Investigations on some Gramineae from Albania and Greece (Chromosome numbers and endophyte infection)

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Investigations on some *Gramineae* from Albania and Greece
(Chromosome numbers and endophyte infection)

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1. INTRODUCTION

In the last few years several field trips have been made to the Balkans, especially to Greece and Jugoslavia, in the course of biosystematic investigations on *Ranunculus* (BALTISBERGER 1980, 1981, BALTISBERGER and MÜLLER 1981, MÜLLER and BALTISBERGER 1984, BALTISBERGER and HESS 1986, HUBER 1988), *Betonica* (BALTISBERGER 1989) and *Stachys* (LENHERR 1983, BALTISBERGER and LENHERR 1984a, LENHERR and BALTISBERGER 1984). In 1982 (see BALTISBERGER and LENHERR 1984b, with map) and 1989 (see BALTISBERGER 1991, with map) two collecting trips to Albania were undertaken, as well. Many additional plant species collected during these trips and not related to the main study groups have been investigated cytologically and chromosome numbers documented (e.g. BALTISBERGER 1984, 1987, 1988a,b, 1990, BALTISBERGER and AESCHIMANN 1988, BALTISBERGER and LIPPERT 1987).
Recently at our institute, interest has also focused on fungal endophytes of *Gramineae*. Many grasses are known to harbor asymptomatic, seed-borne endophytes classified in the form genus *Acremonium* sect. *Albo-lanosa,* which are asexual forms of the related Ascomycete fungus *Epichloë typhina* (Pers.:Fr.) Tul. (CLAY 1989, CLAY and LEUCHTMANN 1989). Research has indicated that in some cases the association between grasses and endophytes is mutualistic in nature (SIEGEL et al. 1987, CLAY 1988). Grass endophytes are

of applied significance because they produce biologically active alkaloids causing toxic syndromes in cattle and providing increased resistance of host grasses to pests (BACON et al. 1986, CLAY 1988). The information on distribution and frequency of infection in wild grasses is still fragmentary except for some economically important species of pastures.

In this paper, chromosome numbers of some *Gramineae* collected as seed samples during the excursions to Albania and Greece in 1989 and 1990 are presented. In addition, endophyte infection of seeds and plants growing from these seeds were studied. The plants have been identified with "Flora Europaea" (TUTIN et al. 1980). The nomenclature used here follows "Flora Europaea", too. The investigated species are arranged in alphabetical order. The name and the chromosome number is followed by site, date of seed collection, collectors and the herbarium specimen number. The voucher number of cultivated plants is given in parenthesis. All herbarium specimens are deposited in the herbarium of the Geobotanical Institute ETH in Zürich (ZT).

2. METHODS

2.1. CHROMOSOME NUMBERS

The chromosome numbers were determined in root tips which had been pretreated for 1/2 hour (*Brachypodium silvaticum, Tragus racemosus*), respectively 2 hours (other species) with colchicine (0.05%), then fixed in ethanol/acetic acid (3:1) and squashed in lacto-propionic orcein (DYER 1963). At least 5 individuals of each species were investigated (see Table 1), and of each individual 5–10 metaphases were scored.

2.2. ENDOPHYTE INFECTION

Endophyte infection was surveyed by isolation of viable endophytes from seeds and by microscopical examination of tissues of plants grown from infected seeds. Thirty seeds bulk collected of several plants of each population were surface sterilized by soaking in 50% sulfuric acid for 20 min., rinsing in sterile water, immersing in 4.5% Na-hypochlorite for 20 min., transferring to 95% EtOH, and rinsing again in sterile water (LEUCHTMANN and CLAY 1990). Seeds were then plated on cornmeal-malt extract-agar (17 g cornmeal agar, 20 g malt extract, 2 g yeast extract, 1 liter distilled water) supplemented with

Tab. 1. Alphabetical list of the investigated species, with chromosome number and indication of infection rate by seed-born *Acremonium* endophytes.

