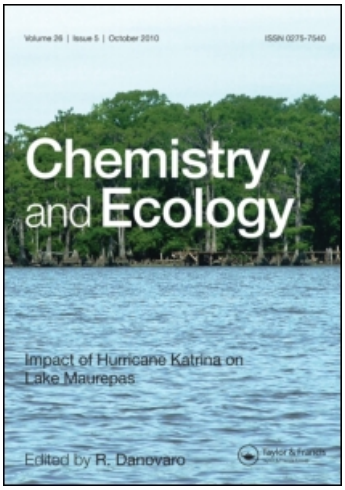

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Ecotypes diversity in autumn olive (*Elaeagnus umbellata* Thunb): A single plant with multiple micronutrient genes

S. D. Ahmad[a]; S. M. Sabir[a]; M. Zubair[a]
[a] Department of Plant Breeding and Molecular Genetics, Faculty of Agriculture Rawalakot, University of Azad Jammu & Kashmir, Pakistan

To cite this Article Ahmad, S. D. , Sabir, S. M. and Zubair, M.(2006) 'Ecotypes diversity in autumn olive (*Elaeagnus umbellata* Thunb): A single plant with multiple micronutrient genes', Chemistry and Ecology, 22: 6, 509 — 521
To link to this Article: DOI: 10.1080/02757540601024819
URL: http://dx.doi.org/10.1080/02757540601024819

Chemistry and Ecology
Vol. 22, No. 6, December 2006, 509–521

Taylor & Francis
Taylor & Francis Group

Ecotypes diversity in autumn olive (*Elaeagnus umbellata* Thunb): A single plant with multiple micronutrient genes

S. D. AHMAD, S. M. SABIR* and M. ZUBAIR

Department of Plant Breeding and Molecular Genetics, Faculty of Agriculture Rawalakot, University of Azad Jammu & Kashmir, Pakistan

(Received 7 December 2006; in final form 14 June 2006)

Elaeagnus umbellata, a member of the Elaeagnaceae family, is native to Pakistan, China, India, Korea, and Japan. It is found commonly at altitudes ranging from 1200 to 2100 m and thrives on eroded and degraded land due to its ability to fix nitrogen. The plant also grows under variable pH (4–8) and drought, and is used locally as fuel wood, fencing, fodder, basket making, and shelterbelts. The fruit of the plant is well known for its essential nutrients and medicinal compounds such as vitamins, minerals, essential fatty acids, carotenoids (lycopene), soluble solids, and sugars. Medicinally, it is widely believed to protect against myocardial infections, pulmonary infections, and various forms of cancers. Ten ecotypes from variable microclimatic conditions were investigated for their morphological, molecular and biochemical diversity improvement and commercialization purposes. Comparisons and disabilities indicated significant variability in terms of morphological (plant height, number of branches, thorn size and number, leaf area, fruit size, 100 fruit weight, and yield), molecular (SDS-PAGE), and micro- and macronutrient (vitamin C, Fe, mg, P, Na, K, essential oils, and sugar) bases among the ecotypes. This variability will be helpful in developing commercial varieties of the plant utilizing the conventional techniques of selection and hybridization for economic activities. The plant has ample quantities of multiple micronutrients, thus indicating their expression through a powerful promoter at one place (fruit mesocarp). Efforts to identify and isolate the micronutrient genes (vitamin A, C, E, and Fe), the deficiency of which causes malnutrition and disabilities within the population of developing countries. Micronutrient genes have also been initiated for their characterization and future transformation into staple food crops for stable bio-fortification.

Keywords: *Elaeagnus umbellata*; Morphological analysis; Poonch District; Vitamin C; Oil content; SDS-PAGE; Intraspecific variability; Minerals

1. Introduction

The land in the Himalayan regions of Pakistan mostly comprises steep hills, at one time covered by conifer forests, but now extensively depleted due to deforestation. The existing limited forests are enriched with timber and fuel wood but also have a large diversity of medicinal plants, wildlife, soil microflora, and fauna of immense value. The topographic nature of the area does not allow extensive production of cereal crops; however, the potential

*Corresponding author. Email: syed.mubasher@gmail.com

Chemistry and Ecology
ISSN 0275-7540 print/ISSN 1029-0370 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/02757540601024819

of medicinal plants and their diversity hold huge commercial promises [1]. Autumn olive (*Elaeagnus umbellata* Thunb), a member of the family Elaeagnaceae [2, 3], is one of the valuable plants from this area with the inherent ability to grow under natural conditions. It is a common medicinal shrub growing wild at a height of 1372–1829 m above sea level in Azad Kashmir [4]. It is abundant in northern Pakistan [5], is native to China, Japan, Korea, Afghanistan, and India [6], and also has been introduced in North America. Autumn olive grows best on deep, relatively coarse-textured soils that are moderately to well drained [7]. It grows on a variety of soils including sandy, loamy, and somewhat clayey textures at pHs ranging from 4 to 8 [8]. It has excellent tolerance to drought and high salt concentrations [2]. Autumn olive forms root nodules induced by symbiosis with actinomycetes (*Frankia* spp.) in the soil. This symbiosis allows fixing and subsequent utilization of atmospheric nitrogen [9, 10]. It is valued for its ability to prevent erosion, to fix nitrogen, and to attract wildlife [6]. Autumn olive is a recommended species for planting as a tall shrub component in windbreaks in the Great Plains [11].

The plants are highly branched, spiny, and deciduous shrubs growing 3–5 m in height [12, 13]. Leaves are alternate [14], simple, variable in size [15], ranging from 1 to 8 cm long and from 1 to 4 cm wide [16], and petiolated in small lateral clusters on twigs [17]. The thorns are several centimetres in length and are formed on spur branches [18]. The fruits are ovoid to globose, 6–8 mm long, 5 mm in diameter [19], single-seeded, produced on pedicels [12, 20], and silvery green with brown scales when immature. They ripen in September to October [18]. The weight of a single fruit is 137 mg, and 353 μl in volume, with a thin epicarp covering the whole fruit; the mesocarp is pulpy and juicy [21]. A mature plant can produce 650 g of fruits in two or three pickings, with the number of seeds ranging from 20 000 to 24 000 [17].

