M. and STANDARDI, A.: Estimating alfalfa somaclonal variation in axillary branching propagation and indirect somatic embryogenesis by RAPD fingerprinting. Int. J. Plant Sci. **158**: 556–562 (1997). — RANI, V., PARIDA, A. and RAINA, S. N.: Random amplified polymorphic DNA (RAPD) markers for genetic analysis in micropropagated plants of *Populus deltoides* MARSH. Plant Cell Rep. **14**: 459–462 (1995). — RUSKORTEKAAS, W., SMULDERS, M. J. M., ARENS, P. and VOSMAN, B.: Direct comparison of levels of genetic-variation in tomato detected by a GACA-containing microsatellite probe and by random amplified polymorphic DNA. Genome **37**: 375–381 (1994). — SABIR, A., NEWBURY, H. J., TODD, G., CATTY, J. and FORD-LLOYD, B. V.: Determination of genetic stability using isozymes and RFLPs in beet plants regenerated in vitro. Theor. Appl. Genet. **84**: 113–117 (1992). — SELBY, C. and COLLIN, H. A.: Clonal variation in growth and flavour production in tissue cultures of *Allium cepa* L. Ann. Bot. **40**: 911–918 (1976). — TIBOK, A., BLACKHALL, N. W., POWER, J. B. and DAVKEY, M. R.: Optimized plant regeneration from callus derived from seedling hypocotyls of *Eucalyptus urophylla.* Plant Sci. **110**: 139–145 (1995). — XAVIER, A., COMÉRIO, J. and LANNELLI, C. M.: Eficiência da estaquia, da microestaquia e da micropropagação na clonagem de *Eucalyptus* spp. In: IUFRO Conference on Silviculture and Improvement of Eucalypts. v.2. EMBRAPA-CNPF Anais. Salvador. pp. 24–29 (1997).

Contribution to the Karyomorphology of Some Species of the Genus *Quercus*

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Summary

In order to contribute to the knowledge of the taxonomic relationships between some species of the *Quercus* genus, the karyomorphology of 4 species (*Quercus ilex* L., *Q. suber* L., *Q. robur* L. and *Q. pubescens* WILLD.) was carried out. The somatic chromosome number 2n = 24 was found in all taxa examined. An evident differentiation in karyotype structure of *Q. ilex* compared to other previous studied species has been found. *Quercus suber*, *Q. robur* and *Q. pubescens* possess moderately asymmetrical karyotypes. *Q. suber* differs from other species of the subgenus *Cerris* on the basis of karyological data showing similarities with *Q. robur* and *Q. pubescens.*

Key words: karyotype, *Quercus ilex*, *Q. pubescens*, *Q. robur*, *Q. suber*.

Introduction

The genus *Quercus* including about 300 species with many varieties and natural hybrids widespread in many regions of the world is one of the most complex taxonomic groups. The systematic relationships in this genus are still not completely clear (NIXON, 1993) although in the literature many data concerning morphological information (SPELLENBERG, 1992; BACILIERI et al., 1995), chemo-taxonomical characters (KNOPS and JENSEN, 1980), enzymes (GUTTMANN and WEIGT, 1989) and DNA (BODENES *et al.,* 1997) studies, ribosomal RNA genes (BELLAROSA *et al.,* 1990) and molecular cytogenetic analysis are available (ZOLDOS *et al.,* 1999). Nevertheless, data on oak chromosomes are considered still insufficient (OHRI and AHUJA, 1990; ZOLDOS *et al,* 1999). D'EMERICO *et al.* (1995) report some data about the karyomorphologies of 8 species of *Quercus* pointing out morphological similarities but also differences in intrachromosomal and interchromosomal asymmetry indices.

In this report, in order to contribute to the knowledge of the relationships between the species belonging to the genus *Quercus,* we have continued the karyological investigation (D'EMERICO *et al.,* 1995), with traditional Feulgen methodology, to describe the karyotype of *Quercus ilex, Q. pubescens, Q. robur, Q. suber.*

Materials and Methods

Acorns of *Quercus ilex* were collected at various sites in Apulia; those of *Q. suber*, *Q. pubescens* and *Q. robur* in Latium (Italy).

