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Evolutionary Relationship and Divergence Based on SDS-PAGE of *Elaeagnus umbellata* (Thunb.) Populations, a Multipurpose Plant from the Himalayas

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Abstract: *Elaeagnus umbellata* (Thunb.), commonly known as autumn olive, belongs to the family Elaeagnaceae and is native to the Himalayan regions of Pakistan, China, and India and is also found in Korea and Japan. The seeds of 8 ecotypes from Azad Jammu and Kashmir, Pakistan, were analyzed at UPM Malaysia for comparisons of their relationship and evolution based on SDS-PAGE of total seed proteins. The results indicated that each autumn olive population can be distinguished by their own specific protein bands with reference to a molecular weight marker included in the gel. The dendrogram based on computer package analysis indicated that populations having the same base of origin fall under 2 simultaneous groups, i.e. P6 and P8 in one group and P1, P2, P3, P4, P5, and P7 in the second group. The distribution of the populations was variable irrespective of their physical location as the seeds of the plant may have been dispersed by birds from distant places. SDS-PAGE thus provided valuable information for the identification of populations and could be utilized for population and varietals discrimination as well as seed quality test in true to type seed producing plants.

Key Words: *Elaeagnus umbellata*, SDS-PAGE, biochemical analysis, evolutionary pattern, Azad Kashmir

Himalayalarda Bulunan Çok Amaçlı Kullanılan *Elaeagnus umbellata* (Thunb.) Populasyonunun SDS-PAGE ile Evrimsel Açıdan İncelenmesi

Özet: *Elaeagnus umbellata* (Thunb.) Himalaya (Pakistan, Çin, Hindistan) bölgesinde ayrıca Kore ve Japonya da bulunan Elaeagnaceae familyasına ait sonbahar zeytini olarak isimlendirilen bir bitkidir. Azad Jammu ve Kashmir Pakistan bölgesinden sekiz tohum Tarım Teknoloji Labaratuvarlarına getirilen örneklerin toplam protein profilleri SDS-PAGE yöntemi ile mukayese edilmiştir. Sonuçlara gore sonbahar zeytini populasyonu kendine özgü protein band profile ile diğerlerinden ayrılmıştır. Jelde moleküler ağırlık markerları kullanılmıştır. Ortalama band sayısı 8-9, fakat jeldeki büyüklük ve yerleri değişkendir. Dendrograma gore aynı menşeye sahip P6 ve P8 aynı grupta yer alırken P1, P2, P3, P4, P5, ve P7 ikinci grupta yer almıştır. Populasyon 6 ve 8 farklı alanlardan alınmış ve aralarında benzerliğin çok fazla olduğu gözlenmiştir. Aynı şekilde populasyon P1 ve P4 fiziksel olarak birbirine yakın olmamasına rağmen çok az varyasyon göstermiştir. Belkide aynı çevreden ekotiplerin dağılımı kuşlar aracılığı ile olmuştur. SDS-PAGE analiz tekniği populasyonların tanımlanmasında oldukça faydalıdır ve populasyon ve varyete ayrımlarında kullanılabileceği gibi tohum kalite denemelerinde de kullanılabilir.

Anahtar Sözcükler: *Elaeagnus umbellata*, SDS-PAGE, biyokimyasal analiz, evrimsel pattern, Azad

Introduction

Autumn olive (*Elaeagnus umbellate* Thunb.) is a valuable plant of the Himalayas with inherent ability to grow under natural conditions. It is a member of the family Elaeagnaceae and is also called cardinal olive or autumn Elaeagnus (1). *Elaeagnus umbellata* is abundantly found in Himalayan regions of Pakistan (2) and is quoted as native to China, Japan, and Korea, and is also found in Afghanistan and India (3). As an exotic species, it inhabits 41 states in the United States of America, southern areas of Canada (4), and Hawaii (5). Autumn olive grows best on deep, relatively coarse-textured soils that are moderately to well drained. It grows on a variety of soils including sandy, loamy, and somewhat clayey textures with pH ranging from 4 to 8 (6). It has excellent tolerance to drought and high salt concentrations.

Autumn olive forms root nodules induced by symbiosis with actinomycetes (*Frankia* spp.) in the soil. This symbiosis permits the fixation and subsequent utilization of atmospheric nitrogen (7). It is valued for its ability to prevent erosion, to fix nitrogen, and to attract wildlife. Autumn olive is a recommended species for planting as a tall shrub component in windbreaks in the Great Plains (8).

