Chromosome studies on Garden pepper
(Capsicum frutescens L.)

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Abstract
Cytogenetic studies to determine the chromosome number and structure was carried out. Attempt was also made to induce polyploidy in the species. Analysis of the cytological behavior of the diploid and polyploidy cytotypes was made. The studies show that the somatic chromosome number in the genus Capsicum is 2n = 24. The karyotype analysis indicates that the chromosomes were generally very small. There were also slight variations in centromeric positions and arm lengths. The significance of this work in the understanding of cytogenetic especially of plants and crop improvement efforts are discussed.

Key words: Cytogenetic study, Capsicum, Chromosome analysis, Mitosis, Solanaceae.

INTRODUCTION:
Capsicum L. is a member of the Solanaceae family and is cultivated in temperate and tropical regions (Lippert et al., 1966; Eshbaugh, 1993; Pozzobon et al., 2005). Fruits of this plants are used as spice, vegetable and herbal remedy. Capsicum has important roles in various aspects of economy, food and pharmacetics. It has the highest content of vitamin C among all plants and has important medicinal properties such as prevention and antioxidant characteristics (Salehi, 2006). Capsicum has at least between 20-30 species, from which five of them have become domesticated: Capsicum annum, C. frutescens, C. chinense, C. pubescens and C. baccatum (Lanteri, 1993; De Teodoro-Pardo et al., 2007).

Studies on Capsicum species have shown that they contain 24 chromosomes (2n=2x=24), similar to many species of Solanaceae family. There are two distinct groups present in the genus: some species have 24 chromosomes (2n=2x=24) while other species have 26 chromosomes (2n=2x=26). The most common chromosome number in the genus is x=12 (Smith and Heiser, 1951; Pickersgill, 1971; Limaye, 1989; Moscone, 1993). Karyotypes in different species with 24 chromosomes are very similar with each other. The species with 24 chromosomes have symmetrical karyotypes. They generally have one pair of acro-centrics and the rest of the species with 26 chromosomes display more asymmetrical complements, with more sub-metacentric chromosomes and often one telo-centric chromosome (Lanteri 1993).

Most species of Capsicum with 26 chromosomes have been found in South America as wild plants but all domesticated Capsicum has 24 chromosomes. Researches on Capsicum in have been concentrated on investigating the performance of landraces pepper and foreign cultivars, genetic variation of genotypes in the country and improving cultivation techniques.

MATERIALS AND METHODS
Collection:
Fruits of capsicum species were collected during field trips to different parts of southern Tamilnadu. The root tips for mitotic studies were obtained from healthy seedlings.

Mitotic studies:
Seeds were germinated on moistened absorbent paper in Petri dishes kept in the dark at temperature of 28-30 °C. Primary roots measuring about 5 to 10 mm long were harvested during late afternoon for slide preparation. Seedlings of capsicum frutescens could not yield enough roots for this protocol. To enhance root production, therefore, their root systems were completely excised just below the soil level and the stems dipped in 10 ppm iodole butyric acid for up to 6 h. They were later transferred to dilute nutrient solution for about 168 h to yield a good crop of roots. Pretreatment in all the species was with 0.002 M aqueous solution of 8-hydroxyquinoline (W/V) for at least 3 h. The root tips were then fixed in 1 part glacial acetic acid and 3 parts ethanol (V/V) for a minimum of 24 h. The roots that were not required immediately for slide
preparation were stored in 70% ethanol in a refrigerator. The root tips were hydrolyzed in 9% hydrochloric acid for a minimum of 5 minutes and squashed in formic-lactic-propionic acid-orcein (FLP-orcein) stain. Chromosomes were examined at x400 magnification using Leitz Labolux microscope fitted with photographic equipment. Good plates with well spread chromosomes were photomicrographed while measurement of chromosomes was done with an ocular micrometer. Chromosomes were studied under oil immersion on a phase contrast microscope at a magnification of 1000x. All slides were made permanent by the venetian turpentine Wilson, (1945). Nomenclature adopted by Levan et al., (1964) was followed for recognizing chromosome types. For the previous chromosome counts, we used the following references: Fedorov (1969), Goldblatt and Johnson (1990).

RESULTS AND DISCUSSION

Mitotic chromosome counts showed that the 2n=24 was the diploid number for Capsicum frutescens L. The chromosomes were generally small in size and differed slightly in their centromeric position and same length. Chromosomes of Capsicum frutescens L. were more or less identical in sizes and their centromeric positions were all metacentric. No secondary constriction was observed in any of these chromosomes.

The basic chromosome number of x=12 reported by Kochhar (1981) was confirmed in these species. The diploid species showed chromosome number 2n=2x=24 but the sizes differed among the species. Such variations among diploid co species arise because of difference in the repetitive DNA sequences, which eventually make up the genome size (Schmidt et al., 1998). Quicke (1997) recorded the possibility of having different chromatin and heterochromatin densities along chromosome arms. This observation was similar to those made in the present studies. In other words, genome evolution may have been directed in the genus by changes in chromosomal organization brought about by different families of repetitive DNA. Zarco (1986) reported the presence of different intercalary tandem repeat units of DNA as responsible for the variation in Hordeum, Aegilops, and Triticum genera. In the case here, the variation in the spread or dispersion of heterochromatin probably mark the location of such families of satellite DNA in the Solanum species. Confirmation of this is only possible with molecular cytogenetic procedures, which can discriminate between the repeat units based on size. The morphology of the chromosomes was mainly of the metacentric to submetacentric types. This symmetry in morphology is a reflection of relatively primitive karyotypes of the members of this genus. Even so, Davis and Heywood (1963) asserted that this view might not be universal since many highly evolved species are also known to show karyotype symmetry. The observed size differences in the species karyotypes could be attributed to cryptic chromosomal structural changes that have brought breeding barriers thus separating the species into the entities seen today. Hartwell et al., (2000) listed chromosomal inversions and translocation as relevant in such cryptic changes. Earlier on Sarbhoy (1977) had noted the extra difficulty encountered in the karyotype analysis of such small-sized chromosomes.

Capsicum frutescens L. Metaphase of mitosis, 2n=24 Chromosomes.

Chromosome condensation and spreading

It is believed that root chromosomes prepared by conventional squashed technique made poor quality spreads. It was either the chromosomes stick together or some are lost or float away between cells during tapping and squashing while the described protocol here optimized for Triticum aestivum allowed the preparation of appropriate metaphase spreads not only for wheat but also for various other cereals including barley and rye without losing the chromosomes. Squeezing out the meristematic tissue on to the surface of the slide presented clean slides. Heating the slide over a heater without boiling the acetic acid helped to break the cell wall and clear appearance of cytoplasm surrounding the chromosomes being almost invisible. The squashing of ice-cold water pretreated materials was easier when compare with colchicines and α-bromonaphthalene giving better metaphase spreads. While colchicines and α-bromonaphthalene was effective in achieving good chromosome morphology, it is advisable to transfer pretreated root tips to ice water for overnight incubation. This addition pretreatment further enhanced chromosome condensation and more importantly, improved the spreading of chromosomes within a cell. Using colchicines requires determination of optimal concentration for different species. Using lower than optimal concentrations do not arrest cells in metaphase. Optimal concentration (usually 0.05% w/v) acts through depolymerization of the microtubular cytoskeleton in all phases of the cell cycle (Caperta et al., 2006). On the other hand unnecessary high concentration may induce excessive chromosome clumping. These characteristics of colchicines are similar to amiprophase methyl (AMP) which is also a spindle inhibitor (Dolezel et al., 1999).

REFERENCES


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