For cytological examination of somatic chromosomes, actively growing root tips were pre-treated in a 0.3% colchicine at 20 °C for 2 h, and then fixed for 5 min in a 5 : 1 : 1 : 1 (volume ratio) mixture of absolute ethanol, chloroform, glacial acetic acid, and formalin (BATTAGLIA, 1957a). Hydrolysis was carried out at 20 °C in 5.5 N HCl for 20 min (BATTAGLIA, 1957b), and then stained with SCHIFF's reagent. Squashes were made in a drop of 45% acetic acid. Chromosomes of 5 to 6 plates were measured in 3 trees of each species.

The nomenclature used for describing karyotype composition followed LEVAN *et al.* (1964). Karyotype morphometric characters were evaluated by calculating haploid complement length and the indices: SYi, which describes the average symmetry of the karyotype (GREILHUBER and SPETA, 1976), A_1, which represents the degree of asymmetry in arm length within the chromosome of the genome, and A_2, expressing the variation in chromosome lengths (ROMERO ZARCO, 1986), as used in D'EMERICO *et al.* (1996).

Results

Karyotype morphometric characters, haploid complement and karyotype formulae are given in *table 1.*

The somatic chromosome number 2n = 24 was found in all taxa examined (D'EMERICO *et al.*, 1995; and references therein).

Q. robur possesses a moderately asymmetrical karyotype, consisting of 14 metacentric and 10 submetacentric chromosomes. Two chromosome pairs (1 and 3) bear evident secondary constrictions on the short arms; pair 7 presents a secondary constriction on the long arm; pair 11 has a satellite on the long arm (*Fig. 1a*).

The detailed karyotype morphology of *Q. pubescens* is reported. The karyotype of this species consists of 18 metacentric and

6 submetacentric chromosomes. Pair 1 has a secondary constriction on the short arm; whereas pairs 6 and 11 showed secondary constrictions located on the long arm (*Fig. 1b*).

The karyotype of *Q. ilex* is described here for the first time and consists of 18 metacentric, 4 submetacentric and 2 subtelocentric chromosomes. Pair 1 shows a secondary constriction on the long arm; pair 2 bears a satellite on the short arm (*Fig. 1c*).

Quercus suber shows a karyotype characterized by 14 metacentric and 10 submetacentric chromosomes. Pair 1 bears an evident satellite on the short arm (*Fig. 1d*).

Figure 1. – Somatic karyotypes of a) *Quercus robur;* b) *Q. pubescens;* c) *Q. ilex;* d) *Q. suber.* Bar: 5 µm.

Discussion

The genus *Quercus* is characterized by complex patterns of genetic change at both the intra- and interspecific levels. Our data clearly established that the basic chromosome number of $x = 12$ is fairly stable in *Quercus* genus. However, BUTORINA (1993) recognised two triploid specimens of *Q. robur* with chromosome number $2n = 3x = 36$. Chromosomes are small in this group as a whole. The absence of chromosome differentiation among different oaks is reported by ZOLDOS *et al.* (1999), who suggest that different species possess a common karyotype. Therefore, *Quercus* species are very different in many morphological, physiological and ecological aspects. In this contest, karyomorphological studies with traditional methodology might bring new knowledge to clarify the *Quercus* genus taxonomy.

Quercus ilex shows a karyomorphology similar to *Q. coccifera* with an identical metacentric number (D'EMERICO *et al.,* 1995), but with higher values in the intrachromosomal asymmetry index and the presence of one subtelocentric chromosome pair. These data indicate an evident differentiation in karyotype structure between *Q. ilex* and the other previously studied species (D'EMERICO *et al.,* 1995) or reported in the present work.

The species *Q. suber* shows the largest intrachromosomal asymmetry (A_1) (*Table 1*); a higher value than the other species mentioned (D'EMERICO *et al.,* 1995), but similar to *Q. robur* and *Q. pubescens.* However, our karyological data seem to be in agreement with previous flow cytometry analyses. In fact, ZOLDOS *et al.* (1998) have found that *Q. suber* has a genome size similar to *Q. robur* and *Q. pubescens.* In this connection, the karyotype structures of *Q. robur* and *Q. pubescens*, belonging to subg. *Quercus,* as described by numerical parameters (*Table 1*) exactly resemble those of *Q. virgiliana* (D'EMERICO *et al.,* 1995) showing the highest degree of intrachromosomal asymmetry (A_1).

