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Karyotypic Studies in Ecotypes of *Hippophaë rhamnoides* L. from Romania

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(Received 30th December 2009)

Abstract

Sea buckthorn is a dioecious Eurasian shrub or small tree with large morphological, biochemical and physiological variability, evidenced by the great number of studies. Cytogenetically, uncertainties exist on species basic number, ploidy level, and sex chromosomes. In this study, detailed cytogenetic measurements were carried out on six Romanian ecotypes belonging to *Hippophaë*

rhamnoides L. ssp. *carpatica* Rousi, in order to establish the features and the symmetry degree of karyotypes, to evidence the sex chromosomes, and to construct the idiogram. The ecotypes have $2n=24$ metacentric and submetacentric chromosomes. An intraspecific variation exists concerning the proportion of these two morphotypes. The karyotypes have similar symmetry patterns ($R=2.57-2.89$; $TF\%=38.54-42.70$; $AsI\%=57.99-61.41$; $A_1=0.27-0.35$; $A_2=0.26-0.36$) and belong to 1B and 2B classes, being relatively high symmetric. Based on obtained results, we presume that the male sex chromosomes are heteromorphic, while in female plants are homomorphic. The Y chromosome is larger than X chromosome.

Key words: *Hippophaë rhamnoides* L. ssp. *carpatica* Rousi, heterosomes, idiogram, karyotype.

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Introduction

The genus *Hippophaë* (Eleagnaceae) is widely but fragmentally distributed in Asia and Europe, between 27°–69°N latitude and 7°W–122°E longitude (ROUSI, 1971; LIAN *et al.*, 2000; BARTISH *et al.*, 2002), but during the last decade, the sea buckthorn became an important subject for domestication in Canada and USA (LI and BEVERIDGE, 2003). There is a notable worldwide increasing interest in exploitation of the nutritive, decorative, medicinal, cosmetic, ecological, and agricultural valences of *Hippophaë rhamnoides* L., the most important species of the genus (ZHAO, 1992; YAO and TIGERSTEDT, 1994; SINGH, 2003).

After 1753, when Linnaeus named sea buckthorn as *H. rhamnoides* L., several other species were found and named by many taxonomists. The data are controversial mainly concerning the classification within the genus which was subjected to numerous modifications (TANG, 2002). Especially the Chinese taxonomists modified it few times over the years by adding new taxa, some of these with possible hybrid origins (BARTISH *et al.*, 2002; SWENSON and BARTISH, 2008). Two moments with repercussions on sea buckthorn classification are the reporting of a new species – *H. neurocarpa* S.W. Liu & T. N. He – (LIU and HE, 1978) and of a new subspecies – *H. rhamnoides* ssp. *wolongensis* Y. S. Lian, K. Sun & X. L. Chen – (LIAN *et al.*, 2003). Originally, the genus consisted of only one species – *H. rhamnoides* – with 3 subspecies: *rhamnoides*, *salicifolia*, and *tibetana*. ROUSI, 1971 divided the species *H. rhamnoides* into 9 subspecies – *carpatica*, *caucasica*, *fluviatilis*, *gyantsensis*, *mongolica*, *rhamnoides*, *sinensis*, *turkestanica*, and *yunnanensis*. LIAN *et al.*, 2000 reconsidered the sea buckthorn taxonomy and raised a new taxonomic system of *Hippophaë*, according to which 6 species and 12 subspecies exist in this genus: *H. rhamnoides* L. (ssp. *carpatica* Rousi; ssp. *caucasica* Rousi; ssp. *fluviatilis* van Soest; ssp. *mongolica* Rousi; ssp. *rhamnoides*; ssp. *sinensis* Rousi; ssp. *turkestanica* Rousi; ssp. *yunnanensis* Rousi), *H. salicifolia* D. Don, *H. goniocarpa* (Lian) X. L. Chen & K. Sun (ssp. *litagensis* Lian & X.L. Chen; ssp. *goniocarpa* Lian), *H. gyantsensis* (Rousi) Lian, *H. neurocarpa* S. W. Liu & T. N. He (ssp. *stellatopilosa* Lian & L. Chen; ssp. *neurocarpa* S. W. Liu & T. N. He), *H. tibetana* Schlecht.