Species	Voucher number	Plants investigated	2n	Infection rate
Aegilops geniculata	12081	9	28	0/30
Aegilops lorentii	12083	9	28	0/30
Aegilops neglecta	12082	13	42	0/10
Aegilops triuncialis	12085	15	28	0/10
Brachypodium distachyon	12066	5	30	0/30
Brachypodium silvaticum	12122	5	18	30/30
Bromus squarrosus	12069	7	14	0/30
	12072	9	14	0/30
Cynosurus echinatus	12079	9	14	0/30
	12363	9	14	0/10
Elymus farctus	11972	6	42	0/30
Festucopsis serpentini	11902	7	14	0/30
Melica ciliata	12170	9	18	30/30
	12171	9	18	30/30
	12364	9	18	17/30
	12365	9	18(36)	10/10
	12366	9	18	10/10
	12367	9	18	30/30
Melica transsilvanica	12121	9	18	30/30
Tragus racemosus	11974	7	40	0/10

Terramycin (50 mg/liter of medium), and infection rate calculated based on the number of seeds with outgrowing mycelia of *Acremonium*. In two species of *Aegilops*, one population of *Cynosurus echinatus*, two populations of *Melica ciliata* and in *Tragus racemosus*, endophyte infection was determined only microscopically in 10 individuals each of greenhouse grown plants by staining and checking for the typical fungal hyphae occurring in the leaf sheaths (CLARK et al. 1983).

3. RESULTS AND DISCUSSION

3.1. Cytology

Aegilops geniculata Roth **2n=28**
Kalidhet e vllejve near Borova, on the road from Erseka to Leskoviku, district Kolonja, Albania; 1000 m; 4.8.1989; leg. E. and M. Baltisberger; (cult. no. 12081).

The tetraploid number of 2n=4x=28 confirms indications in literature (PETROVA in PROKUDIN et al. 1977, DEVESA and ROMERO 1981, LÖVE 1984). According to TUTIN and HUMPHRIES (1980) *A. ovata* L. (pro parte) is a synonym of *A. geniculata*. Therefore some of the numerous indications in literature of 2n=28 for *A. ovata* also refer to *A. geniculata*.

Aegilops lorentii Hochst. **2n=28**
Kalidhet e vllejve near Borova, on the road from Erseka to Leskoviku, district Kolonja, Albania; 1000 m; 4.8.1989; leg. E. and M. Baltisberger; (cult. no. 12083).

With 2n=28 the plants from Albania are tetraploid. This corresponds with the indication of LÖVE (1984), but diploid plants with 2n=14 chromosomes can occurr as well (STRID and FRANZEN in LÖVE 1981).

Aegilops neglecta Req. ex. Betol. (*A. triaristata* Wild.) **2n=42**
Kalidhet e vllejve near Borova, on the road from Erseka to Leskoviku, district Kolonja, Albania; 1000 m; 4.8.1989; leg. E. and M. Baltisberger; (cult. no. 12082).

Two ploidy levels are known for this species, tetraploid with 2n=28 and hexaploid with 2n=42 (see MOORE 1973, FEDEROV 1974, GOLDBLATT 1981, 1985, 1988). With 2n=6x=42 chromosomes, the plants from Albania are hexaploid.

Aegilops triuncialis L. **2n=28**
Rocky slope, Mali i Melenisit, W of Leskoviku, district Kolonja, Albania; 900-1000 m; 4.8.1989; leg. E. and M. Baltisberger; (cult. no. 12085).

Most of the indications in literature (see MOORE 1973, 1974, FEDEROV 1974, GOLDBLATT 1981, 1984, 1985, 1988) correspond with the number 2n=4x=28 found in the Albanian plants.

Brachypodium distachyon (L.) Beauv. (=*Trachymia distachya* [L.] Link) **2n=30**
Rocky roadside on the road from Elbasani to Librazhdi, 16 km after Elbasani, district Librazhdi, Albania; 220 m; 2.8.1989; leg. E. and M. Baltisberger; (cult. no. 12066).

