

## SOME ASPECTS OF SEX DETERMINISM IN HEMP

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**General considerations on hemp sexual phenotype.** Most flowering plant species are hermaphrodite, but a small number of species, as *Cannabis sativa* L., are unisexual. Because the species with male individuals and female individuals have evolved repeatedly from hermaphroditic progenitors, the mechanisms for the control of sex determination in flowering plants are extremely diverse. The sex is principally determined by genotype in all species, but the mechanisms range from a single controlling locus to sex chromosomes bearing several linked loci required for sex determination.

*Cannabis sativa* L. is one of the best studied species under the aspect of genetic determinism of the sex, but the problem is not yet entirely deciphered. The opinions are different, sometimes contradictory. Thus, the hemp is included, according to some authors, in the category of plants with male heterogamy, whereas the others sustain the idea of a complex sex determinism, seen as resultant of interaction between individual hereditary potencies and the environmental factors.

Although the hemp is a dioecious species, with sexual dimorphism occurring in a late stage of plant development, as a consequence of intensive improvement, many varieties with different sexual expressions were produced, and a large scale of sexualization types is observed in culture. The most frequent are the monoecious forms, classified in more categories, on a five-point scale, depending on female flowers/male flowers ratio. The sexual dimorphism in hemp is accompanied by a morphologic dimorphism, but this becomes evident only late in ontogeny, at anthesis. In early ontogenetic phases, the differences between plants are minor, from phenotypical point of view. The dimorphism also is manifest at anatomical level. For example, important differences between sexes were evidenced in respect of palisadic tissue, epidermal stomatic number, secretory hair distribution etc (Toma, 1975, 1977; Zanoschi and Toma 1985). In breeding activity, the early establishment of the sex would be necessary, imposed by the necessity to remove, from agronomic reasons, the male plants or the high masculinized monoecious plants.

Previous personal approaches, dedicated to the biochemical differences between male and female hemp phenotypes, confirmed also the existence of significant differences in isoperoxidase and isoesterase pattern, depending on sexual phenotype, both in number and stain intensity of bands (Truță et al., 2002). For both esterase and peroxidase, the isoenzymatic spectrum is richer for staminate plants (Figure 1). Thus, in female plant, eight multiple isoesterase forms appear, and eleven forms for staminate plant. For isoperoxidase pattern, the female plant has, as in the case of esterase, fewer bands (four), whereas ten fractions for male genotype were evidenced.

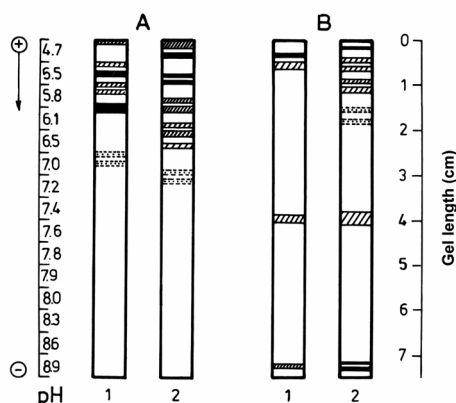


Figure 1. Differences in isoesterase (A) and isoperoxidase (B) zymograms, depending on sexual phenotype (female – 1, male – 2), in hemp (from Truță et al., 2002)

Also, some differences have been observed between the two sexes concerning catalase and peroxidase activities, as well as the level of soluble protein. The level of flavones, polyholozides and polyphenols was different depending not only on sex plant, but also on tested organ.

Because of intensive melioration, of pronounced segregation of the characters of intersexuate forms and because of the possibility to return to the initial dioecious type (the dioecy is dominant over monoecy - Potlog and Velican, 1972) the ways to realize intersexuate hemp varieties with traits of economic importance and to perpetuate them in culture are limited. In practice, difficulties exist in the generalization in culture of monoecious cultivars or of those with simultaneous maturity, because of the impossibility to maintain their genetic purity. They manifest a strong segregation in descendance, and between these forms always appear true male plants which, by pollination of intersexuate forms, determine the issue of a higher number of dioecious plants in descendance. The dioecious trait being dominant, the return to the dioecious type is rapid.

