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Investigation the morphocytological traits and ploidy level in *Iris* species of Iranian native in Zanjan Province

Somayyeh Joz Ghasemi[1*], Vali Rabiei[2], Ali SoleymanI[3], Ahmad Khalighi[4]

[1]*Horticultural Science Department ,College of Agriculture, Science and Research Branch, Islamic Azad University ,Tehran, Iran*

[2]*Horticultural Science Department, College of Agriculture, zanjan University, zanjan, Iran*

[3]*Reasearch Institute of Modern Biologicaltechniques University of Zanjan . Zanjan. Iran*

[4]*Horticultural Science Department, College of Agriculture, Science and Research Branch, Islamic Azad University ,Tehran, Iran*

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Abstract

Determining the chromosomal level is important in breeding programs. Determining the chromosomal levels in Iris species is time consuming and costly by Cytogenetic and DNA Cytometric methods due to the large number and tiny size of chromosomes**.** Therefore, an investigation was performed in 2011-2013 aimed at assessing the relationship between the morphocytological traits and chromosomal level in lris species native to Iran (Zanjan province) and was arranged in a completely randomized block design with10 replications. Results of the statistical analysis showed that the traits of chloroplast number in stomatal guard cells, length stomatal guard cells, stomatal and epidermal cell density, stomatal index and stomatal conductance cannot be an accurate measure for determining the chromosomal level among different species of Iris under this study. During the implementation of test, Clypeate pollen grains were observed in the species *I.persica, I.sp* and *I.psedocaucasica* and the results of the statistical analysis of the data indicated that a significant increase in the pollen grain diameter was observed in species with higher level of chromosome, compared with lris species with lower levels of chromosome, and this trait can be used as a simple and rapid method in determining the chromosomal level of lris species and it is capable of simplifying the implementation of the iris breeding programs.

***Corresponding Author:** Somayyeh Joz Ghasemi ✉ ag2sa@yahoo.com

Introduction

The Irises (*Iris sp*.) are from the family Iridaceae and the categories monocots(Ghahreman , 1805). These plants are resistant to changes in temperature and have long be used as a symbol of beauty, and valued as an ornamental plant in land scaping, cut flowers and potted and due to its medicinal properties, they have been considered as valuable medicinal plants and they are one of the plants native to Iran that we need to be trier in preserving and using it in breeding program (Azimi *et al* , 2011). About 300 species of the wild Iris are found world wide, of which about 20 species and sub-species of them are found in country of Iran. There is a great uncertainty among scientists to identify and classify them (wendelbo , 1704). The native plants are recognized as the valuable genetic resources (Kim *et al* , 2004) and the first step in breeding programs of a species is the knowledge of the amount of diversity in the wild varieties and families of that species.

However, no morphological characters such as plant height, leaf length and width, stem length, flower diameter, and root length are reliable in distinguish different ploidy levels (Cenci *et al* , 1984). So far for the determination of ploidy levels the only on the basis of differences in chromosome number or cytometric DNA estimations (Nordsenkiold, 1945 – Joachimiak , Joachimiak and kula. 1993 and 1996).Rahimi *et al* (2011) to studied the morphological and cytological varation among 5 species of Iranian native Irises. Azizian *et al* (1993) investigated karyotypes of two species of the genus Iris native to Iran, *I.germanica* and *I.pseudacorus*. The chromosome compositions of Iris species are polymorphic among species and their large number and tiny size of the chromosome were the barrier for cytological study (Kim *et al* , 2004) the ploidy level of the species are important in the study of family relation , and of extensive breeding programs the determinate ploidy level by the usual method in root meristematic cells , is difficult and time-consuming. So there is a simple alternative method appears to be essential than the easier and more affordable, such as chloroplast number in stomatal guard cells (lich , 1981).

