958	0	0	0	0	0	0	0	1	0	0	0	0	Unique
65	13.876	0	0	0	0	0	0	0	0	0	1	0	0	Unique
66	13.793	0	0	0	0	0	0	0	0	0	0	1	0	Unique
67	13.753	0	1	0	0	0	0	0	0	0	0	0	0	Unique
68	13.59	0	0	0	0	0	0	1	0	0	0	0	0	Unique
69	13.39	1	0	0	0	0	0	0	0	0	0	0	0	Unique
70	13.31	0	0	0	0	0	0	0	0	1	0	0	0	Unique
71	13.271	0	0	0	0	0	0	0	0	0	0	0	1	Unique
72	12.73	0	0	0	0	1	0	0	0	0	0	0	0	Unique
73	9.57	0	0	0	0	1	0	0	0	0	0	0	0	Unique
Total no; of bands		9	8	7	8	9	8	9	8	9	6	10	9	
Number of unique bands		7	3	3	4	5	5	4	4	6	6	6	4	

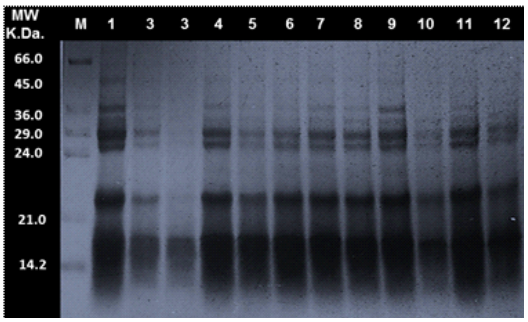

Fig. 1. Electrophoretic banding profiles of seed proteins extracted in Tris-glycineBuffer of the Studied different lettuce Plants for 12 cultivars

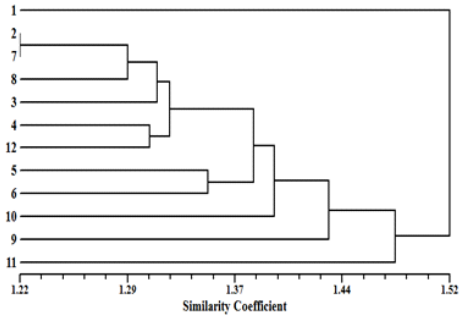

Fig. 2. UPGMA - phenogram constructed for 12 lettuce cultivars based on coding of 73 attributes obtained from SDS-PAGE profiles of seeds proteins extracted in Tris-glycine buffer

The highest molecular weight protein band (71.579 KDa) among the studied samples was recorded in cv. no; (12), while the lowest one (09.570 KDa) was detected in the cv. no;(5).

Seed protein banding patterns as revealed by SDS-PAGE produces reproducible band pattern (profile) when proteins are prepared in a standard method and hence have valid value in taxonomic purposes. Consequently, proteins with identical electrophoretic mobility are deemed to represent the same unit character. Therefore characters derived from seed proteins have been utilized in plant taxonomy at different levels to construct phonetic classifications[43,44,45]**.** Hence they can be considered as traits to study genetic variation among the plant taxa. However, for the study of the taxonomic relationships among species and higher taxonomic ranks more valid assessment should necessarily be obtained when these data are compiled with other lines of evidence from morphology and cytology.

Numerical analysis

The phenogram produced by the analysis of the studied cultivars based on coding of 73 attributes obtained from Tris-glycine extracted proteins are shown in Fig. (2). This phenogram shows that the examined samples have a total similarity coefficient of about 1.52. At this level, cv. no. (1) was split off from the other cultivars, then at 1.48 level cv. no. (11) was split off from the remaining cultivars. At the levels 1.43 and 1.40, cv. no. (9 & 10) were also split off from the remaining cultivars (respectively).

At the level 1.38, the two cultivars (5 & 6) were separated together in a small group from the other cultivars, then they distinguished from each other at the 1.35 level. At the level 1.322, the two cultivars (4 & 12) were separated together from the other cultivars and then distinguished from each other at the 1.31. At the level 1.313, cv. no; (3) was split off from the remaining cultivars. Then at the level 1.29, cv. no; (8) was separated from the remaining cultivars. Finally, at the level of 1.22, cultivars number (2 & 7) were found to be close to each other.

Electrophoresis of proteins is a powerful tool for identification of genetic diversity and the SDS-PAGE is particularly considered as a reliable technology because seed storage proteins are highly independent of environmental fluctuations[46,47]. Seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species[48,49]. However, only a few studies indicated that cultivar identification was not possible with the SDS-PAGE method[50]. The SDS-PAGE is considered to be a practical and reliable method for species identification[51].

According to the results of the SDS-PAGE, the overall pattern of seed storage-proteins showed the diversity of lettuce cultivars. The electropherogram produced from seed protein analysis of the 12 cultivars revealed great polymorphism. The diversity in seed storage proteins has also been reported by Khan *et al.*[52] for wheat varieties. Moreover, identification of three wheat genotypes including ILC-195, CM-2000 and CM-98/99 has also been reported by protein markers[53].

Since in mature seeds, type and amount of proteins are more constant than other plant

tissues[54] therefore, the SDS-PAGE pattern of seed storage proteins of pistachio showed polymorphism based on difference in protein intensity among genotypes. The presence or absence of protein bands has also been applied for detection of polymorphism of *Brassica* cultivars[55].

The present investigation revealed variation in different cultivars of lettuce seeds with regard to their total seed protein profiles. These results of SDS-PAGE seed protein patterns show of a few protein bands (6-10) and identified protein patterns may be used protein marker for lettuce cultivars. Regarding inter specific variation among cultivars this investigation revealed some variations. The genetic affinities within cultivars of the same species generally corroborated the morphological analysis. Similar to our finding the result of differentiation of yellow sarson and brown seeded types of *Brassica* clearly separated the yellow seeded and brown seeded varieties by SDS-PAGE[56]. However, we can conclude that, SDS-PAGE can reveal the differences among seed storage proteins of lettuce cultivars.

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