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ARTICLE

Chromosome banding patterns and localization of 5S and 45S rDNA sites in three shrub-tree species of *Erythrina* L. (Leguminosae: Papilionoideae) from Brazil

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ABSTRACT: (Chromosome banding patterns and localization of 5S and 45S rDNA sites in three shrub-tree species of *Erythrina* L. (Leguminosae: Papilionoideae) from Brazil). *Erythrina* consists of 112 species distributed throughout the tropical and temperate regions. Species of *Erythrina* are of great biological interest because they are pollinated by hummingbirds, which promote a high pollen flow and high chance of homogenization of recombinant forms. The karyotypes of *E. speciosa*, *E. falcata* and *E. mulungu* were cytogenetically analyzed in order to establish relationships among these three species of contrasting habitats. The chromosome count showed $2n = 42$, with small chromosomes, prophasic condensation of the proximal type and non-reticulate to semi-reticulate nuclei with evident chromocenters. Chromosome banding showed AT-rich heterochromatic blocks in the pericentromeric region of most chromosomes and GC-rich regions in the terminal portion of the largest pairs. *In situ* hybridization with 45S rDNA probes exhibited ten terminal signals in *E. falcata* and *E. speciosa* and eight in *E. mulungu*, while the 5S rDNA probe showed only two signals, also terminals in three species. The results show quite conserved karyotypes, with small variations in the size and number of 45S rDNA sites, which can be considered the only elements of karyotype differentiation, independent of plant size and strategies.
Key words: C-CMA/DAPI banding, FISH, 45S and 5S rDNA.

RESUMO: (Padrões de bandamento cromossômico e localização de sítios de DNAr 5S e 45S em três espécies arbóreo-arbustivas de *Erythrina* L. (Leguminosae: Papilionoideae) do Brasil). *Erythrina* consiste de 112 espécies distribuídas nas regiões tropicais e temperadas. Espécies de *Erythrina* têm grande interesse biológico por serem polinizadas por beija-flores, os quais favorecem um grande fluxo de pólen e aumentam a chance de homogeneização das formas recombinantes. Os cariótipos de *E. speciosa*, *E. falcata* e *E. mulungu* foram analisados citogeneticamente para estabelecer relações entre estas três espécies de hábitos contrastantes. A contagem cromossômica mostrou $2n = 42$, com cromossomos pequenos, condensação profásica do tipo proximal e núcleos arreticulados a semi-reticulados com cromocentros evidentes. O bandamento cromossômico mostrou blocos heterocromáticos ricos em AT nas regiões pericentroméricas da maioria dos cromossomos e regiões ricas em GC na porção terminal dos maiores pares. A hibridação *in situ* com as sondas de DNAr 45S exibiram dez sinais terminais em *E. falcata* e *E. speciosa* e oito em *E. mulungu*. A sonda de DNAr 5S mostrou apenas dois sinais, também terminais, nas três espécies. Os resultados mostram cariótipos bastante conservados, com pequenas variações no tamanho e no número dos sítios de DNAr 45S, os quais podem ser considerados os únicos elementos de diferenciação cariotípica, independente do tamanho e do hábito das plantas.
Palavras-chave: bandamento C-CMA/DAPI, FISH, DNAr 45S e 5S.

INTRODUCTION

The genus *Erythrina* L. (Leguminosae: Papilionoideae) includes 112 species distributed throughout the tropical and temperate regions (Krukoff & Barneby 1974). Representatives occur in a wide variety of habitats, from subtropical and tropical rain forests to arid deserts and conifer forests. The genus is mainly represented by trees and shrubs with red to orange flowers, adapted to pollination by hummingbirds (Neill 1988). Some species are ornamental, while some are of economic importance in pharmacology and gastronomy (Krukoff 1941, Burkart 1952).

Erythrina falcata Benth. is a big tree found along riverbanks, with distribution in states of Minas Gerais and Mato Grosso do Sul to Rio Grande do Sul, Brazil (Lorenzi 1992), where it is a very important species in reforestation projects (Carvalho 2003). *Erythrina speciosa* Andrews is a shrub with ornamental interests, occurring in the Atlantic Forest, in states of Espírito Santo and Minas Gerais to Santa Catarina, Brazil. *Erythrina mulungu* Mart. ex Benth. is also a shrub, native to Northeast Brazil and known for its medicinal properties.

