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Recent Progress in Cellular, Biochemical and Genetic Events of *Brassica* Species

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ABSTRACT

Brassica species are playing an even more important role in global agriculture and horticulture. To understand the regulation and evolution of *Brassica* species, the Multinational *Brassica* Genome Project (MBGP) was initiated and various techniques have been developed to characterize the behaviour of the underlying genes, genomic regulatory networks and associated metabolism. By using cDNA library screening and Rapid Amplification of cDNA Ends (RACE), many functional genes related to metabolism have been identified. cDNA microarrays were applied to determine gene expression profiles. Molecular markers were used in marker-assisted selection and breeding, and transformation technology was applied to the introduction of desirable traits. Moreover, quantitative trait loci (QTL) have been identified for a wide range of morphological, physiological and crop traits in the different *Brassica* crop types. Many systems and technologies have also been applied to study self-incompatibility and Cytoplasmic male sterility (CMS) in *Brassica* species. With the development of molecular biology, more and more knowledge at the level of gene expression, biochemistry, metabolism and physiology has been gained in *Brassica* species. Furthermore, as one of the most commonly grown and widely adapted crops *Brassica* species have been used as vegetables. We present a techniques review on cellular, biochemical and genetic events in *Brassica* species.

Keywords: *Brassica rapa*, CMS, metabolism, microarray, molecular marker, QTL
Abbreviations: AFLP, Amplified fragment length polymorphism; **CMS**, Cytoplasmic male sterility; **EMS**, ecotypic male sterility; **ISSR**, intersimple sequence repeat; **GMS**, genic male sterility; **MBGP**, Multinational *Brassica* Genome Project; **QTL**, quantitative trait loci; **RACE**, Rapid Amplification of cDNA Ends; **RAPD**, random amplified polymorphic DNA; **RFLP**, restriction fragment length polymorphisms; **SAGE**, Serial Analysis of Gene Expression; **SI**, self-incompatibility; **SLG**, S locus glycoprotein; **SRK**, S locus receptor kinase

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INTRODUCTION

In the *Brassicaceae* family *Brassica* is the most economically important genus and plays an important role in global agriculture and horticulture. Many species are important oilseed crops and vegetables which related to the lives of people everyday. Because *Brassica* crops contribute both to the economics and health of populations around the world, and some of which contain a large number of novel phytochemicals that could even protect against carcinogenesis, most research has been performed on brassicas to enhance their quality.

Chromosome rearrangements, including fusions and/or fissions, resulted in the present-day chromosome number variation for the three diploid *Brassica* species, *B. rapa* (syn. *campestris*, AA, 2n = 20), *B. nigra* (BB, 2n = 16) and *B. oleracea* (CC, 2n = 18) (Gale and Devos 1998; Lysak *et al*. 2005). The genomes of three allotetraploids, *B. juncea* (AABB, 2n = 36), *B. napus* (AACC, 2n = 38), and *B. carinata* (BBCC, 2n = 34), were derived by spontaneous hybridization among the three diploid species, followed by chromosome doubling (U 1935). The genomic relationships are well characterized, as shown in the 'triangle of U', and these have been exploited to understand the basis of chromosome evolution since divergence from a common progenitor shared with *Arabidopsis*.

The genome size of different *Brassica* species is different. *B. rapa* is the smallest at 529 Mb per haploid, compared with 696 Mb in *B. oleracea* and 632 Mb in *B. nigra* (Johnston *et al*. 2005).The haploid genome equivalent of *B. rapa* is closely related with *Arabidopsis* (125 Mb haploid genome equivalent), the completely sequenced model plant. *Arabidopsis* and *Brassica* are speciated around 14.5-20.4 million years ago from a common ancestor (Bowers *et al*. 2003). Comparative genetic mapping has revealed collinear chromosome segments (Schmidt *et al*. 2001) in the *Brassicacea* family and conserved linkage arrangements between *Arabidopsis* and *Brassica*. Comparative genetic mapping has revealed collinear chromosome segments in the *Brassicaceae* family and conserved linkage arrangements between

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Arabidopsis and *Brassica* (Lukens *et al*. 2003; Town *et al*. 2006).

Genomic regulatory networks and associated metabolism research, which mainly aims to understand the structure and the function of genomes and genes in the target organism, has attracted the attention of the global scientific community. *Brassica* species are especially important in this context as they offer a wealth of opportunities to exploit the inherent variation in genes for their use in crop improvement.