The modern phylogenetic technologies involving molecular markers (BARTISH *et al.*, 2002; SWENSON and BARTISH, 2003) confirmed the hybrid origins of two Chinese taxa – *goniocarpa* and *litagensis* – considered two subspecies of the same species. The correlation of the results on chloroplast DNA analysis with the previous data on isoenzymes and RAPD markers allowed to the authors to conclude that the two taxa are indeed two different species, thus the genus *Hippophaë* comprising seven species and eight subspecies. In spite of these results, RUAN refers in 2006 to the existence of 6 species and 12 subspecies.

The modern techniques of molecular biochemistry and genetics were also used to determine the sea buckthorn genetic variability and to elucidate the genus taxonomy: RAPD (Random Amplified Polymorphic DNA) markers

(JEPPSSON *et al.*, 1999; BARTISH *et al.*, 2000; CHOWDHURY *et al.*, 2000; SHENG *et al.*, 2006; SUN *et al.*, 2006), cp DNA (chloroplast DNA) (BARTISH *et al.*, 2002), ITS (internal transcribed spacer) (SUN *et al.*, 2003), AFLP (Amplified Fragment Length Polymorphism) markers (RUAN and LI, 2005; RUAN, 2006), ISSR (inter-simple sequence repeats) markers (TIAN *et al.*, 2004), DNA *microsatellite loci* (WANG *et al.*, 2008), *intron sequences* (chalcone synthase intron – *Chsi*) (BARTISH *et al.*, 2006). However, in spite of these numerous molecular, taxonomic and phylogenetic studies, there are still undeciphered zones concerning the sea buckthorn classification. The relationships between taxa remain in dispute and require further clarifications especially at species and subspecies levels because some specialists think that some of geographically separated subspecies deserve the rank of species (SMALL *et al.*, 2002).

The phenotypic and genotypic studies revealed a very large variability in all sea buckthorn provenances, and the Romanian sea buckthorn resources are not the exception concerning the high morphological, biochemical and cytogenetic diversity in this species (OLTEANU *et al.*, 2009; OPRICA *et al.*, 2009; TRUȚĂ *et al.*, 2009; ZAMFIRACHE *et al.*, 2009). The high level of variability amplitude is the result of long term evolution and it constitutes the evolutive potential of the species, because it assures the basis for selection and amelioration activities.

H. rhamnoides L. is an important plant in Romania, although its valences are not yet entirely exploited. In the last years, the research interest was focused on the realization of an inventory of Romanian sea buckthorn resources by their complex phenotypic and genotypic characterization, construction of a large theoretical and practical basis for the selection of valuable genotypes, and establishment of a germplasm national fund.

The literature is very rich regarding the sea buckthorn chemical composition, but there is a paucity of data on the chromosome constitution of this species. The cytogenetic studies establish the chromosome number of a species and help to decipher the morphological particularities of chromosomes and the metric characteristics of these, followed by karyotype construction. The pattern of chromosome formula, the presence of ploidy level, the existence of some chromosome anomalies can be discussed in relation with respective phenotypes and can direct the activities of selection and amelioration. *Hippophaë rhamnoides* is a dioecious species with a pronounced sexual dimorphism and the knowledge of cytogenetic indicators is necessary and important.

For these reasons, the main objectives of this study are the following:

- the establishment of chromosome diploid number;
- the analysis of the morphological traits of somatic chromosomes, and the construction of karyotypes of investigated ecotypes in view to evidence the intra-specific variation of karyotypic features;
- the identification of some differences which will permit us to infirm or confirm the existence of heterosomes in this dioecious species;
- the construction of sea buckthorn idiogram.

