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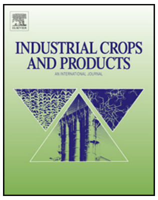

# New developments in fiber hemp (*Cannabis sativa* L.) breeding

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## ABSTRACT

Fiber hemp (*Cannabis sativa* L.) is a sustainable and high yielding industrial crop that can help to meet the high global demand for fibers. Hemp can be grown for fiber, seeds, and/or for dual purpose in a wide range of geographic zones and climates. Currently the main hemp producing regions in the world are China, Europe, and Canada. The number of new cultivars developed for each of these regions has gradually increased, with each region producing its own typical hemp cultivars for different purposes. In this article, the state of the art of fiber hemp breeding programs in Europe, China, and Canada are reviewed. The breeding strategies and tools used in the breeding of hemp cultivars are discussed. We also provide an overview of genetic diversity in hemp for different traits. In addition, the current knowledge of the main breeding goals for fiber hemp, which are an improvement of fiber quality and fiber yield, breeding for specific cannabinoid profiles, control of flowering behavior, male flowering control, and breeding of cultivars for specific environments are evaluated. Lastly, we discuss the inestimable value of next generation technologies to breed new hemp cultivars that are suitable for a biobased economy.

## 1. Introduction

Hemp (*Cannabis sativa* L, $2n = 20$) is one of the world's oldest cultivated annual crops (C3 annual), traditionally grown for its long and strong bast fibers and seeds. In most Western countries the cultivation of hemp vanished or was interrupted for decades as a result of competition with other feedstock's such as cotton and synthetic fibers, high labor costs, and the prohibition of cultivation due to the use of cannabis (*C. indica*) as a narcotic. Only in Eastern Europe, the former Soviet-Union and China has a substantial hemp industry survived (De Meijer, 1995). In the late 1970s and the early 1980s, the average hemp planting area once reached about 160,000 ha in China. After that, the cultivation area declined because of the above-mentioned reasons. The crop can be grown in a wide range of geographic zones of climate, and is well adapted to most regions of the world. China, Europe, and Canada are the three most important hemp planting regions in the world. According to FAOSTAT (excluding Canada), in 2011 hemp was cultivated globally on 61,318 ha, of which 11,400 ha in China, 14,344 ha in The European Union, and 15,720 ha in Canada (Source: Health Canada).

### 1.1. Products

Hemp is involved in a diverse range of products, and has integrated many agro-industrial fields such as agriculture, textile, bio composite, paper-making, automotive, construction, bio-fuel, functional food, oil, cosmetics, personal care, and pharmaceutical industry (Fig. 1). Traditionally, hemp bast fiber is used in textiles, paper pulp, and materials for building and insulation. Hemp hurds (also termed 'shives'), the woody and lignified core tissues of the stems, are used as horse-bedding, pulping, and concreting (Elfordy et al., 2008; Karus and Vogt, 2004). Besides the traditional uses, novel applications for fiber hemp (fibers/biomass) are being developed. The high cellulose content of hemp cell walls (Amaducci et al., 2000) together with the relatively high productivity make hemp biomass an interesting renewable feedstock for energy production (Hanegraaf et al., 1998; Prade et al., 2011, 2012a,b; Ragit et al., 2012), for the production of second generation bio-ethanol (Gonzalez-Garcia et al., 2012) and as a reinforcement in 'green composite' materials (Khalil et al., 2012; Shahzad, 2012) and concrete

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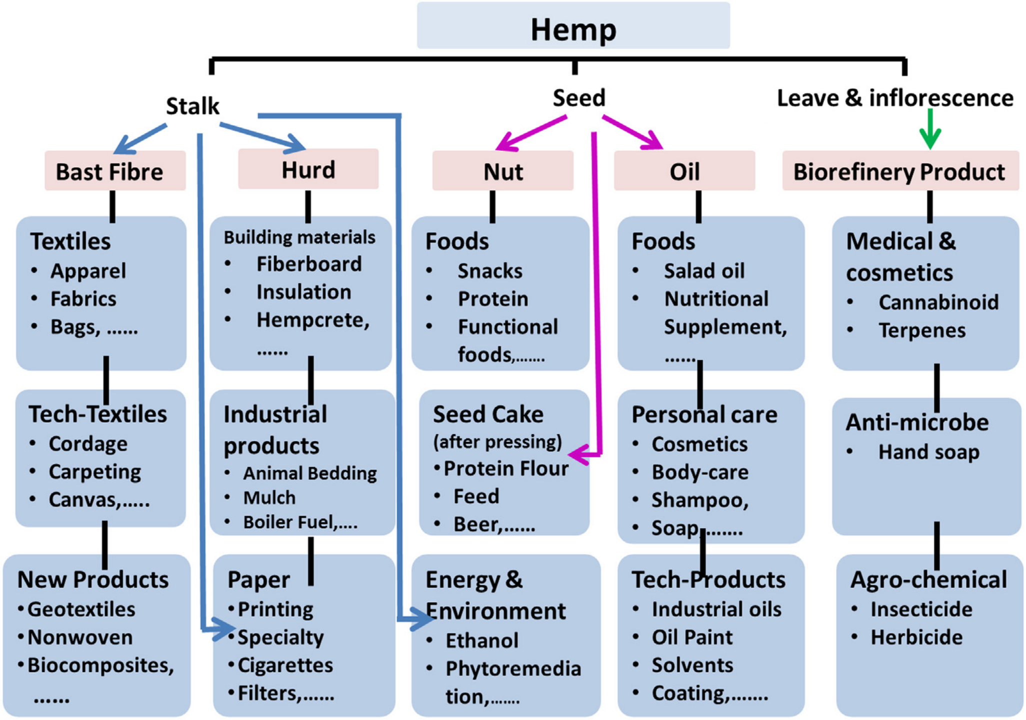

**Fig. 1.** Flowchart of multi-purpose hemp utilization.

(Elfordy et al., 2008). It is estimated that the global market for hemp consists of more than 25,000 products.

