Euphytica **140:** 121–131, 2004.

Current status and future scenarios of hemp breeding

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Key words: genetic advances, hemp germplasm, improved varieties, key genes, metabolic pathways, multi-use crop

Summary

Hemp is a multi-use crop, able to provide fiber, cellulose, seeds and seed oil, cannabinoid, and biomass. Integrating many agroindustrial chains, *Cannabis* is considered a crop model in which insights into specific metabolic pathways and biosynthetic processes are valuable for improvement of the plant for all sets of industrial derivatives. In this review the hemp breeding status is elucidated and many aspects are focused: (i) recovering, maintenance and characterization of genetic resources; (ii) widening of germplasm and genetic variability; (iii) marker-assisted selection and development of breeding programs; (iv) sexual differentiation; (v) monitoring of THC content. Modern hemp varieties for fiber and other specific end uses have been developed and new varieties are entering production. The scenario for the breeding advances in hemp relies on basic and applied research which provides insights to identify a strategy for the design of modified plants with enhanced performance. This is accounted by the dissection of traits into components and the modification of single steps of the related metabolic pathways. These advances are provided by genomic techniques and are able: (i) to identify key genes encoding enzymes and regulatory factors participating in cannabinoid, fiber and oil biosynthesis; (ii) to identify the mode of regulation of these genes; (iii) to characterize the function of the selected genes through higher, lower or specific expression incited by specific promoters. The identification of molecular markers for specific traits, gathered in a saturated linkage map, will have a remarkable impact on hemp breeding. The advances in basic and applied research make it possible to design methods for the identification of superior parents and cross combinations and the development of selection schemes that rely on less labour-intensive and time-consuming methods.

Introduction

The main reasons for considering hemp as a high productivity, multi-purpose non-food crop of increasing importance for Europe are listed below (Ranalli et al., 2003; see also the paper "European hemp industry: cultivation, processing and product lines", by Karus, in this special issue) (Figure 1):

1. it provides raw material for industrial and energy applications; 30–40% of the stem (bark) can be used for several high-value fiber applications, whereas 60–70% of the stem (core) for several industrial applications (paper, composites, automotive industry);
2. it is a potentially profitable crop having the right profile to fit into sustainable farming systems;
3. it offers alternative land use and can be considered a key rotation crop. It provides several direct benefits including the reduction of pesticides, while increasing the yield of crops following it in rotation;
4. it has a high farmer perception. Being an annual crop, hemp does not require a long-term commitment in land use and moreover has a management similar to other conventional crops;
5. it has potentially interesting pharmaceutical applications, for which a market is already existing (Figure 2);
6. it could play a potential role in the remediation of soils contaminated with heavy metals.

Maintenance of valuable varieties and genetic resources

The current hemp varieties are descendant from very few ancestors including three gene pool sections: Northern and Central European ecotypes, Southern European ecotypes, and East Asian ecotypes (de Meijer, 1995). In particular, the 45 currently registered European fiber hemp cultivars were developed starting from a few promising local landraces, unconsciously selected over hundreds of years by peasant farmers in areas of traditional hemp cultivation.

Heterogeneous populations were originally grown throughout the world, as they thrived better, but were less productive in stressful environments, compared to more homogenous ones. Afterward, the crop specialization occurred, the original populations were selected to obtain remarkably uniform landraces.

The *Cannabis* germplasm includes accessions and populations for use in current breeding, research (working collections) and genebank collections. Contrary to genebank collections, accessions of working collections are generally non free available and not intended to be permanently maintained. Examples are the working collections at the Indiana University (Bloomington, U.S.A., 200 populations for biosystematic studies), at CPRO (Wageningen, The Netherlands, 200 original populations and various selections for fiber hemp breeding), at the Ukrainian Research Institute of Bast Crops (Glukhov, Ukraine, an unknown number of original landraces and selections for fiber hemp breeding), at HortaPharm (Amsterdam, The Netherlands, 100 clones and many seed offspring and pollen samples for the breeding of pharmaceutical cultivars; de Meijer, 1999).

