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CHROMOSOME STATUS OF MARSH MARIGOLD, *Caltha palustris* L. (Ranunculaceae) FROM SERBIA

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Marsh marigold, *Caltha palustris* is distributed in the moist, temperate and cold regions of the Northern Hemisphere. This species exhibits considerable amount of intraspecific chromosomal diversity involving hybridization, polyploidy, aneuploidy and B chromosomes. Karyotype analyses of three mountain populations from Serbia were done for the first time. All samples were tetraploid (based number x=8) with 2n=32. In population from mountain Tara presence of one B chromosomes was detected. Tetraploid karyotype consists of 17 median-centromeric (m), 8 submedian-centromeric (sm), 7 subterminal-centromeric (st) chromosomes and one terminal-centromeric (t) B chromosome (2n= 17m+8sm+7st+1B). Studied populations in Serbia belong to the most common cytotype for this species in Europe.

Key words: *Caltha palustris*, polyploidy, B chromosomes

INTRODUCTION

According to revision of SMIT (1973), the genus *Caltha* L. (Ranunculaceae) is composed of ten species. Plants of this genus are low-growing, perennial herbs characterized by simple leaves and actinomorphic flowers. They show general affinity for wet habitats, occupying marshes, fens and other wetlands at lower altitudes. At higher altitudes, they are commonly associated with melt water. Plants of the genus *Caltha* are distributed in the moist temperate and cold regions of both Northern and Southern Hemispheres. In the Southern Hemisphere distribution of the genus is rather restricted, supposedly due to the deficiency of suitable habitats (HOFFMANN, 1999). SCHUETTPELZ and HOOT (2004), based on chloroplast and nuclear DNA

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sequences, proposed that genus *Caltha* most probably originated in the Northern Hemisphere followed by dispersal to the Southern Hemisphere (Gondwanaland).

Marsh-marigold, *Caltha palustris*, as the most widespread species of the genus and the only representative of the genus in Europe, established itself in wet habitats across much of Europe, Asia, and North America. Immense morphological variation featuring this species prompted recognition of many distinct taxa. However, it is proved that majority of this morphological diversity is a product of environmental conditions. Thus, many of the previously recognized segregates are not well supported (SMIT, 1973; WOODELL and KOOTIN-SANWU, 1971).

Caltha palustris is a highly successful species. It exhibits considerable amount of both intraspecific morphological and chromosomal diversity including polyploidy, aneuploidy and hybridization. New chromosome combinations frequently arise in this species and in combination with its obligate out breeding, and its efficient vegetative reproduction, enable quick response to habitat changes. According to present data, distribution of the cytotypes is not strictly geographically defined. Cytotype or race with 2n=32 is the most widespread, ranging throughout the Arctic in North America to Europe with exception of Britain. The 2n=56 race is also frequent in Europe, from Russia to Britain and Iceland, but it is quite rare in North America (SUDA and ARGUS, 1969). In many plant genera climatic changes and migrations that followed Pleistocene glaciations have favoured allopolyploidy. In Europe, the pressures resulting from the predominantly east west orientation of the mountain ranges produced a series of high stress periods, interchanging with periods when the withdrawal of the ice left large areas suitable for rapid colonisation by species like *Caltha palustris*. Therefore, presence of that greatest diversity, either of stable chromosome races or of aneuploidy, is unexpected in European populations. Furthermore, SMIT (1973) has discovered existence of slight ecological differences between the 2n = 32 and 2n = 56 races.

Additionally, presence of B chromosomes, found for the first time by REESE (1954), also characterises this species. Chromosomal data for this species in Serbia are missing, so the aim of this paper is to fill this gap.

MATERIALS AND METHODS

Samples were collected at three mountains in Serbia: Tara Mt (locality Kaluđerske bare, 970m), Povlen Mt. (locality Debelo brdo, 1070m) and Kopaonik Mt (locality Nebeske Stolice, 1800 m). Actively growing roots, about 1-1.5 cm in length, were excised, pre-treated with 0, 2% colchicine for 4-5 h at room temperature, washed in distilled water and fixed in Carnoy's fixative at 4°C for at least 24 h. The fixative was prepared by mixing three parts (in volume) of methanol and one part of glacial acetic acid. Further, the root tips were hydrolysed in 1N HCl for 12 min at 60°C and stained in 2% lacto-propionic orcein overnight. Each of the root tips was carefully transferred on a drop of 45% acetic acid on a slide, covered by cover slip, heated and squashed. Metaphase plates were examined with a light microscope Zeiss Axioscop, under oil immersion (x100), and photographed. From each population at least ten metaphase plates were examined. Chromosome measurements including long arm, short arm, chromosome lengths, arm ratio index and relative chromosome length were made with Micro measure 3.3 software (REEVES, 2001). Chromosomes were sorted in descending order of length and morphology designated according to LEVAN *et al.* (1964).

