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Assessing Potential of Iranian Chicory Genotypes for Industrial Application

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Abstract
Chicory (*Cichoriumintybus* L.) is an important industrial crop which is used for inulin production. Inulin is widely applied as food ingredient due to its health promoting properties. For the first time, attempts were made to investigate thirteen endemic chicory genotypes including three pumilum populations, along with five root chicory cultivars, four witloof chicory varieties and a crispum endive to find their phylogenic relationships based on some diagnostic morphological traits as well as comparing their fresh root yield, total carbohydrate content as indicator of inulin percentage, and inulin yield in RCBD with three replications, 2013-14. In general, with the exception of Firizi landrace which was classified in *C. intybus* class, the other endemic genotypes exhibited the maximum similarity with *C. endivia*, as all formed a monophyletic clade. The highest inulin yield was obtained for 'Orchies', after that for 'Schepens', 'Tilda' and 'Hera', respectively, due to firstly their higher root yield and secondly their high inulin percentage. On the whole, fault of flowering at the first year of life cycle of endemic genotypes made intensive selection and breeding of Iranian genotypes for bolting resistance priority work before applying them to build root chicory varieties.

Keywords: endemic genotypes, endive, inulin yield, phylogenic.

Abbreviations: CRE, chicory root extract; **DP**, degree of polymerization.

Introduction

Chicory (*Cichoriumintybus*) naturally contains a high quantity of inulin in its root. Root of industrial chicory is used as raw material for the extraction of inulin and inulin hydrolysis byproducts (Van den Ende and Van Laere, 1996).Chicory (*Cichoriumintybus* L., 2n = 2x = 18) belongs to the subfamily Cichorioideae, which is a part of the Asteraceae, containing approximately 23,000species (Gemeinholzer and Bachmann, 2005).*C. intybus* and *C. endivia* as two important cultivated species of the *Cichorium* genus are distinctive; the latter is annual and self-compatible while the former is annual and/or biannual with self-incompatibility characteristics. Industrial chicory which belongs to *C. intybus* species is a biannual crop which needs overwintering or cold treatments (<5°C) for vernalization process and, consequently, flowering stimulation (Eenink, 1984; Lucchin *et al*., 2008). Phylogenetically, *Cichorium* genus

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contains high diverse genotypes (Kiers *et al*., 1999 & 2000; Gemeinholzer and Bachmann, 2005; Conti *et al*., 2005), and accurate morphological description had been recorded by Kiers *et al*. (1999). Kiers *et al.* (2000) introduced six species according to the morphological characters and molecular observations, two cultivated and well-known species *C. intybus* and *C. endivia* and the four wild species *C. spinosum* , *C. pumilum*, *C. calvum*, and *C. bottae*. Generally, *Cichorium* species are divided into two groups; annual life span and self-compatible, *C. endivia*, and *C. calvum*, and perennial life span and self-incompatible, *C. intybus*, *C. spinosum*, and *C. bottae* (Lucchin *et al*., 2008). Inflorescence of chicory is capitulum and clusters of 4-6 sessile capitulum (2-4, rarely 8, in *C. intybus*) are placed on the flowering stalk, in axillary position, and single capitulum is brought at the end of peduncles (Lucchin *et al*., 2008). Wagenitz and Bedarff (1989) claimed that outer and inner phyllaries, glands and capitula per head are informative for inter- and intraspecific delimitations while leaf morphology and hairiness are variable due to their plasticity constrained by habitat conditions. Gemeinholzer and Bachmann (2005) studied flower and achene structure as diagnostic characters. According to their study, all the applied molecular methods (i.e., ITS, AFLP, and Microsatellites) failed to distinctively discriminate between *Cichoriumintybus* L. and *C. spinosum* L. species (Gemeinholzer and Bachmann, 2005). Therefore, they used morphological traits for justified species detection because stability and heritability of such features had been approved (Lucchin *et al*., 2008). A comprehensive investigation on chicory classifications was carried out by Conti *et al.* (2005) and this classification was improved by Lucchin *et al.* (2008) in terms of each group usage.