Materials and Methods

Seeds from individuals of six *Hippophaë rhamnoides* ssp. *carpatica* Roussi Romanian ecotypes (noted as HR-L2; HR-S16; HR-C1; HR-L4; HR-B8; HR-Bu2) were used as biological material for cytogenetic investigations. The germination was carried out at 22°C, in dark. At 10–15 mm length, the root tips were pretreated with 8-hydroxyquinoline (0.002 mol/L), for 4 h and were fixed in ethanol-acetic acid mixture (3:1) for 24 h at room temperature. The plant material was stored in refrigerator, at 4°C, in 70% alcohol. In view of analysis, the root tips were hydrolyzed in 50% hydrochloric acid for 8 minutes. A modified carbol fuchsin solution (GAMBORG and WETTER, 1975) was an effective stain for sea buckthorn chromosomes. The squash preparations were obtained in 45% glacial acetic acid. Microscopic investigation was carried out by a Nikon Eclipse 600 microscope. For morphometric analysis, the cells with well-spread metaphase chromosomes were photographed with digital camera Cool Pix Nikon, 1600x1200 dpi, 100x objective. The images were processed by Adobe Photoshop programmer.

Chromosome measurements included *length of individual chromosomes* (C), *long arm length* (L), *short arm length* (S), *arm ratio*, r ($r = L/S$), *centromeric index*, CI ($CI = S/C \times 100$), and *the relative length of each chromosome*, % ($C/\text{length of haploid complement} \times 100$).

Karyotypes were performed according to LEVAN *et al.*, 1964 nomenclature. The chromosome homology and the establishment of the chromosome types were assigned on the basis of centromere position, respectively on CI and r values: the chromosomes are metacentric ($r < 1.70$, $CI = 37.5-50.0$), submetacentric ($r = 1.70-2.99$, $CI = 37.5-25.0$), subtelocentric ($r = 3.00-6.99$, $CI = 25.0-12.5$), and telocentric ($r = 7.00-\infty$, $CI < 12.5$). In karyotypes, the chromosome pairs have been ordered in decreasing size. Idiograms were drawn based on the mean values recorded for all karyotypes.

To evaluate the karyotype asymmetry, the following indexes were analyzed: TF%, AsI%, R, A_1 and A_2 . STEBBINS' classification (1971) was employed to establish the asymmetry classes.

The total form percent (TF%) (HUZIWARA, 1962) is expressed by the ratio between sum of the lengths of the short arms of individual chromosomes to the total haploid complement length: $TF\% = (\sum \text{short arms} / \text{total complement length}) \times 100$.

AsI % index (ARANO and SAITO, 1980) represents the ratio between the sum of the lengths of long arms and total haploid complement length: $AsI\% = (\sum \text{long arms} / \text{haploid complement length}) \times 100$.

R represents the ratio of the longest to the shortest chromosome pair (BAEZA *et al.*, 2007).

The intrachromosomal asymmetry index (A_1) and *the interchromosomal asymmetry index* (A_2) were calculated according to ROMERO ZARCO, 1986:

$A_1 = 1 - [\sum (b/B)/n]$, where b and B are the mean length of short and long arms of each pair of homologues, respectively, n is the number of homologues;

$A_2 = s/x$, where s is the standard deviation, and x the mean chromosome length.

Results and Discussion

Karyotypic features of the studied Romanian sea buckthorn ecotypes

The karyotypes, which describe the complement phenotypes in terms of number, size, arm ratio, and other chromosome specific features, are dynamic structures evolving through numerical and structural changes (LEVIN, 2002). The above mentioned parameters may differ even between closely related taxa. For *Hippophaë*, the karyotypic studies are, with few exceptions, limited to somatic chromosome counts. The seed internal dormancy making heavier the germination, the high chromosome stickiness making more difficult the obtaining of well spread preparations, and the reduced size of chromosomes are the main explanations for the reduced number of cytogenetic approaches. In this research, the detailed analysis of karyotypes evidenced a relatively high degree of intraspecific uniformity for all studied variables in the six studied ecotypes (Table 1, Figure 1). The metaphases of somatic cells displayed 24 chromosomes. $2n=24$ is the diploid number repeatedly reported in the literature, the data being quasi-unanimous to sustain the existence of this chromosome number for all studied varieties, independently of their Asian or European provenance (ROUSI, 1971; SHCHAPOV, 1979; CIREASA and DASCALU, 1983–1984; CAO and LU, 1989; CIMPEANU *et al.*, 2004; RUAN and LI, 2005). Its widespread occurrence suggests that it is probably the true diploid number of *Hippophaë rhamnoides* species (ROUSI, 1971). Some different opinions exist relative to the sea buckthorn ploidy level and basic chromosome number – x (COOPER, 1932; DARMER, 1947; KOVACS and RACZ, 1974; LEVEQUE and GORENFLOT, 1969). In accordance to LIMA-DE-FARIA, 1980, the sea buckthorn chromosomes can be classified as small, they having sizes lower than 4 μm . Only HR-B8 ecotype has one chromosome pair exceeding 4 μm , all the other chromosomes having sizes smaller than 3.5 μm . The mean absolute length of individual chromosomes varies between 4.08 μm (HR-B8 ecotype) and 1.05 μm (HR-Bu2 and HR-L4 ecotypes), with a mean chromosome length/complement ranging from $2.24 \pm 0.23 \mu\text{m}$ to $1.81 \pm 0.16 \mu\text{m}$. Because of the small and very small sizes of chromosomes, it is somewhat difficult to make a very exact determination of centromere position especially for the chromosomes smaller than 2 μm where the details are few distinguishable. The length of haploid complement is comprised between 26.98 μm and 21.73 μm . No secondary constrictions and satellites were evidenced.

