

Chromosome Number Variation in Iranian Populations of *Acorus calamus*

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ABSTRACT

Different ploidy levels, diploid ($2n=2x=24$) to hexaploid ($2n=6x=72$) have been reported for *Acorus calamus*, a perennial medicinal plant. According to available information, there is a significant relationship between the ploidy level and essential oil compositions and medicinal properties of this important genetic resource. However, there is no information about the chromosome number of *A. calamus* that has been recently rediscovered in Iran. This research was conducted to determine the chromosome number and ploidy level of three *A. calamus* populations in Iran. The fresh roots were removed from the rhizome and pretreated in 0.002 Mole of 8-hydroxyquinoline and fixed in Ethanol: acetic acid solution for 24 hours. The fixed roots were macerated in 60°C in 1N HCl for about 30 minutes. A squash technique was used with 2% aqueous aceto-orcin as the stain. The somatic chromosome number and karyotype details were studied at least in five well-prepared metaphase plates for each population. The populations studied showed interesting diversity in cytogenetic features; chromosome number and ploidy level. Arzefon and Pelesk populations had two different chromosome number; $2n=2x=24$ and $2n=3x=36$, while Alandan population had $2n=3x=36$ chromosome number. The haploid total chromosomes length of Arzefon, Pelesk, and Alandan populations were 13.28, 11.33 and 11.3 μm respectively. The longest chromosome was 1.48 μm in Arzefon population and the shortest chromosome with a size of 0.61 μm observed in Pelesk population. The existence of this genetic diversity is important in domestication strategies and the use of appropriate cytotype. Based on the results of this research and available information, the basic chromosome number of *A. calamus* is $x = 12$.

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Introduction

Various chromosomal numbers including; $2n=14-18-21-24-27-36-44-45-48-66-72$ have been reported for medicinal plants sweet flag (*Acorus calamus*) from different geographical regions, especially India, China, Japan, North America and Europe (Mookerjee, 1955; Jervis and Buell, 1964; Larsen, 1969; Marchant, 1973; Ramachandran, 1978; Subramanian and Munian, 1988; Hong *et al.*, 2001; Ogra *et al.*, 2009; Kumar and Singh, 2015; Mittal *et al.*, 2015). The most frequent chromosomal number reports of this plant are $2n = ca 36$. The existence of different cytotypes of this species in different geographical regions and the small

size of chromosomes in this plant are two main reasons about different chromosomal numbers reported in this plant. Based on ploidy level and geographical distribution, *A. calamus* is divided into 4 cytotypes; diploid with chromosome number of $2n= 2x= 24$ distributed in North America, triploid cytotype with chromosome number of $2n = 3x = 36$ in Europe and temperate regions of Asia, tetraploid cytotype with chromosome number of $2n = 4x = 48$ in East Asia, India and Japan and hexaploid cytotype with a chromosome number of $2n= 6x= 72$ in Kashmir (Ogra *et al.*, 2009). The ploidy level influence on the fertility rate, chemical composition of essential oil and



medicinal properties of this species, so that the triploid cytotype, which has a wide distribution in the world in comparison with the other cytotype, has been known to be infertile (Jervis and Buell, 1964). The content of β -asarone in the essential oil of *A. calamus* due to the carcinogenicity of this substance has led to restrictions on the use of this important medicinal plant (Lander and Schreier, 1990). Several studies reported the significant correlation between ploidy level and chemical composition of essential oil especially β -asarone in *A. calamus*. In tetraploid cytotype of *A. calamus*, the content of β -asarone is the highest (70-96%), while the triploid cytotype content and diploid is less than about 5-19% and almost absent, respectively (Lander and Schreier, 1990). Recently *A. calamus* has been rediscovered in Iran, especially in wetland areas of Mazandaran, Golestan, and West Azarbaijan provinces (Gholipour and Sonboli, 2013; Mokarizadeh *et al.*, 2015). In addition, the content of β -asarone in the chemical composition of some Iranian populations of *A. calamus* was reported to be 27 to 53% (Gholipour *et al.*, 2015). According to the content of β -asarone in the essential oil, Iranian populations are likely to be triploid and infertile, reproduced through vegetative reproduction, but the author's observations on the natural habitats of this plant showed that some of its individuals produce a flower in the late of spring. Diploids cytotype of this plant produces flowers with fertile seeds, while polyploids plants often do not produce flowers or fertile seeds (Mittal *et al.*, 2015). However, there is no data about chromosome number and ploidy level of *A. calamus* populations in Iran, therefore the aim of this study was to characterize the *A. calamus* populations in Iran cytologically.

