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Original Article

Variation and Relationships of Shoot Yield, Morphological and Phenological Traits in Chamomile Populations *(Anthemis triumfettii)*

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Abstract

The chamomile species of *Anthemis triumfettii* (L.) All is one of important medicinal plant belong to Compositae family and it grows as wild species in Zagros and Alborz Mountains, Iran. The aim of this study was to evaluation of morphological and phenological traits and their relationships with essential oil. Seeds of seven accessions were sown in jiffy pots and transferred to field using randomize block design with three replications in Alborz research station, Karaj, Iran during 2011-12. Data were collected and analyzed for canopy diameter, plant height, flower number, flowering date, maturity date, shoot fresh and dry weight, Essential oil percentage and GDD to flowering and maturity dates. Results showed significance differences for all traits (P<%1) except of essential oil percent. Populations of Gorgan1 and Gorgan2 had higher values for essential oil yield and shoot yield than other populations. Population of Loshan flowered earlier than other population due to low values of its growth degree days (GDD). Simple correlation analysis showed that shoot dry weight was positively correlated with shoot fresh weight, canopy diameter, flower number, and negatively correlated with flowering and maturity date. Essential oil content was positively correlated with flower number and negatively correlated with flowering and maturity date. Results of stepwise regression analysis for essential oil% as dependant variables and other traits as independent variables showed negative effects of flowering and maturity date and positive effect of canopy diameter on essential oil content. Similarly, for essential oil yield as dependent variables, the shoot dry weight, essential oil%, flowering date and GDD were entered in final regression equation. The results of path analysis showed that maturity date had negative direct effect on oil%. In contrast, both Shoot dry weight and essential oil% had positive and direct effect on oil yield.

Key words: Chamomile, *Anthemis triumfettii,* (L.) All, Morphological traits, Simple Correlation Analysis, Stepwise Regression Analysis, Path analysis

Introduction

Due to different climate condition, Iran has large diversity of plant species which they could be used for medicinal plant domestication and cultivation. Fresh flowering extraction of Chamomile have plenty essential oil which were used for disinfected of moth and gum tissue after draw of teeth, Stomach bloating and colic, relieve insomnia, The healing of wounds, Vascular obstruction, Stop coughing and Tens of discomfort [1]. The Chamomile plant of the genus of Anthemis (L.) Alwhich is related to Composite family and this family is one of the large evaluated families of gymnosperm plant. 1100 genus and 2500 species were identified for this family [2]. In Iran, this genus has 39 perennial and annual plant species

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that 15 plant species is endemic of Iran [3]. According, to Bremer & Humpheries [4], this genus have over of 210 plant species which grows in region including Mediterranean Sea, South west Asia and east Africa. This species is perennial which is cultivated in Europe, Iran and Ghafghaz. This plant species is growing in some part of Iran including: Gorgan, Mazandaran, Gilan, Azarbijan and Semnan province. It goes to flowering and fruiting stages in the end of spring and first of summer [3]. Strengths and weaknesses points of the populations and available potentials would be determined and in other words, the genetic base area of each trait would be determined [5]. In medicinal plant breeding programs using genetic diversity of endemic population, are the optimal ways to obtain the selection criteria [6].

The effect of date of cultivation on vegetative and generative growth of four species of *Anthemis pyrethrum* L., *Anthemis Pseudocotula* Boiss., *Anthemis. tinctoria* L *and Anthemis. triumfetti* (L.) All showed that canopy size, plant height and flower number of *Anthemis pyrethrum* and *A. Pseudocotula* were higher than the other two species. Also, the species of *A. triumfettii* flowered in first cut but it had no flower in second cut [7].

Ggenetic diversity of some chamomile species by morphological and molecular marker in 20 Iranian and 5 European populations using RAPD method. Result showed that economical yield, flower number and essential oil had maximum genetic diversity, but they had no coordinated with geographical diversity [8]. With an research, significant variation found for stem, flower and fruit of yellow chamomile of *tinctoria* in Portland [9]. Evaluation of morphological and agronomical traits of 27 genotype of two genus of Anthemis and Matricaria showed that there was significance differences between all traits [10].