**The genetic determinism of hemp sex.** *Cannabis sativa* L. has a very complex genetic constitution and heredity which explains the dioecy, amplitude of phenotype variability, polymorphism and the great biological plasticity of this species. The flexibility of *Cannabis sativa* L. sexual phenotype often leads to the differentiation of hermaphrodite flowers or bisexual inflorescences (monoecious phenotype) (Molitermi et al., 2004). Therefore, the genetical component of sex determination and of sex control displays, by phenotype expression, a great variability in hemp.

In the plant kingdom, the dioecy is relatively uncommon, and a very low percentage of dioecious plant species have the XY system well determined. In the cases where the XY system was found, the authors sustain that it have evolved recently and independently (Negrutiu et al. 2001). In plants, the structure, the origin and the role of heterosomes in sex determination are poorly understood, despite the number of studies on sex determination in haploid, diploid, and triploid plants (Warmke and Davidson, 1944; Nishiyama et al., 1947, cited by Sakamoto et al., 2005). Ming et al. (2007) consider that sex specificity has evolved in 75% of plant families by male sterile or female sterile mutations, but well-defined heteromorphic sex chromosomes are known in only four plant families. It is possible that an essential event in sex chromosome evolution - suppression of recombination at the sex determination locus - not happened in most dioecious species. However, once the respective recombination is suppressed, an incipient Y chromosome starts to differentiate by accumulating deleterious mutations, transposable element insertions, chromosomal rearrangements, and selection for male-specific alleles.

Based on observations effectuated on sex reversal in hemp, and not on direct analysis of chromosomes, Hirata (1924) concluded that an XY system is implied in sex determination. Soon after the description of X/autosomes system in *Drosophila melanogaster* (Bridges, 1925), Schaffner, 1929, 1931 reconsidered the Hirata supposition and, based on his own studies on sex reversal in hemp, concluded that an X/autosomes system controls the sex determination and that the sexual phenotype is strongly influenced by environment. From the first studies on sex determination in hemp, many different types of sex determination system have been discovered.

A lot of models have been proposed to explain sex determination in *Cannabis sativa* L., species considered as having one of the most complicated mechanism of sex determination and of sex inheritance among dioecious plants (Westergaard, 1958).

McPhee, 1924 and Hirata, 1928 (cf. Arnoux, 1969), Hoffmann 1947, 1952, Sengbusch, 1952, Arnoux, 1963, 1966, 1969, Grant, 1975, Frankel and Galun, 1977 etc. studied some aspects of genetic determinism of the sexual phenotype in hemp. Many of these (Hoffmann, 1947, 1952 and Yamada, 1943 - cf. Arnoux, 1969; Mackay, 1939; Sengbusch, 1952; Panfil, 1984; Molitermi et al., 2004; Sakamoto et al., 2007 etc.) accept the hypothesis of sex determinism in hemp by heterochromosomes, XX in female, respectively XY in male (case in which the stamen development process in male flowers is determined by the dominant influence of the Y chromosome), although these chromosomes are not evidenced in all hemp varieties.

Some of cited authors sustained the size inequality of hemp sex chromosomes. For example, according to Mackay observations (Mackay, 1939), an unequal XY pair of sex chromosomes is present in staminate plants of probably all varieties of *Cannabis sativa*.

Generally, proponents of the XY system state that Y chromosome is larger than both the X chromosome and autosomes, although it is difficult to cytologically discriminate the X from Y chromosome (Yamada, 1943, cited by Sakamoto et al., 2005; Hoffmann, 1961; Peil et al., 2003, Sakamoto et al., 1998). A proof in this direction could be the higher nuclear DNA content of male *C. sativa* plants. This is 47 Mbp larger than that of female plants, possibly owing to the large Y chromosome (Sakamoto et al., 1998). This author found that the genome size of diploid male plants was 1683 Mbp and of diploid female plants 1636 Mbp. This difference is not usually detectable by microscopic techniques, but this very exact measurement confirms earlier observations.