The autumn olive fruit berry is an excellent source of vitamins and minerals (especially vitamins A, C, and E), flavonoids, bioactive compounds, and essential fatty acids [22]. The fruit contains about 913 ± 45 ppm (913 ± 45 mg l^{-1}) of vitamin E [23]. 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 non-reducing sugars, and 12.04 mg of vitamin C [24]. The percentage contents of some of the mineral elements, phosphorus, potassium, calcium, magnesium, and iron, are 0.054, 0.346, 0.049, 0.033, and 0.007, respectively [21]. 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 [25], which is widely believed to protect against myocardial infection [26] and various forms of cancer, including prostrate cancer, cervix, and gastro-intestinal cancer with reversion of growth [20, 27]. The abundant sweet-tart fruit can also be used for preserves, condiments, fruit-rolls, juice, flavouring, and other food products [25] because they have a remarkable keeping quality and can be stored up to 15 d at room temperature. The fruits of the plant are very rich in proteins [21] and much liked by birds and other wildlife for nesting and cover as well as for food [28].

Malnourishment and deficiencies in essential vitamins and minerals cost more than 5 million children's lives every year and cost households in the developing world more than 220 million years of productive life from family members whose lives are cut short or impaired by disabilities related to malnutrition [29]. Vitamin E is a potent antioxidant and is known to have a positive influence on several diseases including heart diseases, some cancers, and cataracts [30] in humans. Lack of vitamin A is probably responsible for the death of 2 million children every year, and its deficiency causes night blindness, which is avoidable [29]. Similarly, vitamin C and folate play a vital role in human health, especially in neuron development and bone formation in foetus development [31]. The widespread lack of inorganic elements such as iron, zinc, selenium, and iodine results in major health problems. Worldwide, iron deficiency affects more than 2 billion people, mostly woman and children in developing countries, but it is also

the major nutrient deficiency in Europe affecting infants, children, adolescents, and women of child-bearing age. The malnutrition problem is expected to increase further with the increase in population as the poor populations in developing countries depend on cereal meals only and cannot afford to balance their diet with vegetables, milk, meat, and fruit supplements. Even in developed Western countries, disadvantaged households suffer from micronutrient deficiencies [31]. Gene traits like vitamin A, C, E, folate, iron, etc. have been successfully transferred in some plants [32–37], however; their source from a single plant and being expressed at a single location will be an additional benefit.

The aim of the investigation was to compare the local ecotypes of autumn olive (*Elaeagnus umbellata* Thunb) on the basis of bio-molecular and morphological characters in order to select the best plants for their commercial value in natural medicine, soil cover, soil reclamation, and other environmental benefits. The selected plants will also be used for varietal improvements in the future and to identify and isolate the multiple genes of medicinal importance for later transformation and bio-fortification of cereal crops.

2. Materials and methods

An investigation was carried out on 10 ecotypes (E1, E2, E3, E4, E5, E6, E7, E8, E9, and E10) of autumn olive from the natural stands of Azad Jammu and Kashmir, from different micro-environments, with a variability in plant height, branching, fruit size, and other plant characters [4]. The ecotypes selected were at least 2–5 km away from each other, thus representing variable micro-environments because of the specific topography of the area.

For morphological comparisons, three plants in five replications were taken randomly from each representative ecotype. Additionally, five lateral branches taken randomly were tagged for average number of thorns/foot length, thorn length, average number of fruit bunches/foot length, average number of fruits per bunch, and leaf area index. Fifteen morphological characters including plant height, plant canopy, stem girth, number of thorns on stem, size of thorns, number of branches per plant, size of branches, number of leaves per branch, leaf area, number of berries per branch, number of berries per bunch, 1000 berries weight, 1000 seed weight, diameter, and longitudinal size of the fruit berries were compared among the ecotypes. The plants had been randomly selected and tagged, indicating the ecotype and replication. Finally, the average data were analysed statistically by following the method of Steel and Torrie [38].

Biochemical components such as vitamin C, oil in pulp, oil in seed, chlorophyll, and reducing and non-reducing sugars were compared among the ecotypes. Mineral components such as potassium (K), sodium (Na), calcium (Ca), magnesium (mg), iron (Fe), and phosphorus (P) were also investigated using standard procedures. Similarly, the molecular comparisons based on SDS-PAGE of total seed proteins were performed as described below.

2.1 *Ascorbic acid content of berries*

Ascorbic acid was determined using the phenol indophenol dye method [39]. Ten grams of fresh berries/fruits was blended with metaphosphoric-acetic acid extracting solution. Five millilitres of the filtrate extract was then titrated with standard indophenol to a pink end-point. The experiment was repeated three times.

2.2 *Lipid content of berries*

The oil content from the berries/fruits of different plants was used for the analysis of lipid content according to the standard methods [40]. Samples were dried in an oven at 105 °C

for 6–12 h. Ten grams of dried sample was used for extraction of oil in a Soxhlet apparatus (30–40 °C) for 6 h using diethyl ether as solvent. The solvent was removed under vacuum, and the residual oil was dried over anhydrous Na_2SO_4. The experiment was repeated three times. Analytical-grade chemicals were used for extraction of oil.

2.3 *Chlorophyll contents*

Chlorophyll was extracted from 1 cm^2 of fresh leaves of *Elaeagnus umbellata*. Chlorophyll was extracted in 80% acetone, and absorbance was measured at 663 and 645 nm with a UV spectrometer for chlorophyll *a* and *b*, respectively. Chlorophyll contents were calculated according to the method of Arnon [41].

2.4 *Estimation of reducing and non-reducing sugars*

The reducing and non-reducing sugars were estimated by the sucrose acid hydrolysis method described previously [39].