### 1.2. Yield

Hemp may potentially yield 25 t $ha^{-1}$ above ground dry matter, 20 t $ha^{-1}$ stem dry matter and 12 t $ha^{-1}$ cellulose (Struik et al., 2000) but in many cases the yield varies. For instance, in Northern Italy total dry matter yield (cv. Futura 77) ranged from 18.7 to 8.3 t $ha^{-1}$ over years and locations (Amaducci et al., 2000). In general cellulose yield is 7–10 t $ha^{-1}$ (Zatta et al., 2012). The yield of the dry bast fiber varies from 1.2 to 3.0 t $ha^{-1}$, and seed yield from 0.7 to 1.8 t $ha^{-1}$. Industrial hemp production statistics for Canada indicate that average yield of seeds is about 0.78 t $ha^{-1}$, and an average 5.9 t $ha^{-1}$ of straw, which can be transformed into about 1.45 t $ha^{-1}$ of fiber (Johnson, 2013).

### 1.3. Hemp fiber

At present, hemp has been re-discovered as an interesting sustainable (Amaducci et al., 2000; Struik et al., 2000; Van der Werf and Turunen, 2008) high yielding industrial fiber crop (Van der Werf et al., 1996) that can help to meet the high global demand for fibers (Shui and Plastina, 2013). Hemp stems can be divided in a 'bark' or 'bast' section, corresponding to the tissue located in the outer part of the stem outside the vascular cambium, and the 'woody core' which is located inside the ring of vascular cambium and consists of lignin rich xylem tissue. The primary bast fibers (elementary fiber about 20 mm. to 50 mm. long) and secondary bast fibers (about 2 mm long) are derived from the vascular bundles in the bark of stems whereas the core fibers are located inside of the vascular cambium in the woody core (0.5–0.6 mm long) (De Meijer and Keizer, 1994; De Meijer, 1994; Mediavilla et al., 2001; Van der Werf et al., 1994). The primary bast fibers of hemp are made up of bundles of pericyclic elementary fibers that are characterized by thick and lignified cell walls. They are composed of cellulose (∼55%), hemi-cellulose (∼16%), pectin (∼18%), and lignin (∼4%) (Garcia-Jaldon et al., 1998).

Although hemp has the potential to produce fiber of excellent quality, the cultivars currently available deliver fiber of variable quality. A better understanding of the key factors determining fiber quality, and how these can be regulated, is of crucial importance for breeding fiber hemp. This article provides an overview of the state of the art with regard to hemp breeding and the possibilities of novel molecular breeding approaches to increase the value of industrial hemp.

## 2. Genetic variation in hemp

Hemp (*C. sativa* L.) can be classified according to different attributes including: (i) population type such as wild and naturalized populations, landraces, and cultivars; (ii) plant-use as fiber cultivars (for long fibers or for pulp), seed cultivars, drug strains, and ornamentals (De Meijer, 1995; De Meijer and Keizer, 1996); (iii) flowering time, which includes early ripening, intermediate-ripening, late-ripening cultivars; (iv) gender, whether they are dioecious or monoecious cultivars, and (v) geographic origin, e.g. North-type and South-type cultivars in China. Hemp is believed to have originated in Central Asia, and several advocate for two centers of diversity, Hindustani and European–Siberian (Zeven and Zhukovsky, 1975). There is still debate over the taxonomic organization of the genus *Cannabis*. Some authors have proposed a monotypic genus, *C. sativa*, while others state that two species can be distinguished, *C. sativa* and *C. indica*, and maybe even a third species *C. ruderalis*. On the basis of allozyme data for 157 accessions from diverse geographic origin, Hillig (2005) recognized three gene pools, *C. sativa*, *C. indica* and *C. ruderalis* and suggested a polytypic concept (=having several variant forms, especially subspecies or cultivars) of Cannabis, with seven putative taxa. The characteristics attributed to such subspecies have generally evolved as a result of geographical distribution or isolation. Russian botanists recognized four 'eco-geographical' groups (ecotypes) of hemp: Northern (Northern Russian, Finland), Central ecotypes (Central Russian, Ukraine), Southern ecotypes (Mediterranean region, Balkan, Turkey, Caucasus), and Far Eastern ecotypes (China, Japan and Korea). The genus is now distributed worldwide from the equator to about 60° N latitude, and throughout much of the southern hemisphere (Hillig, 2005; Mukherjee et al., 2008 and references therein). In order to distinguish drug types from fiber types, Gilmore et al. (2007) used chloroplast and mitochondrial markers to study the phylogeny of a panel of 188 plants derived from 76 populations, representing plant-use groups and geographical regions. Despite the uni-parental (maternal) inheritance and low mutation rate in organelles, six minor haplotypes and three major haplotypes were recognized, that were highly suggestive for both crop-use and geographic region. Two organelle haplotypes (I and II) were observed in populations from Europe, mostly fiber types, whereas the other haplotypes were more indicative *C. indica* drug types from southern Europe and Asia and haplotypes V and VI for drug types from Africa, India, SE Asia, and Mexico. Central Russian and Mediterranean fiber hemp landraces and cross-progenies of these two groups are the ancestors of the present European and west Asian cultivars (De Meijer, 1995). Fiber strains from China (Far Eastern hemp) may be somewhat distinct. In an analysis of the genetic variation in ribosomal and cannabis specific chloroplast DNA regions, ancient DNA from an extinct Chinese hemp showed a close relationship to present day *C. sativa* hemp material from China as well as to *Humulus japonicus* (‘Japanese hop’), whereas *C. sativa* subspecies *indica* formed a separate clade (Mukherjee et al., 2008).

## 3. Hemp breeding in Europe, China, and Canada

### 3.1. Hemp breeding in Europe

The historical importance of hemp cultivation in Europe is well reflected by the abundance of cultivars, traditional landraces, and populations that were selected in the main areas of hemp cultivation throughout Europe. Mass selection was used in the past to select the most important cultivars, such as Carmagnola in Italy (Ranalli, 2004) or Novosasdka konoplia in Yugoslavia (Berenji et al., 2013). In mass selection pollination cannot be controlled and any improvement in fiber content is very slow. A large contribution to the increase of stem fiber content was obtained by the application of the Bredemann method (Bredemann, 1942), that consisted in the individual selection of male plants on the basis of the fiber content, measured on a longitudinal section of the stem.

A great breeding work carried out in Europe by the German researcher Von Segenbuch has yielded the first monoecious cultivars and the first hybrids bred by Professor Bocsa in Hungary had a high fiber content. In Hungary a Chinese accession has been used as a heterosis breeding parent (hybrid breeding) (for references see De Meijer, 1995). In 1995, De Meijer reported that 12 hemp cultivars were registered in the EU of which only seven French cultivars were readily available (De Meijer, 1995). In 2004, the number of registered hemp cultivars increased to 45 (Ranalli, 2004), in 2008 the list contained 46 industrial hemp cultivars (Jankauskienė and Gruzdevienė, 2009) and currently the number of cultivars registered for the EU is 51 (Table 1) reflecting the increased interest in the crop.