THE MULTINATIONAL *BRASSICA* GENOME PROJECT (MBGP)

Although the physiology and developmental biology of *Brassica* and *Arabidopsis* are very similar, the genomes of *Brassica* species are 4-10 times larger and more complex than that of *Arabidopsis thaliana* because of extensive polyploidization of the genome during the course of evolution. Despite the advantages of using information from *Arabidopsis*, and using reference linkage maps both from *Arabidopsis* and *Brassica*, the current challenge remains the need to identify key genes and understand their regulation in *Brassica* crop plants. There is a pressing requirement recently to resolve identified functional loci (major genes and QTLs). Of the cultivated *Brassica* species, *B*. *rapa* ssp. *Pekinensis* is considered as a typical representative of the *Brassica* A-genome. Because of its smallest genome (529 Mb) among the diploid *Brassicas* (Johnston *et al*. 2005) and availability of genomic resources such as mapping population and BAC libraries, this species has attracted the attention of *Brassica* workers world-wide. Then the Multinational *Brassica* Genome Project (MBGP) was initiated. The results from this project are available through a web interface to a *Brassica* database at http://www.brassica.info. In recent years, bioinformatics tools and computational tools have been developed to study DNA sequences of *Brassica* species deposited in the GenBank (Beckett *et al*. 2005; Lim *et al*. 2006; Trick *et al*. 2007). And with the data of genomic information increasing, more and more knowledge at the level of gene expression, biochemistry, metabolism and physiology will be gained.

CLONING OF FUNCTIONAL GENE AND GENE EXPRESSION PROFILES

To study the molecular mechanism of genotype of *Brassica* species, cloning of functional genes is necessary. A variety of strategies can be used to clone a gene such as transposon tagging, screening of a cDNA library, map-based gene cloning, RT-PCR and Rapid Amplification of cDNA Ends (RACE) PCR. Transposon tagging requires that the organism must have well-characterized transposon system such as maize. And so it is not feasible in *Brassica* species. A partial cDNA clone encoding a B-class gene, BnAP3 (*Brassica napus* APETALA3), was isolated from a *B*. *napus* cDNA library (Pylatuik *et al*. 2003) with a probe derived from *A*. *thaliana* AP3. Map-based gene cloning includes target gene mapping, physical mapping, chromosome walking or landing, gene identification four steps. In addition, restriction fragment length polymorphisms (RFLP) or other molecular genetic markers can be used in chromosome walking procedures. PCR is the simple way to clone a gene. In this way, a pair of degenerate primers was designed according to the sequence of GenBank and RT-PCR was conducted on the cDNA template synthesized with RNA of *Brassica* plants. Subsequently, the full-length cDNA sequence of some genes was obtained by RACE technique. Gene expression profiles can be obtained and compared by various methods, such as RNA-DNA hybridization measurements (Northern hybridization), subtractive hybridization, differential display, DNA microarray technique and Serial Analysis of Gene Expression (SAGE). The DNA microarray technique allows large-scale quantitative gene expression analysis. And SAGE allows simultaneous, comparative, and quantitative analysis of gene-specific, 9- to 10-bp sequence tags

Table 1 Genes of *Brassica* species identified by strategies of cloning.