2.5 *Estimation of mineral elements*

Mineral elements including iron, phosphorus, sodium, potassium, and magnesium were estimated by the acid digestion method. A known quantity (0.5–1.0 g) of powdered sample was weighed, transferred to a digestion tube, mixed with 5 ml of concentrated HNO_3, and kept on a treater digester for 30 min at 70 °C. The temperature was raised to 140 °C so that nitrous acid fumes could come out. The tube was cooled to room temperature, and then 3 ml of HNO_3:$HClO_4$ (1:1) mixture was added. The mixture was heated to 200 °C so that any white dense fumes of perchloric acid ($HClO_4$) would disappear. The contents were then cooled and transferred to a 50 ml volumetric flask. The volume was made up to the mark with deionized water. The digest was stored in a refrigerator and used for mineral determination. The samples from the digest were used for the spectrophotometric estimation of minerals using a standard curve for each mineral separately [40].

2.6 *Molecular comparisons based on SDS-PAGE*

Ecotypes were also compared using SDS-PAGE techniques for the comparisons of total seed proteins [42]. A known marker (a wide-range protein marker) was used in each gel for the computation of results in the gel documentation system. The analysis was made using a Fluorchem computer package loaded to a gel documentation system. The gel pictures were taken for reference, and the dendrograms were prepared by taking an ecotype producing maximum bands as a matching reference to determine the population's relationship and differences using the computer package.

3. Results

The results for the comparisons of 10 autumn olive ecotypes are presented in tables 1 and 2. Table 1 lists the values for morphological characters of plant and fruit berries, *i.e.* plant height, plant canopy, stem girth, number of thorns on stem, size of thorns, number of branches per plant, size of branches, number of leaves per branch, leaf area, number of berries per branch,

number of berries per bunch, 1000 berries weight, 1000 seed weight, diameter and longitudinal size of the fruit berries, and number of seeds in 100 g of fruit berries. The plant height varied from 209 to 363 cm among the ecotypes with significant differences. Plant canopy was also found to be variable among the ecotypes, but the variability was not significant. The stem girth was significantly variable among the ecotypes (8.09–21.19 cm). The number of branches on the main stem varied from 8.93 to 18.53 among the ecotypes compared ($p \leq 0.05$). Leaf area comparisons were also significantly variable among the ecotypes ranging from 5.85 to 10.24 cm^2. The plants facing towards north (E1, E2, E3, E5, and E8) indicated a larger leaf area compared with the plants facing towards south (E4, E6, E7, E9, and E10). The number of thorns/sq.ft of the stem was insignificantly variable, ranging from 3.63 to 7.46 among the ecotypes, but was not related to the size of the thorn, which varied from 2.6 to 7.86 cm in length. However, the size of the thorns (1.85–6.59 cm) on lateral branches was smaller compared with the main stem (2.6–7.86 cm).

The average number of fruit bunches on each lateral branch ranged from 11.33 to 14.49 among the genotypes and was not found to be significantly variable. Similarly, the number of fruit berries/bunch ranging from 7.16 to 9.19 was insignificantly variable. The 100 fruit berries weight, pulp weight, and seed weight were in the range of 16.41–22.80 g, 5.53–8.72 g, and 11.47–13.19 g, respectively, among the ecotypes compared. Ecotype 6 produced the maximum fruit weight and pulp weight, while E10 produced the minimum. The number of fruits per bunch, on the other hand, was higher in both E6 and E10. The fruit diameter and length were also found to be variable among the ecotypes ranging from 0.48 to 0.67 cm and from 0.71 to 0.87 cm, respectively. The fruit diameter was highest in E3, whereas the fruit length was greater in E5. The fruit size was not exactly comparable to the fruit weight, but it was greater in ecotypes with a higher fruit weight. The number of seeds in 100 g of fruit was also variable among the ecotypes ranging from 458.9 to 997.08.

Biochemical comparisons based on chlorophyll contents, vitamin C, oil in fruit pulp, oil in seeds, reducing sugars, and non-reducing sugars among the ecotypes are given in table 2. Chlorophyll contents among the ecotypes were variable but were found to be related to the leaf size, *i.e.* the smaller the leaf area, the higher the chlorophyll contents (E7 and E10). Vitamin C was also variable among the ecotypes, with E9 having the highest amount (16.9 mg/100 g) and E6 the lowest (13.8 mg/100 g). The oil in fruit pulp and seed was also variable, ranging from 7.43 to 8.21% and from 5.70 to 6.11%, respectively; however, it was not significantly different among the ecotypes, yet was about 14%, which is a fairly high concentration. The reducing and non-reducing sugars were also variable among the ecotypes, ranging from 7.30 to 8.50% and from 1.70 to 2.30%, respectively.

The concentrations of mineral elements phosphorus, iron, sodium, magnesium, and potassium among the ecotypes are compared in table 2. A closer look at the table reveals the variability in elemental composition among the ecotypes. The phosphorus ranged from 48 to 60 mg l^{-1} in the fruit berry extracts of autumn olive ecotypes. Iron ranged from 157.5 to 190.0 mg l^{-1} of berry extracts with a significant variation among the ecotypes. The presence of high amounts of iron in fruit berries is of great significance; iron is involved in vital body functions in human beings. The variation of sodium was also highly significant among the ecotypes, ranging from 20 to 40 mg l^{-1}. The amount of potassium was highest among all minerals, which was also significantly variable among the ecotypes with range of 175–375 mg l^{-1} of fruit extract. The potassium was higher in the ecotypes from high altitudes compared with the ecotypes from lower altitudes. The amount of magnesium was lower, but the variability among ecotypes is still very clear, ranging from 70.0 to 85.4 mg l^{-1}.

The results for SDS-PAGE after computation and dendrogram preparation are presented in figure 1. The figure indicates the evolutionary relationship and differences among the ecotypes compared. The dendrogram reveals that the autumn olive in the area originated from the same

Table 1. Comparisons of growth, yield, and physical characteristics among 10 autumn olive ecotypes of fruit berries.