To introduce of improved variety chamomile species, study of genetic diversity is important. Since medicinal plant breeding here in Iran is in the early stage therefore, the selection is a tool to obtain the best populations. Also production of synthetic variety, identification and selection of parents with high generic potential, it would be possible to obtain maximum hetrosis. The aim of this experiment was to evaluation of variation and relationship among shoot yield, oil content, morphological and phenological traits and their relationships in some populations of *triumfettii*.

Material and Methods

In this research, populations of *A. triumfettii*, with different provenance (Table 1) were provided from the Natural resource gene bank of Research Institute of Forest and Rangelands, Tehran, Iran. Seeds were sown in Jiffy pots in November 2010. Then, the seedling was transferred in the field in Alborz research station in Karaj, Iran in March 2011. The experiment was randomized completely blocks design (RCBD) with three replicates. Each unit of experiment was consist of six row with 50 cm apart each other with distance of 40 cm. In this research, data were collected for morphological and phenological traits including: canopy diameter, plant height, number of flower, flowering and maturity dates, shoot fresh and dry weight, growth degree days (GDD), essential oil content percentage and essential oil yield. Flowering and maturity date were recorded as number of days from date 21 March till first flower emergence per plant. GDD were recorded by Frank *et al*,[11] as follows:

$$GDD = \frac{T_{min} + T_{max}}{2} - T_b$$

Where:

GDD= Growth degree days

Tmax = Maximum daily temperature

Tmin = Minimum daily temperature

Tbase = the base temperature

For extraction and measuring of essential oil, the shoot samples of plants were collected in fully flowering stage, then 80 g were taken to Measuring of essential oil by Kelvenger Instrument on the base of Hungarian plant pharmocopy letter [12-13] as follow:

$$\text{Essential oil content } \% = \frac{\text{Essential oil weight g}}{\text{Shoot dry matter g}} \times 100$$

Yield of essential oil were calculated by essential oil% x Shoot dry weight/h.

The collected data were analyzed and mean comparisons were made using Duncan method. Phenotypic correlation was determined between traits and two separate stepwise regression equation were developed for essential oil% and essential oil yield as dependant variable and other traits as independent variables. Finally, the correlation coefficients of those traits that were included in regression equation were partitioned to their direct and indirect effects using path analysis. All statistical analyses were conducted by MINITAB 16.

Results

Analysis of variance and mean comparison

A. Canopy Diameter:

There were significance differences between populations, years and populations and years interaction effects for canopy diameter (P<1%) (Table 1). Means comparison of the populations showed that means of canopy diameter of Loshan and Gorgan2 in the first and the second yeas with average values of 58.32, and 75 cm were higher than other populations respectively (Table 1). Also it was proved that canopy diameter of all of populations was increased in the second year. There were significance differences (P<1%), among populations, years and populations by years and interaction effects for plant height (Table 1). Mean comparison of the populations showed that plant height of Loshan and Gorgan2 populations with average values of 40.23 and 48.40 cm had higher stem length in the first and the second yeas, respectively. The minimum plant height was related to Ramian 1 population with average of 25.54 cm over two years.

B. Flowers number, number of days to flowering and maturity:

There were significance differences (P<1%), between populations, years and populations by years interactions effects for both flowering and maturity dates (Table 1).

The flower number of Loshan with average values of 155 in the first yeas and Gorgan2 with 202 flowers per plants in the second year was significantly higher than other populations (Table 2). The minimum flower number was belonging to east Azarbijan population as 11.64 cm (Table1).

The maximum number of days to flowering and maturity with average values of 219 and 261 days respectively were related to Gorgan2 in the first day. The Loshan with average values of 75 and 100 days had the maximum duration of flowering and maturity times in the second year (Table 2). East Azarbijan had minimum value of time of flowering and maturity as 71.17 and 87.33, over two years respectively (Table 2). Mean comparison of flowering and maturity date showed that East Azarbijan population had lowest value of day to flowering and maturity; therefore it was flowered and matured earlier than other population. In contrast, other populations like Gorgan2 had higher value of duration of flowering and maturity date therefore it were flowered later than other populations (Table 4).

This result was confirmed by Alizadeh *et al* (2012). They evaluated effect of planting date on vegetative and reproductive growth on *Anthemis prythrum* L., *Anthemis A. Pseudocotula* Boiss. *AnthemisA. tinctoria* L and *Anthemis A.triumfetti(* L.) All and their result showed that species of *A. pseudocotula* flowered earlier than other populations because of lower GDD.