It is a common medicinal shrub growing wild at a height of 4500 to 6000 ft above sea level in Azad Kashmir (9). Autumn olive fruit berry is an excellent source of vitamins and minerals, especially vitamin A, C, and E, flavonoids, other bioactive compounds and essential fatty acids (10). The fruit contains about 913 ± 45 ppm vitamin E (tocopherol) (11). One hundred grams of autumn olive fruit contains 69.4 g of moisture, 14.5 g of total soluble solids, 1.51 g of acids, 8.34 g of total sugar, 8.13 g of reducing sugars, 0.23 g of nonreducing sugars and 12.04 mg of vitamin C (12). The percentage contents of phosphorous, potassium, calcium, magnesium, and iron are 0.054, 0.346, 0.049, 0.033, and 0.007, respectively (3). The lycopene contents in red pigmented fruits of autumn olive observed in a study ranged from 17 to 48 mg/100 g compared with 3 mg/100 g of tomato (13), which is widely thought to protect against myocardial infarction (14) and various forms of cancer including prostrate, cervix, and gastrointestinal cancer with reversion of growth (10,15,16).

Gardiner et al. (17) and Gardiner and Ford (18) demonstrated that SDS-PAGE of protein extracted from samples consisting of at least 200 seeds produces a composite pattern for the phenotypes in the population analyzed. These authors found that SDS-PAGE can be used to characterize the seed protein banding profiles of species and cultivars in several grass genera, to compare cultivars of different geographical origin, and to provide taxonomically useful descriptors that are substantially free from environmental influence. The technique has also been used by Aiken et al. (19) and Wilson and Liston (20) in the identification of populations within and among the genera and to determine relationships among them. Aiken et al. (21) used SDS-PAGE based results for the classification of Fastuca and Lolium and concluded that the proteins and DNA based results were comparable in the construction of a phylogenetic tree. Ahmad and Kamal (22) used the same technique for the identification of sea buckthorn populations.

The natural populations of autumn olive vary in plant height, fruit size, leaf area, branching pattern, the biochemical composition of the fruits and seeds, and the yield of fruits. The aim of the present investigation was to determine the evolutionary relationship among the populations based on SDS-PAGE marker proteins for quick identification of populations and improvement of this valuable plant for introduction into new potential areas for commercial exploitation.

Materials and Methods

The seed material used in the study was brought from Azad Jammu and Kashmir, Pakistan, which was collected from the natural stands of autumn olive populations at different locations denoted as P1, P2, P3, P4, P5, P6, P7, and P8 (Figure 3). The populations, despite being taken from the same area, were 3-8 km apart. The microclimatic conditions and soil conditions may have had little variation but the general environmental conditions were similar. Each plant population comprising 10 replicate samples was selected for protein analysis. The total seed proteins were extracted from the seeds in the laboratory of agriculture technology UPM, using the method given previously (22). The seeds were ground using a mortar and pestle in an extraction solution (100 mM tris HCl (pH 8.0) + 150 mM NaCl + 1 ul/ml 2-mercaptoethanol) and the extracted proteins were treated with 2X cracking buffer (0.125 M Tris-Cl, 4% SDS, 20% Glycerol, 10% 2-mercaptoethanol, 0.01% bromo-phenol blue) at 100 °C for 90 s before loading to the gel in a reference to a wide range molecular weight marker. The stacking and resolving gel concentrations were maintained at 7% and 12%, respectively (23). The electrophoresis was carried out under submerged mode using a tank buffer (0.025 M Tris pH 8.3, 0.192 M Glycine, 0.1% SDS) at a constant voltage of 70 mA in a mini protein gel apparatus. All chemicals were purchased from Sigma and stock solutions were prepared before making a working solution. The gels were photographed using a gel documentation system and Flourchem computer package.

Results and Discussion

The seed protein banding profile among 8 populations of autumn olive is compared in Figure 1 and the molecular weight and Rf values of various bands are

compared in the Table. Some bands are shared among the populations but their intensity of staining is variable. For example, one band at a molecular weight of 48.71 is shared among P1, P2, and P3, but its intensity of staining is variable (Figure 1). Such fractionation of seed proteins in SDS-PAGE is very common and the discrimination based on either variable banding pattern or the variable staining intensity of the band has been explained earlier (19,22).

