The genus *Zea* is divided into two sections, section *Zea* consisting of the four subspecies *Zea mays* L. ssp *mays*, *Zea mays* L. ssp *mexicana* (Schrad.) H.H. Iltis, *Zea mays* ssp *parviglumis* H.H. Iltis & J.F. Doebley and *Zea mays* ssp *huehuetenangensis* (H.H. Iltis & J.F. Doebley) J.F. Doebley and section *Luxuriantes* consisting of *Z. diploperennis* H.H. Iltis, *Z. perennis* (Hitch.) Reeves & Mangelsd., *Z. luxurians* (Durieu & Asch.) R.M. Bird and the recently discovered *Z. nicaraguensis* H.H. Iltis & B.F. Benz (Molina and Garcia 1999; Iltis and Benz 2000). The wild species of the genus *Zea* have the common name teosinte.

Both cultivated maize and the teosinte species are diploids (2n = 20) with a tetraploid origin (except for *Z. perennis* which is a tetraploid) with the basic chromosome number X = 10 (Molina and Garcia 1999). The chromosome number of *Z. nicaraguensis* has not been reported before. Today, this species is found at only two locations in Pacific Coastal Nicaragua, but was much more common and widespread just some 20 years ago. Young plants of *Z. nicaraguensis* are consumed by cattle while mature plants, which can grow as high as 5 m, are used to make fences and shelters. The species occurs at 6–15 m above sea level, and has the ability to grow in standing or slowly moving water. Because of this ability, due to a high capacity to form root aerenchyma and adventitious roots, it may be useful in wide hybridisations with maize, in order to improve maize growth in water-logged soils (Iltis and Benz 2000; Mano et al. 2006).

*Z. nicaraguensis* is to a large extent an unutilised genetic resource and its properties in terms of adaptation, disease resistance etc. have to be phenotypically and genetically characterized in order to determine its potential in maize improvement. Crosses between maize and *Z. nicaraguensis* have already been performed and hybrids have been obtained in an ongoing project of the authors. Mano et al. (2005) have in teosinte identified Quantitative Trait Loci (QTL) for adventitious root formation and efforts to transfer such QTLs from teosinte into maize are underway (Mano et al. 2006).

The closest relative to Nicaraguan teosinte, *Z. nicaraguensis*, is the Guatemalan teosinte, *Z. luxurians* (Doebley and Iltis 1980). The two species show close morphological similarity but the developmental behaviour differs considerably between the two, supporting a taxonomic segregation. Nicaraguan teosinte has longer and more abundant tassel branches, a larger number of spikelets per branch and a habitat differing from its Guatemalan counterpart (Iltis and Benz 2000).

The aims of this study was (1) to verify the chromosome number of *Z. nicaraguensis*, (2) to compare the C-banding pattern of *Z. nicaraguensis* with that of its close relative *Z. luxurians* as well as with *Z. diploperennis* and cultivated maize in order to see if similarities in banding pattern reflect their relationships.
Table 1. *Species authorities, accession name/number and origin of the material used in the study.*

| Species               | Accession name | Accession number | Origin    |
|-----------------------|----------------|------------------|-----------|
| *Zea nicaraguensis*   | Teosinte       | 4290             | Nicaragua |
| *Zea luxurians*       | Teo: Guate     | 9478             | Guatemala |
| *Zea diploperennis*   | Las Joyas      | 9476             | Mexico    |
| *Zea mays*, ssp. *mays* | Cultivar "Birko" |                 | France    |

**MATERIALS AND METHODS**

**Plant material**

Table 1 lists species authorities, accession name/number and origin of the plant materials used in the study. Seeds of *Z. nicaraguensis* were collected at two different locations in Nicaragua, both in the Chinandega department, in the lowlands of the Gulf of Fonseca. Seeds are preserved in the gene bank of the National Agrarian University in Managua, Nicaragua. Seeds from the other wild *Zea* species were obtained from the CIMMYT Gene Bank in El Batan, Mexico through the Nordic Gene Bank in Alnarp, Sweden. Seeds of the French maize cultivar “Birko” were obtained from Svalöf Weibull AB in Svalöv, Sweden.