Table1 Analysis of variance and means comparisons for canopy diameter and Plant height of the *Anthemis triumfettii*(L.) All. populations grown in field condition over two year (2011-12).

SOV	df	Canopy diameter		Plant height (cm)	
Replication	2	1.58		98.1**	
Population	6	285.0**		182.8**	
Error 1	12	28.6		19.01	
Year	1	288.4**		222.1**	
Year*Population	6	412.4**		342.9**	
Error 2	14	5.88		9.49	
%CV	-	5.34		9.52	
Population		Canopy diameter		Plant height (cm)	
		Year 1	Year 2	Year 1	Year 2
Gorgan2		32.48 d	75.00 a	9.70 c	48.40 a
Orumieh		40.81 c	40.81 c	33.50 b	33.50 b
Ramian2		47.06 b	47.06 bc	38.22 a	38.22 b
Ramian1		42.08 c	42.08 c	25.54 b	25.54 c
East Azarbaijan		38.72 c	38.72 c	35.97 a	35.97 b
Loshan		58.32 a	52.49 b	40.23 a	36.21 b
Gorgan1		39.40 c	39.40 c	27.09 b	24.61 c
		42.70	47.94	30.04	34.64

** Significant at 5% and 1% level, respectively

Means with the same letter in column are not significantly different ($\rho<0.05$).

Table 2 Analysis of variance and means copparions for flower number, days to flowering and maturity of the *Anthemis triumfettii* (L.) All. populations grown in field condition over two year (2011-12).

SOV	df	Flower number per plant	Number of days to flowering	Number of days to maturity
Replication	2	165.33	12.14	22.97
Population	6	18378**	3505**	4446**
Error 1	12	317.61	29.38	38.03
Year	1	6312**	21225**	28855**
Year*Population	6	7527**	4454.7**	6417**
Error 2	14	259.98	20.80	12.48
%CV		32.32	5.43	3.46

Population	Flower number per plant		Flowering date		Maturity date	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Gorgan2	17.97 b	202.3 a	219.8 a	56.67 c	261.0 a	66.03 c
Orumieh	17.89 b	17.80 cd	86.67 b	62.00 b	109.3 b	76.43 b
Ramian2	21.53 b	21.53 cd	90.67 b	62.00 b	112.3 b	75.00 b
Ramian1	20.35 b	28.15 c	97.50 b	63.83 b	110.0 b	76.50 b
East Azarbaijan	11.64 c	11.64 d	87.00 b	55.33 c	105.9 b	68.73 c
Loshan	155.8 a	140.2 b	67.50 c	75.94 a	89.67 c	100.8 a
Gorgan1	18.13 b	13.27 d	95.50 b	54.17 c	110.0 b	67.73 c
Means	37.62	62.13	106.38	61.42	128.32	75.90

** Significant at 5% and 1% level, respectively

Means with the same letter in column are not significantly different ($\rho<0.05$).

Table 3 Analysis of variance and means comparisons for fresh weigh Shoot dry weight and essential oil (%) of the *Anthemis triumfettii* populations grown in field condition over two year (2011-12).

SOV	df	Shoot fresh weigh (g/p)	Shoot dry weight (g/p)	Essential oil (%)
Replication	2	129873.3	44998.1	0.00002
Population	6	1608420**	363401**	0.0005*
Error 1	12	255594.7	75999.9	0.00004
Year	1	2143261**	87001**	0.001**
Year*Population	6	1029225**	279937**	0.0002
Error 2	14	48025.2	5101.1	0.00008
%CV		11.15	7.60	11.06

Population	Shoot fresh weigh (g/p)		Shoot dry weight (g/p)		Essential oil (%)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Gorgan2		837.0 a		403.3 a		0.090 b
Orumie		294.0 c		199.7 b		0.080 b
Ramian2	489.3 b	508.0 b	209.7 b	270.7 ab	0.077 a	0.100 a
Ramian1		588.0 b		291.0 ab		0.103 a
East Azarbaijan	346.7 b	773.0 a	116.3 c	335.0 a	0.080 a	0.103 a
Loshan	878.0 a	790.3 a	440.0 a	396.0 a	0.067 a	0.067 c
Gorgan1	466.0 b	864.7 a	179.0 c	425.0 a	0.070 a	0.093 b
Means	545.1	665.0	236.2	331.5	0.071	0.091

*, **, Significant at 5% and 1% level, respectively

Means with the same letter in column are not significantly different ($\rho<0.05$).