Gene name and function	Strategies of cloning	*Brassica* species	Reference
Genes involved in starch and sucrose, nitrogen metabolism and nitrobenzene degradation	Subtractive PCR and cDNA Microarray	*B. napus* L.	Wu *et al.* 2007
Pathogenesis-related ESTs	Differential Display PCR	*B. oleracea*	Casimiro *et al*. 2006
Cadmium-responsive genes	Fluorescent differential display PCR	*B. juncea* L.	Lang *et al.* 2005
Genes associated with shoot regeneration	Differential Display PCR	*B. juncea*	Gong *et al.* 2004
Fatty Acid Elongase 1 (*FAE 1*) gene	Homolog PCR	*B. campestris*, *B. oleracea*	Das *et al.* 2002
BcMF2, Brassica campestris Male Fertile 2 gene	cDNA-AFLP and RACE	*B. campestris*	Wang *et al.* 2005b
Different expression genes in the CMS line and *B. napus*	cDNA Microarray	*B. napus*	Carlssonl *et al.* 2007
Expressed sequence tags (EST)	SSR-PCR	*B. napus*	Batley *et al.* 2007
The glutathione transporter, *BjGT1*	PCR and RACE	*B. juncea*	Bogs *et al.* 2003
Chaperones, ribosomal or stress-related genes	Screening suppression subtractive hybridization (SSH) library and RACE	*B. rapa*	Bulman *et al.* 2006
Quantitative Trait Loci (tf1,tf2 and tf3) related transformation efficiency	RFLP markers	*B. oleracea*	Cogan *et al.* 2004
QTLs for clubroot resistance	Sequence-characterized amplified region (SCAR) markers	*B. oleracea*	Nomura *et al.* 2005
KCT_2, the gene encoding a membrane-bound protein potassium channel	RACE-PCR	*B. rapa* ssp. *Pekinensis*	Zhang *et al.* 2006
Glutathione reductase gene	Screening cDNA library	*B. campestris*	Yoon *et al.* 2005
BNST4, the gene encoding steroid sulfotransferase	Screening cDNA library	*B. napus*	Marsolais *et al.* 2004
BNCBFs, CBF/Dreb transcriptional activator genes, controlling the expression of cold-induced genes	Screening cDNA library	*B. napus*	Gao *et al.* 2002
Transcript derived fragments (TDFs) related to wound response	cDNA-AFLP	*B. napus*	Sarosh and Meijer 2007
Cytochrome P450 *CYP86MF* gene related to male fertility	Differential Display PCR and RACE	*B. campestris*	Cao *et al.* 2006
Xyloglucan endotransglucosylase/hydrolases (XTHs) associated with cell expansion	Screening cDNA library	*B. campestris*	Shin *et al.* 2006
Androgenesis and embryo development-related ESTs	Subtractive hybridization and differential screening	*B. napus*	Tsuwamoto *et al.* 2007
Pis 30, a pistil-specific proline-rich proteins (PRP) gene	Screening cDNA library and RACE	*B. napus*	Foster *et al.* 2005
A multifunctional polyketide synthase gene	RNA fingerprinting by arbitrarily primed PCR (RAP)	*Plasmodiophora brassicae*	Ito *et al.* 1999
Light responsive genes	cDNA Microarray	*B. rapa*	Zhou *et al.* 2007

(Velculescu *et al*. 1995). By these ways, more and more functional genes of *Brassica* species have been cloned and characterized (**Table 1**).

BRASSICA MOLECULAR MARKERS AND BREEDING

In recent years, Molecular marker-assisted breeding has been widely used to map agronomically important genes in *Brassica* genomes. It is performed directly on genotypes rather than phenotypes. Molecular marker tagging has been reported to select some important agronomic traits and to analyze genetic diversity in *Brassica* crops (Chen *et al*. 1997). And molecular markers have their advantages in breeding including numerous markers, a large number of alleles, codominant mode of inheritance and determining genotypes at early developmental stage.

Actually, 'Markers' are polymorphisms in the nucleotide sequence at homologous (allelic) sites. They can be used to define a multilocus genotype characteristic for an individual or a clone and to be diagnostic for a population or a species. Among using molecular markers in plant genetic studies and breeding programs, RFLPs reflect differences in the length of restriction fragments obtained by restriction enzymes digestion, RAPD (random amplified polymorphic DNA) reflect different length of fragments obtained by PCR. And AFLP (Amplified fragment length polymorphism) combines the advantages of RFLP and RAPD system. It has the advantage of speedy and high resolution, and also requires only a small amount of purified DNA. *Brassica* (including some *Sicilian* populations) have been tested using RFLP and RAPD markers (Lanner-Herrera *et al*. 1997; Lázaro and Aguinagalde 1998). For example, RAPD and ISSR (intersimple sequence repeat) techniques were investigated for the development of molecular markers for genes controlling oleic and/or linolenic acid in spring oilseed rape (*Brassica napus*) (Javidfar *et al*. 2006). Ten RAPD and AFLP markers have been identified to link to the seed colour gene in rapeseed (*B*. *napus* L.). Seven of these were linked to the allele for yellow seed, whereas the other three were linked to the allele for black seed (Liu *et al*. 2005). A RAPD marker linked to the GMS (genic male sterility) gene in *B*. *napus* L. was identified and converted into a SCAR marker (Wang *et al*. 2007a).