**Chromosome preparation and C-banding**

Seeds were placed in water for two days and then put on moist filter paper at 20°C for two to three days. Roots were excised when they were around 10 mm long and placed in 0.02 M hydroxyquinolin for three hours, followed by ice water treatment for 21 h. After fixation in ethanol:acetic acid (3:1) the roots were kept in the freezer. Roots were also taken directly from plants in the greenhouse. Root tip chromosome squash preparations were made using the standard cellulase-pectinase enzyme digestion method (SCHWÄRZACHER and LEITCH 1994).

The C-banding procedure followed the method described by GILL et al. (1991) and good cells were photographed with a Leica CCD digital camera. Ten individuals of each species were examined. Chromosome length, chromosome arm ratio, length of large heterochromatic regions and position of regularly appearing thin heterochromatic bands (in *Zea* species the large regions are often referred to as knobs) were measured on one cell from each of three different individuals per species using the freeware computer program Micromeasure 3.3 (available at http://www.colostate.edu/depts/biology/micromeasure). These measurements were used together with visual examination of pictures from all ten individuals to construct karyograms for the four species. The presence or absence of centromeric heterochromatin was recorded for each chromosome arm but was not included when measuring heterochromatin content, as it usually was small in size and difficult to measure accurately.

**RESULTS AND DISCUSSION**

The measurements of the chromosomes of the four species are summarized in Tables 2 and 3 and can be seen in Fig. 2. The numbering of the chromosomes is following the C-banding pattern described by MOLINA (1982). The banding patterns among individuals within species were the same except for a few minor bands, which were not seen in all cells. This is probably more due to a more or less successful C-banding procedure than to actual differences. Fig. 1a–d show pictures of the chromosomes of the four species after C-banding.

The chromosomes of *Z. mays* are the smallest with an average length of 11.2 μm. The wild species have larger chromosomes with the largest in *Z. nicaraguensis* with an average length of 19.6 μm. The chromosome lengths are not in agreement with the per cent of heterochromatin found in the present study. Differences in chromosome contraction or differences in nuclear DNA content not related to heterochromatin could explain the observed differences in chromosome size. Nucleolus organizing regions (NOR) could be localized in the two species with the longer chromosomes, *Z. nicaraguensis* and *Z. luxurians* but not in the shorter chromosomes of *Z. diploperennis* and *Z. mays*. The average arm ratios are similar between the four species and individual chromosomes vary from 1.0 to 2.5 (Table 2).

In cultivated maize, *Zea mays* ssp. *mays*, the number of heterochromatic knobs has been found to vary in different maize populations and lines. The C-banding pattern hence differs between studies depending on material (HADLACZKY and KALMAN 1975; MOLINA 1982; TITO et al. 1991). However, in all cases, it has mostly subterminal knobs and only a few terminal ones, which makes it different from the wild teosinte species in section *Luxuriantes*. The maize cultivar used in this study has terminal knobs on both arms of chromosome 1, terminal knobs on one of the arms of chromosomes 5 and 7 and subterminal knobs on one of the arms of chromosomes 2, 4, 6, 8 and 9.
Table 2. Chromosome length, chromosome arm ratio and percent heterochromatin of the chromosomes of species *Z. nicaraguensis*, *Z. luxurians*, *Z. diploperennis* and *Z. mays* ssp. *mays*.