RESULTS AND DISCUSSION

Polyploidization has been suggested as a major driving force of plant evolution (BLANC and WOLFE, 2004; CHEN, 2007; SOLTIS and SOLTIS, 2009). Cold and severe climate and nutrient stress in high altitude or latitude areas can induce or stimulate diploid gamete production (LEVIN, 2002). A high temperature environment has the potential to increase gamete ploidy level also (PÉCRIX *et al.*, 2011). This could lead to higher polyploidity level in plants inhabiting extreme environments. Polyploids should possess increased genome flexibility, allowing them to adapt in disturbed or novel niches and persist across heterogeneous landscapes (OTTO and WHITTON, 2000). In the genus *Caltha* 88 cytotypes (82.60%) show polyploidy.

All populations we studied were tetrapolyploid with 2n=32 which is the most frequent chromosome number in this species (TAMURA, 1995). Additionally, in population from Tara Mt. presence of one B chromosome was detected (Fig. 1). B chromosome represented 1, 03% of the tetraploid genome and was nearly half size of the smallest chromosomes in the karyotype. The 32 chromosomes were quite well arranged in 8 groups of four homologues (Fig. 2), except one chromosome in the group 7, where heteromorphy was present. The karyotype consisted of 17 median-centromeric (m), 8 submedian-centromeric (sm) and 7 subterminal-centromeric (st) chromosomes and one terminal-centromeric (t) B chromosome (2n= 17m+8sm+7st+1B).

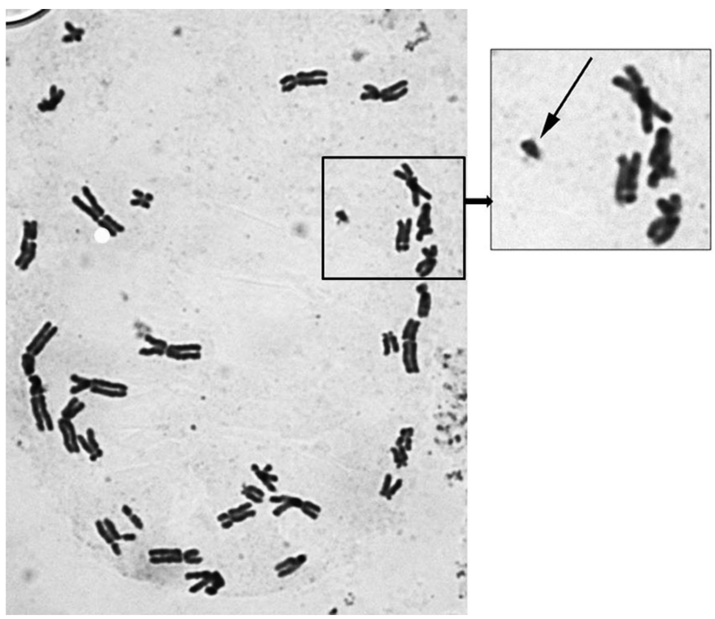

Fig. 1. Metaphase chromosomes of *C.palustris* (arrow designate B chromosome)