Only a few genebanks with no direct commercial interest store a significant number of *Cannabis* seed accessions. Collections occur mainly in connection with recently abandoned or current fiber hemp breeding. By far the largest collection is maintained at the Vavilov Institute (St. Petersburg, Russia). It comprises approximately 500 accessions with a good representation of the entire fiber/seed hemp group including landraces from China. The Hungarian genebank stores about 70 accessions of local hemp. Smaller collections of up to 20 accessions are preserved in genebanks in Germany, Turkey, Japan, and Italy (de Meijer, 1999).

Cannabis germplasm collections serve mainly practical goals, such as breeding and various kinds of agronomical, biochemical, and botanical research which generally require well-specified strains with well-defined characteristics.

Current breeding status

Cannabis is not an easy plant to breed, and presents two major obstacles to the improvement by genetic procedures. It is usually dioecious, that is the plants cannot pollinate themselves, and are therefore outcrossing by nature. The reaction of plant to day length is another constraint of the hemp biology, since it affects the phenological development, length of biological cycle, and yield. Besides, the farmers require the development of a range of varieties to cover different cropping conditions.

Dewey (1927) can be regarded as the first hemp breeder; he raised excellent late-maturing varieties from Chinese landraces (Kymington, Chington, and Arlington). For one of his varieties he used the Italian landrace Ferrara as one of the parents, as indicated in its name (Ferramington). Unfortunately, due to the hemp cultivation prohibition introduced in the United States, these varieties have been lost.

In Europe, in the first quarter of the last century, cultivars were selected from old populations on the basis of the vegetative period length, height, diameter, weight, and in some cases, seed weight. Vegetation period length and stem yield are known to be in strong positive correlation with each other. In this way the population could be homogenized relatively quickly, but the quality traits (fiber) probably were not influenced. This type of family breeding led to the development of varieties in Italy, Hungary, and Romania, all of them coming from the famous Italian landrace Carmagnola.

Carmagnola is the oldest hemp landrace grown in Italy, initially in the North (in the homonymous area located in the Piedmont Region), and subsequently all over the country (Allavena, 1967). This variety was also introduced in other European countries, being used for direct production as well as a breeding parent in crossing programs. Thus, it is present in the pedigree of many current cultivars.

In Italy, Carmagnola was improved by mass selection, leading to the development of “Bolognese”, “Toscana,” and “Ferrarese” ecotypes (the names define the places of cultivation). The predominant breeding approach was continuous mass selection, a cyclic procedure that attempted to upgrade whole populations by directed selection.

The first attempt to breed for an increase in fiber content was made by Bredemann (1924), who began a selection for male plants of dioecious hemp. Selection was based on *in vivo* determinations of their fiber contents, and led to a considerable acceleration of the improvement of this trait.

No selection was made for fiber content before Bredemann, otherwise it should have involved only maternal lines. The importance of his work lies in his discovery that both genders are responsible for an increase in fiber content. He observed that the fiber content of male hemp could be determined before flowering, by splitting the stem lengthwise and determining the fiber content of one half of the stem. Only the best plants were allowed to flower, while the minus variants were destroyed before flowering. With this method he achieved a three-times increase in the fiber content over a period of 30 years. The determination of this quality trait before flowering, thus enabling pollination to be regulated, is only possible for dioecious hemp (Bredemann 1924, 1937, 1953; Bredemann et al., 1961).

As the reservoir of landraces and heterogeneous varieties was gradually depleted, hybridization was performed to create new variabilities. Half-sib family selection was employed, based on the evaluation of progeny from each mother plant. The latter are open pollinated and fertilized by more than one pollen male parent. The selection is based on general combining ability, and the entire population is used as a tester.

In Italy, selection procedures were improved and adapted to different traits and situations. Selection concerned the following traits: phenological development pattern (related to potential stem and seed production), stem quality (characterized by the fractions of wood core, secondary bark fiber and primary bark fiber, as well as by the length of wood core fibers), resistance to soil pathogens (root-knot nematodes), lodging, and suitability for different cultivation regions (Allavena, 1961a). No biometrical studies of these characters were carried out, but the potential inheritance was inferred from the repeatability of results in investigations lasted many years. So, the role of the environment in trait expression was elucidated and the influence of genotypes and environment (year and locations) on yield and quality of hemp was taken into account. The implementation of breeders' skill and the development of improved methods of plant evaluation allowed more efficient crossing methods and selection criteria. This approach allowed the breeding of new varieties like Fibranova, C.S. (Carmagnola Selezionata), Eletta Campana, Superfibra (Allavena, 1967; Barbieri & Tedeschi, 1968).

