

## Fluorescent banding in tropical *Pinus* chromosomes

### Bandeamento fluorescente em cromossomos de *Pinus* tropicais

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**ABSTRACT:** There have been controversies about the taxonomic classification of Tecun Umán pine for about 50 years. Some investigations have shown a close relationship between this conifer and *Pinus patula*, while others showed relationship to the *Pinus oocarpa*. In this study, data on CMA fluorescent chromosomes banding showed that Tecun Umán pine had banding patterns close to *Pinus oocarpa*.

**KEYWORDS:** Tecun Umán pine, CMA banding, *Pinus*

**RESUMO:** Tem havido controvérsias sobre a classificação taxonômica do *Pinus* de Tecun Umán por aproximadamente 50 anos. Algumas investigações mostraram uma relação próxima entre esta conífera e o *Pinus patula*, enquanto que outras mostraram relação com o *Pinus oocarpa*. Neste estudo, dados de bandeamento cromossômico fluorescente CMA mostraram que o *Pinus* de Tecun Umán tinha padrões próximos ao *Pinus oocarpa*.

**PALAVRAS-CHAVE:** *Pinus* de Tecun Umán, Bandeamento CMA, *Pinus*

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#### INTRODUCTION

The great increase in the demand for wood on the world market has encouraged industrial projects to use almost exclusively introduced (non-native) forest species.

Within the tropical conifers introduced in Brazil, the Tecun Umán pine has performed well in several regions (Wright and Osorio, 1992), showing excellent agricultural qualities such as rapid growth, over 50 m height, straight trunk, 1.30 m DBH, and little branch ramification. Therefore, taxonomic classification has not been well established. This is a problem mainly in forest breeding programs, where that information coupled with individuals variability needs to be evaluated to improve the quality of future plantings.

Some studies denominated Tecun Umán pine as *Pinus oocarpa* Schiede var. *ochoterenai* Martinez (Martinez, 1948). Others were called *Pinus tecunumanii* (Schw) Eguiluz and Perry by Eguiluz-Piedra and Perry (1983). Styles (1985) reported that both, *P. oocarpa* var. *ochoterenai* and *P. tecunumanii*, belong to the same taxon, *Pinus patula* Schiede & Deppe ssp. *tecunumanii* (Eguiluz-Piedra and Perry) Styles.

Other earlier studies, Styles and McCarter (1988), showed the difficulties with the identification, because their morphological similarities, mainly in studies using *P. oocarpa* at middle elevations (900 - 1600 m) on sites where both species occur together with Tecun Umán pine.

Some comparative studies have been carried out to show the close relationship among these taxa using wood and morphological characters. They agree on the subspecific category suggested by Styles (Davide and Araújo, 1993; Leão and Davide, 1993). However, such characteristics are usually controlled by several genes, and are greatly influenced by the environment (Strauss et al., 1992).

Using DNA markers and analyzing the phylogenetic relationships between Tecun Umán pine and other conifers, authors suggested a specific category for Tecun Umán pine, and showed an unexpected distinctiveness between *P. patula* and Tecun Umán pine. This study also revealed a similarity at the DNA level between *P. caribaea* var. *hondurensis* and *Pinus oocarpa* (Grattapaglia et al., 1992).

The controversy has lasted for nearly 50 years. The present study make use of characteristics as the CMA fluorescent banding, to assess the relationship between Tecun Umán pine, *P. oocarpa*, *P. patula*, *P. caribaea* var. *hondurensis* and thus to contribute to the taxonomic definition of the Tecun Umán pine.

## MATERIAL AND METHODS

The cytological preparation was carried out using the following botanical material:

- ✓ Tecun Umán pine: provenances which origin is Mountain Pine Ridge in Mexico planted at Agudos city in São Paulo State by the Duratex Company;
- ✓ *Pinus patula* Schiede e Deppe: population from Camanducaia City in Minas Gerais State grown by Melhoramentos Company;
- ✓ *Pinus oocarpa* Schiede: population from Agudos City in São Paulo State grown by Duratex Company;
- ✓ *Pinus caribaea* var. *hondurensis*: population from Agudos City in São Paulo State grown by Duratex Company.

Seeds from those species were placed to germinate for 7 to 10 days in a germination chamber at approximately 27°C. After germination, the roots of approximately 0.5 cm were pre-treated with a 0.05% colchicine solution for 24 hours, fixed in an ethanol-glacial acetic acid-chloroform solution (2:1:1) at 4°C for 24 hours and transferred to 70% ethyl alcohol where they were stored at 4°C until use. The roots were hydrolyzed in 45% acetic acid at 60°C for 10 minutes to prepare the slides. The meristem region was removed and crushed in 45% acetic acid. The cover slides were removed using the dry ice method and the slides containing material to be analyzed were stored in a drying chamber at room temperature until staining.

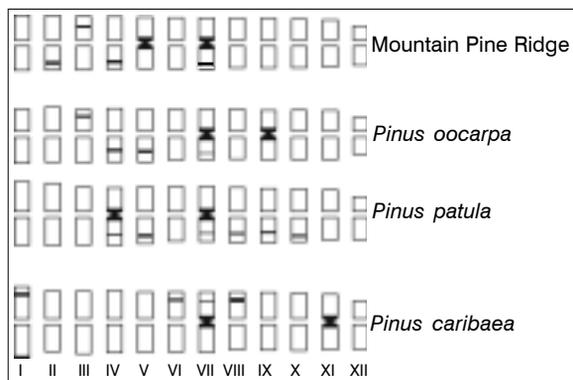
The photomicrographs were made with a Carl-Zeiss fluorescence microscope. The fluorescent staining method with A3 chromomycin (CMA) (Hizume et al., 1989), with modifications was used. The slides were pre-incubated for 10 minutes in McIlvaine buffer pH 7.0 (17.6 ml citric acid 0.1 M and 82.4 ml of sodium monohydrate phosphate 0.2M) and treated with A3 chromomycin solution (12mg/ml) for an hour in darkness, and then washed and mounted in 50% glycerol in McIlvaine buffer MgCl<sub>2</sub> 2%.