### 3.2. Hemp breeding in China

According to archeological finds and ancient records, it has been more than 6000 years since China started cultivating hemp for fiber and seed (Yang, 1991). Due to the long history of cultivation and the wide spread of this fiber crop throughout different geographic zones of climate in China (latitude range about 23–50° N), hundreds of hemp landraces have been established. Examples are ‘Liuan HuoMa’ and ‘Liuan HangMa’ from Anhui province, ‘Laiwu DaMa’ and ‘Laiyang DaMa’ from ShanDong province, ‘Gushi KuiMa’ in Henan province, ‘Wenxian DaBaiPi’ in Hebei province, ‘Liuzhi DaMa’ in Guizhou province, and ‘Dayao DaMa’ and ‘Weishan DaMa’ in Yunnan province. All these landraces are valuable resources of starting material for further hemp breeding and some of these are still being cultivated in certain areas nowadays. Chinese landraces were also used to breed the now extinct ‘Kentucky hemp’ cultivars (Dewey, 1913, 1927) that were cultivated in the United States until the mid-1950s, when the cultivation of hemp was prohibited. Large collections of germplasm resources have been collected and maintained in the Yunnan Academy of Agricultural Sciences, which comprise approximately 350 accessions with a good representation of fiber/seed hemp groups. In 1970s, several cultivars (e.g. 882, 812, 333, 112, 544, 370, and hybrid strain-2) were developed and, although rarely, some are still used in production now. From the 70s till the end of the 20th century limited research on hemp breeding was carried out. In the past decade, many new applications for hemp biomass have arisen and they have been accompanied with the development of related industries and an increase in hemp cultivation area in China. Since 2007, hemp breeding research has continuously received financial support from China Agriculture Research System, and five industrial hemp cultivars (YunMa 1, YunMa 2, YunMa 3, YunMa 4, YunMa 5) have been bred and widely cultivated in China. Other hemp cultivars (LongDaMa 1, JinMa 1, WangDaMa 1, WangDaMa 2) have been registered and used in certain provinces.

### 3.3. Hemp breeding in Canada

Hemp was banned in North America from the late 1930s until March 1998. Since the first commercial licenses for hemp growing were issued in 1998, the Canadian hemp market has gradually developed and Canada has become the main supplier of hemp seed and oil-cake for the United States (Hanks, 2008; Johnson, 2013). Hence, the Canadian hemp oil processing value chain is well established by comparison to the fiber processing chain. Government subsidies were granted to sustain the market, resulting in the development of novel hemp enterprises, improvements in the processing technologies, and development of new hemp cultivars for Canadian environments. To increase the interest for hemp, efforts were made to produce hemp as a certified bio-based crop and in 2012 a food safety accreditation for hemp food was issued to Hemp Oil Canada Inc. (Laate, 2012; Robbins et al., 2013).

Field production was dominated by the cultivars Finola (originating from Finland), Crag (Canada), and USO 14 (Ukrainian) for a long time. Several breeding programs included developing commercial strains from feral Canadian stocks, creating superior oilseed cultivars by increasing seed yield and optimizing fiber use for a variety of regions (Hanks, 2008). Together with the Bast Institute (Summy region, Ukraine) a program to evaluate hemp cultivars for Canada was started, and it included the Ukrainian cultivars USO 14, USO 31 Zolotonoshskaya 11 (Zolo 11), and two Canadian cultivars, Anka, and Carmen (Watson et al., 2012). Since then a number of high yielding cultivars suited to a wide range of growing conditions, including both monoecious and dioecious cultivars grown for seed, fiber or for dual purpose, have been developed and tested. The most common cultivars that are presently being contracted and grown in Canada are Alyssa, Anka, CRS-1, CFX-1, CFX-2, Delores, and Finola (Laate, 2012). The 2013 list of approved cultivars for Canada contains 39 cultivars, of which 24 are kept in Canada and the others are in European countries (http://hc-sc.gc.ca/hc-ps/pubs/precurs/list_cultivars-liste2013/index-eng.php). THC levels are not a problem since they can be kept low and well