Root tip cell chromosome counting is time-consuming and not suited for routine screening of ploidy level. For this purpose, the stomatal cell length can be a rapid and reliable indirect ploidy indicator (Speckman *et al*, 1965 – Mishra , 1997) . Ghanavati and Skandari (2011) reported for *Onobrychis* sp plant, in tetraploid species is the number of chloroplasts in stomatal guard cells almost twice that of chloroplasts in diploid species. Then in *Onobrychis* sp was recommended the chloroplast number in stomatal guard cells as a novel method for the determination of ploidy levels. Abak *et al* (1998) reported in many of plant species, there are correlation between ploidy level and cytogenetic characteristics such as chloroplast number in guard cells, size of stomata cells, stomata density and pollen grain diameter. stomata density and especially the number of chloroplast in guard cells seemed to be reliable for the estimation of ploidy level. Joachimiak and Grabowska-Joachimiak (2000) Stomatal cell length was examined in Phleum and reported that the polyploids have longer stomatal cells than their diploid relatives . stomatal cell length can be a rapid and useful indirect ploidy level indicator and can assist in their identification .

Cramer (1999) reported the tetraploid plants typically have larger pollen grains than diploid plants and diploid plants typically more dense, smaller diameter stomata than tetraploid plants..Omidbeygi *et al* (2010) reported for Basil plants that the tetraploid plants for compared with diploid plants were have larger stomatal and pollen grains and the decrease in stomatal density and has increased chloroplast number in stomatal guard cells. Kery *et al* (2007) Ploidy level was estimated in *Hydrangea inacrophylla* and reported Stomatal guard cell length and pollen diameter of diploid cultivars were smaller than those of triploid cultivars. However, because the range of measurements for the diploids overlapped that of the triploids, neither guard cell nor pollen

grain measurements are recommended for determining ploidy of *H. rnacropylla* cultivars **.** Teare *et al* (1971) investigated the stomatal features in Bromus inermis plants and observed that the epidermal cells size was the main factor determining stomatal frequency.

Murray and standing (1992) in lathyrus plants were observed a significant correlation between the chromosomal level, pollen size and number of chloroplasts in stomatal guard cells . Salisbury (1927) found a highly significant positive correlation between the number of stomata and epidermal cells per unit of leaf area. Mishra (1997) was observed the significant positive correlation between the density of stomata and epidermal cells and the significant negative correlation between stomatal density and stomatal guard cell length and epidermal cell density and stomatal guard cell length of coffee plant and reported that can be based the stomatal guard cells lenght and to the Correlation analysis was assessed the stomatal density and epidermal cells length .

In the present study, for the first time we started to study and investigating on Iris native to Iran (13 species in Zanjan province) about the correlations which exists in the number of chloroplasts in stomatal guard cells and size of these cells, pollen grain diameter, stomatal and epidermal cells density, stomatal index and stomatal conductivity and also for the first time, the appearance of pollen grain of Iris varieties native to Iran (Zanjan province) was examined that in case of effectiveness and existence of a significant correlation and relationship between cytological characteristics mentioned above, these cytological characteristics of the iris plant can be suggested as a simpler, faster and more economical method to determine the chromosomal levels in breeding programs.

Materials and methods

Collection of samples

In 2011, the native varieties of iris (*Iris sp*.) were collected from the cities of Zanjan province (Table 1) and were planted in a farm located in Khorramdarreh city and then ploidy status of all varieties were determined according to the Alishah and Omidi (2008) method. In late March 2012 until late May in 2013, selection was performed for morphophysiologic and cytological traits including pollen grain diameter, length of stomatal guard cells, stomatal index, stomatal conductance, chloroplast number in stomatal guard cells and epidermal and stomatal cells density in 10 replications of each cultivar. Initially 10 plants were selected of each variety and then morphophysiologic and cytological traits were measured follows.