Although the existence of heteromorphic sex chromosomes was reported in staminate plants of Kentucky dioecious variety, they were not found in pistillate plants of respective variety, in individuals of Kentucky monoecious hemp or in an unidentified German cultivar. If Kentucky dioecious variety was assumed to use an XY mechanism, the others noted varieties were assumed to be XX sex determination models (Menzel, 1964). Menzel presented a crude map of hemp meiotic chromosomes. According to the results obtained, the chromosomes of pairs II – VI and IX can be distinguished by the arms length, and the chromosomes of pair I have a large knob on one end and a dark chromomere 1 micron from the knob. The pair VII has very short and dense chromosomes, and the chromosomes of pair VIII are considered by Menzel as sex chromosomes.

The problem of existence of heteromorphic sex chromosomes in hemp is, in spite of some evidences - the most being indirect - yet unsolved, because their sure existence is confirmed especially if these chromosomes are clearly evidenced in a karyotype. Although the hemp was one of the first karyotyped species, it must emphasize that the construction of karyotype was realized with primitive standards. According to Hong and Clarke (1996), no modern karyotype of hemp had been published until 1996.

The chromosome number of *Cannabis sativa* is generally accepted ( $2n=20$ ). Even if a XX/XY mechanism is accepted to control the sex of diploid dioecious varieties, the Y chromosome role is not certainly established. In the experiments on hemp polyploids - forms which raise special problems - Warmke and Davidson, 1944, Rizet, 1946, Nishiyama et al., 1947, and Takenawa, 1953 found that individuals with XXY and XXXY formula are females. Griško, 1937 (cf. Arnoux, 1969) considers that the determinants of phenotype and those of sexualization are independent. The sex, in his opinion, is not determined by particular chromosomes, but by the genome assembly, and by the gene and chromosome interactions. All individuals are, in this manner, able to express one or the other sex. The same author also talks about the existence of a phenomenon of cytoplasmic heredity for feminized males which could inherit in this way the maternal cytoplasmic features.

For Hoffmann, 1952, all types can be XX, XY, even YY. Thus, the hemp plants with male habitus and female flowers may have XX formula. Like Westergaard, 1958, Hoffmann considers that Y chromosome is less active. While Westergaard sustains the idea of a *Drosophila* or *Rumex acetosa* mechanism, Hoffmann supposes a polyfactorial hereditary mechanism in monoecious expression: on X chromosome, the factors of feminizant tendency are placed, and on autosomes those for maleness. Hoffmann and Sengbusch were also interested by heterosomal formula in the plants with female habitus and male flowers appeared in hemp monoecious lines. These plants have, after Hoffmann, genes for monoecy and for feminization of habitus, maybe resulting from a cytoplasmic heredity. For Sengbusch, only one type exists for the forms with female aspect: the true wild male with XY formula. All other sexual phenotypes come from female state, all are XX, and the monoecious state depends on a polyallele gene, with yet unknown placement, resulting from a mutation in the dioecious female.

Menzel, 1964 sustains that some monoecious forms have XX formula, due to the translocation on nucleolar chromosome either of a segment of Y chromosome or rather of a fragment originated in one autosome carrying the factors of male state.

As a first conclusion, we can say that if the heterogametic character of male sex of dioecious forms is generally accepted, the genetic determinism of other sexual forms is yet unknown. Westergaard, 1958 and Grant et al., 1994 concluded that the hemp has a sexual determinism based soon on X/autosomes equilibrium and not on a Y active mechanism.