The chromosomal counts in the genus range from 2n = 16 - 88 with common base number x = 8. KUMAR and SINGHAL (2008) summarized data from different regions of the world: Russia (16, 28, 32, 56, 60, 64), Slovakia (32), Czech Republic (32–34, 44, 47, 55–57, 56–59, 56+6B, 57, 61, 64), Great Britain (32, 32+3B, 42–45, 52, 54, 55, 56, 56–63, 56+6B, 57, 58, 60, 64, 32-56, 56-64), Italy (32, 48, 56, 60), Netherlands (32, 44, 56), Hungary (32), Poland (32), Finland (32), Sweden (60), Arctic-Alpine Scandinavia (56), Belarus (32, 56), north-western Alaska (32, 56–70, 60), western North America (32, 56, 58), northern and Arctic Canada (32, 48, 56, 58, 60), Japan (32, 60, 68, 88), Korea (32) and Nepal (48). In India, (SHARMA *et al.* 1993) polyoidy in *C. palustris* appears as 2n = 32, 40, 42-45, 56-63. Dominant cytotype in British Isles was with 56 chromosomes but 64 cytotype and different aneuploid variants were also present (KOOTIN-SANWU and WOODELL, 1970). In China eight of twelve examined populations contained tetraploid, one hexaploid and three octaploid plants (YANG, 2002). Furthermore, the same author did not find aneuploids and plants with B chromosomes and showed variation in chromosome morphology of tetraploids. Attempts to correlate such great chromosome variability with environmental variables did not produce much success. But CHRTKOVÁ and JAROLÍMOVÁ (1999) divided populations of *Caltha palustris* from the Czech Republic into four subspecies (*cornuta*, *laeta, palustris* and *procumbens*). Only in subsp. *laeta* cytotype with 2n = 32 (34) is found in lowland while cytotype with 2n = 56 (57, 58, 59, 61, 64) is found in mountains. Subspecies *palustris,* with 2n = 32 (34, 35 and 36) chromosomes, do not follow this kind of distribution. Division of species into subspecies explain better distribution of this great variability.

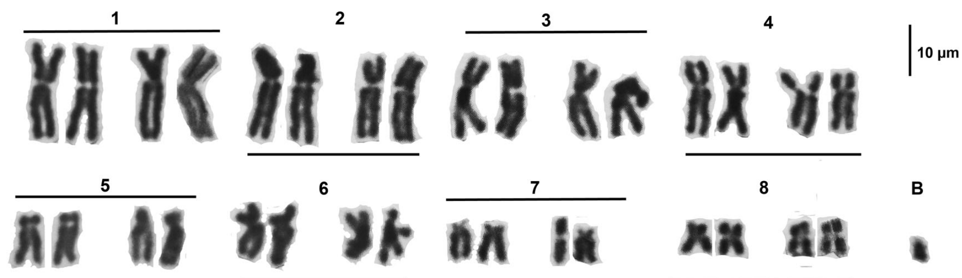

Fig. 2. Karyotype of *Caltha palustris* from Mt. Tara arranged in 8 groups according to chromosome length and morphology

As for presence of B chromosomes, they are most frequently present in plants with 56 chromosomes, and less frequently in plants with 32 chromosomes. In former cytotype the maximal number of Bs is 6 (KOOTIN-SANWU and WOODELL, 1970), while in later cytotype it is 4 (MALIK and MARY, 1970). Furthermore, the great numerical chromosome variation found in *C. palustris* from India, comprising 2n = 16, 28, 32, 35, 38, 40, 42, 44, 48, 50, 52, 54-58, 60, 64, 68, 72 and 88, could partially originate from the presence of different number of B chromosomes (KUMAR and SINGHAL, 2008; GUPTA *et al*., 2009). Data on morphology and size of B chromosomes in marsh marigold are largely missing.

In all three studied populations karyotypes were stable with 32 chromosomes arranged in the way that points out to the autopoliploid origin. Small B chromosome was found only in population from Tara Mt. The extreme environmental conditions increase chromosomal variability, so the level of ploidy decreases from north to south Europe, which could explain stable ploidy found in Serbia.

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HROMOZOMSKI STATUS KALJUŽNICE, *Caltha palustris* L. (Ranunculaceae) IZ SRBIJE

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Izvod

Kaljužnica, *Caltha palustris,* naseljava vlažne, umerene i hladne predele severne hemisfere. Ovu vrstu karakteriše znatna količina intraspecijske hromozomske varijabilnosti uključujući hibridizaciju, poliploidiju, aneuploidiju i B hromozome. Po prvi put je urađena kariotipska analiza tri planinske populacije iz Srbije. Svi analizirani uzorci su bili tetraploidi (osnovni broj n=8) sa 2n=32. U populaciji sa planine Tare detektovano je prisustvo jednog B hromozoma. Tetraploidni kariotip se sastojao od 17 metacentričnih (m), 8 submetacentričnih (sm), 7 subtelocentričnih (st) i jednog telocentričnog (t) B hromozoma (2n=17m+8sm+7st+1B). Analizirane populacije iz Srbije pripadaju citotipu koji je najčešći za ovu vrstu u Evropi.

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