Pollen grain diameter

During flowering, a day after the flowers bloom and according to the method Funamoto *et al*. (2006), were selected 10 plants of each variety and separated two anthers of each flower and fixed in 3:1 absolute ethanol: acetic acid solution for 24 h, and stored in 70% ethanol at 4 °C. After staining with Lacto - Propionic Orsein 45%. Pollen grains examined for optical microscope camera of 100 and 400 magnification and were photographed and then by using the Micro Measure ver3.3 software according to Reeves and Tear (2000) methods , diameter 20 pollen grains was measured from the each plant.

Length of stomatal guard cells

For this purpose, 10 plants of each cultivar randomly were selected. Measurment were performed for two well expanded leaves of each plant. To prevent evaporation, were placed the leaves in plastic bags and were transported to the laboratory. A small area of abaxial side of leaves was covered with a thin layer of clear nail polish and left to dry (Cramer , 1999 and Hamill *et al*., 1992). After drying the polish, it was removed with a tip forceps then placed on a glass slide and observed through the light microscope Camera and were obtained images and then was measured of the 20 pairs of stomatal guard cell length by using software MicroMeasure ver 3.3.

Chloroplast number in stomatal guard cells

According to the method Ghanavati and Eskandari (2011) and by using the scalpel blade was separated sampls of epidermal layer from abaxial side leaves and were placed on the silver nitrate solution. Then this epidermal layer was stained with Loghol soulation ,and and observed by light microscope with a magnification of 400X, the number of chloroplasts was counted in 20 pairs in stomatal guard cells.

Stomatal index

10 plants of each cultivar randomly were selected by using Salisbury (1927) methods and Were counted the number of stomata and the number of epidermal cells per unit leaf by using 400 magnification of light microscope and the stomatal index was calculated by using the following formula

SI = S / (E + S) * 100

S: The number of stomata per unit leaf

E: The number of epidermal cells per unit leaf.

Stomatal conductance

According to the Holand and Richardson (2009) methods and for using 400 magnification an optical microscope were counted the number of stomata per unit leaf and was calculated stomatal conductance for using the following formula.

stomatal conductance = (length stomatal guard cells)^2 * stomatal total capacity * 10^{-4}

Epidemal and stomatal cell density was measured for according to the salisbory (1927) methods and for light microscope by 400 magnification.

Data were arranged in a randomized complete block design with 10 replications and analyzed by using the software MSTATC and was performed mean values by Duncan multiple range test.

Results and discussion

Chloroplast number in stomatal guard cells

In the results of Table 4, a significant correlation between chloroplasts number in stomatal guard cells, epidermal and stomatal cell density, pollen diameter and length of stomatal guard cells were not observed. But the number of chloroplast in stomata guard cells had a significant correlation with stomatal conductance. Hence, it can be concluded that in Iris species, an increase in chloroplast number of stomatal guard cells would cause an increase in stomatal conductance and thus an increases in the stomata efficiency.

Chloroplast number in stomatal guard cells of *I.persica* had a significant difference with other varieties and the larger number of chloroplasts was observed in stomatal guard cells *I.persica* and the lowest in *I.meda*, *I.elegantissim, I.sp var.khorramdarreh, I.pseudacorus* vareiteis. According to the differences in chromosomes amounts (Table 1) in species which have significant difference with other species (species listed above) and have the lowest amount of chloroplast in stomatal guard cells, it was found that this cytological trait in some species of Iris are overlapping and therefore it cannot be a perfect criteria in detecting the ploidy level in different Iris varieties. According to research performed in basil and *Onobrychis sp,* chloroplast number in stomatal guard cells with increasing in ploidy level increased significantly (omidbeigi , 2010 – ganavati and eskandari , 2011), that this result is consistent with the results of the experiment in *I.persica* variety (Table 2) but it is not observed in other varieties because of overlapping (Table 2) this trait is not recommended to determine the ploidy level of the Iris varieties but it is better to study more on *I.persica* variety in breeding works.