According to SCHWARTZ (1964), *Quercus ilex* and *Q. coccifera* are in a different subgenus (*Sclerophyllodrys* O. SCHWARTZ) respect to *Q. trojana*, *Q. suber*, *Q. cerris* and *Q. macrolepis*, which are all attributed to the subgenus *Cerris* (Spach) Örsted and to *Q. robur* and *Q. pubescens*, attributed to the subg. *Quercus.* Our data support the classification of SCHWARZ (1964), but also suggest a divergence on the base of karyological data between *Q. ilex* and *Q. coccifera*, species showing evident differences in ecology and biology. In this contest, we also support divergence of *Q. suber* from other species of the subgenus *Cerris.*

References

BACILIERI, R., DUCOUSSO, A. and KREMER, A.: Genetic, morphological, ecological and phenological differentiation between *Quercus petraea* (MATT.) LIEBL. and *Quercus robur* in a mixed stand of northwest of France. Silvae Genetica. **44**: 1–110 (1995). — BATTAGLIA, E.: A new „5 minutes-fixation" using cold hydrolysis. Caryologia **9**: 368–370 (1957a). — BATTAGLIA, E.: A simplified Feulgen method using cold hydrolysis. Caryologia **9**: 372–373 (1957b). — BELLAROSA, R., DEL RE, V., SCHIRONE, B. and MAGGINI, F.: Ribosomal RNA genes in *Quercus* spp. (Fagaceae). Pl. Syst. Evol. **172**: 127–139 (1990). — BODENES, C., JOANDIT, S., LAIGRET, F. and KREMER, A.: Detection of genetic regions differentiating two closely related oak *Quercus petraea* (MATT.) LIEBL. and *Quercus robur* L. Heredity **78**: 433–444 (1997). — BUTORINA, A. K.: Cytogenetic study of diploid and spontaneous triploid oaks, *Quercus robur* L. Ann. Sci. For. **50**: suppl. 1, 144s-150s (1993). — D'EMERICO, S., BIANCO, P., MEDAGLI, P. and SCHIRONE, B.: Karyotype Analysis in *Quercus* spp. (Fagaceae). Silvae Genetica **44**: 66–70 (1995). — D'EMERICO, S.,

Table 1. – Morphometric parameters (mean ± S.E.) of the karyotypes of four *Quercus* taxa examined. Haploid complement length, A_1, A_2 (ROMERO ZARCO, 1986), SYi (GREILHUBER and SPETA, 1976) and karyological formula.

Taxon	Haploid complement µm	A_1	A_2	SYi	Formula
Quercus ilex	22.92 (±0.47)	0.34 (±0.01)	0.28 (±0.02)	64.54 (±1.41)	14m+4m^{sc}+4sm+2st
Q. pubescens	27.28 (±0.96)	0.34 (±0.01)	0.31 (±0.01)	66.09 (±1.49)	16m+2m^{sc}+2sm+4sm^{sc}
Q. robur	25.41 (±1.50)	0.35 (±0.01)	0.27 (±0.01)	64.11 (±1.26)	12m+2m^{sc}+4sm+6sm^{sc}
Q. suber	22.68 (±1.95)	0.36 (±0.01)	0.30 (±0.01)	62.96 (±1.10)	14m+8sm+2sm^{sc}