For numerous qualitative traits, the development of marker-assisted selection strategies have accelerated the breeding of *Brassica* and led to many responsible genes be cloned. For quantitative traits, however, it has become apparent that traditional mapping of quantitative trait loci is often not sufficient to develop effective markers for trait introgression or for identification of the genes responsible major role in *Brassica* breeding because of the genetic distances between markers flanking a QTL are physically very large. QTL analysis involves selecting and hybridizing parental lines of which are different in quantitative traits and analysing the segregating progeny so as to link the quantitative trait locus to known DNA markers. It not only provides DNA markers for efficient selection, it is also of value in resolving interacting environmental and genetic effects which are common in agronomically important traits such as 'days to flowering', 'stay-green' or tolerance to abiotic stresses.

In *Brassica*, several genetic systems have been developed for the commercial production of hybrid seed, such as cytoplasmic male sterility (CMS), (GMS), self-incompatibility (SI) and ecotypic male sterility (EMS). They are the four main pollination control systems used in China.

Cytopalsmic male sterility (CMS) is a maternally inherited trait whose determinants are located in the mitochondrial (mt) genome. Nuclear genes, termed as fertility restorer (Rf) genes, can restore male fertility by selectively modulating the expression of CMS-associated regions without influencing the other mitochondrial genes. CMS is encoded in mitochondrial DNA and it may result from spontaneous mutations or through substitution of the crop nucleus into the alien cytoplasm, the alloplasmic lines. Cytoplasm from eight wild species, namely *Brassica oxyrrhina* (oxy), *Trachystoma balli* (trachy), *Moricandia arvensis* (mori), *Diplotaxis catholica* (cath), *D*. *siifolia* (sii), *D*. *erucoides* (eru), *Erucastrum canariense* (can) and *Raphanus sativus* (ogu), has been introgressed into *B*. *juncea* using sexual or somatic hybridization (Prakash 2001). In nature, CMS occurs as alloplasmic incompatibility or mitochondrial DNA rearrangements. It has been proposed that such changes lead to the production of defective forms of the essential proteins in mitochondria, thus causing male sterility. CMS occurs infrequently through spontaneous mutations in the mt genome. More often, however, CMS is encountered in alloplasmics derived from interspecific or higher order hybrids (Hanson 1991). Nine mitochondrial genes (*coxII*, *coxIII*, *atpA*, *atp6*, *atp9*, *cob*, *nad3*, *nad6*, and *nad9*) were isolated. The sources and characteristics of these fragments have been reported previously (Yamasaki *et al*. 1998). CMS in alloplasmic *B*. *juncea* carrying *Diplotaxis catholica* cytoplasm exhibited a crooked style and trilocular ovary, and the flowers had smaller nectaries, and anthers were converted into petals or tubular structures (Pathania *et al*. 2003). They found that *atpA* governs fertility restoration. Moreover, AFLP markers linked to genes responsible for fertility restoration have been identified to facilitate restorer breeding for the *tour* CMS system in *B*. *napus* which was developed through the transfer of male-sterile cytoplasm from *B*. *juncea* (Janeja *et al*. 2003).

The CMS system can generate a complete male sterile population economically. However, this system involves the development of three lines: male sterile line (A), maintainer (B), and restorer (R), and it usually takes years to develop A and R lines since most CMS systems have stringent restoring-maintaining relationships. Moreover, many CMS systems have disadvantages such as (1) undesirable pleiotropic effects of the CMS cytoplasm on agronomic quality, (2) increased disease susceptibility, (3) environmentally unstable maintenance of male sterility, and (4) poor floral characteristics of male sterile plants which lead to decreased quantity of hybrid seed (McVetty 1997). On the contrary, some genic male sterility (GMS) systems which can result from mutations in any one of a large number of nuclear genes involved in pollen and/or anther development have great potential in heterosis utilization, with complete and stable male sterility, no negative cytoplasmic effects associated with the CMS system, and easy transference of the male sterility genes to diverse genetic backgrounds. Several kinds of GMS have been used in the breeding of rapeseed hybrids, such as a dominant genic male sterility line (DGMS) and recessive genic male sterility lines (RGMS). DGMS is seldom used in hybrid seed production because of the difficulties in maintaining the sterile lines and finding restorers. The report about this system is few, only dominant epistatic GMS type report can be obtained up to now (Li *et al*. 1990). RGMS attracted the attention of rapeseed breeders because of the wide availability of restorers and the possibility to develop maintainers for this system. Four AFLP markers were found to be tightly linked to the *gms* gene of Chinese cabbage (*Brassica campestris* ssp. *chinensis*) which decided genic male sterility (Ying *et al*. 2003).