**Table 1**
Hemp cultivars registered in the EU on 25-09-2013, 51 entries.

| | Denomination | Type | Maintainers | Country | Admission | Last modification | Deletion | Market extension |
|---|---|---|---|---|---|---|---|---|
| 1 | Armanca | | RO 1002 | RO | 24.11.2010 | | | |
| 2 | Asso | | IT 15 | IT | 23.02.2004 | | | |
| 3 | Beniko | | PL 893 | PL | 23.12.1985 | 16.02.2009 | | |
| | | | NL 613 | NL | | 04.11.2002 | | |
| | | | CH 168 | PL | 07.06.1999 | | 01.06.2009 | 01.06.2011 |
| | | | AT 567 | PL | | | | |
| 4 | Bialobrzeskie | | AT 567 | AT | | | | |
| | | | CZ 1067 | CZ | 31.10.2008 | | | |
| | | | PL 893 | PL | 31.12.1967 | 16.02.2009 | | |
| 5 | Cannakomp | | HU 149424 | HU | 19.02.2004 | 06.01.2012 | | |
| 6 | Carma | | IT 15 | IT | 16.01.2006 | | | |
| 7 | Carmagnola | | IT 15 | IT | | | | |
| 8 | Chamaeleon | | NL 391 | NL | 25.03.2002 | | | |
| 9 | Codimono | | IT 15 | IT | 23.02.2004 | | | |
| 10 | CS | | IT 15 | IT | | | | |
| 11 | Dacia Secuieni | | RO 1018 | RO | 19.12.2011 | | | |
| 12 | Delta-405 | | ES 275 | ES | | 18.01.2012 | | |
| 13 | Delta-llosa | | ES 275 | ES | | 18.01.2012 | | |
| 14 | Denise | | RO 1018 | RO | 25.10.2007 | | | |
| 15 | Diana | | RO 1018 | RO | 14.07.2009 | | | |
| 16 | Dioica 88 | | FR 8194 | FR | | | | |
| 17 | Epsilon 68 | | FR 8194 | FR | | | | |
| 18 | Fedora 17 | | FR 8194 | FR | | | | |
| | | | CH 170 | | 07.06.2002 | | 01.06.2012 | 01.06.2014 |
| 19 | Felina 32 | | FR 8194 | FR | | | | |
| 20 | Férimon | | FR 8194 | FR | | | | |
| 21 | Ferimon | | DE 4668 | DE, FR | | | | |
| 22 | Fibranova | | IT 15 | IT | | | | |
| 23 | Fibrimor | | IT 15 | IT | 17.10.2003 | | | |
| 24 | Fibrol | | HU 149424 | HU | 05.05.2006 | 06.01.2012 | | |
| 25 | Finola | | FI 6157 | FI | 05.02.2003 | | | |
| 26 | Futura 75 | | FR 8194 | FR | | | | |
| 27 | Ivory | | NL 722 | NL | 04.06.2012 | | | |
| 28 | KC Dora | | HU 149424 | HU | 10.03.2009; | 06.01.2012 | | |
| 29 | KC Virtus | | HU 149424 | HU | 12.03.2013 | | | |
| 30 | KC Zuzana | | HU 149424 | HU | 12.03.2013 | | | |
| 31 | Kompolti hibrid TC | Hybrid | HU 149424 | HU | 19.05.1983 | | | |
| 32 | Kompolti | | HU 151322 | HU | 25.04.1954 | 06.01.2012 | | |
| | | | AT 625 | AT | | | | 30.06.2010 |
| | | | CH 173 | CH | | | | 01.06.2010 |
| | | | NL 612 | NL | | 04.11.2002 | | |
| 33 | Lipko | | HU 151322 | HU | 19.02.2004 | | | |
| 34 | Lovrin 110 | | RO 1002 | RO | 22.11.2007 | | | |
| | | | CH 172 | CH | | | | 30.06.2011 |
| 35 | Marcello | | NL 722 | NL | 04.06.2012 | | | |
| 36 | Markant | | NL 722 | NL | 04.05.2012 | | | |
| 37 | Monoica | | HU 149424 | HU | 05.05.2006 | 06.01.2012 | | |
| | | | CZ 666 | CZ | 16.07.2009 | | | |
| 38 | Santhica 23 | | FR 8194 | FR | | | | |
| 39 | Santhica 27 | | FR 8194 | FR | 04.05.2002 | | | |
| 40 | Santhica 70 | | FR 8194 | FR | 30.03.2007 | | | |
| 41 | Secuieni Jubileu | | RO 1018 | RO | 29.02.2012 | | | |
| 42 | Silvana | | RO 1002 | RO | 22.11.2007 | | | |
| 43 | Szarvasi | | HU 108887 | HU | 12.03.2007 | 12.03.2007 | | |
| 44 | Tiborszállási | | HU 105303, | HU | 19.02.2004 | | | |
| | | | IT 1229 | IT | 16.01.2006 | | | |
| 45 | Tisza | | HU 105303 | HU | 10.03.2010 | | | |
| 46 | Tygra | | PL 893 | PL | 12.03.2007 | 16.02.2009 | | |
| 47 | Uniko B | Hybrid | HU 151322 | HU | 29.04.1965 | | | |
| | | | CH 173 | CH | 07.06.1998 | | 27.04.2009 | 01.06.2010 |
| 48 | Uso-31 | | NL 647 | NL | | 31.01.2005 | | |
| | | | CH174 | CH | 07.06.1999 | | 01.06.2009 | 30.06.2011 |
| 49 | Wielkopolskie | | PL 893 | PL | 06.03.2009 | | | |
| 50 | Wojko | | PL 893 | PL | 08.03.2011 | | | |
| 51 | Zenit | | RO 1018 | RO | 14.07.2009 | | | |

Market extension: Certification and marketing of seed of the variety is allowed until the indicated. http://ec.europa.eu/food/plant/propagation/catalogs/database/public/index.cfm?event=RunSearch.

below the Canadian standards of 0.3% of the dry weight. Breeding for stable production of either seed, fiber or for dual purpose in specific environments is the main target for breeding. Another important trait for Canada is increase in gamma linolenic acid (GLA) in the seed oil. This is a highly desirable essential fatty acid component and important for the (healthy) food market. Cultivars with high contents of this fatty acid in the oil have been obtained, e.g. Canda >3.5%, Debby >5%, and Joey >4%.

In 2013, a large decortication plant (parkland industrial hemp processing PIHP) opened in Gilbert Plains, Manitoba. In 2012 several hemp cultivars that may produce a high biomass with a high fiber yield for this new production plant were evaluated. Both mono- and dioecious cultivars were tested for grain and fiber yield, as well as for quality evaluation of oil profiles and percent fiber content. The cultivars were evaluated for their potential as grain-only, fiber-only or dual grain-fiber crop in environments across Canada (Watson et al., 2012).

## 4. Breeding methods in hemp

Methods for breeding hemp have also changed throughout the years (reviewed for Europe by De Meijer, 1995; Ranalli, 2004; Ranalli and Venturi, 2004).

Hemp is open pollinated (wind-pollinated) and is usually a dioecious annual crop, where female and male flowers are on different individuals, indicating that hemp is naturally outcrossing (cross-pollinator). All cannabis strains can inter-cross creating, in some cases, a continuous pattern of variation. In hemp the control of pollination is therefore an important issue. In the case of a dioecious hemp population the male and female plants are intermixed and the female plants are always cross-pollinated. Entirely female populations exist that can be used to produce hybrid cultivars by crossing with a selected pollen donor. In the case of monoecious hemp cultivars the male and female flowers are on the same individual, which enables selfing. The breeding of a cross-pollinator such as hemp requires a specific breeding approach (Posselt, 2010) that comprises three breeding phases: (1) search for the natural variation in the material and create a base population, (2) generate varietal parents through selection and improve the population through recurrent selection steps to create a breeding population, and (3) develop and test experimental cultivars. The hemp cultivars available are mainly population cultivars, such as ‘open pollinated cultivars’ that are the result of recurrent selection and ‘synthetic cultivars’ that are advanced generations of a population initiated by crosses among a restricted number of selected parents and multiplied by a number of random out-crossings in isolation.

The methods commonly used in hemp breeding are ‘mass selection’, ‘cross-breeding’, ‘inbreeding’, and ‘hybrid breeding’, and more recently there are a few examples of the use of molecular markers to assisted breeding (reviewed in Ranalli, 2004).