Four metaphases per taxon were analyzed and the observed bands were localized in their respective idiograms.

## RESULTS

The mitotic metaphases from Mountain Pine Ridge of Tecun Umán pine, *P. patula*, *P. oocarpa* and *P. caribaea* var. *hondurensis* taxa had 2n=24 chromosomes, with 11 metacentric chromosome pair and one submetacentric pair (the XII in Figure 1).

In all analyzed taxa the identification of the 10 first chromosome pairs, made by size and position of the centromere was very difficult, as they were extremely similar (Figure 1).



**Figure 1.** Idiograms of the chromosomes banded by CMA fluorochrome from analyzed taxa.

Six bands were observed in chromosomes of Tecun Umán pine from Mountain Pine Ridge, *P. oocarpa* and *P. caribaea* var. *hondurensis*. Eight bands were observed in *P. patula* (Figure 1). In that figure it is also observed that the Tecun Umán pine from Mountain Pine Ridge had the same number of centromeric (2) and interstitial (4) bands as *P. oocarpa* and *P. caribaea* var. *hondurensis*. *P. patula* had those and two additional interstitial bands.

The band position analysis indicated that the Mountain Pine Ridge provenance of Tecun Umán pine was closer to *P. oocarpa* (4 bands: 1 interstitial on chromosome III (short arm), 1 interstitial on chromosome IV (long arm) and 1 centromeric and another interstitial (long arm) on chromosome VII) followed by *P. patula* (3 bands: 1 interstitial on chromosome IV (long arm), and 1 centromeric and another interstitial (long arm) on chromosome VII), followed by *P. caribaea* var. *hondurensis* (2 bands: 1 centromeric and 1 interstitial (short arm) on chromosome VII). There was also coincidence in the position of 4 bands between *P. oocarpa* and *P. patula*, on chromosomes IV, V, and VII.

## DISCUSSION

Some authors who used morphological and anatomical techniques to solve the taxonomic

classification of Tecun Umán pine have used the category subspecific as *P. patula* subspecies *tecunumanii* (Styles, 1985; Davide and Araújo, 1993; Leão and Davide, 1993). On the other hand, studies using anatomic, morphological and biochemical techniques of terpene analysis (Eguiluz-Piedra and Perry, 1983) and RAPD markers (Grattapaglia et al., 1992; Silva-Mann et al., 1999) have showed the close relationship of this taxon to *P. oocarpa* and have given it a specific category (*Pinus tecunumanii*).

In this study, data from the CMA fluorescent banding confirmed that Tecun Umán pine is closer to *P. oocarpa*. However, it can be seen that there is not a great distance between these two taxa and *P. patula*.

Assessing the results obtained in this and other studies about the Tecun Umán taxonomy there is still doubt about its position as a species (*P. tecunumanii*) or as a subspecies of *P. oocarpa* because the distance between them is less than the distance observed between *P. oocarpa* and *P. patula*, which belong to the same Oocarpae subsection.

*Pinus caribaea* var. *hondurensis* showed less close to Tecun Umán pine than *P. oocarpa* and *P. patula*.

The variation of different characters have been observed in all well studied *Pinus* species, not only among geographically isolated populations but also among individuals obtained of hybridization within the same population (Pederick, 1970). It should be pointed out, however, that the A3 (CMA) fluorochrome chromomycin, which shows regions rich in C and G bases pair, also shows that such regions are variable in conifers, and, therefore, may be used in phylogenetic studies (Hizume et al., 1989).

The variation observed in the banding patterns further suggests that the occurrence of tropical conifers in evolution may be explained by the adaptation of some species to adverse conditions in tropical regions and/or the

emergence of hybrids. These may have undergone chromosomal modification of the paracentric inversion type, already reported in some *Pinus* species, and which seem to be present, if it is not in all, in the majority of the species of this genus. Many fragments of different sizes were observed in cells of different *Pinus* trees indicating the existence of many different inversions in different populations. It seems that these inversions are a characteristic of the *Pinus* genetic system and have a positive genetic value, generating variability among the different species (Pederick, 1970). This may be associated with the similarity observed between the chromosome VII of the taxon that showed 1 centromeric band and 1 interstitial band, only different in *P. caribaea* where this interstitial band appears on short in spite of the long arm.

Some previous studies on some conifers showed that the majority of the interstitial bands coincide with regions of secondary constriction, which show highly repetitive DNA sequences (Hizume et al., 1989). In this investigation this coincidence was observed. Such regions, which have become highly divergent in the course of evolution, justify together with the paracentric inversion phenomenon, the great variability observed in the genus. A series of cytogenetic as well as morphological, anatomic and biochemical characteristics and DNA markers need to be considered to obtain a better taxonomic definition of Tecun Umán pine. Also, geographic distribution and reproductive isolation data may be added to provide a solid base for this definition. The information of secondary constriction on chromosomes act as tool that can be used in association with others characters, as molecular and morphological characters to a better understanding the category taxonomic of Tecun Umán pine. In this investigation the Mountain Pine Ridge provenance of Tecun Umán pine showed close to *P. oocarpa* following *P. patula* and less close to *Pinus caribaea* var. *hondurensis*. In this

investigation, the result makes us to propose the name *Pinus tecunumanii* for the Tecun Umán pine. However, we cannot forget the introgression and alogamy that is frequent in this genus and play an important role for understand the complexity of this classification.

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