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DETERMINATION OF KARYOLOGICAL STAINING METHOD FOR *URTICA DIOICA*

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Abstract

Urtica dioica is one of the medicinal plant that is native in Iran and has many medicinal properties including anticancer and antimicrobial properties. In view of the distribution of different Ecotype of this plant with different medicinal powers in different Ecosystems of Iran, the aim of this research was study of the status of their precise staining of chromosomes and introduction to Karyological researches of different ecotype of this plant, which is a time-consuming by last references. Shoot and root meristem of germinating seedlings and root meristems of stem shoots as measured sampling for chromosomal analysis were evaluated by Squash and then stained. After various stages of squash and different staining methods, finally the most obvious example of chromosomes in root meristems of shoots and stems, were The staining solution of 0.002 Molar 8-hydroxy kinolin as a pretreatment solution and Farmer was as a fixator. Overall, the number of chromosomes in Metaphase stage of cell count was about 40 chromosomes. But all efforts and repeated experiences chromosomal exact count was not possible due to the relatively large number of chromosomes and their size is small.
Keywords: Urtica dioica, karyological and staining.

Introduction

The stinging nettle, *Urtica dioica* L. (Urticaceae), is a perennial and docecius plant, it has basal and quadrupedal, covered with oval and oval leaves, pointed, serrated and with a pair of stipules. *U. dioica* has stinging trichomes on the stem and on the leaves. The flowers are bright green, Male and female flowers are separated (Fig. 1) Inflorescence in a long and branching cluster, the flowers lack petals and have 4 sepals, the female flowers have a stigma shaped like a brush and its fruit is a nut (Johnny Ghorban, 2000; Pignatti, 1982).

Nettle is a plant that has existed since prehistoric times and was used by people at that time to feed it and they were aware of its healing properties. However, in the past, the most attention was paid to the leaves of this plant But nowadays, a study has also been done on the antimicrobial properties of extracts of all organs including leaves, roots, shoots, flowers and seeds, Which showed that among the extracts, nettle seed extract had the most antibacterial effect on Gram-positive bacteria, The leaf extract of this plant had the most inhibitory effect on Gram-negative bacteria and finally the flowers extract had the highest antifungal activity (Majd *et al.*, 2003).

This plant has a variety of climates with different ecotypes, However, different anatomical variations of these ecotypes have been studied (Jafari and Dehghan, 2012), But the karyological study of this plant has not been reported in Iran so far. In view of the distribution of different ecotypes of this plant with different drug potency in different ecosystems of Iran, the aim of this study was to investigate the status of chromosomes and introduce the exact staining method that to be a better way for karyological studies of these ecotypes for other researchers.

It should be noted that due to the bitter secretions of this plant and the very small flowers of this plant, the research on this plant is a bit difficult, Therefore, much research has not been done on the anatomical and genetic structures of this plant.

Materials and Methods

Collection of anther bags from the base of male and seed of plants from female base flowers in May, to karyological studies were performed. It is noteworthy that due to the separation of the female and male flowers, samples were collected from different cities such as Ramsar, Tehran and Mashhad. The staining of the anther bags was not successful. Therefore, 50 seeds were son in dark plates on a wet filter paper in order to study the intensity of germination (Fig. 2). Then use the root of the seedlings and Root meristems were isolated for this purpose. Various methods were used for staining (Deepa *et al.*, 2013; Fukui, 1996) such as Colchicine, Acetocarmine and Hydroxyquinoline.

Fig. 1 : *Urtica dioica* L. (Pignatti, 1982).