Our previous cytogenetic studies (Truță et al., 1988, Truță and Băra, 1994), effectuated on monoecious and dioecious Romanian hemp varieties, revealed the existence of  $2n=20$ . Also, the hemp chromosomes

presented small sizes (under 3 microns) and, according to arm ratio, centromeric index, and relative length we established two chromosome morphotypes - one with centromere in median position and another with centromere placed in submedian position. These parameters state that the hemp karyotype is enough uniform, with a great degree of symmetry, fact that proves a reduced level of evolution (karyotype was not subjected to essential restructurations). In other studies (E. Truță, 1999. Thesis, University of Iași, Romania), secondary constrictions were evidenced in two of the ten pairs of hemp chromosomes, constrictions that delimit satellite regions. For the studied hemp forms, we cannot sustain with certainty the individualization of sex chromosomes. It is possible, as Frankel and Galun, 1977 sustained, that the heteromorphism of sex chromosomes can be reduced or that the morphological differences can be evidenced only in specific meiosis phases.

Some authors express doubts especially on types of agronomic interest, namely of monoecious forms with female phenotype. For example, Hoffmann considers that these forms can be derivable from male, being XY or even XX. Nevertheless, Sengbusch suggests that these forms are originated from a mutation that induces the maleness of the true female and they are XX. However, in 1961, Sengbusch will admit the existence of monoecious forms with female phenotype, with XY formula. Another subject of basic disagreement between these two authors refers to the real genetical nature of male phenotypes with male flowers that appear in descendance of monoecious plants. For Hoffmann, these are XY types, different from the true male, because their crossing with a dioecious female gives in descendance only 28 - 36% male individuals, not 50% as were expected. For Sengbusch, these particular male individuals not exist. In his opinion, these forms are true males resulted from a strange accidental pollination, and the abnormal observed segregation is the result of a sublethality of Y pollen and egg-cells (Dierks and Sengbusch, 1961).

Panfil, 1984 supposes the existence of an allele series of sex factors (F) of different intensities. The XX or XY combinations can produce the same phenotype sex, depending on the degree of each F factor from X chromosome. In any of proposed models, problems as the sex genes placement, their interactions, as well as the individual mode of action are not explained in a satisfactory manner. If Y chromosome is inert, as results from the experiments with polyploids, the sex would depend on X:autosomes ratio (as in *Rumex acetosa*), but nor the possibility that *Cannabis sativa* species constitutes a new type of chromosome balance, yet unknown, can not be excluded. Ainsworth, 2000 describes sex determination in the genus *Cannabis* as using "an X/autosome dosage-type."

Such mechanism is very sensitive to environmental conditions, fact that could explain their significant influence on sexuality expression in hemp, especially on monoecy state (Westergaard, 1958).

As we previously presented, the somatic chromosome number of *Cannabis* is  $2n=2x=20$ , but there is little information concerning chromosome karyotype, genome or DNA.

Regarding the genic determinism of sexual expression in hemp, some authors sustain that female-determining genes and male-determining genes are carried by X and Y chromosomes, whereas the autosomes are not involved in sex determination (Warmke and Davidson, 1944, cited by Sakamoto et al., 2005).

Rath, 1968 suggested the existence of masculinizing or feminizing genes that, by recombinations, could replace the XY mechanism and become predominant and able to induce, for example in the case of masculinizing genes, the appearance of male individuals in the groups of different sexual types. In the opinion of Rath, these genes are placed on autosomes.

Frankel and Galun, 1977 affirm that the males are X/Y, and the females are X/X. Besides that, the allele  $X_m$  exists, that determines the appearance of male flowers in female inflorescence. Thus, X/ $X_m$  plants have female inflorescence, but they can be not strictly females. Depending on genetic and non-genetic additional factors, these plants tend to masculinization, reason for which Köhler named them "subgynoecious". The  $X_m/X_m$  plants will have female inflorescence, but functionally are males. Considering the results obtained in various studies on polyploids by other researchers, Köhler concluded that the masculinizing genes are autosomal and they are balanced by femaleness carrying genes of X chromosome, while the Y chromosome is "empty" under the aspect of sex determining genes.