Materials and Methods

Fresh rhizomes of three populations of *A. calamus* were collected from Arzefon, Pelesk, and Alandan (Sari-Mazandaran) wetlands (Table 1). Herbarium specimens are deposited in Sari Payame Noor University Herbarium (SPNH). The fresh rhizomes were completely rinsed and placed in distilled water at room temperature for rooting. The fresh roots pretreated in 0.002 Mole 8-hydroxyquinolin for 2–3 hours and then fixed in Ethanol: acetic acid solution (3:1) for 24 hours. The fixed

roots were thoroughly washed in distilled water and macerated in 60° C in 1N HCl for about 30 minutes. The sample was stained with 2% aqueous aceto-orcein for about 40-60 minute and then squashed. The somatic chromosome number and karyotype details were studied in at least five well-prepared metaphase plates for each population. The metaphase chromosomes were photographed for each population by a digital camera and the chromosomes long measured by using Image Tools ver. 3 software. Due to being small size and stickiness of chromosome of this species, at least 5-10 well-prepared metaphase extensions of the studied populations were measured.

Results

The karyotype features of the three Iranian populations of *A. calamus* are presented in Table 2 and Figure 1. In this study, two cytotypes (diploid and triploid) were observed in Iranian populations of sweet flag. Arzefon and Pelesk populations had two chromosome number of $2n = 2x = 24$ and $2n = 3x = 36$ with the ploidy level of diploid and triploid, while Alandan population had a chromosome number of $2n = 3x = 36$ as triploid. Based on the results of this study, the basic chromosome number of the sweet flag is $x = 12$. The haploid total chromosome length of Arzefon, Pelesk, and Alandan populations are 13.82, 11.33 and 11.3 μm respectively. The largest chromosome was observed in Arzefon population with 1.48 μm long and the shortest one observed in Pelesk population with 0.61 μm long (Table 2).

Discussion

Chromosome number and ploidy level of three populations of *A. calamus* have been reported for the first time in Iran. Based on the previous study, Iranian populations of this species were expected to be triploid cytotype with $2n = 3x = 36$ chromosome number (Ogra *et al.*, 2009). According to the results of this study, some cells of Arzefon and Pelesk populations and all cells of Alandan population, in accordance with this hypothesis, were triploid (Fig. 1 A, B, D, F), while other cells of Arzefon and Pelesk populations were diploid with chromosome number of $2n = 2x = 24$ (Fig. 1C, E).

Table 1. Localities and herbarium information of studied populations of *A. calamus*.

No	Locality	Hebarium number
1	Mazandaran, Sari, Arzefon village, Malepeshte Abandan, 13 Aug. 2012, 331 m, Gholipour A.	SPNH-285
2	Mazandaran, Sari, Shahid Rajaii dam road, Pelesk village, Abandane Pelesk, , 12 Sept. 2012, 660 m, Gholipour A.	SPNH-286
3	Mazandaran, Sari to Kiasar road, before Kiasar, Alandan, Abandane Alandan, , 29 Aug. 2012, 1350 m, Gholipour A.	SPNH-284

Table 2. Karyotype features of Iranian populations of *Acorus calamus*.

Population	Altitude (m asl)	Chromosome number (2n)	Ploidy level	Chromosome basic number (x)	Shortest chromosome (μm)	longest chromosome (μm)	Total haploid chromosome length
Arzefon	331	24-36	2x-3x	12	0.72	1.48	13.82
Pelesk	660	24-36	2x-3x	12	0.61	1.41	11.33
Alandan	1350	36	3x	12	0.64	1.31	11.3