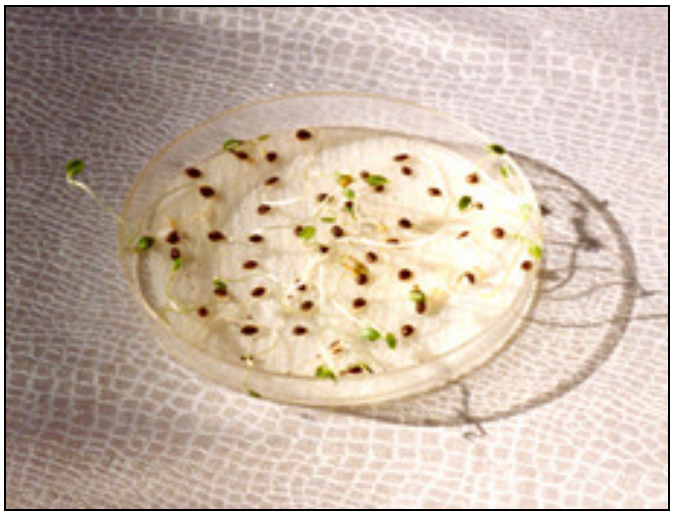

Fig. 2 : Growth of nettle seeds

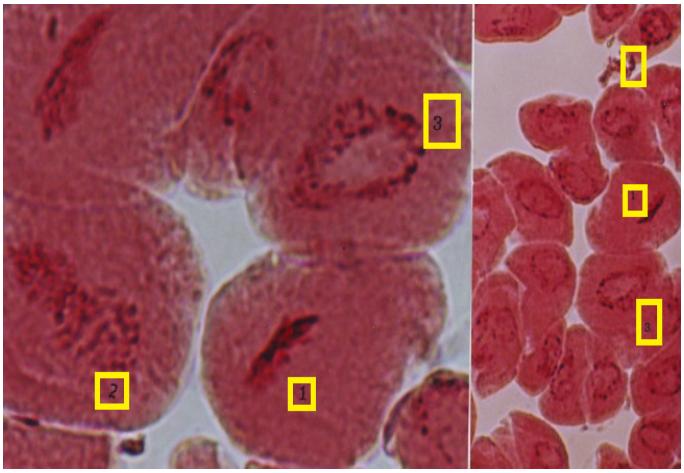

Fig. 3 : Microphotograph of the Prometaphase (1), metaphase (2) and Early Anaphase (3) plate of *urtica dioica* (with magnification of LM x 100 and x 1000).

Results and Discussion

Several methods were used for staining and clarity, all of which failed. Squash and stain anther bags and pollen grains Root meristem from seed growth, then using colchicine did not work and also Squash and stain anther bags and pollen grains and the meristem of the roots resulting from the growth of the seeds and then the use of Aceto kerman did not work.

Finally, after six months of continuous effort on different staining patterns and methods, after removing the root meristem from the seed growth, using 8 M hydroxyquinoline 0.002 M solution as pretreatment and Farmer's solution was used as fixative. Finally, the specimens were stained with Acto Oresein (Figs. 3-6).

The results showed that in the metaphase stage, the total number of chromosomes counted was about 40 chromosomes. But precise chromosome counting was not possible with every effort and repetition of experiments. This is due to the relatively large number of chromosomes and their small size.

Our report on chromosome counting with Coile (1999). reports showing the base number x = 6-14 and with According to Burton's report (Smith *et al.*, 1997), it showed the chromosome base number with n2 = 48 and n2 = 24, It has some alignment.

But with the Nurmi (1980). report showing the base chromosomal number of this plant with n 2= 26 and And does not agree with Maffeif (Corsi *et al.*, 1999) showing the chromosomal base number of this plant with n = 52.

Of course, this difference in the number of chromosomes may indicate differences in gene arrangement or doubling or both in this plant.

Finally, it may be thought that this change in chromosome number may be attributed to ploidy DNA (Mráz, 2006).

Anyway with the help by introduce this staining method, instead of spending too much time identifying different ecotypes, it can be faster to operate and it served other researchers.