PIGNONE, D. and BIANCO, P.: Karyomorphological analyses and heterochromatin characteristic disclose phyletic relationships among 2n = 32 and 2n = 36 species of *Orchis* (*Orchidaceae*). Pl . Syst. Evol. **200**: 111–124 (1996). — GREILHUBER, J. and SPETA, F.: C-banded karyotypes in the *Scilla hohenackeri* group, *S. persica* and *puschkinia* (*Liliaceae*). Pl. Syst. Evol. **126**: 149–188 (1976). — GUTTMANN, S.I. and WEIGT, L. A.: Electrophoretic evidence of relationships among *Quercus* (oak) of eastern North America. Can. J. bot. **67**: 339–351 (1989). — KNOPS, J. F. and JENSEN, R.: Morphological and phenolic variation in a three specie community of read oak. Bull. Torrey Bot. Club **107**: 418–428 (1980). — LEVAN, A., FREDGA, K. and SANDBERG, A. A.: Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201–220 (1964). — NIXON, K. C.: Infrageneric classification of *Quercus* (Fagaceae) and typification of sectional names. Ann. Sci. For. **50**: 25–34 (1993). — OHRI, D. and AHUJA, M. R.: Giemsa C-banded karyotype in *Quercus* L. (Oak). Silvae Genetica **39**: 216–219 (1990). — ROMERO ZARCO, C.: A new method for estimating karyotype asymmetry. Taxon **35**: 526–530 (1986). — SCHWARTZ, O.: *Quercus* L. In: TUTIN, T. G. et al. (Eds): Flora Europaea **1**:61–64. Cambridge University Press, (1964). — SPELLENBERG, R.: A new species of black oak (*Quercus*, subg. Erytrobalanus, Fagaceae) from the Sierra Madre Occidental, Mexico. Am. J. Bot. **79**: 1200–1206 (1992). — ZOLDOS, V., PAPES, D., BROWN, S. C., PANAUD, O. and ŠILJAK-YAKVLEV, S.: Genome size and base composition of seven *Quercus* species: inter- and intra-population variation. Genome **41**: 162–168 (1998). — ZOLDOS, V., PAPES, D., CERBAH, M., PANAUD, O., BESENDORFER, V. and ŠILJAK-YAKVLEV, S.: Molecular-cytogenetic studies of ribosomal genes and heterochromatin reveal conserved genome organization among 11 *Quercus* species. Theor. Appl. Genet. **99**: 969–977 (1999).

Half a Century of Seed Years in Major Tree Species of Poland

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Summary

Data on fruiting estimates for the six main forest species of Poland: Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) KARST.), white fir (*Abies alba* MILL.), European larch (*Larix decidua* MILL.), oak (jointly *Quercus robur* L. and *Q. petraea* LIEBL.), beech (*Fagus sylvatica* L.), that is being regularly collected by the Polish Forest Service through an annual questionnaire sent out to all Forest Districts has been compiled onto a single drawing showing the magnitude and temporal positioning of seed years. A short discussion is given of the nature of recurrence of seed and cone crops in various parts of Poland.

Key words: cone crops, seed crops, seed years, mast, fructification, records.

Introduction

Information about seed years in forest tree species is important not only for the current management of forest nurseries but also for the long term tree improvement programs. Since seed quality is much better during seed years their frequency will affect the area of needed seed orchards and the rate of breeding programs. Thus it is useful from time to time to look back at what records say about crop years. Such long term data is seldom to be found in scientific literature. For half a century the Forest Research Institute (IBL) has been compiling annually the reports from Forest Districts about seed crops. The Forest Districts are required to report on the expected magnitude of the crop, the possibilities of seed collection and seed needs. When making the report in the following year they are also required (since 1962) to verify the data supplied the previous year on the basis of collection experience. On the basis of these reports the Forest Service makes decisions about accumulation of seed reserves for non-crop years or about withholding collections, in spite of good crops, if reserves are sufficient. The Forest Research Institute is an intermediary between the Forest Districts and the General Directorate of State Forests, performing the function of compiler and evaluator of the incoming data. As usual, the veracity of the reports differs from Forest District to Forest District and from year to year. The Forest Research Institute has to use its judgement based on many reports before supplying a country wide evaluation. Each year a communique is issued giving a summary of the pertinent data for the previous year (IBL 1951 to 1999). This work has been continuing for half a century, with various people being involved in the process both of sending the reports and evaluating them. This paper is a joint report for our six main forest tree species for the period 1951 to 1999. Both the end of the century and plans to restructure the mode of reporting justify a backward look at what has already been observed.

Methods

Each year Forest Districts obtain from the General Directorate of State Forests a questionnaire that has to be filled in with information on seed availability for the following species: Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) KARST.), white fir (*Abies alba* MILL.), European larch (*Larix decidua* MILL.), oak (jointly *Quercus robur* L. and *Q. petraea* LIEBL.), beech (*Fagus sylvatica* L.). The questionnaire asks for the following data:

1. Verified information for the previous year concerning:

a) crop magnitude as judged in the autumn, based on the fruiting of trees (since 1962);

b) degree of utilisation of the crop;

c) weight of cones or seed collected;

2. Prognosis for the current year:

a) expected magnitude of the crop;

b) expected collection in weight of cones or seed;

c) seed demand (based on working plan requirements for outplantings).