Pollengrain diameter

I.pseudacorus species has a significant difference compared with other varieties and it possess larger pollen grains.Varieties *I.persica* ، *I.sp* ،*I.acutiloba* ، *I.spuria* ، *I.psedocaucasica* are next, and the smallest pollen grains were observed in 5 different varieties of *I.meda* (Table 3). The results(Table 1and3) showed that by increasing the chromosomes amount in different varieties, pollen grain diameter also was increased significantly. The result was similar and

consistent with the results of Abak *et al* (1998) , Keri *et al* (2007) and Omidbaigia *et al* (2010) that expressed there is a positive correlation between chromosome level and pollen grain diameter in different plants.

Table 1. Iris native species were collected from the different city of Zanjan province.

Taxa	Flower color	Natural habitat	Longitude and Latitude	Chromosome number
I.reticulata	Violet Purple	Khorramdarreh	Longitude: 361845.91 N Latitude : 491414.47 E	20
I.reticulata	Light Blue	Khorramdarreh	Longitude: 36193.93 N Latitude : 491418.62 E	20
I.meda	Yellowish cream with streaks of brown and pink spots	Khorramdarreh	Longitude: 36185701 N Latitude : 49141681 E	20
I.meda	Cream with brown streaks and spots of blue oil	Khorramdarreh	Longitude: 36185995 N Latitude : 49141374 E	20
I.meda	Cream with pale brown streaks	Khodabandeh	Longitude: 35444703 N Latitude : 48335309 E	20
I.meda	White with brown streaks	Mahneshan	Longitude: 365148.75 N Latitude : 473521.71 E	20
I.meda	Yellow and white with blue spots	Mahneshan	Longitude: 365149.84 N Latitude : 473519.94 E	20
I.sp	White with yellow streaks	Khodabandeh	Longitude: 35444818 N Latitude : 48334221 E	30
I.persica	White with blue streaks	Khodabandeh	Longitude: 36172.91 N Latitude : 491430.19 E	38
I.pseudacorus	Yellow	Tarum - Gilvan	Longitude: 36476.48 N Latitude : 49736.97 E	38
I.pseudocaucasica	Clear yellow	Khodabandeh	Longitude: 361735.99 N Latitude : 491516.96 E	40
I. acutiloba	White with purple streaks	Tarum	Longitude: 364649.98 N Latitude : 485130.58 E	42
I.spuria	Yellow	Mahneshan	Longitude: 365150.02 N Latitude : 473515.01 E	44

Table 2. Mean comparison morphocytological traits of Iris species.

TAXA	ECD 0.1mm^{-2}	SCE 0.1mm^{-2}	CN mean	SI %	SC mean
I.reticulata	12.50 cd	6.00 cd	18.40 bc	31.51 b	0.5692 cd
I.reticulata	12.30 cd	6.40 c	20.30 bc	33.21 b	0.5018 cd
I.meda	12.80 cd	5.50 cde	15.00 bcd	31.84 b	0.495 cd
I.meda	12.30 cd	3.70 e	8.70 def	23.33 d	0.3889 d
I.meda	13.00 cd	5.80 cde	3.60 f	31.88 b	0.4418 d
I.meda	14.20 bcd	5.60 cde	20.80 b	24.93 cd	0.4645 cd
I.meda	13.80 bcd	5.60 cde	19.30 bc	28.63 bcd	0.5806 cd
I.sp	11.20 d	5.70 cde	7.40 ef	32.67 b	0.7305 c
I.persica	16.00 bc	11.20 b	39.20 a	41.08 a	1.4354 a
I.pseudacorus	21.2 a	16.60 a	8.40 def	13.36 e	0.6119 cd
I.pseudocaucasica	16.20 bc	11.00 b	20.70 b	40.63 a	1.0166 b
I.acutiloba	11.00 d	4.00 de	8.40 def	26.73 bcd	0.3226 d
I.spuria	17.00 b	6.20 c	13.50 cde	29.94 bc	0.4655 cd

*Means followed by similar letters in each column are not significantly at 1 % level by using Duncan multiple range test.