| Chrom. no. | Chromosome length (µm) | Long/short arm ratio | % Heterochromatin |
|------------|------------------------|----------------------|-------------------|
|            | Z. nic | Z. lux | Z. dipl | Z. mays | Z. nic | Z. lux | Z. dipl | Z. mays | Z. nic | Z. lux | Z. dipl | Z. mays |
| 1          | 24.0   | 20.5   | 13.9    | 11.0    | 1.3    | 1.3    | 1.3     | 1.3     | 22.9   | 31.4   | 33.3    | 41.6    |
| 2          | 21.3   | 18.3   | 12.7    | 10.7    | 1.0    | 1.3    | 1.1     | 1.6     | 24.7   | 23.3   | 25.9    | 23.1    |
| 3          | 22.0   | 17.5   | 12.7    | 13.8    | 1.3    | 1.6    | 1.6     | 1.2     | 19.7   | 23.3   | 0.0     | 0.0     |
| 4          | 13.8   | 13.6   | 9.7     | 9.7     | 2.3    | 1.9    | 1.5     | 2.5     | 20.1   | 23.9   | 20.1    | 26.2    |
| 5          | 21.3   | 18.3   | 13.3    | 11.9    | 1.1    | 1.3    | 1.2     | 1.6     | 12.1   | 17.9   | 11.4    | 22.3    |
| 6          | 17.1   | 15.7   | 11.3    | 11.9    | 2.4    | 2.3    | 1.7     | 1.4     | 17.9   | 20.2   | 19.5    | 20.0    |
| 7          | 18.2   | 15.7   | 14.3    | 10.5    | 1.3    | 1.3    | 1.1     | 1.1     | 16.3   | 21.5   | 2.2     | 19.5    |
| 8          | 17.3   | 16.4   | 13.6    | 11.7    | 1.2    | 1.1    | 1.0     | 1.4     | 17.3   | 21.5   | 16.3    | 22.2    |
| 9          | 25.3   | 20.9   | 19.5    | 11.7    | 1.4    | 1.1    | 1.2     | 1.4     | 11.2   | 8.6    | 9.9     | 21.7    |
| 10         | 16.0   | 18.1   | 10.4    | 9.3     | 1.1    | 1.3    | 1.3     | 1.0     | 2.0    | 17.0   | 2.0     | 2.0     |
| Average:   | 19.6   | 17.5   | 13.1    | 11.2    | 1.4    | 1.5    | 1.3     | 1.5     | 16.4   | 20.9   | 14.1    | 19.9    |

Table 3. Location of heterochromatic knobs on the chromosomes of the four species of *Zea*.

| Chromosome no. | *Z. nicaraguensis* | *Z. luxurians* | *Z. diploperennis* | *Z. mays* |
|----------------|--------------------|----------------|--------------------|-----------|
| 1              | Long arm and short arm terminal | Long arm and short arm terminal | Long arm and short arm terminal | Long arm and short arm terminal |
| 2              | Long arm and short arm terminal | Long arm and short arm terminal | Long arm and short arm terminal | Long arm subterminal |
| 3              | Long arm and short arm terminal | Long arm and short arm terminal | Long arm and short arm terminal | Long arm subterminal |
| 4              | Long arm terminal | Long arm terminal | Long arm terminal | Long arm subterminal |
| 5              | Long arm terminal | Long arm terminal | Short arm terminal | Long arm subterminal |
| 6              | Long arm terminal | Long arm terminal | Long arm terminal | Long arm subterminal |
| 7              | Long arm terminal | Long arm terminal | Long arm terminal | Long arm terminal |
| 8              | Long arm terminal | Long arm terminal | Short arm terminal | Long arm subterminal |
| 9              | Long arm terminal | Long arm terminal | Long arm terminal | Long arm subterminal |
| 10             | Long arm terminal | Long arm terminal | Long arm terminal | Long arm subterminal |
Chromosomes 3 and 10 are lacking heterochromatic knobs. Chromosomes 1, 2, 3, 4, 6 and 9 have centromeric heterochromatin and chromosomes 5, 7 and 10 regularly show a thin intercalary heterochromatic band (Fig. 2a, Table 3). The amount of heterochromatin in a chromosome set, measured as a mean value from the three individuals, is 19.9% (Table 2).

Also in Z. diploperennis, the size and position of heterochromatic knobs have been reported to differ between populations (Kato and Lopez 1990). It has, however, only terminal heterochromatic knobs making it different from cultivated maize. In the population used in this study chromosomes 1 and 2 have terminal knobs on both arms, chromosomes 4, 5, 6, 8 and 9 have terminal knobs on one of the chromosome arms and chromosomes 3, 7 and 10 are lacking heterochromatic knobs. Chromosomes 2, 3 and 4 show centromeric heterochromatin and chromosomes 1, 7, 8 and 10 regularly show a thin intercalary heterochromatic band (Fig. 2b, Table 3). The C-banding pattern is very similar to the one reported by Molina (1982). The amount of heterochromatin in the chromosome set is lower than in cultivated maize, only 14.0% (Table 2).