According to the values of arm ratios and centromeric indexes, all the karyotypes have exclusively metacentric (m) and submetacentric (sm) chromosomes, the metacentrics being more frequent; some differences are present in the proportion of these chromosome morphotypes. Our results on the existence of only m and sm chromosome types in sea buckthorn are in agreement with the reports of CIREASA and DASCALU, 1983–1984 ($2n=8m+16sm$); CAO and LU, 1989; CIMPEANU *et al.*, 2004 ($2n=20m+4sm$).

CAO and LU, 1989 established the karyotype formula for five Chinese *H. rhamnoides* subspecies, as following: *H. rhamnoides* L. ssp. *gyantsensis*

Table 1. – Karyotype features in the six studied sea buckthorn ecotypes. 2n = somatic chromosome number, LHC = length of haploid complement, CL = chromosome length, range = largest chromosome – smallest chromosome, CI = centromeric index, R = ratio of the longest to the shortest chromosome pair, AsI% = asymmetry index, TF% = total form percent, A₁ = intrachromosomal asymmetry index, A₂ = interchromosomal asymmetry index.

Ecotype	2n	Karyotype formula	LHC (μm)	CL (μm) — X ± SE	Range (μm)	CI — X ± SE	R	AsI%	TF%	A ₁	A ₂	Stebbins class
HR-L2	24	2n = 23m+1sm (putative male: 22+XY)	22.41	1.87±0.15	3.29-1.27	40.17±0.32	2.59	59.97	40.07	0.33	0.29	1B
HR-S16	24	2n = 18m+6sm (putative male: 22+XY)	22.20	1.85±0.16	2.93-1.10	39.67±2.16	2.66	59.59	40.00	0.34	0.30	2B
HR-C1	24	2n = 13m+11sm (putative male: 22+XY)	23.97	1.99±0.14	3.40-1.32	38.00±1.17	2.57	61.41	38.54	0.39	0.26	2B
HR-L4	24	2n = 22m+2sm	21.73	1.81±0.16	2.90-1.05	41.89±1.17	2.74	58.12	42.70	0.27	0.30	1B
HR-B8	24	2n = 16m+8sm	26.98	2.24±0.23	4.08-1.43	38.80±2.18	2.84	59.63	40.43	0.35	0.36	2B
HR-Bu2	24	2n = 18m+6sm	22.57	1.88±0.17	3.04-1.05	40.33±2.02	2.89	57.99	41.95	0.31	0.31	2B

2n = 2x = 24 = 18m + 6sm; *H. rhamnoides* L. ssp. *sinensis* 2n = 2x = 24 = 18m + 6sm; *H. rhamnoides* L. ssp. *turkestanica* 2n = 2x = 24 = 20m + 4sm; *H. rhamnoides* L. ssp. *yunnanensis* 2n = 2x = 24 = 14m + 10sm; *H. rhamnoides* L. ssp. *mongolica* 2n = 2x = 24 = 16m + 8sm, but also for other three sea buckthorn species: *H. neurocarpa* 2n = 2x = 24 = 18m + 6sm; *H. tibetana* 2n = 2x = 24 = 14m(2sat) + 8sm; *H. salicifolia* 2n = 2x = 24 = 10m + 14sm.