*ECD : Epidermal cell density - SCD: Stomatal cell density- CN : Chloroplast number - SI : Stomatal index - SC : Stomatal conductance.

Clypeate Pollen grain is observed in *I.persica* ، *I.sp* and *I.psedocaucasica* varieties (fig 1). Clypeate Pollen grains are referred to the pollen grains of medium to large with island-like grooves and contours (eureticulate) (Punt *et al* , 1994), so far has been observed and examined in some varieties of Iris native to Turkey (Donmez and Pinar , 2001). According to the results in Table 3, large pollen grain in these three varieties are observed which possess Clypeate pollen grain, and they are roundel and according to Austin (2005) reports the varieties of Iris *I.psedocaucasica* and *I.persica* are subgenus of Scorpiris. Donmez and Pinar (2001) reported that the all Iris species subgenus of Scorpiris show uniform characteristics in pollen type (clypeate), pollen shape (spheroidal) and exine sculpturing (island-like). Considering the results given in Table3 and Fig1, and interpreting them, it can be concluded that the *I.sp* species also should be Scorpiris. Cramer (1999) , Abak *et al* (1998) , Donmez and Pinar (2001) , Keri *et al* (2007) and Omidbaigia *et al* (2010)reported that the size of pollen grain depends on ploidy level and it increases by increasing the ploidy level. The results in Table1 and 3 also are confirming the idea about Iris that by increasing chromosome level, pollen size increases as well. Sowe can use the pollen grain diameter as a simple and rapid method in determination the chromosome level in different Iris varieties.

Table 3. Mean comparison of pollen grain diameter and stomatal guard cells length of Iris species.

TAXA	PD			SGL		
	M	SD	V	M	SD	V
	mμ		μm	mμ		μm
I.reticulata	85.2 e	0.53±	84.67 -85.73	30.8 bc	0.15±	30.65 – 30.95
I.reticulata	92.2 de	0.23±	91.97 – 92.43	28 bc	0.12±	27.82 -28.12
I.meda	69.8 f	0.056±	69.24 – 70.36	30 bc	0.09±	30.09 – 29.91
I.meda	85.2 e	0.66±	84.54 – 85.86	32 ab	0.18±	31.82 – 32.18
I.meda	80.8 ef	0.47±	80.33 – 81.27	27.6 c	0.17±	27.43 – 27.77
I.meda	85.6 e	0.33±	85.27 – 85.93	28.8 bc	0.12±	28.68 – 28.92
I.meda	85.6 e	1.03±	84.57 – 86.63	32.2 ab	0.20±	32 – 32.4
I.sp	100 d	-	-	35.8 a	0.18±	35.62 – 35.98
I.persica	113.6 bc	0.30±	113.3 – 113.9	35.8 a	0.09±	35.71 – 35.89
I.pseudacorus	139.2 a	0.43±	138.77 – 139.63	19.2 d	0.05±	19.15 – 19.25
I.pseudocaucasica	100.8 d	0.30±	100.5 – 101.1	30.4 bc	0.36±	30.04 – 30.76
I.acutiloba	117.2 b	0.46±	116.74 -117.66	28.4 bc	0.13±	28.27 – 28.53
I.spuria	116.6 b	0.49±	116.1 – 117.09	27.4 c	0.14±	27.26 – 27.54

*Means followed by similar letters in each column are not significantly at 1 % level by using Duncan multiple range test.

