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

Bo Zhou\(^1\) • Yuhua Li\(^1*\) • Fei Zhao\(^2\) • Saneyuki Kawabata\(^3\)

**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* (BCC, 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 *Brassicaceae* 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
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 polyplidization 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.

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

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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, BrAP3 (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.
were linked to the allele for yellow seed, whereas the other fertility gene in 2005). A RAPD marker linked to the GMS (genic male sterility gene in rapeseed (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 (QTL) 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 (SMT). They are the four main polleniation control systems used in China.

Cytoplasmic 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 oxisyrrhina (oxy), Trachystoma balti (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 leads to the production of defective forms of the essential proteins in mitochondria, thus causing male sterility. CMS occurs in different environments, with 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, atpB, cob, nad3, nad5, 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 restoration for the 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 rapeseeded 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 episitic GMS type report can be obtained up to now (Li et al. 1990). RGMS attracted the attention of rapeseeded 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 (Yang et al. 2003). An ecotyope 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 was tightly linked to the gms gene of Chinese cabbage. CMS 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 (Dodd 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 assumed 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. 1987). During selecting self-compatible 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 *Brassicaceae* vegetables including Chinese cabbage (*B. rapa* ssp. *pekinesis* 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 out-crossing.

**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 binant vectors containing heterologous genes of interest into *Agrobacterium* (Ren et al. 2000). The development of transformation protocols 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 intragenic 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 of *Brassica 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* were obtained by treating the protoplasts of *A. thaliana* with iodoacetamide (1OA) 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.
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| 1-acyl-2-oleoyl-sn-glycero-3-phosphocholine desaturase (FAD2) gene | pSE (the original plasmid RD400) | kanamycin
(amp/I) | 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 AgNO₃ + 500 mg/l pVPyridol + 500 mg/l MES + 500 mg/l Carb + 25 ml/l or 50 mg/l Kan. Shoot elongation medium: MS + 0.05 mg/l 6-BA + 0.03 mg/l GA + 150 mg/l chlorogenic acid + 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 | pCAMBI A1300 | Hygromycin
(npt) | 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 g/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, Western, Southern blot analysis | | 2 |
| The Turnip mosaic virus (TuMV) replicase (Nb) gene | pBIN- pROK | Free | Flower buds of Chinese cabbage (B. campestris spp. pekinensis) (Lour) Olsen | 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 10³ pa | PCR, ELISA, Southern, TuMV resistance analysis | 2.09-2.53% | 3 |
| GFP and cry1Ac Bacillus thuringiensis (B) gene | pBIN19 | kanamycin
(amp/I) | Hypocotyls of B. napus cv. ‘Westar’ | Pre- and co-cultivation medium: (MS + 1 mg/l 2,4-D) Selection medium 1: (MS + 4 mg/l 6-BA + 2 mg/l Zeatin + 5 mg/l AgNO₃ + 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.) 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, Western blot, Fluorescenc e spectrophotometry, insect analysis | 3.5% | 4 |
| Cry1Ac and Cry1C Bacillus thuringiensis (B) gene | pNS6 and pMON10517 | kanamycin
(amp/I) | Hypocotyls and cotyledonary petals of Collards (B. oleracea var. acephala) | 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 10³ pa | PCR, Western, Southern blot analysis | 0.8-3.6% | 5 |
| betaA gene for biosynthesis of glycinebetaine | pRC-cdh (the original plasmid pH101) | kanamycin
(amp/I) | 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 kethin + 0.8% agar). Regeneration medium SI: (MS + 2% sucrose + 0.5 mg/l IAA + 2 mg/l 6-BA + 3.5 mg/l AgNO₃ + 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: Cao and Earle 2003) | PCR, Southern, mRNA, expression, NaCl stress analysis | | 6 |
| Late embryogenesis abundant (LEA) gene isolated from Brassica napus | pGIG121– LEA | kanamycin sulphate
(amp/I) | Hypocotyls with the cotyledons of Chinese cabbage (Brassica campestris spp. pekinensis) | Pre-and co-cultivated medium: MS medium + 2 mg/l 6-BA + 1 mg/l NAA + 16 g/l Phytagar Selection medium: MS medium + 2 mg/l 6-BA + 1 mg/l NAA + 4 mg/l AgNO₃ + 300 mg/l Tim. + 10 mg/l Kan. + 16 g/l Phytagar Rooting medium: MS medium + 300 mg/l Tim. + 10 mg/l Kan. + 16 g/l Phytagar (Following the protocol: Bhattacharya et al. 2002) | PCR, Southern, Northern, salt tolerance analysis | 2.2% | 7 |
| Basta resistance (Bar) gene | pDHB- Nla1 | Basta (Bar) | Plants of B. rapa spp. chinensis var. utilis, cv. ‘49 Caixin’ | Soak in IM medium under negative pressure at 10³ pa for 25 mins | PCR, Southern, Northern, basta resistance analysis | | 8 |

Abbreviations: 2,4-D, 2,4-Dichlorophenoxyacetic Acid; 6-BA, 6-benzylaminopurine; AS, acetylsyringone; bar, phosphinothricin acetyltransferase gene; Carb, carbencillin; Cef, cefotaxine; 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; nptII, neomycin phosphotransferase II; TDZ, thidiazuron; Tim, timetinin.

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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