Inulin with diverse applications in food industry is known as bioactive prebiotic compound, fat replacer, sweetener, food stabilizer and thickener agent (Roberfroid, 2007, 2005), so intensive breeding of industrial (root) chicory (*C. intybus* var. *sativum*) and increasing its cultivation area since 1990havebeendone (Baert and Van Bockstaele, 1993; Baert, 1997). Traditionally, chicory was cultivated in North-Western Europe (Belgium, The Netherlands and the north of France). There are now about 4,000 ha of industrial chicory fields in the Netherlands, 2,000 ha in France and 8,126 ha in Belgium. Recently, new regions such as Poland, Puerto Rico, and Serbia have been trying to introduce industrial chicory into their cropping systems (FAO, 2013).Although different factors such as harvest date, plant density and temperature can affect inulin yield, root yield and inulin percentage are two major components to increase inulin yield (Baert, 1993). Narrow genetic back ground basis of root chicory is the fundamental issue on inulin yield which hampers its root yield improving (Baert, 1997;Frese *et al.*, 1991).Also, maintenance and extension of the genetic variability is the key characteristic for better adaptation in a changing environment and also for breeding purposes (Lucchin *et al*., 2008; Baert, 1993). On the other hand, inulin content and quality is influenced molecularly by the endogenous factors such as degradation enzymes (1-FEHII, 1-FEHI; Van den Ende, 1996; Maroufi *et al.*, 2012). Hence, to assess variability, potential yield and taxonomic positions, some Iranian heterogeneous collections and wild genotypes for further uses in breeding or commercial programs were studied in comparison with some European industrial cultivars. The results would be helpful in improving the chicory genotype sources for further uses in breeding industrial chicory.

Materials and Methods

Plant materials

A wide range of Iranian chicory genotypes along with different cultivars of root,

witloof, and endive genotypes were selected for evaluation, as summarized in Table 1.

Experimental design and sampling

All genotypes were grown in a randomized complete block design (RCBD) with three replicates in the research farm of Agricultural and Natural Resources College of Tehran University (latitude: 35.480783 and longitude: 50.574147, average rainfall 280 mm and 1290 m elevation) at the end of April, 2013. Each plot had five rows with 0.5 m distance and 2 m length and 8 plants were grown per square meter. Soil texture was silty loam with pH 7.8 and EC 1.45 ($d.sm^{-1}$) and moderate levels of NPK, and sono fertilization was applied. Hand pulling of weed was conducted three times, without using any herbicides. Roots were harvested from a 0.5 square meter at the end of September, 2013. After weighing the harvested roots per unit area, they were frozen at -70°C for further analysis.

Table 1. Genotypes used in this experiment

Cultivar group	Varity	Place of collection
Crispum endive	Very fine vegetable 3	France
Root chicory	'Hera'	Belgium
	'Tilda'	Sugar beet Ins., Iran
	'Melci'	Belgium
	'Orchies'	Sugar beet Ins., Iran
	Hungarian population	Research Ins. of Food Sci. and Tech. (RIFST), Iran
Witloof	Yellowstar	Tabriz Uni., Iran
	Belgian endive	Karaj, Iran
	' Schepens'	Belgium
	Novipia	Belgium
Pumilum (Iranian genotypes)	pumilum	Research Ins. of Food Sci. and Tech. (RIFST), Iran
	Khorassan 1	Research Ins. of Food Sci. and Tech. (RIFST), Iran
	Khorassan 2	Research Ins. of Food Sci. and Tech. (RIFST), Iran
Iranian genotypes	Kazeron 2	Research Ins. of Food Sci. and Tech. (RIFST), Iran
	Khorramabad	Research Ins. of Forest and Regions (RIFR), Iran
	Isfahan	Research Ins. of Forest and Regions (RIFR), Iran
	Semnan	Research Ins. of Food Sci. and Tech. (RIFST), Iran
	Kazeron 1	Research Ins. of Food Sci. and Tech. (RIFST), Iran
	Ramin	Research Ins. of Food Sci. and Tech. (RIFST), Iran
	Bushehr	Research Ins. of Food Sci. and Tech. (RIFST), Iran
	Frizi, Chenaran	Research Ins. of Food Sci. and Tech. (RIFST), Iran
	Zanjan	Sugar beet Ins., Iran
	Ardestan	Research Ins. of Forest and Regions(RIFR), Iran