Table 4 Analysis of variance and means comparison for essential oil yield, GDD of flowering and maturity date of the *Anthemis triumfettii* populations grown in field condition over two year (2011-12).

SOV	df	Essential oil yield (gh^{-1})	GDD of flowering	GDD of maturity
Replication	2	0.03*	4084	537587
Population	6	0.13**	1802657**	5338107**
Error 1	12	0.06	16678	648804
Year	1	1.45	5182729**	13460782**
Year*Population	6	0.30**	1913462**	348178
Error 2	14	0.005	15130	483992
%CV		9.23	7.16	34.02

Population	Essential oil yield (gh-1)		GDD of flowering		GDD of maturity	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Gorgan 2	261.00 a	66.03 c	4547 a	1362 c	5067 a	3006 a
Orumie	109.33 b	76.43 b	1617 b	1403 b	2255 b	1445 b
Ramian 2	112.33 b	75.00 b	1776 b	1403 b	2388 b	1450 b
Ramian 1	110.00 b	76.50 b	1958 b	1392 b	2314 b	1449 b
East Azarbaijan	105.93 b	68.73 c	1673 b	1362 c	2195 b	1412 b
Loshan	89.67 c	100.87 a	1175 c	1442 a	1712 c	1182 c
Gorgan 1	110.00 b	67.73 c	1897 b	1362 c	2350 b	1410 b
Means	128.31	75.90	2091.9	1389.4	2611.6	1479.1

** Significant at 5% and 1% level, respectively

Means with the same letter in column are not significantly different ($\rho<0.05$).

Table 5 Simple correlation between different traits among different populations of *Anthemis triumfettii* (L.) All.

Traits	Canopy diameter	Plant height	Flower number per plant	Flowering date	Maturity date	Fresh weight	Dry weight	Essential oil %	Essential oil yield	GDD flowering
Plant height	0.67**	-	-	-	-	-	-	-	-	-
Flower number per plant	0.93**	0.64**	-	-	-	-	-	-	-	-
Flowering date	-0.19	-0.06	-0.18	-	-	-	-	-	-	-
Maturity date	-0.16	-0.04	-0.11	0.95**	-	-	-	-	-	-
Shoot fresh weight	0.50**	0.19	0.56**	-0.44**	-0.35*	-	-	-	-	-
Shoot dry weight	0.54**	0.20	0.60**	-0.56**	-0.47**	0.94**	-	-	-	-
Essential oil percentage	0.26	-0.18	0.40*	-0.59**	-0.68**	0.01	0.03	-	-	-
Essential oil yield	0.33*	0.08	0.31	-0.74**	-0.71**	0.84**	0.88**	0.48**	-	-
GDD of flowering	-0.33*	-0.18	-0.41**	0.86**	0.73**	-0.49**	-0.63**	-0.25	-0.61**	-
GDD of maturity date	-0.15	-0.01	-0.04	0.15	0.11	-0.10	-0.21	-0.04	-0.17	0.27

*, ** significant at 5% and 1% level, respectively

Table 6 Stepwise regression analysis in the case of essential oil percentage as depended and other are independent

Traits	Steps		
	Step 1	Step 2	Step 3
Constant	13.63	14.63	12.05
Maturity date	-0.060	-0.064	-0.062**
Flowering date	-	-0.011	-0.020**
Canopy diameter	-	-	0.061*
R-Square	45.59	67.37	70.69

** Significant at 5% and 1% level, respectively

Table 7 Stepwise regression analysis when the essential oil yield as depended and other traits are independent

Traits	Steps			
	Step 1	Step 2	Step 3	Step 4
Constant	1.29	-26.19	-35.01	-30.57
Shoot dry weight	0.081	0.080	0.084	0.083**
Essential oil percentage	-	3.280	3.410	2.960**
GDD of flowering date	-	-	0.004	0.010**
Flowering date	-	-	-	-0.116**
R-Sq	77.29	98.48	98.85	99.08

** Significant at 5% and 1% level, respectively

Table 8 Results of path analysis for partitioning of total correlation to direct and indirect effects of various traits to essential oil percentage in *Anthemis triumfettii* (L.) All.