The mean values of asymmetry indexes of karyotypes are close: R ranges from 2.57 to 2.89, AsI% has values comprised between 57.99 and 61.41, and TF% varies from 38.54 to 42.70. The detailed and comparative analysis shows that HR-C1 ecotype has the most asymmetric karyotype having a smaller TF% (38.54%) and a greater A₁ index (0.39) than the other five karyotypes (Table 1).

Also, this karyotype has the most increase value of AsI% (61.41%) and the lowest value of interchromosomal index A₂ (0.26). It has approximately 55% metacentric chromosomes with median placed centromere. The most symmetric karyotype is HR-L4, with the highest value of TF% (42.70%) and the lowest A₁ index (0.27). According to STEBBINS' classification (1971), the karyotypes HR-L2 and HR-L4 are of 1B category - they have all the chromosomes with r < 2:1, while R is 2.59, respectively 2.76. Also, these karyotypes have more than 90% metacentric chromosomes with median centromere. The other four karyotypes belong to 2B class, with 75–83% metacentric chromosomes having r < 2:1 and R varying between 2.57–2.89. The 1B and 2B classes of the Stebbins' classification are considered relatively primitive; 2B karyotypes are considered more evolved than those included in 1B class.

The karyotypes having chromosomes smaller than 4 μm and predominantly of m and sm type are considered as being primitive, little evolved, because they have not been supported significant restructurations and rearrangements during evolution (STEBBINS, 1971; ACOSTA *et al.*, 2005; PASZKO, 2006). STEBBINS, 1971 stated that the tendency to karyotype asymmetrization by the increase of the number of telocentric chromosomes in spite of those of metacentric and submetacentric type represents a progressive step in karyotype evolution and has repercussions on species evolution. According to pre-

vious considerations and on the basis of our results, the ecotypes of *Hippophaë rhamnoides* ssp. *carpatica* Rousi have rather symmetric karyotypes, being relatively little evolved. By comparing them, the most asymmetric and, thus, the most evolved is HR-C1 ecotype, while HR-L2 and HR-L4 ecotypes have the most symmetric, therefore more primitive karyotypes.

Sex chromosomes in sea buckthorn

Another interesting problem is that of sea buckthorn sex determination. In dioecious species, the sex is genetically determined, either by morphologically differentiated sex-chromosomes, either by allele expression at one or several autosomal loci, placed on morphologically non-distinguishable chromosomes. For commercial production of sea buckthorn berries, only 7–12% of male individuals (sex ratio of 1♂:6♀ – 1♂:8♀) are necessary as producers of fertile pollen and pollinators (JANA *et al.*, 2002; LI, 2002). The breeding efforts are mainly orientated towards the development of valuable female phenotypes and to removal of undesired males by early sex discrimination of plants. Unfortunately, the plant sex can not be established before flowering.

If for certain dioecious plant species the sex determining chromosomal system is well known, for sea buckthorn the thoroughgoing studies are fewer and without details regarding sex chromosomes. Generally, in dioecious plants with dimorphic sex chromosomes, the male is heterogametic (XY) and female is homogametic (XX), like in mammals (AINSWORTH, 2000; CHARLESWORTH, 2002). In contrast to the typically small mammalian Y chromosome, in angiosperms the heteromorphic Y chromosome is often the largest chromosome in the male genome (*Silene latifolia* – CIUPERCESCU *et al.*, 1990; NAKAO *et al.*, 2002, *Cannabis sativa* – SAKAMOTO *et al.*, 1998, *Rumex acetosa* – SHIBATA *et al.*, 1999, *Coccinia indica* – PARKER, 1990). For example, in hemp, a dioecious plant with strong sexual dimorphism, it is accepted the existence of heteromorphic sex chromosomes in male plants: Y chromosome is telocentric, X chromosome is metacentric. The size genome – 1636 Mbp for female, 1683 Mbp for male – additionally confirms the larger dimensions of Y chromosome in hemp (SAKAMOTO *et al.*, 1998).