Morphological traits

Some flower characteristics of genotypes such as average number of axillary sessilecapitula per bunch of flowers in three records and average number of flowers per capitulum in three records were documented during the flowering of each genotype. Also, maximum length of outer phyllaries, maximum width of outer phyllaries, maximum length of inner phyllaries, maximum width of outerphyllaries and petal length were measured with digital caliper when genotypes produced flower stalk. Fresh root weight ($g\ plant^{-1}$) and fresh shoot weight ($gplant^{-1}$) were measured in early September. Leaf characteristics such a shair density at three levels (low=0, medium=1 and high=2), maximum rosette leaf length and maximum rosette leaf width were recorded at the end of July. The number of years for each genotype which completed its vegetative and reproductive cycle was

recorded as life cycle (1= annual and 2= biannual) and the number of years in which each genotype plant was alive after overwintering was recorded as life span (1=annual and 3=perennial). The number of seeds which were produced after obligate selfing by packing each capitulum before pollination and fertilization was recorded as selfing seeds.

Measurement of inulin percentage

HPLC method, involving water extraction of inulin and its indirect determination by HPLC-ELSD (Evaporative Light-Scattering Detection) of UNICAM Company after hydrolysis, was used. Reference sugars of glucose and fructose were from Sigma (carbohydrates kit, CAR- 11).Sample preparation was done by adding 10 g of chopped harvested roots in a flask filled with 120 cc of distilled water (1 to 12 ratio). This mixture was kept for 40 min on a water bath 80°C. Afterwards, samples were shaken well, kept at ambient conditions to reduce heat, and then treated by filtering the solution through a Whatman no. 1 filter round paper and followed by centrifuging in 4500 g for 10 min at 30°C to obtain chicory root extract (CRE). Nearly1 ml of 5% phenol solution was added to 1ml of CRE and then 5ml of sulfuric acid 98% were added. Prepared samples were kept for 20 min at 30°C (Paseephol *et al*., 2007). Automatic injection of 20 microliters of prepared samples to Bio-Rad Aminex-HPX-87Pcolumn was done at ambient temperature. After detection of peaks on the basis of standards, concentration of fructose and glucose were calculated according to retention time and standard curve (Zuleta and Sambucetti, 2001; Waes *et al*., 1998). Carbohydrate content was calculated on the basis of equation 1 which gives a good prediction of inulin content (UPOV, 2003). The degree of polymerisation (DP) can be obtained according to equation 2 (UPOV, 2003).

$$\text{carbohydrate content} = \frac{(\text{fructose} + \text{glucose}\%)}{1.1} \quad (1)$$

$$\text{degree of polymerisation} = \frac{\text{fructose}\%}{\text{glucose}\%} + 1 \quad (2)$$

Statistical analysis

Genotypes were compared using ANOVA (F-Test). All statistical analyses were done with SAS9.1.3 software and means comparison was done using Duncan test at 5% level. Phylogenic tree was drawn using PAST software on the basis of neighbor joining (NJ) method using all the measured and morphological traits.

Results and Discussion

Phylogenic relationships

Endemic genotypes which are related to *C. intybus* species can be easily detected on the basis of their inability to produce seed at the obligate selfing condition and more than one year of their life span (Lucchin *et al*., 2008) because *C. intybus*is the only perennial species of chicory in Iran (Shoorideh *et al*, 2015). In conforming to life span and production of obligate selfing seed just Firizi genotype can be from *C. intybus* species (Table 2). Most studied genotypes had between 2 to 8 capitulaper bunch of flower except pumilum genotypes which had about 10sessile capitula (Table 2). The lowest number of flowers per each capitulum (about 10 flowers) was observed in pumilum genotypes and the highest one (about 20 flowers) was observed in only crispum endive genotype. Also, petal length was shortest for endive and pumilum genotypes (Table 2). There was no significant difference between genotypes for size of other parts of flower.