The cluster analysis picture on the base of dendrogram generated through the Flourchem computer package is given in Figure 2, which indicates a very clear picture of the species' evolutionary pattern in relationship and variability. The dendrogram shows that the 2 groups of the populations separated very early from each other and thus originated in separate ways. The 2 populations P6 and P8 fall very close in the cluster but were separated from each other in the very near past to form discrete populations. On the other hand, the 6 populations P1, P2, P3, P4, P5, and P7 originated from the same ancestor shared by P6 and P8 but separated further into 2 groups represented by P1, P2, and P4 in one group and P3, P5, and P7 in the other group during the process of evolution of discrete populations. The

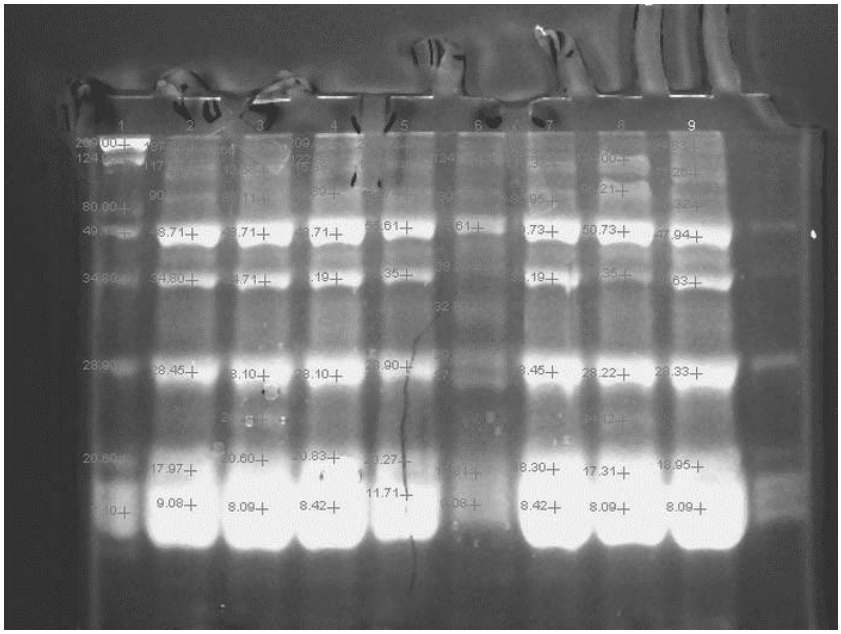

Figure 1. The banding pattern of seed proteins from autumn olive populations in SDS-PAGE. Lanes are 1 marker, lane 2 is P1, lane 3 is P2, lane 4 is P3, lane 5 is P4, lane 6 is P5, lane 7 is P6, lane 8 is P7, and lane 9 is P8

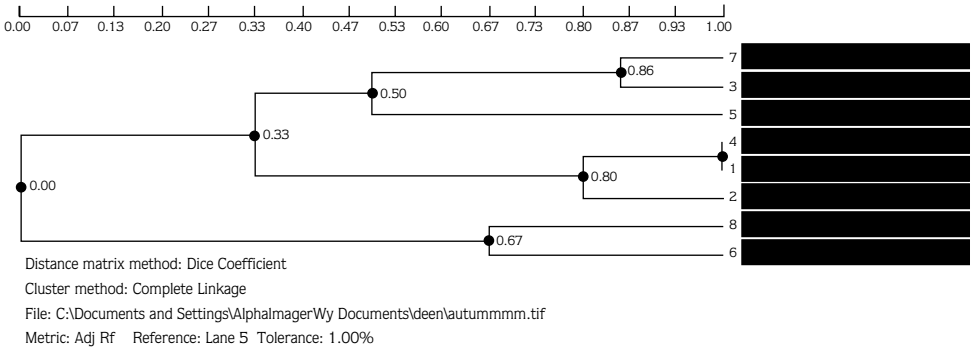

Figure 2. Cluster analysis based on the dendrogram obtained through banding comparisons among 8 populations of autumn olive in the Flourchem computer package.

