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Cytogenetic studies in *Phaseolus* L. (Fabaceae)

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Abstract

A review of the cytogenetic studies carried out on *Phaseolus* as well as the different proposals that have been suggested to explain the chromosomal changes in the group are presented. The importance of including wild species in cytogenetic studies and the collaboration between taxonomists and cytogeneticists in order to draw better conclusions are emphasized.

INTRODUCTION

The family *Fabaceae* (*Leguminosae*) contains the sub-family *Papilionoideae* of which the tribe *Phaseoleae* is one of the most important groups because it contains genera such as *Glycine* (soybean), *Phaseolus* (American beans) and *Vigna* (Asiatic beans), which are economically important due to their role in human nutrition and their use as cattle forage and ornamental (Lackey, 1981).

The genus *Phaseolus* is mainly found in the Mexican mountains (Sousa and Delgado, 1993), and contains approximately 50 species, with four (Delgado-Salinas, 1985) or five (Debouck, 1991) cultivated ones: *P. vulgaris*, *P. coccineus*, *P. acutifolius*, *P. lunatus* and *P. polyanthus* (= *P. coccineus* subsp. *darwinianus*).

In his original description of *Phaseolus*, Linnaeus (1753) included eleven species, but with time the number grew to 200, distributed both in the Old and the New World. In 1970, Verdcourt redefined *Phaseolus*, considering it exclusively of New World origins, with approximately 50 species whose characteristics are similar to those of *P. vulgaris* (generitype). This redefinition was confirmed as valid and refined by a series of studies by other researchers (Maréchal *et al.*, 1978 and Lackey, 1981, 1983).

The last revision of the genus was made by Delgado-Salinas (1985), who recognized only 36 species in North and Central America. Despite the taxonomic studies carried out on the genus that have led to its clear delimitation, neither the number of taxa of which the genus is composed nor the genetic relationship between species has been well established (Debouck, 1991). Delgado-Salinas (1985) estimates that the genus contains 36 species, in North and Central America, some of them with subspecific divisions, while Debouck (1991) includes 52 especies, without subspecific divisions.

Although the importance of cytogenetic studies have been noted by several authors (Thomas, 1973; Green *et al.*, 1980; Almeda and Chuang, 1992), most studies have dealt with economically important species, ignoring the potential of wild species and relating only to cultivated species such as *Phaseolus*.

CHROMOSOMAL STUDIES

The first reports on chromosome numbers in *Phaseolus* go back to 1925, when Karpetschenko obtained $2n = 22$ for *P. acutifolius* A. Gray, *P. coccineus* L., *P. lunatus* L. and *P. vulgaris* L. From then on, a large number of cytogenetic studies have focused mainly on the determination of chromosome numbers, establishing $x = 11$ as the basic number.

Prior to 1996, of the approximately 50 species recognized in the genus *Phaseolus*, only 9 species and 4 subspecies had been chromosomally counted. Mercado-Ruaro and Delgado Salinas (1996, 1998) increased the number of taxa analyzed to 31. Based on the published literature Lackey (1979), Goldblatt (1981) and Mercado-Ruaro and Delgado Salinas (1996, 1998) propose that, as in the tribe *Phaseoleae*, the basic chromosome number in the genus is $x = 11$, with a haploid number of $n = 10$ in three species (*P. leptostachyus* Benth., *P. micranthus* Hook. & Arn., and *P. macvaughii* A. Delgado, ined. (Mercado-Ruaro and Delgado-Salinas, 1998)). The number of species that have been analyzed is very low, and the analyses have been restricted mainly to cultivated species (Sarbhoy, 1977; Joseph and Bouwkamp, 1978; Sinha and Roy, 1979a; Zheng *et al.*, 1991). Mercado-Ruaro and Delgado-Salinas (1998) reported the karyotypic analysis of 10 wild species, that represent on average 20% of those comprising the genus. The lack of karyologic studies in the genus has been attributed to the reduced size of the chromosomes, which makes the analysis difficult (Hucl and Scoles, 1985; Zheng *et al.*, 1991). Nonetheless, the available information has shown that there is a predominance of metacentric and submetacentric chromosomes, which translates into very symmetrical karyotypes.