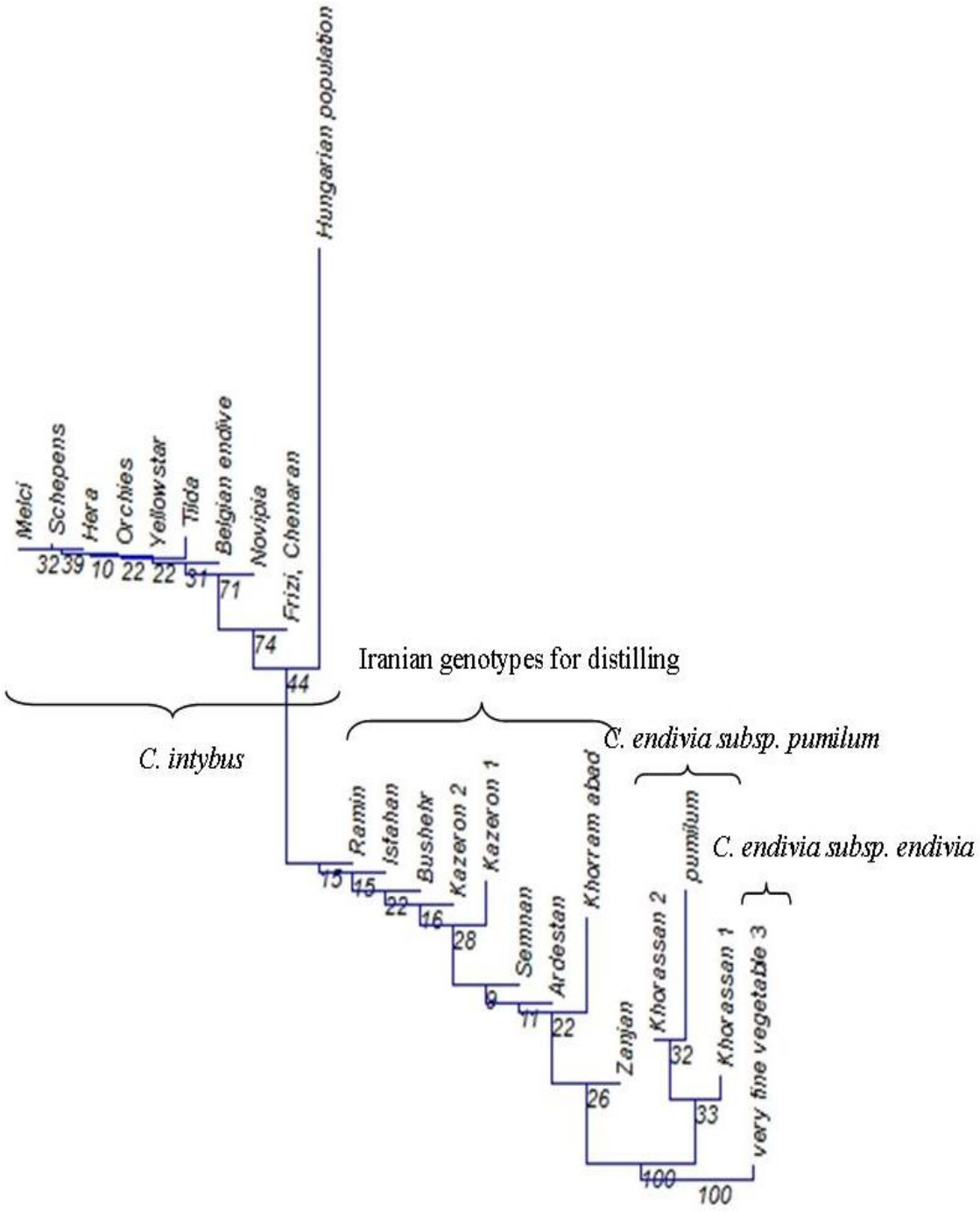

Fig. 1. Phylogenic tree of studied genotypes based on morphological characteristics and their root and inulin yield.