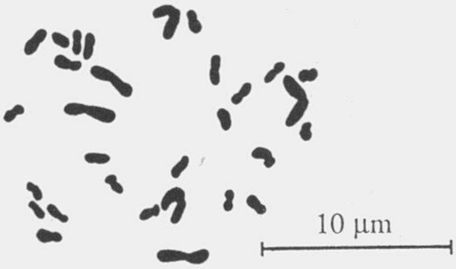

Fig. 1. Somatic metaphase of *Brachypodium distachyon*.

In *B. distachyon*, different chromosome numbers are known: 2n=10, 20, 28, 30 (see MOORE 1973, 1974, 1977, FEDEROV 1974, GOLDBLATT 1981, 1984, VAN LOON 1987). All plants from Albania, however, showed 2n=30 chromosomes with 24 small and 6 larger chromosomes (Fig. 1) as indicated by FERNANDES and QUEIROS (1969).

Brachypodium silvaticum (Hudson) Beauv. **2n=18**
Ancient Butrint, 15 km S of Sarandë, district Sarandë, Albania; 5-30 m; 7.8.1989; leg. E. and M. Baltisberger; (cult. no. 12122).

The chromosome number 2n=18 corresponds with the numerous indications in literature (see MOORE 1973, 1974, 1977, FEDEROV 1974, GOLDBLATT 1981, 1984, 1985, 1988, MOORE 1982, VAN LOON 1987).

Bromus squarrosus L. **2n=14**
- Rocky place near the river Cem, on the road from Shkodër to Vermosh, 40 km N of Shkodër, district Shkodër, Albania; 700 m; 13.8.1989; leg. E. and M. Baltisberger; (cult. no. 12069).
- Kalidhet e vllejve near Borova, on the road from Erseka to Leskoviku, district Kolonja, Albania; 1000 m; 4.8.1989; leg. E. and M. Baltisberger; (cult. no. 12072).

The chromosome number 2n=14 found in all plants from the two sites confirms earlier indications in literature (see MOORE 1973, FEDEROV 1974, GOLDBLATT 1981, 1984, 1985, 1988, VAN LOON 1987, GOLDBLATT and JOHNSON 1990).

***Cynosurus echinatus* 2n=14**
- Rocky slope, Mali i Melesinit, W of Leskoviku, district Kolonja, Albania; 900-1000 m; 4.8.1989; leg. E. and M. Baltisberger; (cult. no. 12079).
- Roadside in Delphi, district Fokis, Greece; 550 m; 15.9.1989; leg. M. Baltisberger; (cult. no. 12363).

All plants of both sites revealed the chromosome number 2n=14. These correspond with numerous indications in literature (see MOORE 1973, 1977, FEDEROV 1974, GOLDBLATT 1981, 1984, VAN LOON 1987).

Elymus farctus (Viv.) Runemarke ex. Melderts (=*Agropyron junceum* [L.] Beauv.) **2n=42**
Sand-dunes near Golem, 10 km SE of Durrësi, district Durrësi, Albania; 0-2 m; 15.8.1989; leg. E. and M. Baltisberger; no. 11972 (cult. no. 12350).

E. farctus s.l. is a polyploid complex of races ranging from diploid (2n=2x=14) to dodecaploid (2n=12x=84) with the following known numbers: 2n=14, 28, 35, 42, 49, 56, 84 (see FEDEROV 1974, MOORE 1982, GOLDBLATT and JOHNSON 1990). Since chromosome numbers are not always correlated with morphological characters, the taxa within *E. farctus* s.l. are treated as subspecies (MELDERIS 1980). The Albanian plants belong to *E. farctus* subsp. *farctus* and are hexaploid (2n=6x=42).

Festucopsis serpentini (C.E. Hubbard) Melderis **2n=14**
Rocks on the road from Elbasani to Librazhdi, 22 km after Elbasani, district Librazhdi, Albania; 250 m; 2.8.1989; leg. E. and M. Baltisberger; no. 11902.