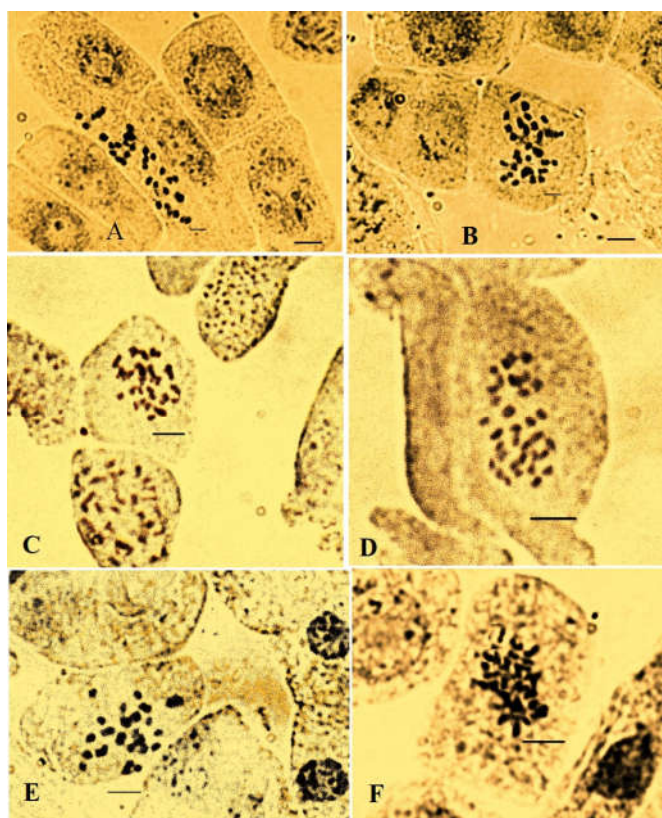


Fig.1. Karyotype of Iranian populations of *A. calamus*: A-B. Triploid cytotype ($2n=3x=36$) of Alandan population, C. diploid cytotype ($2n=2x=24$) of Pelesk population, D. triploid cytotype ($2n=3x=36$) of Pelesk population, E. diploid cytotype ($2n=2x=24$) of Arzefon population, F. Triploid cytotype ($2n=3x=36$) of Arzefon population (Scale bar = 5 μm).

Orga *et al.* (2009) reported the presence of diploid cytotype in India for the first time, and confirming that two populations of the western temperate areas of Himalayas had $2n = 2x = 24$ chromosome number with diploid level, and the populations of tropical and subtropical regions were triploid. In general, there are two main reasons for reporting different chromosome numbers from different populations of this species. The first reason is the presence of various cytotypes of this species in different geographical regions (Orga *et al.*, 2009). Another important reason is the cytological characteristics of this species as a primitive plant in monocotyledonous angiosperms. Primitive angiosperm plants have very small genome size (Soltis *et al.*, 2003). The genus *Acorus* is considered as a primitive plant in the basal of the monocotyledonous phylogenetic tree as the sister group of another monocotyledon (Soltis *et al.*, 2003). *A. calamus* has small size of chromosomes ranging from about 0.51 μm to 3.4 μm , on the other hand, the chromosomes of this plant tend to be adhesive (Marchant, 1973; Subramanian and Munian, 1988; Kumar and Singh, 2015), subsequently it is difficult to provide appropriate metaphase extension. These problems make it difficult to detect chromosomes from each other and count them easily. To resolve this problem, the study of a large number of metaphase samples and precision in the cytological procedures are necessary. These problems caused the reporting of several chromosome base number including; $x = 7, 9, 11, 12$ and heterogeneous chromosome numbers; $2n = 14, 18, 21, 24, 27, 36, 45, 48, 66, 72$ of this species (Mookerjee, 1955; Jervis and Buell, 1964; Larsen, 1969; Marchant, 1973, Ramachandran, 1978; Subramanian and Munian, 1988, Hong *et al.*, 2001; Orga *et al.*, 2009; Kumar and Singh, 2015; Mittal *et al.*, 2015). It is noteworthy that the hexaploid chromosome number of this species was reported by Hong *et al.* (2001) as $2n = 6x = 66$, while in fact, it is $2n = 6x = 72$ (Orga *et al.*, 2009). Therefore, the chromosome base number of this species appears to be $x = 12$.

The results of this study confirmed the correlation between ploidy level and the

content of β -asarone, so that the triploid cytotype of Alandan population have the most content of β -Asaron in comparison with pelesk, and Arzefon populations (Gholipour *et al.*, 2015).

Conclusion

Iranian populations of *A. calamus* are cytologically composed of two cytotypes: diploid ($2n = 2x = 24$) and triploid ($2n = 3x = 36$). In this study, the presence of two ploidy levels in a population of *A. calamus* has been reported for the first time. Due to the correlation between ploidy level and the content of chemical composition of this species and its diverse medicinal, health and industrial applications, the existence of this genetic diversity is important in domestication strategies and the use of appropriate cytotype.

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