Table 2. Morphological traits studied at different chicory genotypes

Cultivar group	Traits / Genotypes	Selfing seeds	No. of capitulum per bunch of flowers	No. of flower per capitulum	Fresh root weigh (gplant^{-1})	Fresh shoot weigh (gplant^{-1})	Hair density†	Max. rossette leaf length (mm)	Max. rossette leaf width (mm)	Max. length of outer phyllaries (mm)	Max. width of outer phyllaries (mm)	Max. length of inner phyllaries (mm)	Max. width of inner phyllaries (mm)	Petal length (Mm)	Life span	Life cycle
Crispum endive	Very fine vegetable3	17	5	21	10	170	0	170	80	9.44	3.32	10.36	1.86	12.01	1	1
Root chicory	'Hera'	0	2	14	220	221	0	210	70	5.53	2.49	9.87	2.63	15.82	3	2
	' Tilda'	0	3	14	230	190	0	240	70	5.5	2.46	9.79	2.64	15.81	3	2
	' Melci'	0	2	13	110	130	0	290	100	5.57	2.53	9.99	2.63	15.83	3	2
	' Orchies'	0	2	15	140	200	0	230	70	5.53	2.49	9.88	2.63	15.82	3	2
	Hungarianpopulation	0	3	14	70	560	0	380	80	5.41	2.37	9.52	2.546	15.8	3	1 & 2
Witloof	Yellow star	0	2	15	80	160	0	220	65	5.58	2.54	10.03	2.62	15.82	3	2
	Belgian endive,Karaj	0	2	14	110	125	0	160	60	5.49	2.46	9.77	2.64	15.81	3	2
	'Schepens'	0	2	13	110	120	0	280	70	5.47	2.43	9.7	2.64	15.81	3	2
	Novipia	0	2	15	30	80	0	230	65	5.64	2.61	10.33	2.61	15.83	3	2
Pumilum (Iranian genotypes)	pumilum	11	10	9	5	840	1	320	70	6.53	3.02	8.08	1.63	11.22	1	1
	Khorassan1	7	7	9	10	430	1	350	70	5.61	2.91	7.97	2.47	7.65	1	1
	Khorassan2	11	11	11	30	570	1	360	70	7.17	2.71	9.21	2.27	11.1	1	1
Iranian genotypes	Kazeron2	13	4	14	20	240	2	500	110	9.66	11.07	11.07	3.08	16.4	1	1
	Khorramabad	13	11	14	10	450	2	300	100	8.42	3.24	11.7	2.76	18.71	1	1
	Isfahan	13	5	14	15	195	2	400	110	7.42	2.85	10.13	2.64	13.91	1	1
	Semnan	15	7	13	30	700	2	380	110	6.91	4.39	11.15	2.77	16.78	1	1
	Kazeron1	14	11	14	20	830	2	470	105	7.44	4.57	10.56	3.15	13.73	1	1
	Ramin	11	4	14	20	480	2	350	110	8.97	3.87	10.05	2.88	17.6	1	1
	Bushehr	13	6	14	40	1000	2	360	100	8.35	3.72	10.17	2.4	16.59	1	1
	Frizi ,Chenaran	0	2	14	30	330	0	300	60	5.72	2.69	10.46	2.6	15.84	3	1 & 2
	Zanjan	13	3	15	40	460	2	330	100	7.76	4.7	10.7	3.2	17.24	1	1
	Ardestan	13	5	14	20	210	2	360	100	7.96	4.04	10.03	2.67	14.9	1	1

† Hair density at three levels (low=0, medium=1 and high=2).