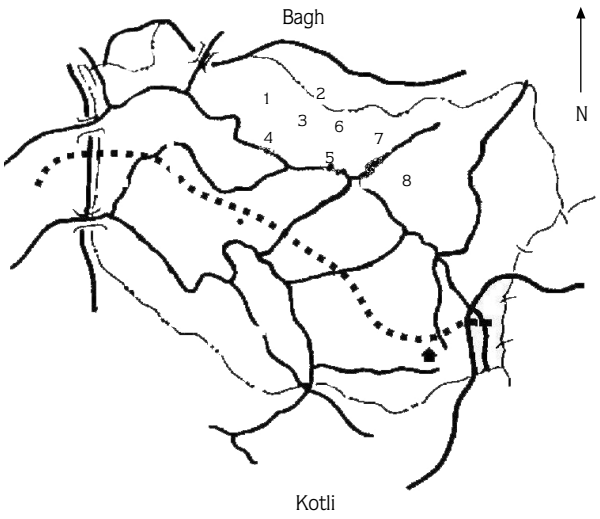

Figure 3. Map of District Poonch (Rawalakot), Azad Kashmir, Pakistan, indicating the places of populations (1-8) of autumn olive.

phenomenon of very close clustering of P6 and P8 and P1 and P4 as well as P3 and P7 was not expected as the populations are physically quite distant from each other. Considering the seed dispersal of autumn olive, which mostly depends on birds (1,25), it is assumed that the commonality of origin of populations physically distant from each other may be the result of such seed dispersal by fruit-eating birds. Sabir and Riaz (24) reported biochemical and elemental variations among different ecotypes of *E. umbellata* from Pakistan. The plant can withstand various climatic odds including soil pH (6), soil temperature and low fertility levels (7), drought and salt concentration, and is rich in valuable phytochemicals (1,3,10,12,13), and therefore its evaluation for population diversity and evolutionary studies based on SDS-PAGE seed protein will have great implications for its improvement and commercialization.

Conclusion

SDS-PAGE provided valuable information for the identification of populations and could be utilized for population and varietal discrimination as well as seed quality tests in true to type seed producing plants.

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Table. SDS-PAGE fractionation pattern of 8 autumn olive (*E. umbellata*) populations' seed proteins.

NO	Lane 1		Lane 2		Lane 3		Lane 4		Lane 5		Lane 6		Lane 7		Lane 8		Lane 9	
	MW	Rf	MW	Rf	MW	Rf	MW	Rf	MW	Rf	MW	Rf	MW	Rf	MW	Rf	MW	Rf
1	209.00	0.0210	187.75	0.0299	166.50	0.0389	209.00	0.0210	201.92	0.0240	124.00	0.0569	152.33	0.0449	124.00	0.0569	194.83	0.0269
2	124.00	0.0569	117.05	0.0749	113.58	0.0838	122.84	0.0599	118.21	0.0719	89.26	0.1467	119.37	0.0689	96.21	0.1287	111.26	0.0898
3	80.00	0.1707	90.42	0.1437	88.11	0.1497	115.89	0.0778	92.74	0.1377	55.61	0.2156	86.95	0.1527	50.73	0.2246	82.32	0.1647
4	49.10	0.2275	48.71	0.2305	48.71	0.2305	93.89	0.1347	55.61	0.2156	38.28	0.3114	50.73	0.2246	36.35	0.3263	47.94	0.2365
5	34.80	0.3383	34.80	0.3383	34.71	0.3413	48.71	0.2305	36.35	0.3263	32.89	0.4042	35.19	0.3353	28.22	0.5599	34.63	0.3443
6	28.90	0.5419	28.45	0.5539	28.10	0.5629	35.19	0.3353	28.90	0.5419	29.59	0.5180	28.45	0.5539	24.12	0.6677	28.33	0.5569
7	20.60	0.7605	17.97	0.7844	24.24	0.6647	28.10	0.5629	20.27	0.7635	27.99	0.5659	18.30	0.7814	17.31	0.7904	18.95	0.7754
8	7.10	0.8832	9.08	0.8653	20.60	0.7605	20.83	0.7545	11.71	0.8413	17.31	0.7904	8.42	0.8713	8.09	0.8743	8.09	0.8743
9					8.09	0.8743	8.42	0.8713			9.08	0.8653						

Note: The lanes indicated in the table denote the 8 populations (2-9) respectively whereas Lane 1 represent marker, MW is the molecular weight of the bands and Rf is the relative fractionation pattern

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