Trait	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	*SL
Plant height (cm)	363.68 ± 16.6	351.6 ± 8.6	353.33 ± 11.2	310.1 ± 7.6	307.6 ± 9.6	305.46 ± 5.2	281.46 ± 16	270.2 ± 7.1	261.79 ± 7.2	209.46 ± 2.1	**
Plant canopy (cm)	120 579.7 ± 16.2	169 670.9 ± 22.1	187 611 ± 29.1	73 380 ± 13.2	11 121.7 ± 12.5	88 220.6 ± 29.8	87 208.2 ± 16.1	65 439.39 ± 24.5	85 452.7 ± 26.5	32 696.47 ± 18.1	**
Stem girth (cm)	15.55 ± 0.3	17.59 ± 1.8	21.19 ± 1.2	14.33 ± 0.5	13.17 ± 1.6	15.75 ± 0.69	15.59 ± 1.3	17.66 ± 0.25	16.19 ± 3.1	8.09 ± 0.15	NS
No. of branches on the main stem	8.39 ± 0.25	13.53 ± 0.6	12.33 ± 0.01	13.33 ± 0.5	9.39 ± 1.2	7.86 ± 1.6	11.19 ± 0.05	9.13 ± 1.3	11.46 ± 0.26	18.53 ± 0.31	**
Leaf area (cm^2)	10.24 ± 0.71	9.12 ± 0.29	12.71 ± 1.2	8.10 ± 1.5	9.23 ± 1.58	7.32 ± 1.22	7.12 ± 0.26	10.8 ± 0.18	8.23 ± 1.56	5.85 ± 0.04	**
No. of thorns ft^{-2} on the main stem	4.39 ± 0.1	5.53 ± 0.16	3.63 ± 0.02	7.46 ± 0.18	6.79 ± 1.1	6.53 ± 0.03	7.19 ± 0.24	7.19 ± 1.2	5.86 ± 0.35	4.59 ± 0.11	**
Size of thorns on the main stem (cm)	3.19 ± 0.21	4.91 ± 0.06	4.05 ± 0.08	7.86 ± 0.4	2.91 ± 0.05	5.52 ± 0.21	5.99 ± 0.32	4.93 ± 0.05	5.16 ± 0.14	2.60 ± 0.36	**
No. of thorns ft^{-2} on the lateral branches	6.26 ± 0.012	5.53 ± 0.03	6.13 ± 1.56	9.46 ± 1.3	6.26 ± 0.9	7.13 ± 1.21	8.06 ± 0.59	5.73 ± 0.48	6.46 ± 1.2	6.39 ± 0.21	**
Size of thorns on the lateral branches (cm)	1.95 ± 0.05	5.64 ± 1.1	3.23 ± 0.04	5.25 ± 1.3	1.91 ± 0.08	2.56 ± 0.013	5.29 ± 1.24	6.59 ± 1.15	2.23 ± 0.21	1.85 ± 0.01	**
Average no. of fruit bunches/branch	12.66 ± 2.5	14.46 ± 2.8	12.86 ± 6.1	12.06 ± 5.1	14.06 ± 6.32	11.39 ± 2.14	13.26 ± 5.8	11.33 ± 3.26	14.66 ± 5.58	14.59 ± 7.8	**
No. of fruit berries per bunch	7.16 ± 1.25	8.79 ± 2.54	7.79 ± 3.26	8.06 ± 3.58	9.06 ± 5.1	7.93 ± 3.1	9.96 ± 2.5	8.33 ± 2.13	9.79 ± 1.54	9.19 ± 2.9	NS
100 Fruit berries weight (g)	19.07 ± 5.2	18.57 ± 2.6	21.13 ± 5.14	20.46 ± 4.9	19.16 ± 3.5	22.08 ± 3.6	16.83 ± 2.8	19.83 ± 2.54	17.85 ± 0.8	16.41 ± 3.4	**

100 Fruit berries pulp weight (g)	6.91 ± 1.8	6.67 ± 0.65	8.61 ± 0.95	7.46 ± 0.8	6.90 ± 0.54	8.72 ± 0.87	5.26 ± 0.25	7.80 ± 1.1	5.89 ± 1.5	5.53 ± 1.68	**
100 seed weight (g)	12.15 ± 2.5	11.89 ± 3.6	11.98 ± 2.5	12.99 ± 2.9	12.26 ± 3.5	13.19 ± 3.9	11.57 ± 6.1	12.03 ± 3.54	11.95 ± 6.3	11.47 ± 0.64	NS
Diameter of fruit berries (cm)	0.54 ± 0.02	0.60 ± 0.03	0.67 ± 0.015	0.62 ± 0.04	0.58 ± 0.08	0.65 ± 0.012	0.48 ± 0.042	0.54 ± 0.04	0.55 ± 0.021	0.480 ± 0.04	**
Length of fruit-berries (cm)	0.82 ± 0.03	0.79 ± 0.01	0.91 ± 0.042	0.84 ± 0.1	0.87 ± 0.003	0.83 ± 0.21	0.71 ± 0.47	0.79 ± 0.51	0.78 ± 0.32	0.75 ± 0.05	**
No. of seeds in 100 g of berries	583.8 ± 6.2	676.2 ± 7.4	636.58 ± 11.2	997.8 ± 5.3	591.94 ± 13.2	628.46 ± 15.1	666.68 ± 16.9	458.9 ± 19.1	600.6 ± 16.2	536.06 ± 18.2	**

Note: **Significant at the 0.05 level of probability; NS: non-significant at the 0.05 level of probability.

Table 2. Biochemical composition based comparisons of 10 autumn olive ecotypes.