### 4.1. Mass selection

Mass selection is performed by selecting seeds after harvest (method I) and selecting seeds from the best plants in the field (method II). Initially selections from old naturalized (weedy) populations resulted in the establishment of landraces. In the case of fiber hemp mass selections or single plant selections of such landraces selections were performed to produce more genetically uniform breeding material, cultivars and ecotypes. For example, in the first quarter of the 20th century cultivars were selected from landraces using selection characteristics such as the length of the vegetative period length, height, diameter, weight, and in some cases seed weight. The predominant approach to improve the material was continuous mass selection, a cyclic procedure that attempted to upgrade whole populations by directed selection (Ranalli, 2004). This type of ‘family breeding’ led to the development of cultivars in Italy, Hungary and Romania, all of them coming from the famous Northern Italian landrace Carmagnola. In Italy, Carmagnola was further improved for specific regions by mass selection, leading to the development of ‘Bolognese’, ‘Toscana’, and ‘Ferrarese’ ecotypes (the names define the places of cultivation). In China some cultivars (e.g. YunMa 1 and YunMa 5) were bred by this method from the local landraces. Mass selection is a common breeding method in hemp for traits with high heritability (Hennink, 1994). One example of a high heritable trait is bast fiber content and mass selection proved to be efficient to breed for this parameter. Characteristics such as date of flowering, plant height, and stem diameter, which are not directly related to bast fiber yield, were shown to have disadvantages as selection criteria for the improvement of bast fiber yield (Hennink, 1994).

### 4.2. Cross-breeding

In later stages, more efficient crossing methods and selection criteria were employed. Dedicated crosses were made to breed for improved characteristics such as fiber content, stem and seed yield, gender, phenological development, stem quantity, low $\Delta$9-tetrahydrocannabinol (THC) content, resistance to pathogens (such as root-knot nematodes) and lodging, as well as suitability for different cultivation regions taking into account the effects of genotype and environment (GxE) on the yield and quality of hemp. 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 (GCA; the average performance of an individual in a particular series of test crosses), with the entire population used as a tester. Furthermore, hybridization (between cultivars) was performed to create new variability (Ranalli, 2004 and references therein). The cultivars YunMa 2 and YunMa 4 are good examples of the application of this method in China.

### 4.3. Inbreeding and hybrid breeding

Dioecious hemp populations also contain monoecious or intersexual plants. These populations were used to develop monoecious hemp. Monoecious plants and subdioecious plants (female plant with induced male flowers) enabled self-fertilization and inbreeding. Often the progeny of such plants is exclusively female, which enables heterosis and hybrid breeding. The unisexual female character can be considered an analog for male sterility and allows for large scale hybrid seed production with limited labor. Tetraploid forms of hemp were developed ($2n = 40$) that were completely fertile but showed reduced fiber quality.

Heterosis breeding of hemp has been used in Hungary and China and has resulted in several F1 hybrid cultivars, including Uniko-B, Kompolti hybrid TC, and YunMa 3. Uniko-B is a single cross hybrid (Kompolti (female) × Fibrimon 21 (male)). The F1 of this cross is almost unisexual female and is used to produce an F2 containing 30% males that is cultivated for fiber production. Kompolti hybrid TC is a three way-cross hybrid (1° Kinai dioecious, female × Kinai monoecious, male = Kinai uniszex, female; 2° Kinai uniszex (female) × Kompolti (male) = Kompolti hybrid TC, sex ratio 50/50). The Kinai material is of Chinese origin (Ranalli, 2004 and references therein). Finally the YunMa 3 from China is one of the F1 hybrid cultivars. Interestingly, the single cross hybrid can only be produced in winter in the low latitude region like Yunnan Province because its parents cannot cross in the normal growth season (Summer) due to distinctly different flowering times.

### 4.4. Marker assisted breeding

Next generation sequencing technologies opened the gate toward genotyping by sequencing (Elshire et al., 2011) and defining causal relationships between genetic polymorphism’s and phenotypic differences on a large scale. The expansion of the genetic and phenotypic data and the development of molecular markers are of inestimable value for plant-breeding. Genome wide genetic polymorphisms can be used to explore the genetic diversity of the available germplasm or breeding populations and genetic maps with markers linked to a trait of interest can assist the selection for complex traits. The history of marker-assisted selection in hemp was reviewed by Mandolino and Carboni (2004). Molecular markers have mostly been used to exploit hemp for forensic applications, to trace back the origin of illegal hemp material and recognize the presence of cannabis in materials. Five main conclusions have been made regarding the genetic structure of hemp: (1) It has a high genetic variation and a high level of heterozygosity due to open-pollination and the out-crossing character of hemp. The majority of the alleles occurred in low frequency in the population (<0.30) with only a few major alleles being observed in cultivars with a high level of inbreeding, such as cultivar Fibrimon; (2) it has a low discriminative power of marker loci to distinguish among cultivars or accessions; a high degree of variation within cultivars or accessions was observed, even in female inbred lines; (3) There is no clear split between drug types and fiber types; (4) Hemp has a widely shared gene pool, with limited cultivar boundaries and little segregation between populations; and finally, (5) The practical use of a genetic maps and molecular markers is limited in cannabis because of the high level of variability.

## 5. Breeding goals

Traits that are important in fiber hemp breeding comprise: high fiber yield and fiber quality, cannabinoid content and composition, degree of monoecy, length of vegetative cycle, and resistance to diseases and pests. A great deal of attention is given to high fiber yield and quality together with low THC content. Some traits show a high plasticity, especially cannabinoid content and phenological development. Because hemp is very sensitive to environmental conditions, such as day length and temperature, cultivars are typically developed for specific environments and cropping conditions. However, the ranking of cultivars for most traits in different environments are expected to be fairly stable. Furthermore, the suitability of certain cultivars to a given environment also depends on the purpose for which they are cultivated (Ranalli, 2004; Ranalli and Venturi, 2004).