Some authors (Sarbhoy, 1977, 1980; Sinha and Roy, 1979a,b) have pointed out that the main factors involved in the karyotypic evolution of the genus are pericentric and paracentric inversions, translocations and the loss or gain of chromatin. They have also proposed that the karyotype

of *Phaseolus* has evolved towards an asymmetry, with a decrease in the total chromatin content. Mercado-Ruaro and Delgado-Salinas (1998), after encountering three aneuploid species with $2n = 20$, have pointed out that aneuploidy has also played a role in the evolution of the karyotype.

GENOMIC HYBRIDIZATION

Studies of *Phaseolus vulgaris* by Frediani *et al*. (1993) using *in situ* hybridization have shown the position of the genes that code for polygalacturonase-inhibiting protein (PGIP) and stablished that the coding sequences are located in the heterochromatic pericentromeric region of metacentric chromosome 10, while Schumann *et al.* (1990) and Nenno *et al*. (1993) have documented the position of the phaseolin gene. In *P. coccineus* Avanzi *et al.* (1972) have located the ribosomal cistrons in the nucleolar and satellite regions of chromosomal pairs I and V using tritium-labelled rRNA. These studies, all employing polytene chromosomes, show the potential of *in situ* hybridization for chromosome mapping.

The application of genomic *in situ* hybridization to taxonomy and the elucidation of genetic relationships are exemplified by the studies of Mercado-Ruaro on the *Phaseolus vulgaris-P. coccineus* complex, which investigated the possible hybrid origin of *P. coccineus* subsp. *darwinianus* Hernández X. & Miranda C. (= *P. polyanthus* Greenm.) as well as the genetic relationships between the species and subspecies that make up the complex. The results of this study have shown the high degree of genetic homology between the members of this group, and because of this it was not possible to establish whether or not *P. coccineus* subsp. *darwinianus* is the result of a cross between *P. coccineus* and *P. vulgaris*, although it was possible to establish that *P. glabellus* is a taxon only distantly related to other members of the complex.

STUDIES OF NUCLEAR DNA CONTENT

There is much variation in the reported DNA content of the *Phaseolus* species studied by different authors. The DNA content of cultivated *P. vulgaris* has been reported as being 1.56, 1.63, 1.69, 1.79 pg by Castagnaro *et al*. (1990), 2.7 pg by Bennett (1982) and 3.7 pg by Ayonoadu (1974), while that of the wild-type *P. vulgaris* var. *aborigineus* has been reported to be 1.71 pg by Castagnaro *et al*. (1990). These differences may be attributable to the source of the material, the type of control used or to errors inherent in the technique. Other species studied for their DNA content include *P. coccineus*, containing 3.5 pg according to Ayonoadu (1974) and 1.98 pg according to Castagnaro *et al*. (1990); *P. lunatus* with 2.5 pg (Ayonoadu, 1974); *P. dumosus* with 3.8 pg, and *P. leucanthus* with 3.3 pg (Ayonoadu, 1974).

The latter two species are probably *P. coccineus* subsp. *darwinianus*, since both names have always been nomenclaturally associated with this subspecies.

As is the case in cytogenetic studies, there are reports of species referred as *Phaseolus* when they actually belong to other genera, for instance, *P. angularis* is really *Vigna angularis*, with a DNA content of 2.8 pg, while both species *P. geophilus* (2.6 pg) and *P. lathyroides* (2.3 pg) belong to genus *Macroptilium.*

Ayonoadu (1974) found a positive correlation between the nuclear DNA content and the nuclear volume, nucleolar and nuclear dry mass and total dry mass, i.e., high DNA content indicates high values for volume and dry mass parameters. Castagnaro *et al*. (1990), studying *P. coccineus* and several cultivars of *P. vulgaris*, along with *P. vulgaris* var. *aborigineus*, also found a positive correlation between seed weight and DNA content, with the exception of *P. vulgaris* var. *aborigineus*, which presented a negative correlation. Even so those authors conclude that varieties with a high DNA content are better adapted to cold or temperate regions, while those varieties with a lower DNA content are adapted to hot, dry environments.

We are now in the process of analyzing the DNA content of wild species of *Phaseolus* to determine if there is any relationship between DNA content and taxonomic relationships between the species and/or karyotype.

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