*PD : Pollen grain diameter - SGL : Stomatal guard cell length - SD : Standard deviation - M : mean - V: variation

Stomatal guard cells length

A negative and significant correlation was observed between stomatal and epidermal cells density and length of stomatal guard cells (Table 4). Then by increasing the length of stomatal guard cells and stomatal being larger, the epidermal and stomatal cells density decreases. The results in Tables 1 and 3 showed that by increasing the number of chromosomes in *I.persica* (2n=38) and *I.sp* (2n=30) varieties, stomatal guard cells length increased significantly and in *I.reticulata* (2n=20) , stomatal guard cells length decreased significantly. But the results in Table3 revealed that the stomatal guard cells length was overlapping in some species with different chromosomes level (Table 1) and there were no significant differences in stomatal guard cells length by changing in levels of chromosome. These results show that there is not an exact relationship in

all Iris varieties about the variability of stomatal guard cells length, and considering this, it can be said that length of stomatal guard cells is most affected by environmental conditions and it cannot be used as perfect and precise criteria for determining the ploidy level in different varieties of Iris. Joachimiak and Grabowska- joachimiak (2000) reported in Phluem that the length of stomatal guard cells are overlapping in some species with different levels of chromosome and is not suitable for determining the ploidy level of the plant Phluem, that this resultis similar to the results of our experiment, however, given that *I.persica*, *I.sp* and *I.reticulata* varieties, along with increasing levels of chromosome, a significant increase was observed in stomatal guard cells length. The cytologic trait could be used as an immediate measure to determine the ploidy level in breeding programs of each variety individually which needs more investigation.

Table 4 . Correlation coefficients between morphocytological traits of Iris species .

FEATURES	ECD	SCD	CN	PD	SI	SC
SCD	0.811**	-	-	-	-	-
CN	- 0.158ns	0.102ns	-	-	-	-
PD	0.637*	0.615*	0.060ns	-	-	-
SI	- 0.674*	- 0.134ns	0.332ns	- 0.327ns	-	-
SC	0.033ns	0.536*	0.536*	0.199ns	0.615*	-
SGL	- 0.748**	-0. 451*	0.382ns	- 0.455ns	0.684**	0.443ns

* and ** significant at the 5 and 1 % levels of probability , respectively . ns = not significant

*ECD : Epidermal cell density - SCD: Stomatal cell density- CN : Chloroplast number - SI : Stomatal index - SC : Stomatal conductance - PD : Pollen grain diameter – SGL : Stomatal guard cell length.

Epidermal and stomatal cell density

The results in Table 2 revealed that by increasing the chromosome level, a significant increase occurs in epidermal and stomatal cells density. This result is in consistent with the results of Mishra (1997) who stated about coffee plant that epidermal and stomatal cells density decrease significantly by increasing the levels of chromosome. The reason may be for this reason that different Iris varieties are resistant and compatible to different environmental conditions and calcareous soils to salinity and dehydration (Austin , 2005). Also structurally and morphologically, it has a high variability and is divided in two major groups of bulbous and rhizomes and types off lowers bearded or Pogon Iris and beardless or Apogon and botanical and physiological are divided into two major and different groups of Juno and Oncocyclus (wendelbo , 1704). This kind of high variability varieties of Iris in the structure, consistency and resistance to varying environmental conditions caused the epidermal and stomatal cells density have been affected by environmental and genetic factors and cannot create perfect and accurate criteria for assessing the ploidy level among different varieties. It is better to evaluate these two cytological traits in breeding programs each Iris species separately.

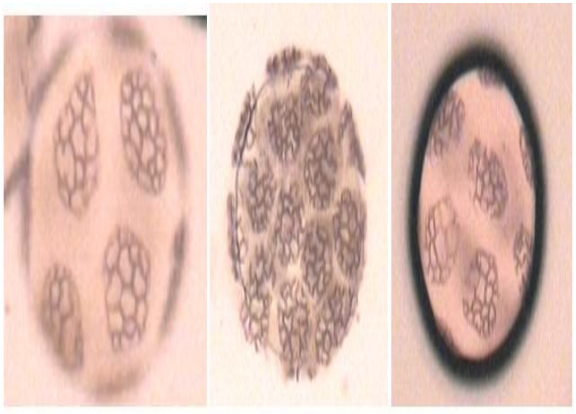

Fig. 1. Clypeate pollen grain from left to right, *I.pseudocaucasica -I.persica-* I.*sp.*

Stomatal index and stomatal conductance

The results(Table 4) of correlation among traits clarified that there is a negative and significant

correlation between stomatal index and epidermal cell density which show that by increasing the epidermal cell density, the stomatal index decreases significantly. But according to the results of Table 2, the stomatal index between some varieties have overlapping which may be due to being in different environmental conditions and environmental effects on this cytological trait.