The basic chromosome number of the genus is $x = 21$ and the karyotypes have a predominance of $2n = 42$ (Lewis 1974, Goldblatt 1981a, 1981b), as previously described in most species of *Erythrina*, and *E. speciosa* and *E. falcata* (Atchison 1947). The aneuploidy is unknown and the polyploidy is rare (Lewis 1974; Goldblatt 1981, 1984), reported for *E. acanthocarpa* E. Mey, *E. amazonica* Krukoff (Atchison 1947) with $2n = 84$, *E. burana* Chiov. (Neill 1988) with $n = 84$ and *E. burttii* with $2n$ = ca.126 (Atchison 1947) and $2n$ = ca. 168 (Goldblatt 1981a). This last author suggested that the formation of viable hybrids is common among diploid species of *Erythrina*. Conventional cytogenetics has been very useful in taxonomic and evolutionary studies of

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different plant groups (Pedrosa *et al*. 1999), but the use of chromosome banding (Guerra 2000) and fluorescence *in situ* hybridization has expanded karyotype affinity studies in Brazilian species, such as those carried out with *Lobelia* L. (Vanzela *et al*. 1999), *Cestrum* L. (Fregonezi *et al*. 2006, Fernandes *et al.* 2009) and *Solanum* L. (Rego *et al*. 2009).

Taking into account the relevance of this kind of analysis and the scarcity of cytogenetic studies in Brazilian species of *Erythrina*, this study aims to analyze comparatively cytogenetic aspects of three species of genus that present contrasting habits. In the present study, several cytogenetic tools were employed to determine if these distinct species of *Erythrina* also have very distinct chromosome characteristic.

MATERIAL AND METHODS

Seeds of *Erythrina falcata* (LABRE 468), *Erythrina speciosa* (LABRE 315) and *Erythrina mulungu* (LABRE 475) were obtained from the biological collection of Laboratório de Biodiversidade e Restauração de Ecossistemas – LABRE, CCB, UEL, Londrina, Brazil. At least five individuals of each species were used for the karyotype analyses. Samples are kept in the experimental garden of LABRE.

Roots were pretreated with 2 mM 8-hydroxyquinoline for 5 h at 14°C and fixed in absolute ethanol:glacial acetic acid (3:1, v:v) for 12 h and kept at -20°C until used. Afterward, the samples were digested in a mixture of 4% cellulase and 40% pectinase (w/v) at 37°C for 3 hours, and further hydrolyzed in 1 M HCl at 60°C for 10 min. The root tips were dissected in a drop of 45% acetic acid and squashed. The coverslips were removed after freezing in liquid nitrogen. The material was stained with 2% Giemsa and permanent slides mounted in Entellan (Merck).

Chromosome banding was performed as described by Vanzela & Guerra (2000). Root tips were digested in an enzyme solution composed of 4% cellulase and 40% pectinase (w/v) at 37°C and dissected in a drop of 45% acetic acid. After removal of the coverslips, the slides were incubated in 45% acetic acid for 10 min at 60°C, followed for 10 min in 5% barium hydroxide at room temperature, and 2x SSC, pH 7.0, for 80 min at 60°C. The staining was performed with fluorochromes: 0.5 mg/mL CMA_3 for 1.5 h and 2 µg/mL DAPI for 30 min. Slides were mounted with a medium composed of glycerol/McIlvaine buffer (pH 7.0) 1:1, plus 2.5 mM $MgCl_2$.

For fluorescent *in situ* hybridization (FISH), slides were prepared as described for banding and immediately used for FISH, as described by Vanzela *et al*. (2002). The probes p*Ta*71 (45S rDNA) and p*Ta*794 (5S rDNA) were labeled with biotin-14-dATP and digoxigenin-11-dUTP, respectively, both by nick translation. Each slide was treated with 34 µL of hybridization mixture containing 100 ng of labeled probe, 50% formamide, 50% polyethylene glycol, 20x SSC, 100 ng of calf thymus DNA and 10% SDS. Samples were denatured at 90°C for 10 min, and hybridization was performed overnight at 37°C in a humidified chamber. Post-hybridization washes were carried out in 2x SSC with 70% stringency. The probes were simultaneously detected with avidin-FITC conjugate and antidigoxigenin-rhodamine conjugate in 5% BSA (1:1:100, v:v:v), and the baths were carried out in 4x SSC/0.2% Tween 20 at room temperature. Slides were mounted with 25 µL of a medium composed of 23 µL of antifade solution (1,4-diaza- bicyclo(2.2.2)-octane (2.3%), Tris HCl 20 mM, pH 8.0 (2%) and glycerol (90%), in distiled water), 1 µL of 2 µg/mL DAPI and 1 µL of 50mM $MgCl_2$ (Fernandes *et al*. 2009).

All the images were acquired with a Leica DM 4500B microscope equipped with a DFC 300FX camera and Leica IM50 4.0 software. All the images were optimized for best constrast and brightness with iGrafx Image software.