Traits	Direct effect	Indirect effect via			Correlation
		Canopy Diameter	Flowering date	Maturity date	
Canopy diameter	0.159	-	-0.121	0.201	0.239
Flowering date	0.636	-0.031	-	-1.197	-0.590
Maturity date	-1.26	-0.026	0.604	-	-0.681

Residual effects: 0.693

Table 9 Results of path analysis for partitioning of total correlation to direct and indirect effects of various traits to essential oil yield in *Anthemis triumfetti* (L.) All.

Traits	Direct effect	Indirect effect via				Correlation
		Flowering date	Dry weight	Essential oil %	GDD of Flowering	
Flowering date	-0.202	-	-0.504	-0.231	0.195	-0.741
Shoot dry weight	0.898	0.112	-	0.011	-0.143	0.879
Essential oil %	0.391	0.118	0.026	-	-0.057	0.479
GDD of flowering	0.226	-0.174	-0.567	-0.098	-	-0.611

Residual effects: 0.105

C. Shoot Fresh weight, Dry weight and Essential oil percentage

There were significance differences (P<1%) among populations, years and population and years interaction effects for shoot fresh and dry weight (Table 3). In the first year three populations of Gorgan2, Orumie and Ramian1produced no shoot yield. The population of Loshan with average values of 878 and 440 g/plant had higher fresh and dry shoot weight in the first year respectively.

In the second year, Gorgan1 Gorgan 2, East Azarbaijan and Loshan with range of 773 to 864 g/plant fresh weight and 335 to 425 g/plant of dry weight had maximum shoot production than to other population. The minimum shoot fresh and dry weight of average of two years was belonged to Ramian2 (Table 3). Mean comparison showed that the population of Gorgan2 had essential oil yield due to high shoot dry yield (Table 4). This result was similar with result of Kohan Moo *et al*. (2011), Golparvar and Ghasemi Pirbalouti (2011) reported that essential oil yield would be affected by plant density, date of cultivation, temperature, light and fertilization.

There were significance differences among

populations (P<5%) and years (P<1%) for Essential oil percentage (Table 3). In the first year, three populations of Gorgan2, Orumieh and Ramian1 don't produce any shoot enough for oil extraction. However there were no differences between other four populations for oil% with range of 0.07 to 0.08%. In the second year, Ramian1, Ramian2 and East Azarbaijan with range of 0.100 to 0.103% had maximum oil content than to other population. The minimum average value was related to Loshan population as 0.067% (Table 3).

D. Essential oil yield, Growth degree days (GDD) for flowering and maturity

There were significance differences among populations and years and population interaction effect (P<1%) for essential oil yield (Table 4). Average Essential oil yield of the first year for Gorgan1 population was as 261 g/ha was significantly higher than other populations, In contrast in the second year Loshan with average values of 100 g/ha had higher oil production (Table 4). The minimum average essential oil yield of two years was related to Esat Azarbaijan population as 87 g/ha (Table 4). There were significance differences, among populations, years and populations and years for GDD of both flowering and maturity date (P<1%) (Table 4). Mean comparisons of populations showed that maximum average value of the first year was as 4547 and 5067ºC of GDD of flowering and maturity, respectively for population Gragan2. In the second year, the minimum average value of GDD of 1182ºC were related to Loshan in maturity but in flowering time, average value of GDD of 1442ºC was maximum (Table 4). The maximum values of GDD were related to population of Gorgan2 as 3006 ºC in maturity (Table 4).

Correlations between traits

Result of simple correlation analysis showed that essential oil yield was positively correlated with canopy diameter, shoot dry weight and essential oil percentage and negatively correlated with flowering and maturity date (Table 5). Shoot dry weight positively correlated with canopy diameter, flower number and fresh weight and it was negatively correlated with flowering and maturity date. Flower number positively correlated with canopy diameter and plant height. Regarding of result of simple correlation analysis showed that essential oil yield was positively correlated with canopy diameter, shoot dry weight and essential oil percentage and negatively correlated with flowering and maturity date (Table 5).