In Hungary, Fleischmann (1931, 1934) bred varieties which were also grown in the neighboring countries; his basic stock originated from Italy. In Germany, Heuser (1927) pioneered anatomical and agronomical questions of hemp fiber content. Hemp breeding started relatively late in Russia (Vedenski, 1929), but soon a new line of research was initiated: Grishko & Malusha (1935) and Grishko et al. (1937) bred monoecious and simultaneously ripening dioecious hemp varieties.

Sex differentiation

Grishko was the first to realize that all dioecious stands contain monoecious or intersexual types from which monoecious hemp can be bred (Figure 3). Later on, the question of sex was the research subject of McPhee (1925). Photoperiodic treatment enabled the development of subdioecious forms, i.e. to induce the development of a small number of male flowers on female plants. If these plants were self-fertilized, exclusively female progeny would have been obtained. This means that habit was inherited independently of sex. Grishko & Malusha (1935) and Grishko et al. (1937) described and isolated the first monoecious forms, from which inbreeding could be attempted and new varieties developed. The inheritance of intersexual forms of hemp, together with heterosis breeding and the development of hemp hybrids with various sexual forms (monoecious × dioecious hybrids), brings us up to the present day.

The study of the inheritance of various properties of the hemp plant (hypocotil color) started early in the course of which chiefly morphological mutations were described (Crescini, 1953).

Research on hemp cytology took place relatively early: Hirata (1928) not only established the $2n = 20$ chromosome number, but also demonstrated the presence of the heterochromosomes and proved that in dioecious hemp the inheritance of sex was based on the XY mechanism.

It also became clear at an early date that cells with $2n = 40$ chromosomes occur quite frequently in various root tissues due to endomitosis (Breslavets, 1926). Tetraploid forms were also developed (Warmke & Blakeslee, 1939; Ribin, 1939; Hoffmann, 1953) and proved to be completely fertile, with larger seeds and seed yields, but their fibers were coarser, so their production was of no economic interest.

Hybridization breeding

Probably the first intervarietal crosses took place between the hemp varieties Kymington and Ferrara, carried out by Dewey (1927) These were geographically and phylogenetically distant forms. The F1 had extremely good properties, especially in regard to fiber content and fiber yield.

Hungary is the only country where heterosis breeding of hemp was implemented. This resulted in several F1 hybrid cultivars. A single cross hybrid cultivar is "Uniko-B" (registered in 1969). It is a hybrid progeny of Kompolti × Fibrimon 21 where the monoecious Fibrimon 21 acts as pollen spender. The F1, being almost unisexual female, is used to produce an F2, containing about 30% males, which is cultivated for fiber (Bócsa & Karus, 1998).

Kompolti Hybrid TC (registered in 1983) is a three way-cross hybrid in which two selections from Chinese origin, Kinay Ketlaki (dioecious) and Kinai Egylaki (monoecious), and Kompolti are combined. The first step of the crossing (Kinai dioecious × Kinai monoecious), where the monoecious parent acts pollen spender, gives an unisexual, almost pure female F1, called "Kinai Uniszex". This unisexual progeny can be considered analogous to male sterile breeding lines. Afterwards it is used as female parent in the crossing (Kinay Uniszex × Kompolti) which produces the commercial three way cross hybrid Kompolti Hybrid TC, which is again 50/50 sex ratio.