An ecotypical male fertile-sterile line AB1 was selected out from the progeny of a cross between two *B*. *napus* lines. AB1 was male fertile when it was sown at Wuhan in Autumn, but it became sterile when it was sown at Kunming or Xining in Summer (Yang *et al*. 1999). They found that light period had probably no considerable influence on the fertility of AB1 and its hybrid, and temperature was the main environmental factor influencing the fertility of AB1 and its hybrid, the higher the temperature, the complete the male sterility. They also suggested that the influence of temperature on the fertility be related to the genotypes or the number of the temperature sensitive genes in the nuclei of the materials. In some range, the more the number of the temperature sensitive genes, the weaker the influence of the temperature (Yang *et al*. 1999).

Self-incompatibility in which pollen-tube growth of self-pollen is inhibited after a self-recognition reaction between the pollen and the pistil is controlled genetically by multiple alleles of the *S* locus in *Brassica* and the pollen phenotype is determined by the parental genotype (Dodds *et al*. 1996). Several S locus genes have been isolated such as S locus glycoprotein (SLG) gene and S locus receptor kinase (SRK) gene which are required for the SI phenotype. It is reported that the recognition specificities of the pollen and the stigma are determined by S-locus protein 11/S-locus cysteine-rich (SP11/SCR) (Schopfer *et al*. 1999; Suzuki *et al*. 1999) and S-locus receptor kinase (SRK). SLG and SRK which function as the female determinant of SI are exclusively expressed in pistil, whereas SP11/SCR of the male determinant is expressed in pollen (Takasaki *et al*. 2000). According to the similarity of nucleotide sequences, they can be divided into two classes. Class I exhibits strong self-incompatibility, while class II is relatively weak. Genetically, class I is pollen dominant over class II (Nasrallah and Nasrallah 1993; Hatakeyama *et al*. 1998). Gene conversion from SLG to SRK would result in self-compatibility in *B. rapa* (Fujimoto *et al*. 2006). The genotypes with class II alleles showed great variation in compatibility, from highly self-incompatible to fully self-compatible (Ruffio-Chable *et al*. 1997). During selecting self-incompatible lines, the class II alleles should be avoided, because it will lead to a highly frequent contamination of selfed seeds in F1 hybrids. The model for SI in *Brassica* proposes that the pollen-borne ligand interacts with SLG and SRK, activates the SRK protein kinase and triggers a signal cascade that leads to the rejection of self-pollen (Nasrallah *et al*. 1994; Watanabe *et al*. 2006). Presently, seventy-two genotypes of five crops of *Brassica* vegetables including Chinese cabbage (*B. rapa* ssp. *pekinensis* L.), purple flowering stalk (*B. rapa* ssp. *chinensis* var. *purpurea* Hort.), cauliflower (*B. oleracea* var. *botrytis* L.), cabbage (*B. oleracea* var. *capitata* L.), mustard (*B. juncea* L.) have been used to investigate the diversity and distribution of S haplotypes by PCR-RFLP comparing *SLG* and *SRK* (Wang *et al*. 2007b). It was found that S haplotypes were more diverse and they exhibited obvious regional distribution except cabbage and purple flowering stalk in which most genotypes from different regions carried the common S haplotype, Self-incompatibility is a natural mechanism in plants that prevents inbreeding and promotes outcrossing.