Analysing these arguments and hypotheses, the following models were established:

- male plants with male inflorescence: X/Y,  $X_m/Y$ ;
- male plants with female inflorescence,  $X_m/X_m$ ;

- variable phenotypes, from true females to monoecious plants, but all with female inflorescence,  $X_m/X$ ;
- female plants with inflorescence of female type,  $X/X$ .

**Molecular markers in sex identification of hemp plants.** The development and applications of molecular markers to hemp breeding are relatively recent (after 1990). For the practical purpose, important is the development of molecular markers closely linked to the male sex and to some of the most relevant chemotypes. DNA-based markers as AFLP - amplified fragment length polymorphism - markers have been exploited in the field of forensic science, to discriminate licit from illicit hemp and to separate drug and fiber strains unequivocally (Datwyler and Weiblen, 2006).

Random amplified polymorphic DNA (RAPD) is widely used in genetic mapping, detection of phenotypic variation, and evolutionary studies. It is also very useful in studies of sexual differentiation and identification of dioecious plants. Using these approaches, researchers have identified molecular markers of female and male plants in various species, including hemp (Sakamoto et al., 1995; Mandolino et al., 1999; Flachowsky et al., 2001; Törjék et al., 2002; Peil et al., 2003; Rode et al., 2005)

The Y chromosome has a high affinity for Giemsa and DAPI (Sakamoto et al., 1998), suggesting that the Y chromosome has abundant heterochromatic regions including tandem and dispersed repetitive sequences such as transposable elements. The majority of the identified sex-related RAPD markers in *C. sativa*, therefore, seem to encode unknown or retrotransposon-like sequences (Mandolino et al., 1999; Sakamoto et al., 2000; Törjék et al., 2002). Some AFLP markers are also linked to sex chromosomes (Peil et al., 2003) and can contribute to the knowledge of the Y chromosome structure.

Mandolino et al., 1999 identified, by RAPD analysis, a DNA marker, named OPA8<sub>400</sub>, male associated, quasiabsent in female plants and absent in the monoecious plants. Because the marker was present in both staminate and carpellate plants, by modern techniques it was converted into a SCAR marker (Sequence-Characterized Amplified Region) 390rbp in length and male-specific, used for a precise, early and rapid identification of male plants during breeding programs of dioecious and monoecious hemp. The results can be useful in marker-assisted selection in hemp genetic improvement (Mandolino and Carboni, 2004).

The study of the hemp sex - as an important trait for genetic improvement - and of the sexual differentiation, is of great interest in hemp research. A morphological and molecular study of *Cannabis sativa* sexual differentiation, at the level of male and female apices of the Italian dioecious cultivar Fibranova, carried out by Moliterni et al., 2004, revealed that their reproductive commitment may occur as soon as the leaves of the fourth node emerge. The genetic expression of male and female apices at this stage has been compared by cDNA-AFLP. The authors developed a rapid method for the early sex discrimination in hemp plants.

The RAPD markers can be converted to sequence characterized amplified region (SCAR) markers, based on their DNA sequence, which could be detected through polymerase chain reaction (PCR) with longer sequence-specific primers. In this situation, the analysis was based on the PCR amplification of a male-specific SCAR marker directly from a tissue fragment. Five of the identified polymorphic fragments have been confirmed to be differentially expressed in male and female apices at the fourth node. They belong to nine different mRNAs that were all induced in the female apices at this stage.