Root and shoot weights were distinctively different among genotypes. Commercial Iranian genotypes which are mainly cultivated for distilling industry had high shoot weight and low root weight but European cultivars which are mainly cultivated for inulin production or chicon salad had low shoot weight and high root weight at the first year of cultivation. In addition, industrial chicory cultivars had lower maximum rosette leaf length and leaf width than commercial Iranian genotypes (Table 2). Hair density was the other studied morphological trait which was high for all endemic genotypes except Firizi and pumilum genotypes (Table 2). Though wild genotypes of chicory and landraces showed more variability for morphological and physiological traits, there was low variation among them for root weight (Table 2). Also, industrial chicory cultivars are characterized by a strong uniformity mainly due to their very narrow genetic basis (de Proft *et al*., 2003), this matter is almost correct in wild types according to the results of this research which made them useless to improve root yield of commercial genotypes, but theymay contain useful genes controlling some major quality or quantity traits such as disease or insect resistance. Phylogenic tree (Fig. 1) showed that commercial Iranian chicory genotypes which are cultivated conventionally for distilling industry were more related to *C. endivia* species than *C. intybus*and all of the commercial Iranian genotypes formed a monophyletic clade except wild type genotype of Firizi genotype which is classified in *C. intybus* category. Thus, like *pumilum* genotypes which are classified as subspecies of *C. endivia* (Conti*et al*, 2005; Lucchin *et al*, 2008), it seems that these endemic genotypes which are selected for distilling industry in Iran must be another subspecies of *C. endivia* (Fig. 1). Hence, using endemic genotypes in breeding program of root chicory for inulin production would be useless unless more wild type genotypes such as Firiziwhich are from *C. intybus*are screened for selection of bolting resistant genotypes. In general, this investigation on endemic genotypes helped us to effectively detect the taxonomic position of local genotypes based on diagnostic morphological characteristics including life span and selfing seed, and it also aided us in finding their potential root yield and inulin productivity.

Inulin assay

Analysis of variances demonstrated that the effect of genotypes was significant on all traits at 1% level. Coefficient of variation (C.V.) of traits which was obtained from the main data without any transformation showed high variation for total carbohydrate content as indicator for inulin percentage. It seems that high variation of fructose (C.V= %34) due to inulin degradation among genotypes caused fluctuation of inulin percentage among the studied genotypes. Presence of *C. endivia* genotypes such as *pumilum* genotypes due to their low content of carbohydrate and fructose in this study can be the main cause of high C.V. for this trait. Another reason for that can be activation of inulin degradation enzymes such as fructan 1-exohydrolase enzymes (1-FEHs)due to bolting of endemic genotypes which led to the removal of fructose from the end of inulin chain and reduction of polymerization degree of inulin and their low quality (Shoorideh *et al*., 2014). Fresh root yield as another component of chicory inulin yield (Baert and van Bockstaele, 1993) showed lower variation than total carbohydrate content which proves the little variation of this trait similar to the other researchers' results (Baert, 1997; Frese *et al*., 1991). The cause of this variation for root weight is again the existence of annual genotypes in this study and bolting of endemic genotypes which led to the remobilization of stored carbohydrates in root to complete generative growth phase and so the roots are shrunken (Shoorideh *et al*., 2015).