In *H. rhamnoides* L., according to several data, the male could be heterogametic and the female homogametic, but the existence of sex chromosomes never was proved in an unquestionable manner. As we previously presented, the somatic chromosome number of *H. rhamnoides* L. is $2n=24$, but there is little information concerning karyotype, genome or DNA, and sex chromosomes. SHCHAPOV, 1979 reported the presence of male heteromorphic chromosomes and the existence of an X/Y system determining sea buckthorn sexual phenotype. CAO and LU, 1989 published data on chromosome size and morphology in Chinese varieties, but they not identified heteromorphic sex chromosomes. In the studied ecotypes, we find two categories of karyotypes: one with twelve homologous chromosome pairs and another with eleven homologous chromosome pairs and one pair with heteromorphic chromosomes. These data permit us to presume, on the basis of their size and morphology, the existence of sex chromosomes in sea buckthorn (Figures 1 and 2) – see section Idiogram in *H. rhamnoides* L. for details.

Obviously, the mechanism governing gender determination in sea buckthorn still needs further research, especially at molecular level, in view to solve all uncer-

tainties concerning this important problem of large practical interest. Till now, utilization of molecular markers – especially RAPD – closely linked to sex determination is irrelevant. For example, in the research of PERSSON and NYBOM, 1998, although in the F1 descendancy of one cross, the RAPD marker was present both in male parental and in all male descendants and was absent in all female individuals, it can not be considered universal, because in F1 progenies of another cross it was present in only one of the males. If sex chromosomes exist in *Hippophaë*, it is plausible that they are very similar in DNA composition, since it was not discovered a general sex specific marker although 80 primers were tested (PERSSON and NYBOM, 1998). Anyhow, in dioecious plants without identified sex chromosomes, the markers for maleness indicate either the presence of cytologically not evidenced sex chromosomes either that the respective markers are tightly linked to genes involved in sex determination (AINSWORTH, 2000).

Sea buckthorn idiogram

Idiograms were constructed for both female (Figure 2A) and male individuals (Figure 2B) on the basis of the mean values calculated for the six ecotypes of sea buck-

Figure 1. – Metaphases and karyotypes in sea buckthorn Romanian ecotypes: A. HR-L2 ecotype (male); B. HR-S16 ecotype (male); C. HR-C1 ecotype (male); D. HR-L4 ecotype (female); E. HR-B8 ecotype (female); F. HR-Bu2 ecotype (female).

Figure 2. – Sea buckthorn idiograms: female (A) and male (B). Both idiograms are at the same scale.

Table 2. – Average values of cytogenetic parameters of the six studied sea buckthorn ecotypes, calculated for idiogram construction.

chromosome pair		chromosome length (μm)		long arm length (μm)		short arm length (μm)		arm ratio $\bar{X} \pm SE$	centromeric index $\bar{X} \pm SE$	relative length (%) $\bar{X} \pm SE$
		$\bar{X} \pm SE$	range	$\bar{X} \pm SE$	range	$\bar{X} \pm SE$	range			
I		3.11±0.19	4.08-2.78	1.67±0.1	2.17-1.45	1.23±0.09	1.91-1.25	1.17±0.05	46.20±1.13	13.31±0.44
II		2.86±0.1	3.50-2.64	1.56±0.04	1.77-1.44	1.29±0.08	1.73-1.08	1.23±0.07	45.07±1.43	12.28±0.27
III		2.35±0.17	3.13-1.95	1.42±0.08	1.83-1.05	0.92±0.12	1.33-0.55	1.67±0.23	38.69±3.17	10.05±0.52
IV		1.99±0.07	2.39-1.69	1.19±0.06	1.51-0.99	0.80±0.04	0.94-0.67	1.50±0.09	40.23±1.69	8.58±0.24
V		1.88±0.07	2.26-1.68	1.13±0.05	1.33-0.99	0.74±0.06	1.09-0.58	1.59±0.16	39.30±2.45	8.05±0.14
VI		1.76±0.05	1.99-1.60	1.10±0.06	1.33-0.94	0.66±0.03	0.74-0.53	1.70±0.17	37.62±1.99	7.56±0.17
VII		1.68±0.05	1.94-1.56	1.04±0.05	1.29-0.90	0.63±0.03	0.94-0.44	1.71±0.16	37.65±2.18	7.21±0.1
VIII		1.58±0.03	1.74-1.49	0.97±0.06	1.24-0.83	0.61±0.03	0.72-0.43	1.63±0.21	38.90±2.71	6.80±0.08
IX		1.48±0.05	1.65-1.27	0.96±0.06	1.25-0.83	0.52±0.02	0.61-0.43	1.86±0.19	35.47±1.91	6.35±0.14
X		1.45±0.06	1.70-1.22	0.89±0.06	1.05-0.66	0.55±0.02	0.65-0.48	1.63±0.13	38.47±1.91	6.22±0.15
XI		1.36±0.06	1.63-1.10	0.78±0.03	0.88-0.66	0.58±0.03	0.74-0.44	1.35±0.06	42.58±1.16	5.83±0.17
XII	X	1.45±0.21	2.29-1.05	0.94±0.11	1.56-0.70	0.51±0.10	0.56-0.31	1.85±0.19	35.73±2.2	6.23±0.99
	Y	3.02±0.32	3.40-2.38	1.74±0.24	1.84-1.27	1.28±0.14	1.56-1.10	1.37±0.2	42.64±3.4	13.2±1.24