Contents	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Chlorophyll ($mg\,cm^{-2}$)	6.40 ± 0.25	5.90 ± 0.23	5.40 ± 0.61	6.40 ± 0.5	6.70 ± 0.21	6.50 ± 1.3	7.10 ± 1.24	5.30 ± 1.3	6.80 ± 1.5	6.80 ± 0.65
Vitamin C (mg/100 g)	15.50 ± 0.9	14.40 ± 1.2	16.2 ± 1.1	15.50 ± 1.4	15.6 ± 1.8	13.80 ± 2.5	14.70 ± 0.3	15.70 ± 3.2	16.90 ± 0.6	15.30 ± 2.1
Oil in pulp (g/100 g)	7.63 ± 0.3	7.80 ± 0.6	8.21 ± 0.5	7.63 ± 0.56	7.60 ± 0.31	7.62 ± 0.2	7.51 ± 0.8	8.11 ± 0.54	8.06 ± 2.1	7.43 ± 0.9
Oil in seeds (g/100 g)	6.06 ± 0.08	6.00 ± 0.02	5.91 ± 0.8	6.06 ± 1.5	5.91 ± 1.4	5.7 ± 1.69	5.98 ± 0.65	5.84 ± 1.9	6.10 ± 1.3	6.11 ± 0.1
Reducing sugar (%)	8.10 ± 0.9	8.20 ± 1.6	8.30 ± 1.2	8.10 ± 2.1	8.40 ± 0.6	7.40 ± 0.15	7.30 ± 2.1	6.80 ± 1.4	7.40 ± 1.3	8.50 ± 1.8
Non-reducing Sugar (%)	1.70 ± 0.01	1.60 ± 0.01	2.00 ± 0.05	1.70 ± 0.6	2.10 ± 1.5	1.80 ± 0.02	1.70 ± 0.05	1.90 ± 0.06	2.20 ± 0.4	2.30 ± 0.05
Phosphorus	52.0 ± 2.5	48.0 ± 2.5	54.4 ± 3.2	48.0 ± 3.2	60.0 ± 4.2	58.7 ± 2.6	49.5 ± 2.4	52.7 ± 2.8	52.1 ± 3.3	59.6 ± 3.6
Iron	175.0 ± 6.9	190.0 ± 6.1	176.2 ± 6.8	172.5 ± 5.4	170 ± 3.0	189.4 ± 8.5	188.7 ± 6.5	180.1 ± 7.7	157.5 ± 7.1	179.3 ± 6.5
Sodium	40.0 ± 0.6	20.0 ± 0.21	38.5 ± 1.4	25.0 ± 2.5	35.0 ± 1.6	34.7 ± 1.5	36.5 ± 2.4	38.3 ± 2.1	25.0 ± 1.8	37.9 ± 1.2
Potassium	340.0 ± 9.5	185.0 ± 3.6	351.7 ± 9.2	375.0 ± 8.2	375 ± 12.5	326.6 ± 6.9	278 ± 13.2	258.6 ± 11.1	175.0 ± 12	288.6 ± 14.1
Magnesium	73.7 ± 1.6	83.9 ± 0.12	74.8 ± 2.1	72.4 ± 2.9	70.0 ± 3.8	85.4 ± 4.1	75.7 ± 2.9	82.8 ± 1.5	86.6 ± 6.4	79.4 ± 5.2

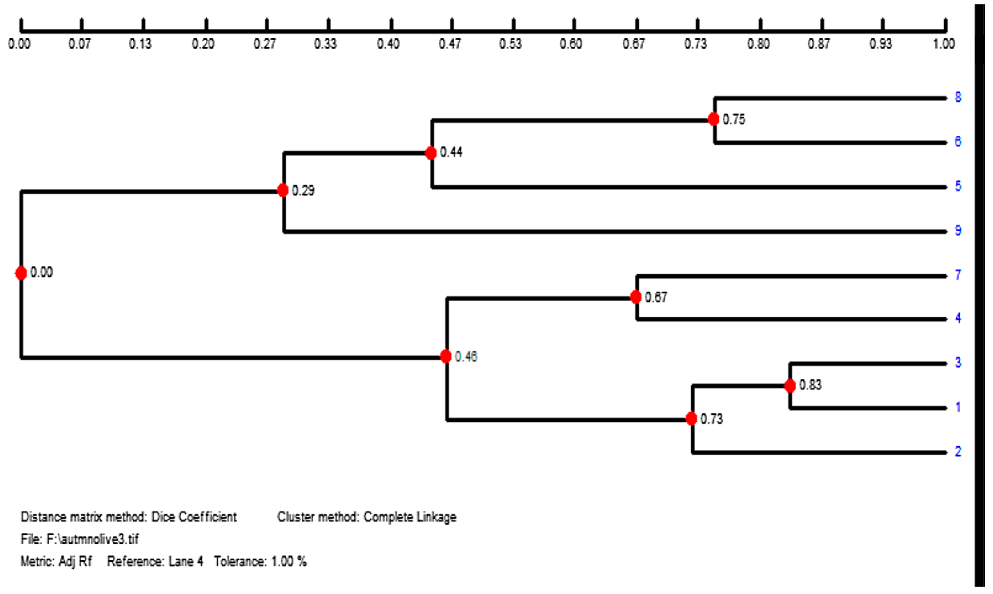

Figure 1. Evolutionary relationship and variation among the ecotypes of autumn olive from Azad Jammu and Kashmir. The numbers in the dendrogram are 1 (E10), 2 (E5), 3 (E3), 4 (E4), 5 (E9), 6 (E1), 7 (E6), 8 (E2), and 9 (E7).

source; however, it split immediately into two groups comprising E1, E2, E7, and E9 in one group and E3, E4, E5, E6, and E9 in the other group, with further bifurcations into individual ecotypes. The ecotype E8 was not included in the analysis, as only nine ecotypes were accommodated in one 10-valve gel with a marker. However, it was included in SDS-PAGE using a second gel by excluding E1 or E2. It always showed a similar banding pattern to that of E3.