GENETIC TRANSFORMATION

There are several techniques for the introduction of recombinant vectors containing heterologous genes of interest into plant cells, and the subsequent regeneration of plants from such cells. The target DNA can be introduced using *Agrobacterium* or particle bombardment (Christou 1996; Siemens and Schieder 1996). Another relatively new and simple procedure for transformation is '*Agrobacterium* vacuum infiltration', which was widely used in the transformation of *Arabidopsis thaliana* but has only had limited success in *Brassica* (Cao *et al*. 2000; Wang *et al*. 2003). By the method of infiltration with *Agrobacterium*, Cao *et al*. obtained 2 transformants out of about 20,000 seeds harvested from infiltrated of Pakchoi (*B. rapa* L. ssp. *chinensis*). The two transformants had a different foreign gene insertion site, and both were hemizygous. Wang found that the successful conditions to produce transgenic *B. napus* plants were: vacuum infiltration at 25-27 in. Hg twice for longer than 5 min either 1 week apart or consecutively and treatment at induced small flowering plants (ISFP, the plants with buds before flowering) and adult flowering plants (AFP, the plants with flowers in flowering). The transformation efficiency was only 0.05%-0.18%. Compared with *A. thaliana*, the transformation frequency was lower. But research is ongoing to improve its feasibility.

Transformation technology has already greatly expanded the genetic diversity of the *Brassica* crop and offered an array of opportunities for basic scientific research and for producing commercial genetically modified crops. A variety of genes affecting agriculturally important traits have been transferred into *Brassica* species: for improved oil quality (Jadhav *et al*. 2005; Jha *et al*. 2007), disease resistance (Yu *et al*. 2007), insect resistance (Halfhill *et al*. 2001; Cao *et al*. 2005), salt and drought tolerance (Bhattacharya *et al*. 2004; Park *et al*. 2005), herbicide resistance (Qing *et al*. 2000) (**Table 2**).

Another important advancement in the transformation of *Brassica* crops is the development of male-sterile lines and the development of a restoration system. In *B. juncea*, the male-sterile plants were obtained by introducing the *barnase* gene with TA-29 specific promoter (Jagannath *et al*. 2001). The male-sterile line could be restored by crossing it with a *barstar*-containing transgenic line (Jagannath *et al*. 2002). And this male sterility/fertility restorer system has tremendous application in hybrid breeding.

The development of transformation protocols of *Brassica* by *Agrobacterium* had been delayed because shoot regeneration was problematic and the main obstacle in the 1980s. More recently, the problem was overcome by using silver nitrate in the culture medium during shoot regeneration of hypocotyls and cotyledon explants (Chi *et al*. 1990; Hachey *et al*. 1991; Palmer 1992). Although a wide range of target explants were used in the transformation of *Brassica*, including hypocotyls (Radke *et al*. 1988), cotyledonary petioles (Moloney *et al*. 1989), thin cell layers (Charest *et al*. 1988), stem segments (Fry *et al*. 1987), microspores (Pechan 1989) and protoplasts (Wang *et al*. 2005a), the transformation of *Brassica* is still hindered by genotype restrictions (Ono *et al*. 1994; Zhang *et al*. 1998). Previous studies have demonstrated that a number of key factors affecting transformation including the susceptibility to *Agrobacterium* and the response of tissue culture *in vitro*.

Protoplast fusion has allowed not only intrageneric hybridizations, but the production of intergeneric hybrids and cybrids as well. In addition, applying protoplasts as recipients for DNA introduction do not have the restriction of susceptibility to *Agrobacterium*, but production of transgenic plants through protoplasts requires suitable regeneration systems. By protoplast fusions between *B. napus* and *Orychophragmus violaceus*, 257 somatic hybrid plants were obtained (Hu *et al*. 2002). Variation in fatty acid composition was found in the hybrid plants and their offspring. In addition, several male-sterile plants with high female fertility were recovered. As a conclusion, male sterility most-likely originated from alloplasmic recombination and would be of great potential for the development of a new CMS system. Protoplast fusion has produced disease-resistant hybrids (Ren *et al*. 2000). Twelve somatic hybrids (synthetic *B. napus*) were obtained and confirmed. The offspring populations of the somatic hybrids (F1–S1 and F1–BC1) showed more disease-resistant than the parents; the percentage of resistant plants increased from 21% (average of parents) to 36% (F1–S1) and 48% (F1–BC1). Male-fertile and male-sterile plants were also obtained (Yamagishi *et al*. 2002). In their study asymmetric hybrids between *A. thaliana* and *Brassica napus* were obtained by treating the protoplasts of *A. thaliana* with iodoacetamide (IOA) and *B. napus* protoplasts with UV-irradiation before fusion with polyethylene glycol (PEG).