RESULTS AND DISCUSSION

Conventional analysis showed similar karyotypes with $2n = 42$ (Figs. 1D, 1E and 1F), confirming the numbers described for *E. falcata* and *E. speciosa* (Atchison 1947, Neill 1988), as well as the basic chromosome number of *x=21*. This is the first chromosome count for *E. mulungu.* Stebbins (1966) suggested that ancestral number of woody angiosperms were $x = 6$ and 7, and other numbers were resulted of chromosome rearrangements and polyploidy. Although most species of *Erythrina* present $n = 21$ as predominant number, *Erythrina vogelii* Hook F. reported $n = 12$ (Gill & Husaini 1982), suggesting the ancestral base number for the genus may be less than 21.

According to Goldblatt (1981a), the genus *Erythrina* may have originated by allopolyploidy based on $n = 11 + n = 10$ or $n = (11 \times 2) - 1$, where it would be considered a paleopolyploid genus. High polyploid numbers were reported only for *E. acanthocarpa* and *E. amazonica*, both with $2n = 84$ (Atchison 1947), for *E. burana* with $n = 84$ (Neill 1988) and for *E. burttii* $2n =$ ca.126 (Atchison 1947) and $2n =$ ca. 168 (Goldblatt 1981a). These numbers showed that polyploidy is very important in the karyotype evolution of *Erythrina*, as well as being important in the evolution of tropical woody species (Morawetz 1986).

These three species of *Erythrina* showed similar chromosome shapes (meta- and submetacentrics) and sizes (less than 2 µm) (Figs. 1D, 1E and 1F). The interphase nuclei were of the non-reticulate to semi-reticulate type, with several well-defined chromocenters (Figs. 1A and 1B), and prophasic condensation was proximal in the three species (Fig. 1C). Other legumes, such as those of *Phaseolus* L., although having different diploid numbers, also showed small chromosomes (from 1.70 to 3.50 µm) and karyotype with a predominance of meta- and submetacentrics (Moscone *et al.* 1999). Species of the subfamily Caesalpinioideae, as those of *Apuleia* Mart. and *Senna* Mill., also showed small chromosomes of about 2 µm and non-reticulate nuclei (Biondo *et al.* 2005). According to Guerra (2000), species with small chromosomes, less