Hemp breeding in Italy

Hemp breeding at the Research Institute for Industrial Crops in Bologna (ISCI) focused on many aspects (Ranalli, 2003): (1) recovery, maintenance and characterization of genetic resources. Italian germplasm and outside accessions have been collected, restored, characterized and used as a donor of valuable characters (resistance to diseases, greater fiber content and quality) (Faeti et al., 1996). This germplasm was investigated genetically and molecular markers suited to its genetic characterization were identified. Fingerprint was developed in hemp and provided a reliable varietal characterization (finding helpful for the advisory service). The germplasm collection also implied the development of breeding populations with a huge reservoir of genetic variation available and a wide range of useful traits, and the availability of sexual habits (dioecious or monoecious) needed for a generation of new varieties; (2) widening of germplasm and genetic variability. It is possible to induce undifferentiated tissue (callus) from different explants of hemp; the subsequent proliferation differs according to genotypes, source of explant and culture media. Further insights into the morphogenetic process, aiming at the widening of the regeneration capacity of initial explants, make it possible to use this approach for the isolation of new forms of hemp with a number of valuable characteristics, as well as forms resistant to extreme factors of environment (somaclonal variation; Zottini et al., 1996; Feeney & Punja, 2003); (3) development of new varieties. In 1995, a breeding program aimed at enhancing the yield, textile suitability, fiber quality and THC content reduction started, funded by the Italian Ministry of Agriculture. The program was carried out through an integration of conventional procedures, a better understanding of constraints and how to overcome them, and the application of molecular approach (marker-assisted selection). The breeding work led to the release of three dioecious varieties (Red Petiole, Asso, and Fibrimor) and four monoecious varieties (Ermes, Carmono, Carma, Codimono) (Di Candilo et al., 2002; Ranalli, 1998). These new varieties offer growers the opportunity to choose among a wider range of genotypes. The basic information generated is useful to address the future breeding work for the development of special varieties with increased seed and seed oil yield, modified seed oil fatty acid profiles, specific cannabinoid profiles, suitability for equatorial cultivation and resistance to specific pest and diseases; (4) propagation: i*n vivo* and *in vitro* propagation systems have been developed and performed for the propagation of valuable genotypes and long-term collection samples storage (Bizzarri & Ranalli, 1995); (5) marker-assisted selection: it was based on the identification of markers linked to valuable traits (Mandolino & Ranalli, 2002; see also the paper "Potential of marker-assisted selection in hemp genetic improvement", by Mandolino & Carboni, in this special issue). A marker, discriminating the male plants from both female and monoecious individuals was developed, using primers able to generate a SCAR marker 390 bp in length and fully male-specific. This SCAR is suitable for a reliable, early and rapid identification of male plants during breeding programs of dioecious and monoecious hemp (Carboni et al. 1998; Mandolino et al., 1999); molecular markers associated to chemotype have been also identified (de Meijer et al., 2003; Mandolino et al., 2003). A model involving one locus, B, with two alleles, B_D and B_T, was proposed, with the two alleles being codominant (de Meijer et al, 2003). The F2 segregating populations were used in a bulk segregant analysis of the pooled DNAs for screening RAPD primers and for developing chemotype-associated markers; (6) sexual differentiation: the gene expression of male and female plants was compared by cDNA-AFLP (see the paper " The sexual differentiation of *Cannabis sativa* L.: a morphological and molecular study", by Moliterni et al., in this special issue): several polymorphic fragments were identified as differentially expressed in male or female apices in the transition to flowering. Their DNA sequences and putative coding functions, and to their possible roles in the earliest step differentiation were identified; (7) monitoring of THC content. Serological methods for the identification of THC have been developed. The immunological tests appear to be a valid alternative to traditional THC analysis by gas-chromatography, and provide a convenient approach to meet the large amount of work needed in a hemp selection programme aimed at reducing THC content. A monoclonal antibody was raised and serological evaluation is currently performed (Grassi & Ranalli, 1999).

Future breeding advances

European hemp breeders have developed many highly productive fiber cultivars with low-THC content. Breeders in France, Germany, Poland, Romania and the Ucraine worked mainly on the development of low THC monoecious varieties, while those in Hungary and Italy concentrated primarily on low-THC dioecious cultivar development.

Until recently, there have been modest incentives for the development of new *Cannabis* varieties. In the past, hemp was used almost entirely for fiber production and there were few commercial uses for the seed. During the 1980s, hemp cultivation nearly ceased in eastern Europe and there was little need for sowing seed of existing cultivars, much less the costly development of new ones.

The resurgence of interest in hemp is accompanied by a wide range of new products and potential uses for hemp. Besides, breeding of hemp, as well as many other crops, nowadays requires high expenses in terms of labor, time, proficiency and means to develop new varieties.

Presently, what scenarios can we depict for the breeding advances in hemp?