4. Discussion

Autumn olive is a very important plant in the mountainous regions of Himalayas because of its traditional medicinal uses, ability to thrive in variable pHs, soil moistures, and temperatures, as well as its ability to rehabilitate degraded lands by biological nitrogen fixation. Its medicinal importance has not been exploited on a commercial scale until very recently [11], although is being used in its native environment by local people to cure urinary-tract infections, prostate cancer, and high blood pressure, and to control cough [43]. Various species of birds have been reported to feed on the fruits of autumn olive because of its attractive colour and presence of sugar compounds [36]. Morphological comparisons based on growth and yield characters have revealed significant variability among ecotypes. Plant height has shown a very clear positive trend in relation to altitude above sea level. E1 from high altitudes indicated the highest plant height, while E10 from lower altitudes indicated the shortest plant height. The trend does not support the findings of another plant species (sea buckthorn) of the same family, where altitude affects plant height in reverse order [44]. We only compared ecotypes differing in altitude from 1676 to 1981 m above sea level, and the positive trend with altitude may not be a true picture for such an explanation. The plant height in the present study ranged from 209 to 364 cm, which is already very much lower than in earlier investigations, *i.e.* 3–5 m [2, 12]. The other morphological characters did not show any such trend, but the variability was quite prevalent

in all parameters compared among the ecotypes. The leaf area was found to be greater in ecotypes facing the north side of mountains (E1, E2, E3, E5, and E8) compared with ecotypes facing towards the south side (E4, E6, E7, E9, and E10). Such adaptations in plant leaves may be due to the lower availability of light for north-facing ecotypes, which have their energy requirements compensated by having an increased leaf area. Simple and alternate leaves of variable sizes have been reported in the literature [14, 15, 45], ranging in size from 1 to 8 cm [12], which is similar to our results where the leaf area was in the range of 5.8–10.8 cm^2. The leaf structure was also similar to the description of Eckardt and Sather [17], *i.e.* petiolated in small lateral clusters on twigs. The distribution and length of the thorns (1.85–6.59 cm) were found to be variable among the ecotypes, but generally these were similar to the findings of Sternberg [18], *i.e.* thorns were several centimetres in length and mostly found on spur branches.

The fruit was 4.8–6.7 mm in diameter and 7.1–9.1 mm in length in the present investigation, whereas earlier studies reported a length of 6–9 mm and a diameter of 5 mm [19] which is on the positive side to the earlier reports but not significantly different in the ecotypes investigated. The colour of the fruit at maturity was bright red and they mature during September and October. Similar information regarding the colour and maturity has been reported in earlier investigations [12, 20, 46]. The number of seeds in 1 kg ranged from 4500 to 10 000 in the present investigation, which is somewhat lower than the 20 000–24 000 reported by Eckardt and Sather [17]. The fruit weight on average ranged from 160–220 mg in the present investigation which was higher than that previously reported [13] and may be important as most of it goes towards important biochemicals. The average number of fruits per plant was lower (334–500 g) though in the present investigation compared with the 0.9–3.4 kg [17] and 650 g [21] reported earlier.

The cluster-analysis picture on the base of the dendrogram generated using the Flourchem computer package is shown in figure 1. The dendrogram shows that the two groups of the populations separated very early from each other and thus originated separately, although these were based on the same single source. The two groups comprise E1, E2, E7, and E9, and E3, E4, E5, E6, and E10. The ecotypes further split into near relatives and discrete populations, indicating that E1 and E2 are closer to each other and share some similarity with E9, whereas E9 separated from the group in a discrete manner at an early stage. E6 and E4 were placed very much closer but separated from the other members of the group by a distance rendering E3 and E10 closer to each other with some commonality to E5. The phenomenon of very close clustering of E5 and E3 and E10 with E4 and E6 as well as E3 and E2 with E7 was not expected, as the ecotypes were physically quite distant from each other. However, this is understandable if we consider the dispersal of autumn olive seeds by birds [28] and expect their role in seed dispersal at distant places in earlier periods of ecotype establishment.

The biochemical constituents compared among the ecotype also indicated significant variation among the ecotypes. The chlorophyll contents were higher in the south-facing ecotypes (E4, E6, E7, E9, and E10) but comparatively lower in the north-facing ecotypes (E1, E2, E3, E5, and E8), the significance of which is not known at present. However, the possibility of the smaller leaf area in the south-facing ecotypes (high-intensity sunlight) being compensated by a higher chlorophyll content cannot be ruled out. The vitamin C and sugar contents reported by Graham [24] in 100 g of autumn olive were 12.04 mg of vitamin C and 8.34 g of total sugar, comprising 8.13 g of reducing sugar and 0.23 g of non-reducing sugar. In the present investigation, vitamin C was found to be in the range of 13.80–16.20 mg/100 g among the ecotypes, and total sugar was in the range of 8.70–10.80 g, with reducing sugars 6.80–8.50 g and non-reducing sugars 1.90–2.30 g/100 g of fruits. Both vitamin C and sugars were found to be higher in the fruits of autumn olive investigated, indicating a better potential for medicinal value and food materials. Sugars can increase the shelf-life and keeping quality of the fruit; hence, more sugars can help better marketing of the fruits [25]. The presence of larger amounts

of sugar in autumn olive is also important for its direct use as a fruit or in the form of juice and other by-products.

The variable and higher amounts of pulp (7.43–8.11 g/100 g) and seed oil (5.84–6.11) found in autumn olive ecotype may be of significant value in medicines. It has been reported earlier that these oils contain certain vitamins such as vitamin E or tocopherol [23] and phytosterols [5] which have some value in protecting against myocardial infection [26] and various forms of cancers, *i.e.* cervix, prostrate, and gastrointestinal [22, 27, 47] as reported for autumn olive fruits. The percentage contents of phosphorus, iron, sodium, potassium, and magnesium in current studies are 48–60 mg l^{-1}, 157–190 mg l^{-1}, 20–40 mg l^{-1}, 175–340 mg l^{-1}, and 70–84 mg l^{-1}, respectively. The mineral contents reported in earlier studies were lower [21]; the amount of iron was significantly higher in the ecotypes investigated from Kashmir. The role of these minerals is well known in human nutrition and thus means that autumn olive can be of great value for human health. High lycopene contents were reported to be 6–16 times higher then tomato in autumn olive (17–48 mg/100 g) may have an additional value for its fruits [25]. The value of autumn olive fruit as a bird food has also been reported in various studies [28]. Clinical trials of fruit berries have indicated its role in the efficient control of high blood pressure and hypertension [5].