DNA genomic isolated from male and female hemp plants was subjected to RAPD (random amplification of polymorphic DNA) analysis, cloning, gel blot analysis, hybridization. These analyses established the existence of a male-associated DNA sequence in *Cannabis sativa*, named MADC1 (Sakamoto et al., 1995). Subsequently, Sakamoto et al., 2000 found that fluorescence *in situ* hybridization (FISH) with MADC1 as probe generated a clear doublet signal at the end of the long arm of the Y chromosome. FISH using pachytene chromosomes of pollen mother cells at meiotic prophase I revealed that pairing of X and Y chromosomes occurred at the short arm of the Y chromosome where MADC1 was not present. Therefore, the accumulation of a specific male-associated DNA sequence at the terminal region of the long arm of the Y chromosome might be one cause of heteromorphism of sex chromosomes. Until now, six male-specific RAPD markers, named male associated DNA sequences in *C. sativa*, and noted from MADC1 to MADC6. Several other research groups have reported identification of sex-associated markers using RAPD and AFLP in *C. sativa* (Mandolino et al., 1999, 2002; Törjék et al., 2002; De Meijer, 2003;

Sakamoto et al., 1995, 2005), some of which are interpreted as indirect evidences of a male chromosome existence.

Flachowsky et al., 2001 described several AFLP markers showing fragments only in male plants. Peil et al. 2003, studying AFLP markers on X and Y chromosomes in male and female progenies of a single cross, detected five markers located on both chromosomes and showed that polymorphism on sex chromosomes was not restricted only to differences between X and Y, but was also found between X chromosomes. These observations confirm the earlier data on existence of such a polymorphism published by Mandolino et al., 1999 and Törjék et al., 2002.

These results were commented by Ainsworth 2000 in the following manner: "It is not surprising that male-associated markers are relatively abundant. In dioecious plants where sex chromosomes have not been identified, markers for maleness indicate either the presence of sex chromosomes which have not been distinguished by cytological methods or that the marker is tightly linked to a gene involved in sex determination."

**The influence of some epigenetic factors on hemp sexualisation.** The sexual expression in flower plants is under the control of genetical factors, as well as of epigenetic factors such as environmental conditions, phytohormones, photoperiod etc. (Tanurdzic and Banks, 2004; Manoj et al., 2005).

Many researchers have suggested that sex in *Cannabis* is determined or strongly influenced by environmental factors. The studies confirmed the role of endogenous hormones in maintenance of genetic sex, as well as the possibility to modify it by exogenous growth regulators, especially in polymorphic sexual systems, like hemp. The gibberellins, auxin, ethylene and cytokinins favour the expression of male or female sex in many monoecious and dioecious systems. It has been reported that sex can be reversed also in *Cannabis* using chemical treatment. It must emphasize that the exogenous treatment with these hormone regulators determines only a phenotypic modification of floral sexual expression, without affect chromatin structures.

Ainsworth (2000) reviews that in *Cannabis sativa* L. the auxins and ethylene have feminizing effects, whereas the cytokinins and gibberellins have masculinizing effects, but exist other contrary opinions. Chailakhyan and Khryanin, 1978, 1979 argued the masculinizing effect of gibberellins, but sustained the feminizant effect of treatment of hemp de-rooted cuttings with 6-benzylaminopurine (cytokinin), whereas Galogh, 1978 (cited by Dellaporta and Calderon-Urrea, 1993) accepted the feminizant effect of cytokinins in hemp, in the sense of induction of pistillate flowers on genetically male individuals of dioecious hemp.

Heslop-Harrison, 1963 a, b found that auxin induces the feminization of male hemp plants. Mohan Ram and Jaiswal, 1974, Mohan Ram and Sett, 1982, Grabowska et al., 2004 reported modifications in sexual expression, after the treatment with variable doses of 2-chloroethylphosphonic acid and the possibility to obtain a male sterility of hemp with this compound. The Mohan Ram group concluded that morphactin affects the sexual flower differentiation, in the sense of feminization of flowers. Thus, doses of 250 and 500 ppm induced a flowering delay, as well as the issue of stigmatic anthers and of carpellar structures in anthers. Experiences with GA<sub>3</sub>, GA<sub>4+7</sub> and GA<sub>9</sub> also determined modifications in sexual expression of hemp.

The external factors exert a strong modifying action especially on sex of monoecious plants, in which a middle level of ovary inducing substances exists, inducing both staminate and pistillate flowers, in variable proportions.