### 5.1. *Fiber quality and fiber yield*

Fiber quality strongly depends on the morphology of the fiber bundles and on the chemical composition of the cell wall of the elementary fiber (Rowell et al., 2000). In polymer reinforcement or biocomposites, surface characteristics and finesses are important fiber traits that influence the interfacial bond strength between the fibers and the matrix (Gamelas, 2013), as well as the fiber tensile strength (Placet, 2009). The variability of natural fiber properties, moisture absorption, and processing costs are weak factors of natural fibers for composite applications (Deyholos and Potter, 2014). The variability of fiber characteristics is a consequence of the high heterogeneity of hemp cultivars, but it is also due to an unavoidable interplant heterogeneity. In fact, fiber maturity decreases from bottom to top of the stem (Amaducci et al., 2008b) and from the outer to the inner fiber layers in the same internode (Amaducci et al., 2005), and the fiber yield and quality changes with time during plant development; for instance cellulose increased up to 56–65% until late flowering. Furthermore, cultural techniques such as plant density, nitrogen fertilization, and harvest time are also important factors that affect fiber yield and quality (Amaducci et al., this issue).

A high cellulose content, a low degree of lignification and a reduced number of cross links between the pectins and the structural components of the cell wall are important characteristics for a suitable extractable fiber for both paper- and textile industries (Mandolino and Carboni, 2004). Fiber extraction by mechanical decortication and scutching has a strong influence on fiber quality and it is often the main cause of damage to the fiber properties (Hänninen et al., 2012). Breeding for genotypes that are easier to process would provide an advantage in terms of fiber quality and a reduction of costs related to fiber extraction (Van den Broeck et al., 2008). These objectives seem to be met by cultivars recently released in The Netherlands: Chamaeleon (Toonen et al., 2004), Markant, Ivory, and Marcello.

From studies in Arabidopsis and other model plants it is known that many genes are involved in cell wall related processes. Using microarrays a random set of hemp cDNAs ($n = 3414$ cDNAs) derived from fiber material was tested for the differential expression in between bast fiber tissue and core tissue (Van den Broeck et al., 2008) across developmental stages of two hemp cultivars, cv. Chamaeleon and cv. Felina 34. Hemp genes that were highly expressed in core tissue were coding for proteins involved in lignin biosynthesis and C1 metabolism, a process closely connected to lignin biosynthesis. Genes coding for arabinogalactan related proteins, lipid transfer proteins, lipoxygenase, and endoxyloglucan transferases were highly expressed in the bast fiber tissue (Van den Broeck et al., 2008).

### 5.2. *Cannabinoid profiles*

Breeding for low delta-9-tetrahydrocannabidol (THC) has been a main target in fiber hemp breeding and levels below <0.2% THC have been reached for some cultivars. Government regulations that allowed a THC content of only 0.2% were implemented in the European Union in 2001. Since then, a further and stable reduction of THC gained importance as a breeding goal. It was necessary to start the difficult task of further decreasing the THC content while keeping the productivity and other positive characters intact. In the former USSR a successful breeding program for the reduction of cannabinoids was initiated in the 1970s. Cultivars completely lacking THC were obtained (Hennink, 1994; Mandolino and Carboni, 2004). In a joint effort between scientists in France and Ukraine several new monoecious cultivars were developed. These cultivars have very low THC levels (THC < 0.07%) and lack the typical hemp aroma (e.g. USO-45) (Holoborodko et al., 2014 report at: www.interchanvre.com/docs/article-Laiko.pdf).

The distinction between fiber and drug accessions can only be made on the basis of the cannabinoid profile (chemotype). Three major 'chemotypes' are recognized in hemp based on the ratio in the inflorescence dry matter between the two major cannabinoids of hemp, delta-9-tetrahydrocannabidol (THC) and cannabidiol (CBD): (i) drugs type with THC prevalent, (ii) intermediate type with similar amounts of both THC and CBD, and (iii) fiber type with CBD prevalent. Two alleles at the B locus ($B_T$ and $B_D$) are controlling the trait (Fig. 2, De Meijer et al., 2003). The fourth and fifth chemotype are minor chemotypes that are not frequently found. In the fourth chemotype, cannabigerol (CBG), the precursor of THC and CBD is the major cannabinoid. This chemotype is most likely to be controlled by a $B_0$ allele, a mutant form of the $B_D$ locus (De Meijer and Hammond, 2005). The fifth chemotype has undetectable amounts of cannabinoids (zero cannabinoid). This trait is controlled by is single locus (O) that operates upstream of the B locus. This zero cannabinoid chemotype is most likely due to a block in the metabolic pathway leading to the production of cannabinoids and not to an alteration in the glandular trichomes (De Meijer et al., 2009a,b).

A sixth chemotype is found in plants with a specific morphological phenotype 'prolonged juvenile chemotype' that produce cannabichromene (CBC). The genetic factors controlling this trait (termed locus C or $B_C$) are independent from the B loci that encode THC- and CBD synthase (De Meijer et al., 2009a,b) (Fig. 2). Cannabis chemotypes having no cannabinoids, or only CBG or CBC are interesting because of their pharmaceutical value. CBC dominates the

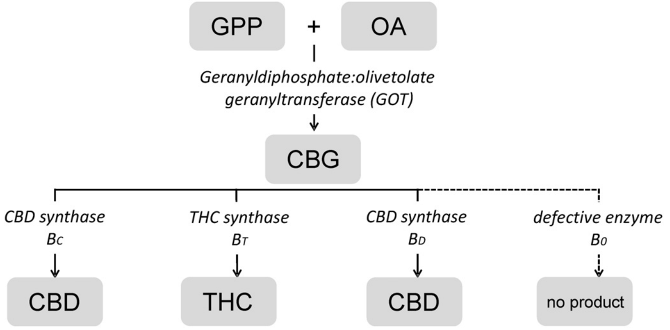

**Fig. 2.** The biosynthetic pathway of the most common cannabinoids in cannabis. Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabichromene (CBC). The first step in the cannabinoid biosynthesis pathway, the condensation of geranylgeraniol diphosphate (GPP) with olivetolic acid (OA) is catalyzed by the enzyme geranyldiphosphate:olivetolate geranyltransferase (GOT), leading to the production of (CBG). The next step is the action of THC-, and CBD-synthases that are converting CBG into THC ($B_T$ locus), CBD ($B_D$ locus), and in some cases CBC-synthase which catalyzes the conversion of CBG into the cannabinoid cannabichromene (CBC). $B_C$, $B_T$, $B_D$ and Bo are alleles of the B locus that account for the different chemotypes.

cannabinoid fraction of juvenile cannabis plants and declines with maturation.