The autumn olive ecotypes compared provide very good information regarding the selection and direct use of ecotypes for commercial purposes as well as for future improvements and introduction of new varieties in the area. Most of Azad Jammu and Kashmir is hilly with bare peaks, which were once covered with thick forest. Extensive deforestation and soil erosion have degraded the land considerably. The autumn olive plants investigated have a very good potential to rehabilitate the land due to their nitrogen fixation value and source of additional income to mountain communities. As ecotypes E3 and E6 show good plant growth, they are better for soil reclamation and reforestation as good growth is an indication of better nitrogen fixation. The ecotypes also have a larger fruit size and more pulp in the fruit, which are an indication of higher biochemical yield. The two ecotypes (E3 and E6) although indicated good distance in their evolutionary distribution and variability in various growth, yield and biochemical constituents but excelled other ecotypes on the whole. These ecotypes will be hybridized using a conventional breeding programme for the production of commercial varieties.

The presence of several nutrient genes in autumn olive makes the plant more valuable, as it has all the genes for vitamins A, C, and E, folate, and iron expressed in a single place (fruit mesocarp). By contrast, staple food cereals are deficient in these micronutrients and do not provide the deficient nutrients in populations in developing countries inflicted with disabilities related to malnutrition [29–31]. Single gene traits such as vitamins A, C, and E, folate, and iron individually have been successfully transferred in some plants [32–37]. The transfer of multiple genes in one plant for variable traits seems to be difficult but not impossible [48]. Future investigations therefore will help to isolate these genes and transfer these into the staple food cereals for bio-fortification and to help provide a balanced diet for poor communities in developing countries.

5. Conclusion

This investigation has provided very important information regarding the presence of certain phytonutrients, *e.g.*minerals, oil, vitamin, and sugars in autumn olive fruits, and highlighted their medicinal importance in various ailments including myocardial infection, certain types of cancer, and anaemia. The ecotypes E3 and E6 were found to be the best in terms of plant vigour, fruit yield, and important biochemical constituents. The variability and evolutionary

relationship among the ecotypes thus will help in further improvements of the plants for commercial use and economic development of mountain communities in the region and elsewhere. The most important findings in autumn olive are the presence of multiple micronutrient genes (vitamins A, C, and E, and Fe) in one place (fruit mesocarp), and the deficiency of these causes many disabilities in malnourished populations of developing countries. These genes may work under the same promoter and, once isolated and transferred to staple cereals, could help in bio-fortification and provision of balanced diets to poor populations in developing countries.

References

[1] S.D. Ahmad, M.Q. Khan, K. Mahmood. Biodiversity in Pakistan: opportunities, prospects and threats. *Sci. Tech. Dev*., **17**, 1–9 (1998).

[2] M.A. Dirr. *Manual of Woody Landscape Plants. Their Identification, Ornamental Characteristics, Culture, Propagation and Uses*, Stipes, Champaign, IL (1989).

[3] W. Wunderlin, P. Richard. *Guide to the Vascular Plants of Florida*, University Press of Florida, Gainesville, FL (1998).

[4] S.M. Sabir, S.D. Ahmad, M.K. Tahir. Antibacterial activity of *E. umbellata*, a medicinal plant from Pakistan. *Saudi Med J*., (in press).

[5] S.M. Sabir, S.D. Ahmad, N. Lodhi. Morphological and biochemical variation in Sea buckthorn (*Hippophae rhamnoides* L. ssp *turkestanica*), a multipurpose plant for fragile mountains of Pakistan. *South Afr. J. Bot*., **69**, 587–592 (2003).

[6] B. Edgin, E.E. John. Control of autumn olive (*Elaeagnus umbellata* Thunb.) at Beall Woods Nature Preserve, Illinois, USA. *Natural Areas J*., **21**, 386–388 (2001).

[7] Natural Food Institute, Wonder Crops. Physical characteristics of *Elaeagnus umbellata.* Available online at: http://www.comp.leeds.ac.uk/cgi-bin/pfaf/arr.html (accessed October 2001).

[8] W.R. Reed. *Elaeagnus umbellata.* In *The Fire Effects Information System*, W.C. Fischer (Ed.), p. 588, US Department of Agriculture, Station, Intermountain Fire Sciences Laboratory, Missoula, MT (1992).

[9] S.C. Kim, K.U. Chang, D. Park, M.C. Kim, C.H. Song, S.D.C. Sun. Isolation of symbiotic Frankia Eulk 1 strain from root nodule of *E. umbellata. Korean J. Bot*., **36**, 177–182 (1993).

[10] M.W. Paschke, J.O. Dawason, M.B. David. Soil nitrogen mineralization under black walnut interplanted with *Elaeagnus umbellata* or Black Alder. *J. Plant Soil*, **118**, 33–42 (1989).

[11] J.R. Hays, F. James. Wildlife considerations in windbreak renovation, paper presented at *Great Plains Agricultural Council, Complier, Windbreaks: Living with the Wind: Proceedings, Windbreak Renovation Workshop Hutchinson, Ks. Great Plains Agriculture Council Publ. No. 133*, pp. 35–41, Kansas State University, Cooperative Extension Service, Manhattan, KS, 23–25 October (1990).

[12] H.A. Gleason, A. Cronquist. *Manual of Vascular Plants of Northeastern United States and Adjacent Canada*, 2nd ed., New York Botanical Garden, New York (1991).

[13] M.A. Dirr. *Manual of Woody Landscape Plants*, Stipes, Campaign, IL (1983).

[14] N.J. Plainfield, Moldenke. *The Flora of New England*. 2nd ed., Phytologia Memoirs (1982).

[15] R.K. Godfrey. *Trees, Shrubs, and Woody Vines of Northern Florida and Adjacent Georgia and Alabama*, The University of Georgia Press, Athens, GA (1988).

[16] A. Clewell, F. Andre. *Guide to the Vascular Plants of the Florida Panhandle*, Florida State University Press, Tallahassee, FL (1985).

[17] S. Eckardt, A. Sather. *The Nature Conservancy Element Stewardship Abstract for* E. umbellata *Practice. Preliminary Report 111*, Department of Conservation Arlington, Virginia (1987).

[18] G. Sternberg. *Elaeagnus umbellata* – autumn olive. In *Invasive Plants: Weeds of the Global Garden. Handbook No. 149*, J.M. Randall, M. Janet (Eds), p. 54. Brooklyn Botanic Gardens, Brooklyn, NY (1996).