Fig. 2. From left to right, *I.sp* -I.*persica* - *I.pseudocaucasica*.

Fig. 3. from left to right, *I.reticulata- I.reticulata - I.spuria*.

Stomatal conductance has a positive and significant correlation with stomatal cell density, chloroplast number in stomatal cells and stomatal index. Considering this issue that the stomatal is related to physiological functions of the plant, it is more influenced by environmental conditions of plant growth place and with regard to results in Table 2 it cannot be a detailed and perfect criterion among different varieties of Iris for determining the ploidy level.

Fig. 4. From left to right, *I.meda- I.meda- I.acutiloba*.

The general conclusions

Considering that determining the chromosomal level is so important in breeding programs and determining the chromosomal level is very time consuming and costly by cytogenetic and DNA cytometric method with regard to the large number and small size of chromosomes in the Iris. According to the results of this test, morphological and cytological characteristics including chloroplast number in stomatal guard cells, epidermal cell density, stomatal cell density, stomatal index, stomatal conductance and stomatal guard cell length regarding to adaptation to diverse environmental conditions in Iris plant and considering that the stomatal conditions is associated with physiological functions of the plant and they are more influenced by environmental conditions, it cannot be an accurate measure for determining the chromosomal level between different varieties, but the determination of chromosomal level is less influenced by environmental conditions by measuring the pollen grain diameter of the varieties of lris, according to the results presented in Table 3 and way of standing anthers in the flower of lris varieties and by increasing the chromosomal level, a significant increase is observed in pollen grain diameter which can be used as a simpler and faster method in determining the chromosomal level in different Iris varieties and would make Iris breeding program easier.

References

Abak K. Comlekcioglu N, Buyukalaca S,

Sari N. 1998. Use of stomatal characteristics to estimate ploidy level of haploid and diploid pepper plants. Tenth EUCARPIA meeting Capsicum and Eggplant. **12,** 179-182.

Alishah O, Omidi M. 2008 . laboratory Methods of Cytogenetics. University of Tehran Press.

Austin C. 2005. Irises a Gardeners Encyclopedia. Portland. U.S.A.

Azimi MH, Moradi Ashori B, Hoseininia A. 2011 . Genetic variation and heritability of Iranian *Iris* species characters. The Quarterly Journal - Plant Breeding and Genetic Research of pasture and forest of Iran. **19,** 153-166.

Azizian D, Sheidai M, Jahanmiri Z. 1993. An anatomical and cytological study of three Iranian Iris species. Pajohesh va Sazandegi. **29,** 44-47.

Cenci CA, Pegiatti M, Falistocco E. 1984. Phleum pretense (Gramineae) chromosomal and biometrical analysis of Italian populations. *Willdenowia*. **14,** 343-353.

Cramer CH. 1999. Laboratory techniques for determining ploidy in plants. Hort technology. 594-596.

Donmez EO, Pinar NM. 2001. The clypeate pollen grains of Turkish Iris L. (Iridaceae): subgenus Scorpiris sach. Turk J Bot. **25,** 57-62.

Funamota T, Kondo K, Tatarenko Gontcharov IV. 2006. Intraspecific polyploidy of *Parnassia palustris* Var.multiseta (*Saxifragaceae* s. l.) collected in Primorye and Altai Territories. Russia. Chromosome botany. **1**, 23-26.

Ghahreman A. 1805. Iran floor. Anestitue Science jangal v marta. 7.