Because hemp is a multi-purpose crop, the development of plants for specialized end-products needs an enhanced understanding of metabolic control, both in terms of genetic and molecular mechanisms in order to promote the remodeling of metabolic pathways. This is accounted by the dissection of traits into components and the modification of single steps of the related metabolic pathways. Advances in basic and applied research will provide insights to identify a strategy for the design of modified plants with enhanced performance. Future research activities have to deal with physiological, genetic and molecular aspects.

Physiological tasks

Under favorable growing conditions, the biomass production of crops depends on the amount of light intercepted by the active photosynthesizing plant parts and on the efficiency of the conversion of radiation energy to dry matter. The radiation use efficiency (RUE) and the average value over the growth cycle of dry matter production, per unit of intercepted photosynthetically active radiation (PAR), have been used to analyze hemp productivity and to compare the production of hemp to that of other species. Before flowering, the RUE of the hemp crops was 2.0 g MJ^{-1} at high-plant density; after flowering it dropped to 1.1 g MJ^{-1} (de Meijer et al., 1995).

The RUE values of hemp are at the lower end of the range of values found for other C3 crops. For many species a RUE of 2.2 to 2.0 is reported and for the subtropical fiber crop kenaf a value of 2.4 g MJ^{-1} was reported (Carberry & Muchow, 1992).

The RUE value depends on the composition of dry matter produced from the primary assimilates, on the proportion of the biomass being lost during the growing season and on the canopy photosynthesis rate. The analysis of experimental data led to the following factors reducing the RUE of hemp: the planophile character of the canopy hampers radiation distribution within the canopy; growth respiration is relatively high in hemp, because the stems contain about 16% lignin and because, after flowering, fat and protein are synthesized in the seed. The cost and weight losses associated with the conversion of assimilates to these constituents are high compared to those associated with sugars, starch, and cellulose. Finally, losses of dry matter are huge in hemp crop, particularly after flowering: dead leaves are shed rapidly and many plants die as a result of inter-plant competition. The contribution of the above factors to the relatively low RUE in hemp were calculated: planophile canopy decreases the RUE by 3% when compared with canopies of cereals or potatoes; in comparison with cereals, the extra lignin in the stem reduces the RUE by 6% and the oil and protein formation in the seed gives a 3% reduction; the biomass losses related to self-thinning were 4–7%, depending on initial plant density (see also the paper "Potential productivity of fiber hemp in Southern Europe", by Di Bari et al., in this special issue).

Figure 1. A hemp field close to maturity. Many traditional dioecious ecotypes can reach heights up to 4 meters.

Dry matter production and partitioning

Fiber hemp is grown for the production of stem dry matter; within the stem, the bark is more valuable than the core (van der Werf et al., 1994). Therefore, both a high proportion of stem in the above ground dry matter and a high proportion of bark in the stem dry matter are sought after (Figure 1). Both levels of dry matter partitioning will be examined below.

Flowering date, plant density, and the proportion of male plants are the main factors affecting the proportion of stem in the aboveground dry matter of a hemp crop. The later a cultivar flowers, the smaller the fraction of the inflorescence and the larger the fraction of the leaves and the stem in the aboveground dry matter will be. The resulting effect of the flowering date on the proportion of the stem is variable so that, relative to an early cultivar, a late cultivar may contain a similar, smaller or larger proportion of stem, depending on genotype and the kind of sexual habit (monoecious or dioecious) (Figure 3). In order to obtain a high proportion of stem in the aboveground dry matter the crop should be grown at a the highest possible density not causing self-thinning. The more male plants are present, the larger the stem proportion.

Cultivar and plant density are the main factors affecting the proportion of bark in the stem. In order to maximize bark content a high-fiber cultivar should