[19] A. Wagner, L. Warren, R.H. Derral, S.H. Sohmer. Contributions to the flora of Hawaii. II. Begoniaceae–Violaceae and the monocotyledons. *Bishop Museum Occasional Papers*, **29**, 88–130 (1989).

[20] F. Mohlenbrock, H. Robert. *Revised Edition; Guide to the Vascular Flora of Illinois*, Southern Illinois University Press, Carbondale, IL (1986).

[21] C. Parmar, M.K. Kaushal. *Elaeagnus umbellata* Thunb. In *Wild Fruits of Sub-Himalayan Region*, pp. 23–25, Kalyani, New Delhi (1982).

[22] V. Matthews. *The New Plantsman*, Royal Horticultural Society, London (1994).

[23] G.C. Willmoth, J.G. Foster, J.L. Hess, M. Phillips, D.T Belesky, J. Berdhal. Tocopherol (Vitamin E) content in three invasive, woody species on underutilized Applachian farmland. In *Proceeding Reports of American Forage and Grassland Council, 37th North American Alfalfa*, pp. 86–90, Improvement Council, Madison, WI (2000).

[24] G. Graham. The Elaeagnaceae in the Southeastern United States *J. Arnold Arbor*., **45**, 274–278 (1964).

[25] I.M. Fordham, B.A. Clevidence, E.R. Wiley, R.H. Zimmerman. Fruit of autumn olive; A rich source of lycopene. *Hort-Science Alexandria*, **36**, 1136–1137 (2001).

[26] L. Kohlmeier, J.D. Kark, E. Gomez-Garcia, B.C. Martin, S.E. Steck. Lycopene and myocardial infarction risk in the EURAMIC study. *Am. J. Epidemiol*., **146**, 618–626 (1997).

[27] S.K. Clinton. Lycopene, chemistry, biology, and implications for human health and disease. *Nutr. Rev.* **56**, 35–51(1998).
[28] R.J. Mackie, R.F. Batchelor, M.E. Majerus, J.P. Weigand, V.P. Sundberg. *Publications and Information. Animal and Range Sciences, Extension Service*, Montana State University, Bozeman, MT (2003).
[29] B. Sofi. *FAO's Annual Hunger Report, the State of Food Insecurity in the World* (2004).
[30] R. Winklhofer, M. Michaela, M. Johannes, D. Hiller. Effects of vitamin E and carotenoid status on oxidation stress in health and disease. Evidence obtained from human intervention studies. *Mol. Aspects Med*., **24**, 391–402 (2003).
[31] S. Poletti, W. Gruissem, C. Sautter. The nutritional fortification of cereals. *Curr. Opin. Biotechnol*., **15**, 162–165 (2004).
[32] P. Beyer, S. Al-Babili, Ye X, P. Lucca, P. Schaub, R. Welch, I Potrykus. Golden rice: introduction the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *J. Nutr*., **132**, 506S–510S (2002).
[33] S. Romer, P.D. Fraser, J.W. Kiano, C.A. Shipton, N. Misawa, W. Schuch, P.M. Bramley. Elevation of the provitamin A contents of transgenic tomato plants. *Nature Biotech*., **18**, 666–669 (2000).
[34] D. Shintani, D. Dellapenna. Elevating the vitamin E content of plants through metabolic engineering. *Science*, **282**, 2098–2100 (1998).
[35] S Eva, S. Williams, D. Keen, P. Christou. Molecular characteristics of transgenic wheat and the effect on transgene expression. *Transgen. Res*., **7**, 463–471 (1999).
[36] A. Kohli, R.M. Twyman, R. Abranches, E. Wegel, E. Stoger, P. Christou. Transgene integration, organization and interaction in plants. *Plant Mol. Biol.*, **52**, 247–258 (2003).
[37] J.K.C. Ma, P.M.W. Drake, P. Christou. The production of recombinant pharmaceutical proteins in plants. *Nature Rev. Genet*., **4**, 794–805 (2003).
[38] R.G.D. Steel, H. Torrie. *Principles and Procedures of Statistics: A Biometrical Approach*, 2nd ed., McGraw-Hill, New York (1980).
[39] A.O.A.C. Vitamins and other nutrients. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, S. William (Ed.), pp. 838–841, AOAC, Arlington, VA (1984).
[40] A.A.C.C. *Approved Methods of American Association of Cereal Chemists*. The American Association of Cereal Chemists, St. Paul, MN (1983).
[41] D.I. Arnon. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris. Plant Physiol.*, **4**, 1–9 (1994).
[42] S.D. Ahmad, M. Kamal. Morpho-molecular characterization of local genotypes of *Hippophae rhamnoides* ssp. *turkestanica* a multipurpose plant from northern areas of Pakistan. *Online J. Biol. Sci*., **2**, 351–354 (2002).
[43] S.D. Ahmad, N. Lodhi, M. Sabir. Morphological and biochemical comparison of *Hippophae rhamnoides, Elaeagnus umbellata* and *crataegus oxycantha* intra and interspecifically. *South Afr. J. Bot*., **71**, 232–238 (2005).
[44] S.D. Ahmad, M. Sabir, J. Mir, A.H. Shah. Morphological and biochemical variation in *Elaeagnus umbellata* Thunb from mountains of Pakistan. *Acta Bot. Croat*., 64, 121–128 (2005).
[45] A. Radford, E. Albert, E.A Harry, B.C. Ritchie. *Manual of the Vascular Flora of the Carolinas*. The University of North Carolina Press, Chapel Hill, NC (1968).
[46] G. Sternberg. *Elaeagnus umbellata* in Illinois conservation practice. Preliminary Report 111, Department of Conservation (1982).
[47] E. Giovannnucci, A. Ascherio, E.B. Rimm, M.J. Stampfer, W.C. Colditz. Intake of carotenoids and retinol in relation to risk of prostate cancer. *J. Nat. Cancer Inst*., **87**, 1767–1776 (1995).
[48] P. Christou, R.M. Twyman. The potential of genetically enhanced plants to address food insecurity. *Nutr. Res. Rev*., **17**, 23–42 (2004).