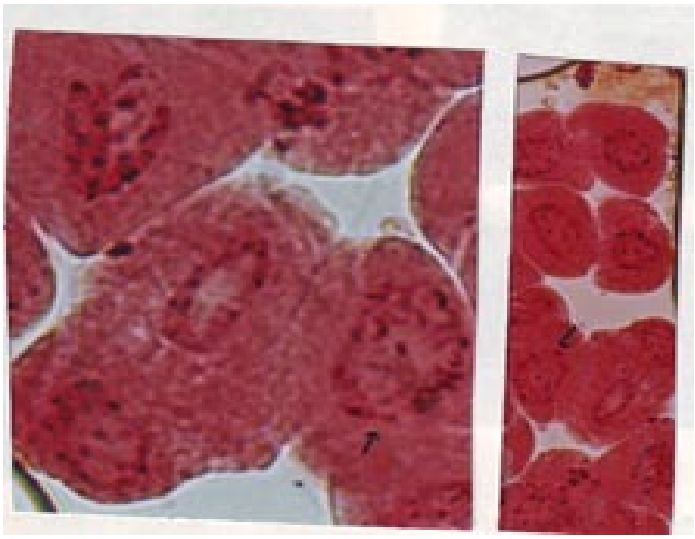

Fig. 4 : Microphotograph of metaphasic chromosomes in Pre-metaphase plate of *urtica dioica* (with magnification of LM x 100 and x 1000).

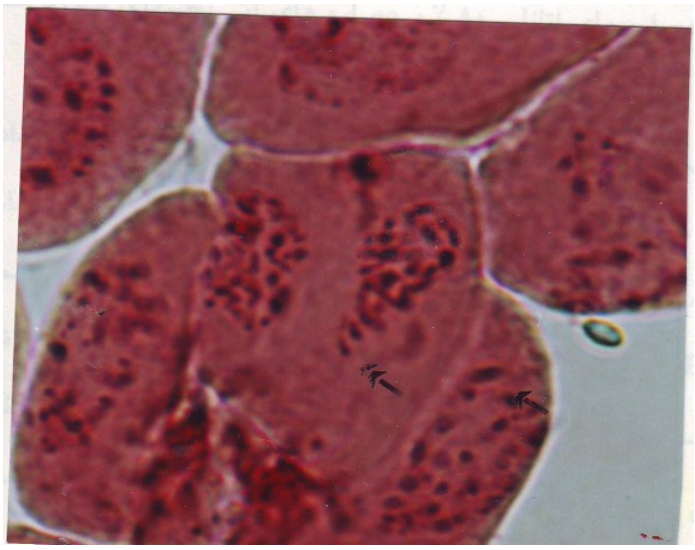

Fig. 5 : Microphotograph of the Late anaphase and telophase stage plate of *Urtica dioica* (with magnification of LM x 1000).

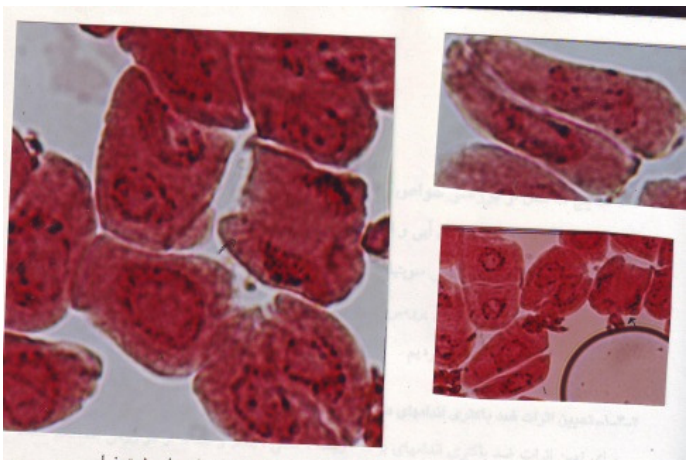

Fig. 6 : Microphotograph of the Mid-anaphase plate and middle lamella formation stage of *Urtica dioica* (with magnification of LM x 100).

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