Chemotype-associated molecular markers could well be used to assist the selection of THC producing plants for elimination. To define such markers a cross was made between inbred lines with contrasting chemotypes (THC x CBD). The F1 was almost entirely hybrid (CBD/THC; chemotype II), while the F2 segregated in a Mendelian way 1:2:1 for the chemotypes, THC [$B_T$,$B_T$]: CBD/THC [$B_D$,$B_T$]: CBD [$B_D$,$B_D$], which was in agreement with one gene with two codominant alleles ($B_T$ and $B_D$). The F2 population was used to identify RAPD markers that resulted in a SCAR marker (B190/B200) that was useful for chemotype identification in that particular population, but unfortunately was not linked to chemotype in other populations (Mandolino and Carboni, 2004). The genes for THC and CBD synthases have been isolated (1635 bp, 89.3% identity, deletion of a Serine coding triplet at position 757–759 in the nucleotide sequence for THC synthase) and allele specific primers have been developed that allow the identification of the allelic composition at the B locus (Mandolino and Carboni, 2004). Based on a genetic model with two codominant alleles $B_T$ and $B_D$ on the same locus, drug types are homozygous for BT (BT/BT) and expected to harbor only the THC synthase. With the publication of the draft genome of the drug strain ‘Purple Kush’ in 2011 (Van Bakel et al., 2011) analysis of the genes involved in cannabinoids biosynthesis was facilitates. In this hemp genome only a single THC synthase gene (*THCAS*) with a full open reading frame was identified (with 99% nucleotide identity to the published sequence PK29242.1, genbank: JP450547) whereas no intact CBD synthase gene (*CBDAS*) was found. However, the genomic organization of the locus is more complex because pseudogenes with pre-mature stop codons and frame-shift mutations (*THCAS-like* pseudogenes and *CBDAS-like* pseudogenes, genbank) for both genes were observed indicating that the cannabinoid synthase genes are part of a small gene family (Van Bakel et al., 2011). In a study based on chemotype and genotype of cannabinoids in hemp landrace, Chen et al. (2013) speculated that there is probably another ($B_T$/$B_T$)-like locus involved. RNAseq analysis (Van Bakel et al., 2011) of the hemp cultivars ‘Finola’ (fiber type), Purple Kush (drug type), and ‘USO-31’ (fiber type) showed that besides cannabinoid synthase other genes involved in the cannabinoid pathway were up-regulated in the drug type Purple Kush and that the differences between drugs and fiber strains are not only due to changes in the coding DNA-sequence of the genes themselves but are either due to cis-or trans-regulatory transcription factors that regulate the expression from the cannabinoid genes. For instance, CBD-synthase [genbank: AB292682] is predominantly expressed in Finola ($B_T$:$B_D$) (Van Bakel et al., 2011).

### 5.3. Flowering behavior

Hemp, *C. sativa* L., is a dioecious annual with separate male and female plants, and occasionally monoecious individuals can be found within a population. As a “short day” plant hemp has a critical photoperiod, under which flowering is induced, at approximately 14 h (Amaducci et al., 2008a, 2012; Lisson et al., 2000). Variation for the timing of the transition from vegetative to reproductive growth (=flowering time) is present among cultivars and heterogeneity in hemp stands is partly due to the differences in flowering time and the dioecious nature of hemp (Amaducci et al., 2008c). These characteristics of hemp are reviewed in Hall et al. (2012). Three developmental stages are recognized in hemp; the juvenile-, photosensitive-, and flowering-stage. Based on the flowering time, three main groups of genotypes can be distinguished; early (flowering after 40–60 days), intermediate (60–90 days), and late (90–120 days) cultivars (Zatta et al., 2012). Early and intermediate groups are selected under Northern conditions (short growing seasons with long day-lengths in the summer). If grown under Southern conditions such cultivars start flowering even earlier as the critical day-length for hemp flowering in the South comes earlier than in the North and the biomass yield is therefore lower. On the contrary, late ripening cultivars should be selected in the low latitude regions in order to get high fiber yield (e.g. the south of China, Thailand, Australia, Southern Europe). The fiber yield can increase if the cultivar from a low latitude location is grown in a higher latitude location. This effect of increase in yield becomes obvious when the value of latitude change is above 2 degrees (Guo et al., 2013). There are differences among hemp cultivars for sensitivity to changes in photoperiod: ‘Felina 34’ and ‘Futura’, and Chinese landrace ‘Huoqiuzi’ are regarded low sensitive, whereas ‘Tiborszallasi’ and most Chinese landraces are very sensitive. The Italian landrace ‘Carmagnola’ and the cross-bred variety ‘Fibranova’ are intermediate sensitive. The flowering behavior is, besides the length of the photoperiod, depending on temperature and light quality. Amaducci et al. (2008a, 2008b, 2012) generated a model to estimate the flowering time of hemp that is useful to make decisions on the sowing time and harvesting time and cultivar choice. The basis for a good fiber quality and yield is the use of a cultivar in combination with good environmental conditions and crop management (Struik et al., 2000) whereby each application also has its own demand on fiber characteristics and processing of fibers.

### 5.4. Control of gender (male flowering control)

Monoecious hemp cultivars have a higher seed yield and higher uniformity compared to dioecious cultivars and therefore mechanical harvesting of such cultivars is easier. Drawbacks are the narrower genetic base, necessity to maintain the monoecious trait (including the selfing of a monoecious plant and elimination of male plants), strict isolation of propagations and seed batch control for male plants. In dioecious cultivars, selection of males before pollination and pollination only with the best-scoring male is a common practice in breeding. Selection for sex is therefore important in hemp breeding. The determination of the gender in *C. sativa* L. is influenced by both genetic and environmental factors. In cannabis, two sex chromosomes, X and Y are present, whereby the Y chromosome is much larger than X chromosomes and autosomes. True male plants have one X and one Y chromosome, females have two X chromosomes resulting in a difference in genome size between male and female plants as determined by flow cytometry (respectively 1683 Mbp and 1636 Mbp for the diploid genome). The long arm of the Y chromosome differed from the other chromosomal regions by showing early condensation at the metaphase stage (Sakamoto et al., 1998). However, as is the case in many other plant species, in *C. sativa* L., determination of gender is not only controlled by sex-chromosomes (Van der Werf and Van den Berg, 1995) and can be altered by chemicals such as growth regulating hormones or silver thiosulfate; Gibberellic acid induces male characters while auxins, ethylene and cytokines induce femaleness, silver thiosulfate promotes male flowers on female (XX) plants and is a useful tool for producing seeds that give rise to only female plants (Ram and Sett, 1982; Kaushal, 2012). In dioecious hemp cultivars differences in growth rate and development between male and female plants are evident whereby the male plants tend to flower and senesce earlier (Meijer et al., 1995; Struik et al., 2000). This generates variation in the crop and also the competition between plants may lead to the suppression of smaller ones which may result in self-thinning of the field (Van der Werf et al., 1995). As such, compounds that control gender are useful tools to achieve uni-sexuality and uniformity in hemp (Hall et al., 2012; Ram and Sett, 1982; Kaushal, 2012).