thorn (Table 2). The mean chromosome length calculated for the species as a whole is 1.94 µm, this parameter having a range of variability from 3.11±0.19 µm to 1.36±0.06 µm. The eleven pairs of autosomes are represented by 8 pairs of m-type chromosomes and 3 pairs of sm-type chromosomes.

Concerning the sex chromosomes, the mean values of total chromosome lengths calculated for idiogram construction establish that the putative Y chromosome is longer than X chromosome (3.02±0.32 µm, comparatively to 1.45±0.10). On the basis of mean r and mean CI, the two putative sex chromosomes are classified as following: X chromosome is submetacentric, with submedian placed centromere (r=1.85±0.19; CI=35.73±2.2), Y chromosome is metacentric, with median placed centromere (r=1.37±0.20; CI=42.64±3.4).

Therefore, a diploid chromosome formula of 2n=22+XX (16m+8sm), with two putative homomorphic sex chromosomes, was described for female individuals, and 2n=22+XY (17m+7sm), with two putative heteromorphic sex chromosomes, was established for male plants in *H. rhamnoides* ssp. *carpatica* Rousi.

Conclusions

Relying on the available data, we conclude that the six Romanian sea buckthorn ecotypes are similar concern-

ing the chromosome diploid number (2n=24), the presence of two chromosome morphotypes, and the reduced size of these, but also exists an intraspecific karyotype variation, especially at the level of proportion of metacentric and submetacentric chromosomes. The karyotypes are rather symmetric (1B and 2B types) and relatively little evolved. In our studies, we find karyotypes with twelve homologous pairs of chromosomes, and karyotypes with eleven homologous chromosome pairs and one pair of heteromorphic chromosomes. On the basis of their size and morphology, we presume the existence of sex chromosomes in sea buckthorn, homomorphic in female, and heteromorphic in male. No satellites were evidenced.

Acknowledgements

This research was realized in project CEE-BIOTECH 109 from National Research Program of Romania, with the financial support of Ministry of Education and Research.

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Genetic Correlations Among Field Trials of Norway Spruce Clones Representing Different Propagation Cycles

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(Received 14th January 2010)

Abstract

Serial cutting propagation means that clones are produced in consecutive propagation cycles, where a new round of cuttings are taken from the previously rooted cuttings, etc. The objective of this study was to judge the importance of propagation cycle on height growth in field trials. In a field trial series with Norway spruce clones, comprising nine trials in four cutting propagation cycles, propagation cycle only had a minor effect on height growth compared to the site effect. For inter-site

correlation models where all trials were included, the best fit was obtained by an unstructured model, while the most parsimonious model included constraints on the correlations depending on cycle structure. Model constraints based on grouping of the trials by site productivity did not improve the fit. Testing for generally good performers over a range of different site conditions appears to be a good approach, unless drivers of genotype by environment interaction can be identified.

Key words: cuttings, field performance, genotype x environment interaction, Norway spruce, propagation effects.

Introduction

An important purpose of vegetative propagation is to identify and clonally propagate superior genotypes in order to increase the genetic gain compared to a sexual-

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