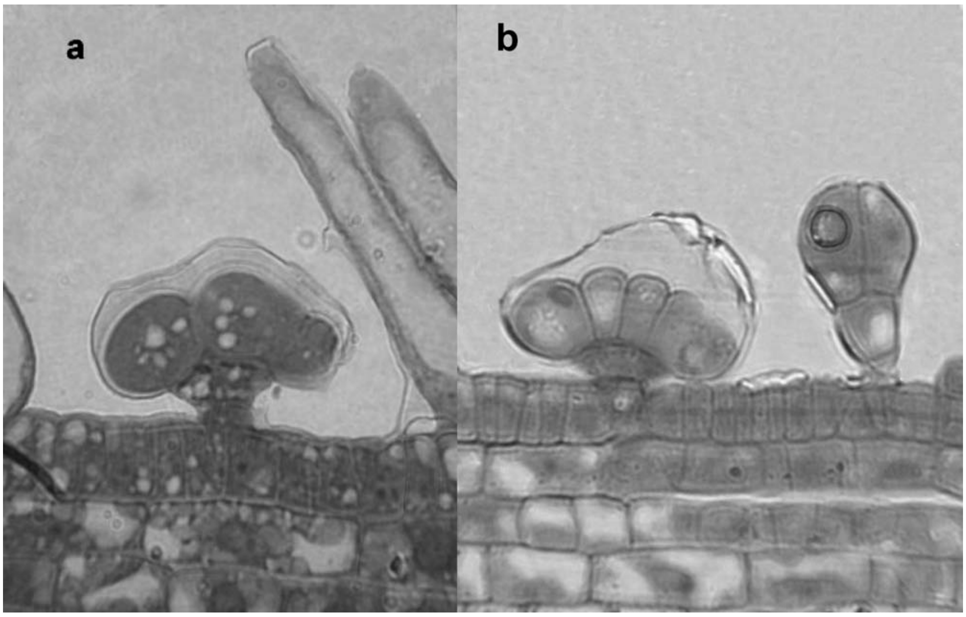

Figure 2. Micrographs of Cannabis' trichomes. a), a glandular trichome and, immediately to its right, a group of non glandular trichomes from leaves. b), different types of trichomes on the surface of a hemp leaflet. The glandular trichomes are supposed to be the main site of production and accumulation of the cannabinoids (photo by V.M.C. Moliterni, ISCI).

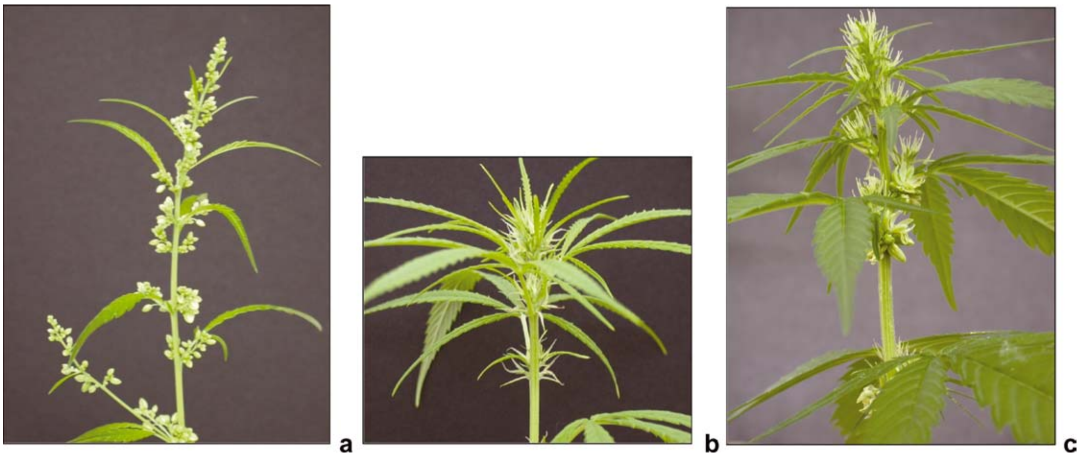

Figure 3. Cannabis sativa sexes. A male inflorescence (a), a female one (b), and a monoecious plant (c). Sex-related traits are of paramount importance in hemp breeding of the dioecious and monoecious cultivars (photo by V.M.C. Moliterni, ISCI).

be grown at the highest possible density not causing sel-thinning. In Kompolti Hybrid TC, the bark content increased from 33% at 10 plants m^{-2} to 36% at 90 plants m^{-2}, at 35% at 270 plants m^{-2} (Ranalli, 1999). The level of soil nitrogen affected the bark content: it was 36% at 80 kg ha^{-1}N and 34% at 200 kg ha^{-1}N (van der Werf et al., 1995). The effect of soil nitrogen probably resulted mainly from a difference in plant density which had arisen as a result of self-thinning: plant density was 129 m^{-2} at 80 kg ha^{-1}N and 92 m^{-2} at 200 kg ha^{-1}N.