This species is endemic to southern Albania. The chromosome number of 2n=14 (Fig. 2) is in agreement with the indication by JONES (in DARLINGTON and WYLIE 1955). This is the same chromosome number as found for the other European species of *Festucopsis*, *F. sancta* (Janka) Melderis (STRID and FRANZEN in LÖVE 1983, LÖVE 1984; in KOZUHAROV and PETROVA [in LÖVE 1973] there is no chromosome number of *Festucopsis* [as indicated in VAN LOON 1987]).

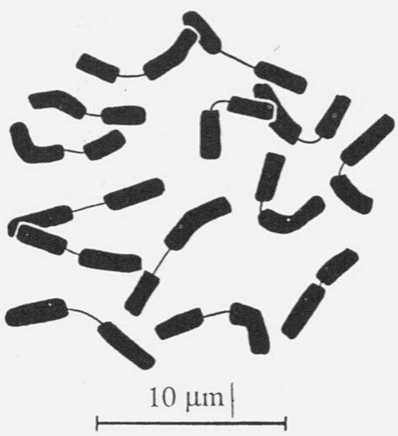

Fig. 2. Somatic metaphase of *Festucopsis serpentini*.

The karyotype consists of 14 meta- to submetacentric chromosomes (nomenclature of chromosome morphology according to LEVAN et al. 1964), and the chromosomes are rather large (more than 4.5 µm). Consistent differences in chromosome number and size in species of *Festucopsis* compared to *Brachypodium* (e.g. *B. distachyon* 2n=30, *B. silvaticum* 2n=18, both with very small chromosomes) confirm the validity of the recently established segregation of *Festucopsis* from *Brachypodium*, based on morphological characters (MELDERIS 1978).

Melica ciliata **L. 2n=18 (36)**

- Rocky place near the river Cem, on the road from Shkodër to Vermosh, 40 km N of Shkodër, district Shkodër, Albania; 700 m; 13.8.1989; leg. E. and M. Baltisberger; (cult. no. 12364).
- Rocky roadside, on the road from Elbasani to Librazhdi, 16 km after Elbasani, district Librazhdi, Albania; 220 m; 2.8.1989; leg. E. and M. Baltisberger; (cult. no. 12367).
- Rocky slope near Spilios Agapitos, mount Olympus, district Pieria, Greece; 2000-2100 m; 18.9.1989; leg. M. Baltisberger; (cult. no 12170).
- Rocky slope near Kataphygion E of Paleokastron, Vourinos, 20 km NE of Grevena, district Grevena, Greece; 1350-1550 m; 9.8.1990; leg. M. Baltisberger and U. Schäppi; (cult. no. 12365).
- Rocks, mount Vounassa, NNW of Deskati, 35 km ESE of Grevena, district Grevena, Greece; 1500-1600 m, 9.8.1990; leg. M. Baltisberger and U. Schäppi; (cult. no. 12366).
- Rocky roadside, on the road from Arachova to mount Parnassos, ENE of Delphi, district Voiotia, Greece; 1150 m; 13.9.1989; leg. M. Baltisberger; (cult. no. 12171).

In all 6 populations, the chromosome number was 2n=18, confirming the very numerous indications by other authors (see MOORE 1973, 1974, 1977, FEDEROV 1974, GOLDBLATT 1981, 1984, 1985, 1988, VAN LOON 1987). In some metaphases of plants from Vourinos, Greece (cult. no. 12365), 36 chromosomes occurred which indicates endemitosis frequently occuring in plants at this site.

Melica transsilvanica **2n=18**

Rocks near the coast, S of Sarandë, district Sarandë, Albania; 10-30 m; 7.8.1989; leg. E. and M. Baltisberger; (cult. no. 12121).

The chromosome number 2n=18 corresponds with the indications in literature (see MOORE 1973, 1977, FEDEROV 1974, GOLDBLATT 1981, 1984, 1985, 1988).