CONCLUSIONS AND FUTURE PROSPECTS

Development of cellular and molecular biology in plant accelerated the breeding of *Brassica* species and integrated information from agronomy, breeding, genetics and genomics. Genomic research on these crops will have gained impetus after completing the MBGP. The availability of the whole genome sequence of *Brassica* will facilitate the isolation of specific genes to aid in crop improvement and to improve economic use in *Brassica* species. Functional gene analysis, marker-assisted breeding, protoplast fusion, tissue culture, and genetic transformation together can be of use in the development of *Brassica* species with novel desirable traits.

Table 2 Functional genes transferred into *Brassica* species.

Functional gene	Expression vector	Selector/ marker gene	Explants	Conditions of tissue culture/transformation	Level of detection	Transformation efficiency	Ref.
1-acyl-2-oleoyl-sn-glycero-3-phosphocholine desaturase (FAD2) gene	pSE (the original plasmid RD400)	kanamycin (*npt*II)	Cotyledons of *B. carinata*	Regeneration medium: MS + 2 mg/l 6-BA+ 0.05 mg/l NAA+ 30 g/l sucrose + 7 g/l agar Selection medium: Regeneration medium + 5 mg/l $AgNO_3$ + 500 mg/l polyvinylpyrrolidone + 500 mg/l MES + 500 mg/l Carb. + 25 mg/l or 50 mg/l Kan. Shoot elongation medium: MS + 0.05 mg/l 6-BA+ 0.03 mg/l GA + 150 mg/l phloroglucinol + 25 mg/l Kan. (or 5 mg/l L-ppt) + 500 mg/l Carb. + 20 g/l sucrose + 9 g/l agar Rooting medium: 1/2 MS + 0.05 mg/l NAA + 10 g/l sucrose + 7 g/l agar + 500 mg/l Carb. + 50 mg/l Kan. or 5 mg/l L-ppt (Following the protocol developed by Babic *et al*. 1998)	PCR, Southern, Northern analysis	/	1
Acyl carrier protein (ACP) gene	pCAMBIA1300	Hygromycin (*hpt*)	Apical meristems of *B. juncea*	Pre- and co-cultivation medium, MSB5H: (MSB5 (MS salt + B5 vitamins + 30 g/l sucrose) + 1.5 mg/l 6-BA + 0.01% 2,4-D) Regeneration medium: (MSB5H + 200 mg/l Cef. + 60 mg/l augmentin) Selection medium: (Regeneration medium + 40 mg/l hygromycin) Multiple-shoots induction medium: (MSB5 + 2 mg/l 6-BA+ 2 mg/l TDZ) Rooting medium: (MS salt + B5 vitamins + 20 g/l sucrose + 5 mg/l IBA)	PCR, Southern, Western blot analysis	/	2
The Turnip mosaic virus (TuMV) replicase (NIb) gene	pBIN-pROK	Free	Flower buds of Chinese cabbage (*B. campestris* ssp. *pekinensis* (Lour) Olsson)	Soak in IM medium (MS + vitamin B5 + 6-BA 0.04 mg/l + AS (acetosyringone) 19.6 mg/l + Silwet-77 0.02 mg/l + sugar 50 g/l, pH 5.5) under negative pressure at 10^4 pa	PCR, ELISA, Southern, TuMV resistance analysis	2.09-2.53%	3
GFP and cry1Ac *Bacillus thuringiensis* (Bt) gene	pBIN19	kanamycin (*npt*II)	Hypocotyls of *B. napus* cv. 'Westar'	Pre- and co-cultivation medium: (MS + 1 mg/l 2,4-D) Selection medium: (MS + 1 mg/l 2,4-D + 400 mg/l Tim. + 20 mg/l Kan.) Regeneration medium 1: (MS + 4 mg/l 6-BA + 2 mg/l Zeatin + 5 mg/l $AgNO_3$ + 400 mg/l Tim. + 20 mg/l Kan.) Regeneration medium 2: (MS + 4 mg/l 6-BA+ 2 mg/l Zeatin + 400 mg/l Tim. + 20 mg/l Kan.) Shoot development medium: (MS + 0.5 mg/l 6-BA + 400 mg/l Tim. + 20 mg/l Kan.) Rooting medium: (MS + 0.1% IBA + 400 mg/l Tim. + 20 mg/l Kan.	PCR, Western blot, Fluorescence spectrophotometry, insect analysis	3.5%	4
Cry1Ac and Cry1C *Bacillus thuringiensis* (Bt) gene	pNS6 and pMON10517	Hygromycin (*hpt*) kanamycin (*npt*II)	Hypocotyls and cotyledonary petioles of Collards (*B. oleracea* var. *acephala*)	Induction medium: MS + B5 vitamins + 5 mM arginine + 5 mg/l 6-BA + 0.5 mg/l NAA + 3% sucrose + 0.8% Phytagar + 50 mg/l Kan. Multiplication medium: MS + 100 mg/l *myo*-inositol + 1 mg/l thiamine HCl + 2 mg/l 6-BA + 3% sucrose + 0.8% Phytagar + 50 mg/l Kan. Rooting medium: MS + 100 mg/l *myo*-inositol + 1 mg/l thiamine HCl + 1% sucrose + 1.1 g/l Gelrite + 4 g/l Agar gel (Following the protocol: Cao and Earle 2003)	PCR, Southern, ELISA, insect analysis	0.8-3.6%	5
betA gene for biosynthesis of glycinebetaine	pRC-cdh (the original plasmid pBI101)	kanamycin (*npt*II)	Hypocotyl of cabbage (*B.oleracea* var. *capitata*) cv. 'Golden Acre'	Pre- and co-cultivation medium: (MS + 2% sucrose + 1 mg/l 2,4-D + 0.5 mg/l kinetin + 0.8% agar). Regeneration medium SI: (MS + 2% sucrose + 0.5 mg/l IAA + 2 mg/l 6-BA + 3.5 mg/l $AgNO_3$ + 250 mg/l Cef. + 0.25% phytagel) Selection medium: (SI medium + 25 mg/l Kan.) Rooting medium (MS + 0.1 mg/l IAA + 250 mg/l Cef. + 25 mg/l Kan.) (Following the protocol: Bhattacharya *et al*. 2002)	PCR, Southern, mRNA expression, NaCl stress analysis	/	6
Late embryogenesis abundant (LEA) gene isolated from *Brassica napus*	pIG121–LEA	kanamycin sulphate (*npt*II)	Hypocotyls with the cotyledons of Chinese cabbage (*Brassica campestris* ssp. *pekinensis*)	Pre-and co-cultured medium: MS medium + 2 mg/l 6-BA + 1 mg/l NAA + 16 g/l Phytagar Selection medium: MS + 2 mg/l 6-BA + 1 mg/l NAA + 4 mg/l $AgNO_3$ + 300 mg/l Tim. + 10 mg/l Kan. + 16 g/l Phytagar Rooting medium: MS + 300 mg/l Tim. + 10 mg/l Kan. + 16 g/l Phytagar	PCR, Southern, Northern, salt tolerance analysis	2.2%	7
Basta resistance (Bar) gene	pDHB-NIa1	Basta (*Bar*)	Plants of *B. rapa* ssp. *chinensis* var. *utilis*, cv. '49 Caixin'	Soak in IM medium under negative pressure at 10^4 pa for 25 mins	PCR, Southern, Northern, basta resistance analysis	0.01%	8