Ghanavati F, Eskandari H. 2011. Relationship between the chloroplast number in stomatal guard cells, flow cytometry and ploidy level in *Onobrychis* spp. Journal Behnejadi Nahal and Bazr **3,** 1-27.

Hamill SD, Smith MK, Dodd WA. 1992. In vitro Induction of banana autotetraploids by colchicine treatment of micropropagated diploids. Australian Journal Botanical. **40,** 887-896.

Joachimiak A, Kula A. 1993. Cytotaxonomy and karyotype evolution in *Phleum* sect. *Phleum* (Poaceae) in Poland. Plant Systematics and Evolution. **188,** 17-30.

Joachimiak A, Kula A. 1996. Karyo system ati cs of the *Phleumalpinum* polyploid complex (Poaceae). Plant Systematics and Evolution. **203,** 11-25.

Joachimiak A, Grabowska-Joachimiak A. 2000. Stomatal cell length and ploidy level in four taxa belonging to the *Phleum sect. Phleum*. Acta Bolologica Cracoviensia . Ser Botanica. **4,** 103-107.

Keri DJ, Reed SM, Timothy A, Rinehart A. 2007. Analysis of Ploidy Level and Its Effects on Guard Cell Length, Pollen Diameter, and Fertility in Hydrangea macrophylla. Horticultural Science. **42,** 483-488.

Kim HH, Park YW, Yoon PS, Choi HW, Bang JW. 2004. Karyotype analysis of eight Korean native species in the genus Iris. Korean .Journal Medicinal Crop Science **12,** 401-405.

Leech RM. 1981. Observation of the mechanism of chloroplast division in higher plants. New Phytologist. **87**, 1-9.

Mishra MK. 1997. Stomatal characteristics at different ploidy levels in *Coffea* L. Annals of Botany. **80,** 689-692.

Murray H, Standing L. 1992. Genomic constancy during the evelopment of *Lathyrus odoratus*

cultivars. Heredity **68,** 321-327.

Nordenskiold H. 1945. Cytogenetic studies in the genus Phleum. Acta Agriculturae Suecana. **1**, 1-137.

Omidbaigia R, Mirzaeea M, Hassanib ME, Sedghi Moghadam M. 2010. Induction and identification of polyploidy in basil (*Ocimum basilicum* L.) medicinal plant by colchicine treatment. Plant Production **4,** 1735-6814.

Punt W, Blackmore S, Nilson S, Le TA. 1994. Glossary of pollen and spore terminology. Utrecht. LPP Foundation.

Rahimi V. 2008 . Investigate Variety of morphological and chromosomal syndromes Iranian native Iris. Master's thesis. Department of Plant Production.Tehran university.

Rahimi V, Sadat-Hosseini M, Solymani A, Bahermand N, Meftahizade H. 2011. Assessment of cytological and morphological variation among Iranian native Iris specie. African Journal of Biotechnology **10,** 8805-8815.

Reeves A, Tear J. 2000. MicroMeasurc for Windows, version 3.3. Free program distributed by the authors over the Internet from

Rinehart A. 2007. Analysis of Ploidy Level and Its Effects on Guard Cell Length, Pollen Diameter, and Fertility in Hydrangea macrophylla. Horticultural Science **42,** 483-488.

Salisbury KE. 1927. On the causes and ecological significance of stomatal frequency with special refrence to the woodland flora. Philosophical transactions of the Royal Society. **216,** 575 – 578.

Speckman GJ, POST J, Dijkstra H. 1965. The length of stomata as an indicator for polyploidy in rye-grasses. Euphytica. **14**, 225-230.

Teare ID, Peterson CJ, law AG. 1971. Size and frequency of leaf stomata in cultivars of Triticum aestivum and other Triticum species. Crop Science. **11,** 496-498.

wendelbo P. 1704. Tulips and Iris by Iran and neighboring species. Botanical Institute. Iran. 83.