Tragus racemosus **(L.) All. 2n=40**

Sand-dunes near Golem, 10 km SE of Durrësi, district Durrësi, Albania; 0-2 m; 15.8.1989; leg. E. and M. Baltisberger; no. 11974 (cult. no. 12190).

The chromosome number 2n=40 confirms the numerous indications in the literature (see FEDEROV 1974, MOORE 1977, GOLDBLATT 1981, 1984, 1988, VAN LOON 1987).

3.2. ENDOPHYTE INFECTION

Among the 13 species investigated in this study, three species (*Brachypodium silvaticum* and two species of *Melica*) were found to be infected by seed-born *Acremonium* endophytes (Table 1). The samples examined of the other species showed no sign of infection, neither in seeds nor in the plant tissues. In *M. ciliata* five populations were infected with an estimated infection rate of 100 % and a sixth population from Albania (no. 12364) with a rate of 56 %. The single populations of *M. transsilvanica* and *B. silvaticum*, respectively, were infected by a rate of 100%.

Both *Melica* species have previously been reported as hosts of symptomless *Acremonium* endophytes (WHITE 1987), and infection was always present in samples of these species from several locations in Switzerland (LEUCHTMANN, pers. observation). Thus, the endophytes seem to be obligately asssociated with *M. ciliata* and *M. transsilvanica*. The sexual stage of *Acremonium*, the chockeinducing form *Epichloë*, however, is not known in these species, but is documented in another *Melica* from North America, *M. bulbosa* (SPRAGUE 1950).

B. silvaticum is already known as host to *Epichloë typhina* (Pers.:Fr.) Tul. (KOHLMEYER and KOHLMEYER 1974), but not to symptomless *Acremonium* as reported here. At several sites in Switzerland *B. silvaticum* was also observed to be infected by *Epichloë*. However, only a few plants exhibited stromata of *Epichloë*, while virtually all plants of a population were actually infected (LEUCHTMANN, unpubl.). This represents a pattern known of several other host species in North America as well (CLAY and LEUCHTMANN 1989). The mechanisms and factors inducing stroma formation are not well understood, but there seems to be a strong genetic component of the individual fungal strain determining whether stromata are formed or not.

To our knowledge, the ten species which turned out to be endophyte free in our samples have never been reported to be hosts to grass endophytes. However, other species of *Bromus* and *Elymus*, not examined here, are common hosts of *Epichloë* and *Acremonium* endophytes (KOHLMEYER and KOHLMEYER 1974; CLAY and LEUCHTMANN 1989; LEUCHTMANN, pers. observ.). There is a tendency for *Epichloë* and its *Acremonium* anamorphs to infect primarliy cool-season grasses of the subfamily *Pooideae*, where all the investigated genera belong (with the exception of *Tragus*, placed in the subfamily *Chloridoideae*) (CLAY1989).

The consistantly high frequency of endophyte infection in *Brachypodium silvaticum* and species of *Melica* raises the question whether the plants benefit from being infected. In strictly seed-borne endophytes, theory predicts that such endophytes should not be able to persist in host populations without increasing the fitness of their hosts (CLAY 1991). Therefore, it is likly that the relationship of these three host species with their endophytes is mutualistic. In several other plant species experiments have shown that plant growth and seed production can be increased by infection, in addition, endophytes are known to produce alkaloids in the tissues of their hosts, providing protection against herbivores (for a review see CLAY 1988). *Melica decumbens* Thunb. known as "dronk gras" in South Africa exhibits strong toxic qualities when infected, causing staggers condition in cattle feeding on it (MEREDITH 1955). In other infected grasses, enhanced resistance to insect herbivores has been demonstrated (CLAY 1988; CHEPLICK and CLAY 1988). These factors may provide the basis for mutualistic symbiosis between endophyte and plant.

SUMMARY

The chromosome numbers of 13 species (20 sites) of *Gramineae* from Albania and Greece were investigated. Additionally, infection by seed-born *Acremonium* endophytes were examined, revealing 3 of the 13 species being infected.

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