Zea luxurians has only terminal heterochromatic knobs. Chromosomes 1, 2 and 3 have terminal knobs at both ends while the remaining chromosomes have terminal knobs on one of the arms. Chromosomes 1, 2, 3, 4, 6, 8 and 9 have centromeric heterochromatin and chromosomes 1, 5, 6, 7 and 10 regularly show a thin intercalary heterochromatic band (Fig. 2c, Table 3). There is no pair lacking heterochromatic knobs and the mean amount of heterochromatin in a chromosome set is as high as 20.9% (Table 2). Tito et al. (1991) reported about a relationship between the number of C-bands and the DNA content where Z. luxurians showed a higher DNA content and more C-bands than both Z. diploperennis and Z. mays. Also

Fig. 1. 1a–d. Photos of the four Zea species after C-banding. (a) Z. mays ssp. mays, (b) Z. diploperennis, (c) Z. luxurians, (d) Z. nicaraguensis. Scale bar represents 10 \( \mu \)m.
in this study, the number of C-bands on the chromosomes is higher in *Z. luxurians* (13 terminal knobs) than in *Z. diploperennis* (9 terminal knobs) and *Z. mays* (9 terminal or subterminal knobs).

*Zea nicaraguensis*, like most other *Zea* species, is a diploid with 2n = 20 chromosomes. It has a C-banding pattern similar to *Z. luxurians*, and the two species are therefore probably closely related. Chromosomes 1, 2 and 3 have terminal knobs on both arms, chromosomes 4-9 have terminal knobs on one of the arms while chromosome 10 is lacking heterochromatic knobs. Chromosomes 1, 2, 3, 4, 6 and 9 have centromeric heterochromatin and chromosomes 1, 5, 7 and 10 regularly show a thin intercalary heterochromatic band (Fig. 2d, Table 3). The biggest difference compared to *Z. luxurians* is that chromosome 10 is knobless and this is the case in all 10 individuals studied. This leads to a lower heterochromatin content compared to *Z. luxurians*, a mean value of 16.4% (Table 2).

From the C-banding pattern it is clear that *Z. nicaraguensis* is more similar to *Z. luxurians* than to *Z. diploperennis* and cultivated maize. The similarity in C-banding pattern most likely reflects a closer relationship between the two species. The small differences in banding pattern that exist between them neither contradict nor support regarding them as subspecies as the C-banding pattern can vary considerably even between populations of the same species (as in maize and *Z. diploperennis*). Whether they should be regarded as subspecies or separate species is thus not possible to conclude from this study.

**REFERENCES**

Doebley, J. F. and Iltis, H. H. 1980. The taxonomy of *Zea* (Gramineae). I. Subgeneric classification with key to taxa. – Am. J. Bot. 67: 982–993.

Gill, B. S., Friebe, B. and Endo, T. R. 1991. Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). – Genome 34: 830–839.

Hadlaczky, G. Y. and Kálmán, L. 1975. Discrimination of homologous chromosomes of maize with Giemsa staining. – Heredity 35: 371–374.

Iltis, H. H. and Benz, B. F. 2000. *Zea nicaraguensis* (Poaceae), a new teosinte from Pacific coastal Nicaragua. – Novon 10: 382–390.

Kato, T. A. and Lopez, R. 1990. Chromosome knobs of the perennial teosinte. – Maydica 35: 125–141.

Mano, Y., Muraki, M., Fujimori, M. et al. 2005. Identification of QTL controlling adventitious root formation during flooding conditions in teosinte (*Zea mays ssp. huehuetenangensis*) seedlings. – Euphytica 142: 33–42.

Mano, Y., Omori, F., Takamizo, T. et al. 2006. Variation for root aerenchyma formation in flooded and non-flooded maize and teosinte seedlings. – Plant Soil 281: 269–279.

Molina, M. 1982. Cytogenetic studies on *Zea diploperennis*. – Nucleus 26: 1–7.
Molina, M. and Garcia, M. D. 1999. Influence of ploidy level on phenotypic and cytogenetic traits in maize and *Zea perennis* hybrids. – Cytologia 64: 101–109.

Schwarzacher, T. and Leitch, A. 1994. Enzymatic treatment of plant material to spread chromosomes for in situ hybridisation. – In: Isaac, P.G. (ed.), Methods in molecular biology 28. Protocols for nucleic acid analysis by nonradioactive probes. Totowa, Humana Press Inc, p. 153–160.

Tito, C. M., Poggio, L. and Naranjo, C. A. 1991. Cytogenetic studies in the genus *Zea*. 3. DNA content and heterochromatin in species and hybrids. – Theor. Appl. Genet. 83: 58–64.