All external factors seem to influence in an indirect manner the sexual phenotypisation, by the control of endogenous auxins. The auxins have the role of an inducer or of an effector for the genes which exert a direct control on the synthesis of specific substances implied in sexual organs differentiation. In this system, it is possible that the gibberellins act as corepressors. The ability to reverse the sex hormonal treatments suggests that the floral primordia are sexually bipotent and that sex determination genes regulate alternative programs of sexuality, possibly through a signal transduction mechanism that modifies the endogenous hormonal levels.

It is very important to establish the endogenous hormonal level and the auxin/cytokinin ratio in the sex modifying experiences with various hormones. For example, in dioecious hemp, femaleness was found to be associated with high endogenous auxin levels; female plants contained up to 30 times more auxin than

male plants. In the same hemp, high endogenous levels of gibberellins in either vegetative or reproductive parts have been associated with maleness (Galoch, 1978, cited by Dellaporta and Calderon-Urrea, 1993).

The species has also an extremely important role in the phenotypisation of a certain sexual expression in this kind of approaches; the genotype is essential, as well as the fact that the species is monoecious or dioecious. It seems that the feminizing or masculinizing action of a certain growth regulator is species - dependent, the same hormone acting completely different in two different species. These facts explain why it is a low consensus on the influence of these compounds on sex expression in plants. Additionally, in hemp, the insufficient knowledge of the sex determination mechanism sometimes makes difficult and inconsistent the attempts to act on the sexual expression with external factors and can result in irrelevant conclusions.

Another factor that intervenes in sex modification is photoperiod, which has a masculinizing or feminizing effects, depending on day duration, whereas a nitrogenous rich nutrition induces a more or less phenotypic masculinization (Arnoux, 1963, 1966, 1969). Thus, in hemp, the long day and the increased temperature are favourable to male sex phenotypisation, and the short day and the decreased temperature to female sex phenotypisation. This fact is realizable by the regulation of endogenous hormones level. The environmental action is very important, because it can make unobservable the real genetical nature of a certain phenotype, a fact that amplifies the difficulties in analysis and interpretation of *sex ratio* in studied descendance.

The facts are additionally complicated by the coexistence in hemp of the two reproduction modalities - amphimixis and apomixis (by the presence of diembryony). This aspect confers a selective advantage to hemp. In our observations, in the variants which displayed the polyembryony phenomenon, the percentage of diembryony ranged from 0.5% to 5.0% (Truță and Băra, 1994). The elimination of practical difficulties supposed by hemp sexual dimorphism implies the obtainment of monoecious or even hemaphrodites cultivars, or the sex ratio modification in favour of female plants. The spreading of monoecious cultivars is possible only by assuring their biological purity, and the polyembryony by partenogenetic development of some cells could resolve this problem, because the descendance will represent the characteristics only of one parent (mother). It is also important the obtainment of haploid embryos and this is possible when diembryos develop from a seed. By their subsequent diploidization, homozygous plants, isogenous lines can be obtained and these constitute start points in the creation of valuable hybrids. In our study, the diembryony not implied in all cases the uniformity of the two descendants, the sexual phenotype and, implicitly, the whole complex of characteristics being different. Therefore, it is possible that the two embryos have different origin.

One of the principal criticisms directed to the chromosomal theory of sex determination is that a complex character like the sex can not be an exclusive monopoly of the “special” sex chromosomes. Additionally, not always a concordance exists between the heterosome formula and the sex of respective individual. The diversity of theories and of proofs found in their reasoning result from the fact that genotype is not only the information placed on chromosomes, but also the information from various cell organelles which contain nucleic acids. Consequently, to affirm that the sex is determined only by chromosomes or only by environmental conditions constitutes, if not a total mistake, then at least a narrow, limited approach of an extremely important biological phenomenon, with special implications for live world evolution. Therefore, we consider that the sex, like to any other phenotype character, is a resultant of the flow of hereditary information on genetic channel, but in concrete environment conditions and the hemp is a good example in this direction.

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