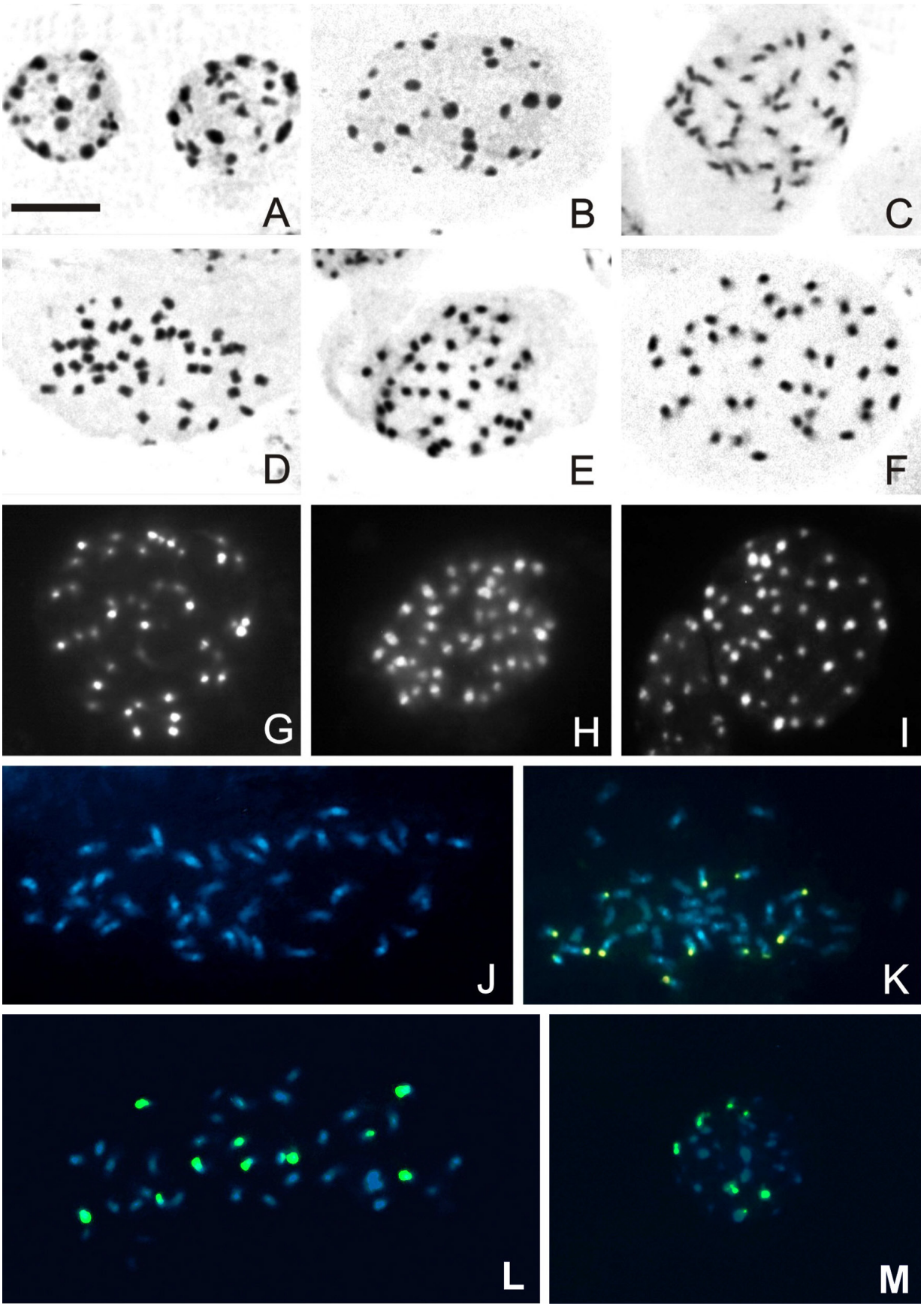

Figure 1. Conventional staining and chromosome banding with fluorochromes in *Erythrina* species. Interphase nuclei of *E. falcata* (A) and *E. mulungu* (B). Note the evident chromocenters. Prometaphase of *E. falcata* (C). Note the proximal condensation. Mitotic metaphases of *E. falcata* (D), *E. speciosa* (E) and *E. mulungu* (F). C-DAPI banding in *E. falcata* (G and J), *E. speciosa* (H) and *E. mulungu* (I). Note the C-CMA/DAPI banding in *E. falcata* (K), *E. speciosa* (L) and *E. mulungu* (M). Bar represents 5 µm.

than 3 µm, usually have non-reticulate nuclei with proximal prophasic condensation, as found here in *Erythrina*.

Chromosome banding showed two heterochromatin distribution patterns in the three species. In the first case, AT-rich blocks (C-DAPI$^+$) were found in the proximal region (Figs. 1G, 1H, 1I, 1J and 1K). In *E. falcata* and *E. speciosa,* larger and brighter C-DAPI$^+$ blocks were observed in about ten largest chromosome pairs, while weaker signals were visualized in the remaining chromosomes (Figs. 1G, 1J and 1H, respectly). *Erythrina mulungu* exhibited approximately eight pairs with brighter C-DAPI$^+$ blocks in proximal regions of the largest chromosomes (Fig. 1I). The occurrence of DAPI$^+$ bands at proximal regions was also reported in *Phaseolus* L. species by Moscone *et al.* (1999). The AT-heterochromatin accumulation at proximal regions seems to depend at least in part on chromosome size in these three species. According to Guerra (2000), this distribution pattern is more common in karyotypes with small chromosomes, as observed here in *Erythrina*. In the second case, GC-rich blocks (C--CMA$^+$) were found in the terminal region of the largest chromosome pairs (Figs. 1K, 1L and 1M). In *E. falcata* and *E. speciosa,* there were up about 12 terminal signals with more intense brightness in the larger chromosomes (Figs. 1K and 1L, respectly), while *E. mulungu* had no more than eight terminal GC-rich blocks (Fig. 1M). The distribution of C-CMA$^+$ blocks preferentially at terminal regions is also common in most plants; however, there are examples of GC-rich blocks in proximal regions, as described in *Crotalaria juncea* L. (Mondin *et al.* 2007). In both cases, the distribution of heterochromatin seems to follow the equilocal model proposed by Schweizer & Loidl (1987).

FISH with 45S rDNA probe localized ten terminal signals for *E. falcata* and *E. speciosa* (Figs. 2A and 2B) and eight terminal signals for *E. mulungu* (Fig. 2C), in chromosomes of distinct sizes. The terminal location of rDNA sites was also evidenced in other Leguminosae, such as *Phaseolus* L. (Moscone *et al.* 1999), *Camptosema* Hook. & Arn. and *Galactia* P. Br. (Sede *et al.* 2006). Despite the stable location of terminal 45S rDNA sites, the number and size of blocks were variable in *Erythrina*, as in other plant groups. Species of *Phaseolus* showed three to seven sites (Moscone *et al.* 1999) and species of *Galactia* showed four to eight hybridization signals (Sede *et al.* 2006). This variation can be the result of differential accumulation/dispersion of 45S rDNA segments (see Schubert & Wobus 1985, Adams *et al.* 2000). However, it is important to mention that the dispersion of rDNA segments occurs in an independent manner when comparing 45S and 5S rDNA, since FISH with the 5S rDNA probe always showed two hybridization signals in the three species of *Erythrina* (Figs. 2C, 2D, 2E and 2F).