Table 3. Means comparison of measured traits among chicory genotypes

Cultivar group	Genotype	Traits					
		Fructose (%)	Glucose (%)	Total carbohydrate content (%)	Average DP	Fresh Root yield (gm^{-2})	Inulin yield (gm^{-2})
Crispumendive	Very fine vegetable	$4.9^{g\dagger}$	1.3^{d}	5.6^{f}	4.8^{d}	106.88^{k}	6^{i}
Root chicory	'Hera'	18.3^{b}	1.9^{b}	18.4^{b}	10.4^{ab}	2968.88^{d}	546.3^{c}
	'Tilda'	17^{c}	1.9^{b}	17.2^{c}	9.9^{b}	3562.66^{b}	612.1^{b}
	'Melci'	18.9^{b}	2^{b}	19^{b}	10.3^{ab}	1855.54^{f}	353.1^{d}
	'Orchies'	19.5^{b}	2^{b}	19.5^{ab}	10.8^{a}	4453.33^{a}	869.6^{a}
	Hungarian population	8.3^{e}	1.3^{d}	8.8^{e}	7.2^{c}	267.20^{jk}	23.4^{f}
Witloof	Yellow star	17.9^{c}	1.9^{b}	18^{bc}	10.7^{a}	1603.20^{g}	287.8^{d}
	Belgian endive, Karaj	13.3^{d}	1.9^{b}	13.8^{d}	8^{c}	2493.86^{e}	344.6^{d}
	'Schepens'	21.9^{a}	2.2^{a}	21.9^{a}	10.9^{a}	3340.00^{c}	732.1^{b}
	Novipia	18.6^{b}	1.9^{b}	18.6^{b}	10.8^{a}	356.26^{ij}	66.4^{ef}
Pumilum (Iranian genotypes)	pumilum	4.3^{h}	1.4^{d}	5.2^{f}	4^{d}	160.32^{jk}	8.4^{h}
	Khorassan 1	3.3^{h}	1.2^{d}	4.1^{f}	3.7^{d}	222.66^{jk}	9.2^{h}
	Khorassan 2	5.5^{g}	1.6^{c}	6.4^{f}	4.5^{d}	200.40^{jk}	12.8^{g}
Iranian genotypes	Kazeron 2	5.6^{fg}	1.3^{d}	6.2^{f}	5.5^{d}	587.84^{h}	36.6^{f}
	Khorramabad	5.3^{g}	1.3^{d}	6^{f}	5^{d}	346.38^{ijk}	20.9^{f}
	Isfahan	5.1^{g}	1.3^{d}	5.8^{f}	5^{d}	235.05^{jk}	13.7^{g}
	Semnan	5.5^{g}	1.3^{d}	6.2^{f}	5.2^{d}	195.94^{jk}	12.1^{g}
	Kazeron 1	6^{f}	1.3^{d}	6.7^{f}	5.5^{d}	534.40^{hi}	35.7^{f}
	Ramin	5.7^{fg}	1.7^{c}	6.7^{f}	4.4^{d}	279.92^{jk}	18.8^{f}
	Bushehr	6.1^{f}	1.4^{d}	6.8^{f}	5.4^{d}	385.94^{hij}	26.3^{f}
	Frizi, Chenaran	8.6^{e}	1.3^{d}	9^{e}	7.5^{c}	326.58^{ijk}	29.5^{f}
	Zanjan	6^{f}	1.4^{d}	6.7^{f}	5.4^{d}	163.28^{jk}	10.9^{h}
	Ardestan	6.1^{f}	1.3^{d}	6.8^{f}	5.6^{d}	247.40^{jk}	16.7^{fg}

† Similar letters for genotypes in each column show no significant difference

Means comparison revealed that 'Schepens'cultivar from witloof group had the highest total carbohydrate content and, therefore, inulin percentage (Table 3). After that 'Orchies', 'Hera', and 'Melci' from industrial chicory cultivars as the main source for inulin production and Novipia from witloof group had the highest total carbohydrate content. Only Firizi genotype from endemic genotypes as Hungarian population had intermediate inulin percentageand other endemic genotypes had the lowest inulin content (Table 3). Similar total carbohydrate content of Firizi genotype and Hungarian population ascertain phylogenetic tree results which were classified as Firizi genotype in *C. intybus*group (Fig. 1). Lower carbohydrate content of Firizi genotype and Hungarian populations is due to their bolting and breaking down of inulin (Shoorideh *et al*., 2015). Also, bolting of these genotypes and their inulin degradation led to intermediate DP of their inulin in comparison with root chicory and witloof cultivars (Table 3). The highest fresh root yield was observed for 'Orchies' cultivar (equal to 4.4 kg of fresh root per square meter) from industrial (root) group. Endemic chicory genotypes also exhibited low root yield because of their bolting (Table 3; Fig. 2). Consequently, the highest inulin yield was obtained for 'Orchies', after that for 'Schepens', 'Tilda' and 'Hera', respectively. In general, the most limiting factor for high production of inulin among industrial chicory genotypes is root yield because they will have, to some extent,the same inulin percentage with the same quality if they are cultivated and harvested at the right time (Shoorideh *et al*., 2015; Schittenhelm, 2001).