Abbreviations: 2,4-D, 2,4-Dichlorophenoxyacetic Acid; 6-BA, 6-benzylaminopurine; AS, acetosyringone; *bar*, phosphinothricin acetyltransferase gene; Carb, carbenicillin; Cef, cefotaxime; GA, gibberellic acid; *hpt*, hygromycin phosphotransferase gene; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; Kan, kanamycin; L-ppt, L-phosphinothricin; MES, morpholinoethane sulfonic acid; MS, Murashige and Skoog medium; NAA, α-naphthalene acetic acid; *npt*II, neomycin phosphotransferase II; TDZ, thidiazuron; Tim, timentin.

1, Jadhav *et al*. 2005; 2, Jha *et al*. 2007; 3, Yu *et al*. 2007; 4, Halfhill *et al*. 2001; 5, Cao *et al.* 2005; 6, Bhattacharya *et al*. 2004; 7, Park *et al*. 2005; 8, Qing *et al*. 2000

With developing DNA microarray and SNP genotyping technologies, and using the enormous progress already made in *Arabidopsis* as a foundation, it will provide a functional understanding of the molecular genetics of *Brassica* crops. And genetic transformation will bring the highest impact on genetic improvements of *Brassica* species. Thus, it can be expected that practical *Brassica* breeding will reap considerable benefits from molecular genetic research, technology and marker development in the next decade.

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