To conclude, *E. falcata*, *E. speciosa* and *E. mulungu* are well-defined species, contrasting in morphology and ecological habits. However, the chromosome number and shape, interphase nucleus type and prophasic condensation show similar karyotypes, except for minor variations in the banding patterns and 45S rDNA regions. In fact, these karyotype similarities could indicate a few occurrences of chromosome rearrangements and that the most important mechanism to karyotype evolution in *Erythrina* could be the polyploidy.

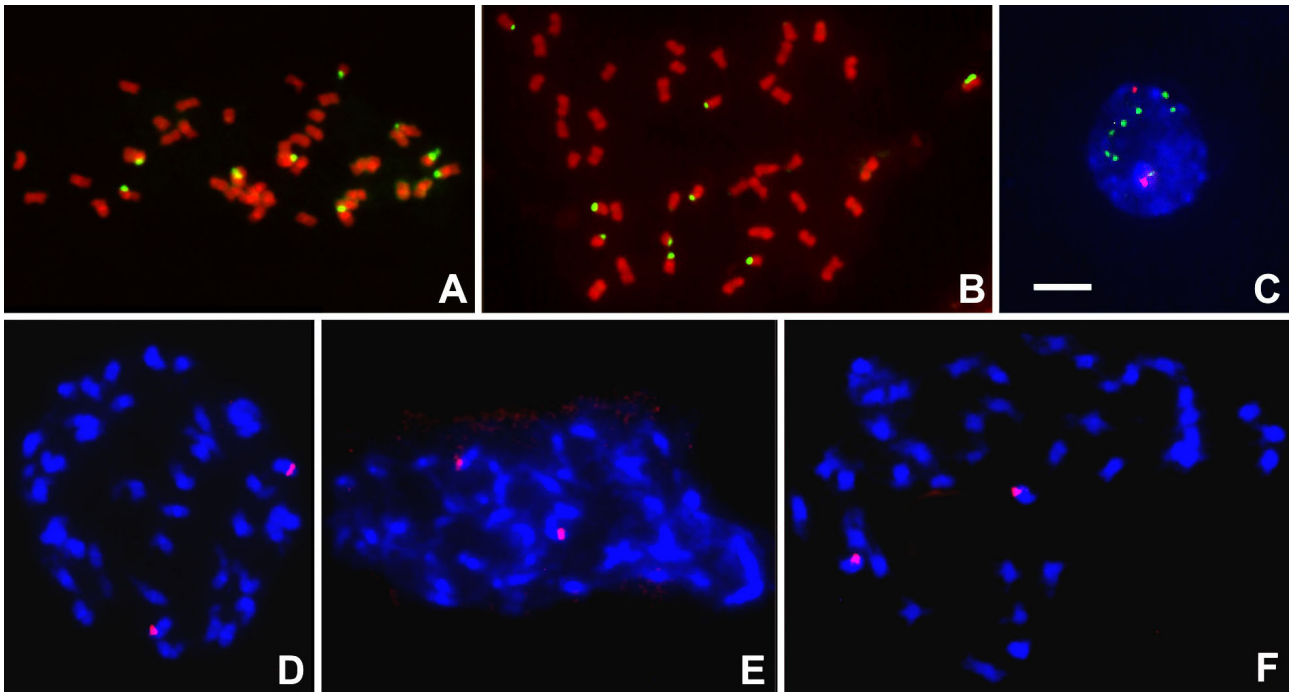

Figure 2. FISH with the p*Ta*71 (45S rDNA) and p*Ta*794 (5S rDNA) probes in *Erythrina* species. Metaphase hybridized with 45S rDNA probe in *E. falcata* (A) and *E. speciosa* (B) always showing ten hybridization signals. Interphase nucleus simultaneously hybridized with 45S and 5S rDNA probes in *E. mulungu* (C). Note the occurrence of eight signals of 45S rDNA. Metaphase hybridized with 5S rDNA probe in *E. falcata* (D), *E. speciosa* (E) and *E. mulungu* (F), always showing two terminal signals. Bar represents 5 µm.

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