Identification of polytene chromosomes of *Phaseolus coccineus* on the basis of centromeric heterochromatin morphology

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Introduction

Since the discovery of polytene chromosomes in the embryo suspensor of *Phaseolus coccineus* (Nagl 1962a) attempts were made to accurately describe each chromosome (Nagl 1962b, 1965). A preliminary idiogram based on total chromosome length and the distribution of euchromatic and heterochromatic segments was first presented by Nagl (1967). Later, Nagl (1974) pointed out, however, that the length can vary considerably depending on the level of endopolyploidy, the state of condensation and the degree of stretching during preparation. Therefore it was necessary to improve the description of the karyotype and to find a more reliable and constant feature in order to identify each chromosome of a given nucleus. We report here the first results of the identification of the polytene chromosomes of *P. coccineus* based on the analysis of centromeric heterochromatin morphology after pepsin pretreatment and DAPI staining.

Material and methods

Seeds of *Phaseolus coccineus* cv. Preisgewinner were grown in the Botanical Garden of the University of Kaiserslautern. Embryo suspensors were taken from seeds of unripe pods and preparations of polytene chromosomes were made from the biggest basal suspensor cells (Nagl 1996). After enzymatic maceration the nuclei were gently squeezed out from single cells to get rid of the cell wall. Additionally, the extracted nuclei were incubated in 45% acetic acid before squashing in order to dissolve the cytoplasm to some extent, and thus improving the spreading of chromosomes. After removing the cover slip and air drying, the preparations were pretreated in the same way as was carried out for fluorescence *in situ* hybridization (Nenno et al. 1994). Chromosomes were then stained with DAPI. Pictures from chromosomes of ten well preserved nuclei were taken by a cooled CCD camera at high resolution and analyzed by using an image analysis system (IPLab Spectrum).

Results and Discussion

The analysis of polytene chromosomes from the embryo suspensor of *P. coccineus* after pepsin pretreatment and DAPI banding made it possible to give a better description of individual chromosomes (Fig.1). The new key feature is the centromeric heterochromatin morphology (CHM). Two characteristics make the CHM useful for chromosome identification. Firstly, it is less sensible to variations in level of endopolyploidy and the degree of stretching during preparation. Secondly, the CHM of each of the 11 chromosome pairs can be individually identified by its pattern of at least one bright DAPI band as well as by the position of the centromere and the presence of secondary constrictions.

For the construction of an idiogram, traditionally, the total length and the ratio of the short and the long arms are relevant. In the polytene chromosomes of *P. coccineus* these parameters, however, vary significantly due to the state of condensation and the degree of stretching during preparation. Especially, the euchromatin behaves like a elastic band. For this reason, the arrangement of chromosomes labeled C-J in Fig. 1 is arbitrary, and the euchromatic parts are marked with dashed lines. Theses chromosomes are metacentric to submetacentric. Chromosome A is the longest and is telocentric. B is the next longest chromosome and the only one which is generally submetacentric. Chromosome K is the smallest of the complement and is acrocentric. Since the telomeres mostly appear as clusters of heterochromatin knobs they are symbolized by triple dots in Fig. 1.

Besides the new characterization of the chromosomes by the CHM there are two striking differences between the new idiogram and that previously proposed (Nagl 1967). The new idiogram shows three chromosome carrying a NOR (Fig. 1 A, C, K). This was also demonstrated by silver-staining (Schweizer and Ambros 1979) and by fluorescence *in situ* hybridization (Nenno et al. 1994). Furthermore, the existence of the formerly named 'SAT-chromosomes' 1 (S_1) and 5 (S_2) (Nagl 1965, 1967) could not be confirmed. Our present results indicate, that chromosome fragments sticking to the

nucleolar material were obviously misinterpreted as 'chromosome satellites'. These fragments seem to arise from the small centromeric heterochromatin block close to the NOR on the short arm of chromosome K, from which the long arm readily detaches (Fig.1).