Regression analysis

Results of stepwise regression analysis for essential oil% as dependant variables and other traits as independent variables showed negative effects of flowering and maturity date and positive effect of canopy diameter on essential oil content in the final model with R^2=70.69% (Table 6). Similarly, for essential oil yield as dependent variables, the Shoot dry weight, essential oil% , Flowering date and GDD of flowering date were entered in final regression equation in the final model with R2=99.08% (Table 7).

There was a good agreement between the results of correlation and regression analysis. With regard of stepwise regression analysis for essential oil percentage as dependant variables and other traits as independent variables showed significant effects of shoot dry matter, GDD, plant height, canopy diameter and flowering date in essential oil content. Similarly, Pirkhezri *et al*., (2009) reported that there were positive correlation between essential oil% and flowers number per plant, shoot fresh yield, flower and plant height but there were negative correlation with phenological traits.

Path analysis

The results derived from path analysis for essential oil% indicated that maturity date had negative direct effect on oil%. Since the sign and amount of direct effects was in the same trend of correlation coefficient suggested that improvement of oil% was possible by selection of early flowering populations (Table 8). The results derived from path analysis for essential oil% indicated that improvement of oil% was possible by selection of early flowering and early maturity populations.

The results of path analysis for essential oil yield indicated that both Shoot dry weight and essential oil% had positive direct effect on oil yield. Since the sign and amount of direct effects was in the same trend of correlation coefficient suggested that improvement of oil yield was possible by selection of shoot dry weight and essential oil% (Table 9). It was concluded that for improvement of oil yield selection of shoot dry weight coupled with essential oil% will be effective (Table 9).

Discussion

Regarding to canopy diameter, two populations of Gorgan2 and Loshan were superior than other

populations (Table 1). Also results showed that mean flower number and plant height of Loshan population were higher than other population (Table 2). This result was similar with result of Dadkhah, and coworker [14], because their result showed that date of cultivation had significantly effect on growth traits and number of flower.

Mean comparison of flowering and maturity date showed that East Azarbijan population had lower value of day to flowering and maturity, therefore it was flowered and matured earlier than other population. Other population like Gorgan2 had higher value of duration of flowering and maturity date therefore it was flowered later than other populations. This result was confirmed by Alizadeh *et al* [15], they evaluated effect of planting date on vegetative and reproductive growth *Anthemis pseudocotula*, . *A. tinctoria* and *A. triumfettii*, and their result showed that species of *A. pseudocotula* flowered earlier than other populations because of lower Growth degree days. Regarding of shoot fresh and dry weight, two populations of Gorgan2 and Loshan had higher mean value over two years compare with other populations. Mean comparison showed that the population of Gorgan2 had essential oil yield due to high shoot dry yield.

This result was similar with result of Kohan Moo *et al*. [16], Golparvar and Ghasemi Pirbalouti [17], they reported that essential oil yield would be affected by plant density, date of cultivation, temperature, light and fertilization.

Simple correlation analysis showed that essential oil yield was positively correlated with canopy diameter, shoot dry weight and essential oil% and negatively correlated with flowering and maturity date. With regard of stepwise regression analysis for essential oil percentage as dependant variables and other traits as independent variables showed significant effects of shoot dry matter, GDD, plant height, canopy diameter and flowering date in essential oil content. Similarly, Pirkhezri *et al*., [10] reported that there were positive correlation between essential oil% and flowers number per plant, shoot fresh yield, flower and plant height but there were negative correlation with phenological traits.

The results derived from path analysis for essential oil% indicated that improvement of oil% was possible by selection of early flowering and early maturity populations. Similarly for improvement of oil yield selection of Shoot dry weight coupled with essential oil% will be effective.

Conclusion

Regarding result of this research work some points were concluded: 1) Two population of Gorgan2 and Loshan had higher value of vegetative and generative traits like canopy diameter, flower number than other populations, 2) Fresh and dry shoot yield, essential oil yield of Gorgan1 population were higher in first year but in second year, fresh and dry shoot yield, essential oil yield of Gorgan2 population were higher, 3) With regard of GDD in flowering and maturity time, the population of Gorgan2 considered as later population because of high values of flowering and maturity time but Loshan population was considered as earlier population because of low values for flowering and maturity time.

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