Genetic and breeding aspects

The future research will focus on the following aspects.

1. Early canopy closure. Increasing the total amount of intercepted radiation during the growing season

might improve crop productivity. Plants with limited branching are naturally superior than irregularly branching plants. They fully and uniformly occupy the field, and use the maximum of solar radiation. Earlier sowing may be an effective way of obtaining earlier canopy closure. The introgression of "wintering" trait in new varieties allows the resistance to early spring cold and the sowing of hemp in earliest spring time (early germination). Sowing and harvesting dates both have consequences for the potential stem yield: delay of the harvest date can increase stem yields, but requires the breeding of very late-flowering cultivars.

2. Composition of the cell wall. The elucidation of genes implicated in the structure and chemical composition of the cell wall allows to breed hemp varieties with a lower lignin content in the stem; what would also reduce the conversion loss and improve the quality of stem dry matter as a raw material pulp and paper.
3. Late blooming. Using cultivars which flower later than the current ones would probably improve the stem yield, as no assimilates would be invested in the inflorescence, and new leaves continue to be formed, thereby prolonging the maximum photosynthetic capacity of the canopy.
4. Low sensibility to day length. This trait reduces the photoperiod effect on plant height and yield, resistance to drought, frost, cold, and diseases. It must be the main trait of perfect modern variety.
5. Sexual differentiation. It will be possible to move forward with a transcriptome analysis of differentially expressed clones, the identification of their putative coding function and the possible roles in the earliest step of sexual differentiation.
6. Cannabinoid profile. This implies the identification and selection of plants or strains able to synthesize new cannabinoid-type molecules. These cultivars will be used to prepare a variety of pure cannabinoid extracts that will be tested in clinical trials for medical efficacy for several indications (see the paper "Pharmacological and therapeutic targets for Δ^9-tetrahydrocannabinol and cannabidiol", by Pertwee, in this special issue). The varieties produce single cannabinoids, such as CBD and THC, at very high levels (over 10%), often with only negligible amounts of any other cannabinoids (see also the paper "Cannabinoids for the pharmaceutical industry", by Guy & Stott, in this special issue). In the near future the most recent developments of molecular biology should allow the identification of the genes controlling the synthesis of all cannabinoids and of other valuable secondary metabolites. The study of the structure of these genes, and of the relative controlling sequences (promotors, enhancers) might lead to an alteration in the constitutive level of secondary products synthesis.
7. Fatty acid profile. Hempseed offers a unique nutritional package, in terms of dietary oil, protein, vitamins and minerals, which can be produced at high latitudes ($>50^\circ$latitude; see also the paper "Hemp seed as a unique nutritional resource", by Callaway, part of this special issue). Hempseed oil is highly unsaturated and contains both essential fatty acids (linoleic acid and alpha-linolenic acid) in a nutritional balanced ratio, in addition to considerable amounts of biochemically important gamma-linoleic acid (GLA) and stearidonic acid (SDA). The identification and selection of genes which participate in seed oil biosinthesis underlying the increase or modification of seed oil content have to be attempted.
8. Fiber quality. Identification and selection of genes involved in the fiber composition and quality. Efforts of breeding and molecular biology should focus on the characterisation of the cell wall components (see also the paper "Histochemical and supramolecular studies in determining quality of hemp fibers for textile applications", by Meneghini Bonatti et al., in this issue), and of the genes involved in cell wall composition, in the biosynthesis of lignin, cellulose, hemicellulose, pectin, and cell wall proteins (see the paper "Predicting the chemical composition of fiber and core fraction of hemp (*Cannabis sativa* L.), by Toonen et al., in this issue).

These goals will be reached if they are supported by advances in the identification and characterization of genes underlying the different analyzed pathways: genes involved in the biosynthesis of cannabinoids, in the quality of seed oil, fiber and co-products. This research will provide a knowledge base that may facilitate the development of *Cannabis* as a high-value oilseed crop and the creation of *Cannabis* varieties containing modified or zero psychoactive cannabinoid levels.