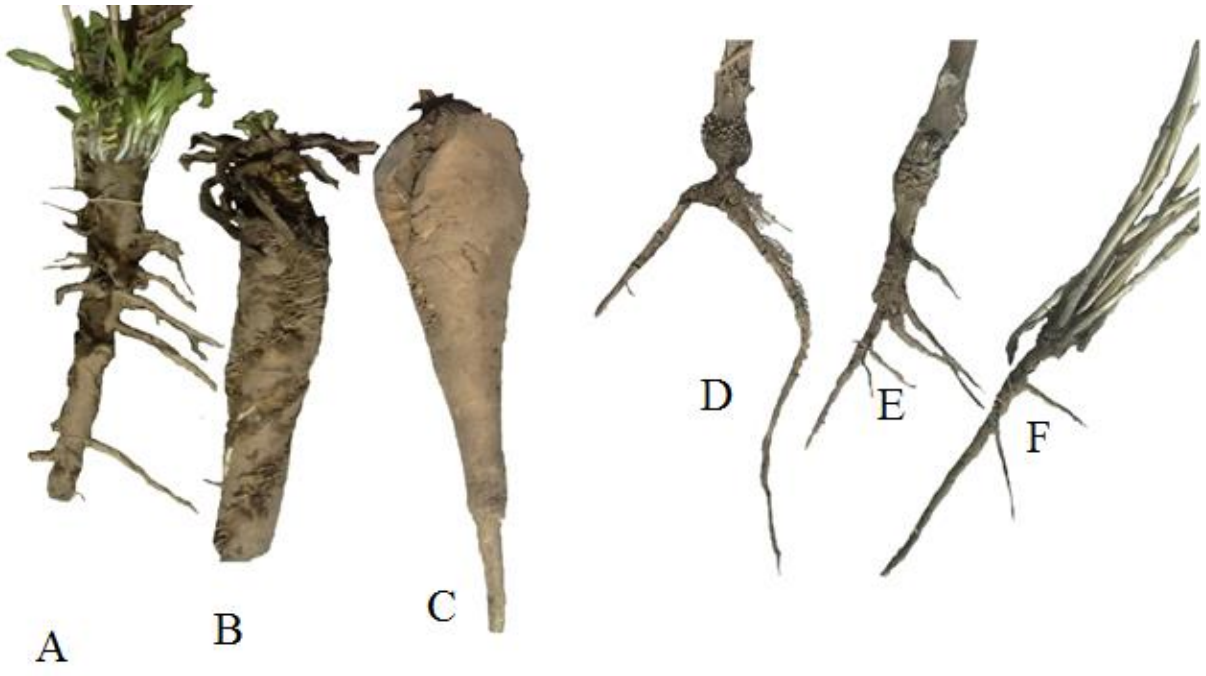

Fig. 2. A. Iranian wild genotype (Frizi),B. witloof genotype (Yellow star), C. root or industrial chicory ('Hera'), D. curly endive (var. *Crispum*), E. Commercial Iranian genotype for distilling, and F. Iranian pumilum genotype (this picture was taken on March 9, 2014).

Conclusion

Among endemic genotypes only Firizi genotype (Fig. 1A), collected from Chenaran mountainous region in Khorassan province, Iran, continued its growth for the following year. Other endemic genotypes such as commercial Iranian genotypes (Fig. 1E), with a long time period of cultivation in Iran for distilling industry, were passed away after overwintering. According to the phylogenic analysis, it can be concluded that commercial Iranian chicory genotypes are from *C. endivia*and might be classified as its subspecies. Thus, commercial Iranian chicory genotypes aren't appropriate for improving root yield of chicory as industrial cultivar for inulin production. Also, it can be concluded that the first step to enrich the genetic basis of root chicory for industrial chicory breeding program is to find biannual chicory genotypes which are resistant to bolting since all inulin fractions (quantity and quality)were affected adversely by this important characteristic.

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