The problem, however, that not all of the eleven pairs can be recognized equally well is not yet solved. Although, eight pairs of homologous can now be recognized quite easily, the identification of chromosome H, I, and J is more difficult. Nevertheless, the preliminary results presented here are encouraging and suggest that in a near future it will be possible to produce a new, high resolution karytotype for *Phaseolus coccineus*.

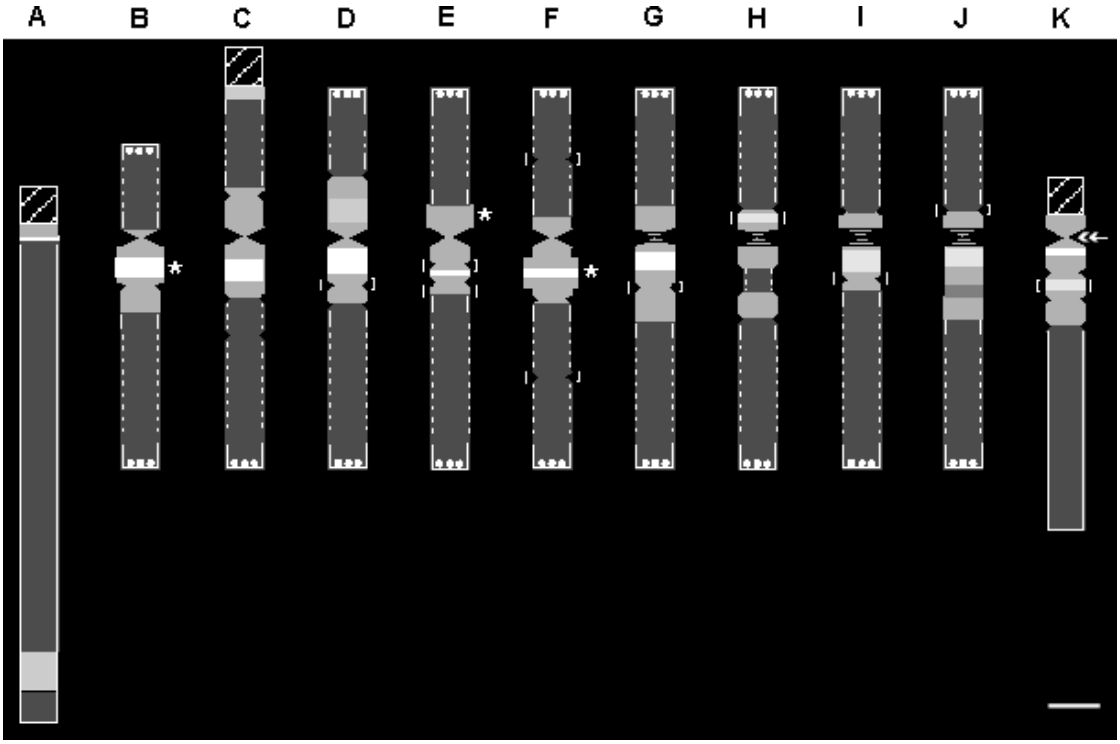

Figure 1: Idiogram of the polytene chromosomes of *Phaseolus coccineus* on the basis of centromeric heterochromatin morphology (CHM). *Hatched boxes*:NORs; *Triple dots*: Telomeric heterochromatin: *Horizontal hatching*: Centromeres which are not always visible; *Dark* : Euchromatin; Centromeric *Dark gray*: heterochromatin (CH); *Gray* or *white*: DAPI-bands within the CH, according to their relative staining intensity. *Square brackets*: structures which are not always present; *Double headed arrow*: the position in K, where the long arm detaches readily; *Asterisks*: regions which normally are wider than the rest of the chromosome. Bar represents 10 µm.

The idiogram and a complete karyotype for the polytene chromosomes is available in BeanRef (Nenno and Nagl 1996) on the WWW at the URL: http://www.ba.cnr.it/Beanref/karyop/

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