Several markers for maleness have been identified. SCAR markers fragments were present in both female, male and monoecious plants but a single band was specific for male plants. These are thus not the primers themselves but feature in the region amplified that is male specific. A specific marker (SCAR, OPA08; developed on the MADC2 region; 391 bp fragment in male plants and two larger fragments in females and monoecious plants) allows the safe identification of male plants in dioecious and monoecious cultivars at all developmental stages with a quick and easy, direct PCR method (Mandolino and Carboni, 2004 and references therein). Three other male specific markers were developed on the MADC5, MADC6, and MADC400-S208 loci (Törjék et al., 2002; Li et al., 2012). A marker for the monoecious trait is still required to fully characterize the sexuality in hemp. Obstacles are the environmental influences altering the expression of male flowers in monoecious plants that can change the female:monoecious rate.

### *5.5. Hemp cultivars for specific environments and end-uses*

Given the strong influence of the environment on hemp biomass yield and quality, hemp cultivars were developed for specific environments and end-uses. As result of the efforts in hemp breeding specific cultivars were designed for cultivation in Italy (Ranalli, 2004), France, Hungary, Poland, Romania, Bulgaria, Russia (former USSR), former Yugoslavia, Spain, former Czechoslovakia, Germany, The Netherlands, Finland (De Meijer, 1995; Ranalli, 2004), Canada (Watson et al., 2012), and China. For example, Ukrainian cultivars and French cultivars differ in the length of their vegetative period. For seed production, flowering, and seed ripening is required. Therefore early ripening cultivars are more suitable for seed production in Northern Europe, where the growing season is short, and late ripening cultivars are suitable for the same use in the South of Europe. As the fiber formation finishes already a month before ripening, late cultivars are often grown in Northern regions, for the production of stem and high quality fibers. In the Northern regions late flowering cultivars have a prolonged vegetative phase and a higher stem yield, in situations where flowering and seed ripening is not required. The harvest can be performed at different developmental stages depending on the use.

Hemp is a promising phytoremediation crop for clean-up of heavy metal pollutions, different genotypes demonstrate distinct differences in their abilities to tolerate and accumulate heavy metals (Zeng et al., 2013). Besides, different hemp cultivars have variable tolerance abilities under low temperature, drought and saline stress, and resistance for lodging and diseases (Guo et al., 2010, 2011).

Currently, hemp-based products are increasingly being diversified and developed on industry-specific goals, which is a striking feature for this crop. Breeding of cultivars for specific end-uses will become critical. For making functional foods, the seed yield, nutrient composition and content will be the most important targets of breeding. For the oil-type cultivar breeding, the major objectives include high seed yield and the content and composition in fatty acids. For paper making, both bast fiber and woody core can be used but the quality of paper made from the bast fiber is higher. Therefore, breeding for paper production has primarily aimed at improved bast fiber production (Hennink, 1994). The recent increasing demand in the market for cannabinoid-CBD will result in a rapid growth of production for medicine-type hemp (high CBD content but less than 0.2% THC in Europe Union or 0.3% in other countries). Thus new cultivars need to be developed. In 2013, about 800 ha hemp with cultivation licenses were grown for fiber and by-product CBD in Yunnan province of China, which greatly benefitted the hemp farmers economically. With this trend, two new industrial hemp cultivars with high CBD content are expected to be registered and released by Yunnan Academy of Agricultural Sciences by the end of 2015.

Studies were done on the improvement of hemp for specific industries, such as textile (Amaducci, 2003, 2005), papermaking (Italian paper and pulp organization), biobuilding compounds, and plywood. Such initiatives paved the way for further industry-driven studies and the selection of new genotypes for specific uses (Zatta et al., 2012). For example, new genotypes especially for textile uses (Toonen et al., 2004; Di Candilo et al., 2010).

## 6. Perspectives of ‘Genomics’ for hemp breeding

In 2011 the result of the first genome sequence of *C. sativa* was published (Van Bakel et al., 2011). The size of the haploid hemp genome is 818 Mbp (female) to 843 Mbp (male). A major part of the hemp genome, 534 Mb, encompassing the vast majority of the non-repetitive genome and the individual genes was sequenced and an assembled draft genome of hemp became available including DNA sequences of three cultivars, the drug type Purple Kush (clonal propagated) and the industrial types Finola and USO-31. The genomic sequences from Finola, Purple Kush, and USO-31 were compared and elucidated many SNPs that supported the separation of drug strains from the fiber types. Whole genome re-sequencing and genome comparisons can provide information about the amount of genetic variation that is present in the hemp germplasm. A physical map of the hemp genome saturated with DNA polymorphisms will provide targets for the development of molecular markers for breeding. In hemp however, due to the high level of genetic variation within and among accessions, the high number of minor alleles and the plasticity of traits in different environments, such genomics studies require a specific approach in which the effect of the environment on the phenotype is included, and in which a correction is made for variation in the genetic background of populations (false positive correlations in specific populations). In the ‘Multihemp’ project (www.multihemp.eu) a large mapping panel including wild material, landraces, and cultivars are being genotyped by whole genome re-sequencing. Upon phenotyping of the mapping panel in three different environments, genome wide association mapping (GWAS; reviewed in Korte and Farlow, 2013) is being performed to provide information about important alleles, quantitative trait loci, and linked DNA polymorphisms in the underlying genes. Next generation sequencing technologies increase the possibilities of mutation detection by facilitating the screening of large numbers of plants for rare, induced or natural genetic variation in specific target genes known to be involved in important traits. The targeted mutations eliminate or alter the functionality of genes and selected mutant genotypes are directly useful as breeding material (Comai et al., 2004; Elshire et al., 2011; McCallum et al., 2000; Metzker, 2010).

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