The approaches listed above will make an increasing use of the techniques of genomics and post-genomics, in order: (i) to identify key genes encoding enzymes and regulatory factors participating in cannabinoid, fiber and oil biosynthesis, (ii) to identify the mode of regulation of these genes: RNA analyses by cDNA-AFLP or microarrays analyses. The functional characterization of the selected genes will be obtained through higher expression incited by over-expression, lower expression by antisensing, specific expression using specific promoters.

Expression profiling using arrays has been used to complement EST sequencing to facilitate gene discovery. Array analysis involves the spotting of DNA fragments on a solid support and then the hybridization of radioactively or fluorescent-labeled probes to the spotted samples. This allows the spatial or temporal expression of cDNAs to be compared with the biosynthetic process under analyses.

Molecular markers

The possible applications of molecular markers to hemp breeding have been dealt with in detail by another contribution in this special issue. Molecular markers, and particularly DNA-based markers, have been largely used to highlight the genetic structure of several crop species, to identify molecular profile characteristics of a given variety (fingerprint), or to track specific traits of interest. Isozymes, RFLP, RAPD, AFLP, and microsatellite have been used for these purposes; each has advantages and disadvantages. The reintroduction of hemp cultivation in most European countries since 1996 has led in the past few years to the application of many molecular biology techniques to this species, with a particular aim to the specific problems and the traits of value for this crop. Since the beginning, the study at the molecular level of hemp mainly focused on three aspects of biology and physiology: fiber, sex, and secondary metabolism. These traits are of paramount importance in hemp breeding, and many of the efforts devoted to the application of molecular tools to this ancient and traditional plant are aimed to understand, exploit and, in the future, modify these characters.

The availability of molecular markers for specific traits, gathered in a saturated linkage map could have a remarkable impact on hemp breeding. The close association found for male phenotype and cannabinoid types with specific molecular markers, even in absence of a molecular map, provides rapid diagnostic tools in breeding. However, saturated molecular maps will be needed to find markers associated with other important traits, probably quantitative in their genetic control, such as fiber content and fiber quality, retting parameters and monoecy. In these cases, the degree of heritability will influence the selection method to be chosen. Mapping these QTL is certainly the most important task for the future.

Exploitation of basic and applied research

Advances in basic and applied research will provide useful insights to the identification of strategies for designing modified hemp plants with differentiated and enhanced industrial end-uses. They also make it possible to design methods for the identification of superior parents and cross combinations, and for the development of selection schemes relying on less labor-intensive and time-consuming methods. New breeding programs could be promoted, implying: (i) planning of mating designs; (ii) selection of progenies, performed through an integration of conventional procedures, a better understanding of constraints and how to overcome them, and the application of molecular approach (marker-assisted selection); (iii) evaluation of developed lines in different environmental conditions; (iv) release of new modern varieties for specific end uses suitable to grow in areas with different photoperiod, lands, environmental conditions and crop management (see the papers "Copper localization in *Cannabis sativa* L. grown in a copper-rich solution", by Arru et al., and "Life cycle analysis of field production of fiber hemp, the effect of production practices on environmental impacts," by van der Werf, in this special issue).

Modern hemp varieties for fiber and other specific end uses have been developed and several new varieties are entering in production. "FIN-314," the first hemp cultivar strictly for grain seed production, was developed in Finland from germplasm accessioned in the Vavilov Research Institute Gene Bank and entered in commercial production in Canada in 1998. The seed oil of this cultivar contains high levels of the essential fatty acids gamma-linolenic acid (4.4%) and stearidonic acid (1.7%), which are valuable as nutraceuticals and dietary supplements (Laakkonen & Callaway, 1998). The short stature of "FIN-314" makes it readily harvestable by machine combine and seed yields are expected to be high.

Besides, textile fiber crops are difficult to harvest, so considerable investments are in progress in different countries for the design of a suitable harvesting machine. Water retting, essential in some areas (like Italy), requires much time and labor: advanced innovations have been obtained in this field, they need the scale-up (Tamburini et al., 2002; Di Candilo et al., 2000; see also the paper "Exploitation of bacterial pectinolytic strains for improvement of hemp water retting", by Tamburini et al., in this special issue).

The optimization of crop cultivation methods and the application of refined fibers in various high-added value applications will promote hemp for the production of renewable materials, and could provide a significant opportunity to improve diversification in EU agriculture.

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