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INVITED REVIEW: *BRASSICA* BIOTECHNOLOGY: PROGRESS IN CELLULAR AND MOLECULAR BIOLOGY

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SUMMARY

Considerable progress has been accomplished in the cellular and molecular biology of *Brassica* species in the past few years. Plant regeneration has been increasingly optimized via organogenesis and somatic embryogenesis using various explants; with tissue culture improvements focusing on factors such as age of the explant, genotype, and media additives. The production of haploids and doubled haploids using microspores has accelerated the production of homozygous lines in the *Brassica* species. Somatic cell fusion has facilitated the development of interspecific and intergeneric hybrids in the sexually incompatible species of *Brassica*. Crop improvement using somaclonal variation has also been achieved. The use of molecular markers in marker-assisted selection and breeding, transformation technology for the introduction of desirable traits, and a comparative analysis of these as well as their future prospects are important parts of the current research that is reviewed.

Key words: biotechnology; *Brassica*; genetic transformation; marker-assisted selection; tissue culture.

INTRODUCTION

Brassica is the most economically important genus in the *Brassicaceae* family (syn. Cruciferae). Several species and types of *Brassica*s are significant oilseed crops, vegetables, forage crops, and are used in the production of condiments, such as mustard. *Brassica* species are widely used in the cuisine of many cultures and recognized as a valuable source of dietary fiber. *Brassica* vegetables contain little fat, and are sources of vitamins, minerals, and fiber. They also contain a large number of novel phytochemicals, some of which protect against carcinogenesis (Steinmetz and Potter, 1996). Hence, *Brassica*s are believed to be useful in the prevention of cancer.

Among the *Brassica* crops, oilseeds have the highest economic value. The oilseed *Brassica*s are found within *Brassica juncea*, *Brassica carinata*, *Brassica rapa* (syn. *Brassica campestris*) and *Brassica napus* collectively, and are commonly called oilseed rape. When *Brassica* oils are low in aliphatic glucosinolates and erucic acid, the varieties are increasingly commonly referred to as canola, a more pleasant-sounding name. Canola, which is most often *B. napus*, has received much attention worldwide and may soon be the most popular oilseed crop. There are now also canola-quality *B. rapa* and *B. juncea* varieties. Canola oil is widely used in cooking since it is very low in saturated fat, making it appealing to health-conscious consumers. *Brassica nigra* is mainly used as a mustard condiment in addition to oil. Vegetable *Brassica* are an important and highly diversified group of crops grown worldwide that belong mainly to the species *Brassica oleracea*, as well as *B. rapa* and *B. napus*. This group includes plants such as broccoli, Brussels sprouts, cabbage, cauliflower, collards, kale, kohlrabi, rutabaga, and turnip.

While most research in *Brassica* crops has been performed on oilseed and vegetable biotypes, rapid-cycling *Brassica* biotypes of various species have gained attention in recent years. These rapid-cycling types were genetic selections having short life cycles of 20–60 d and small sizes (Williams and Hill, 1986). Rapid cycling *Brassica*s are attractive model laboratory plants because of their small genome sizes, in some cases just 3–4-fold larger than *Arabidopsis thaliana* (Arumuganathan and Earle, 1991). Other desirable qualities are their high female fertility, rapid seed maturation, and absence of seed dormancy.

In addition to *Brassica* crops and rapid-cycling models, the genus contains several weedy species, of which the most important is *B. rapa*. Weedy *B. rapa* is considered one of the two most important weeds in the world (the other is wild rice) that is closely related to and sexually compatible with prominent row crops (Holm et al., 1997). Therefore, there is considerable interest in better understanding its genetics and genomics because of concern of introgression from transgenic canola to weedy biotypes (e.g., Halfhill et al., 2003; Stewart et al., 2003). *B. rapa* is also receiving attention as an important candidate for those interested in weed genomics to better understand the molecular basis of weediness as a syndrome (Basu et al., 2004).

Considerable research has been conducted in tissue culture, transformation and molecular breeding of the *Brassica*s. Transformation in *Brassica* has been reviewed by Poulsen (1996), Earle et al. (1996), and Earle and Knauf (1999). While information is available on genetic transformation in *Brassica*, there is no review on the other aspects of recent progress in cellular and molecular biology of

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the genus. In this review, we present pertinent research with an eye towards improving *Brassica* biotechnology. This includes organogenesis, somatic embryogenesis, microspore culture and doubled haploids, somatic cell fusion, molecular markers for genetic fidelity of *in vitro*-grown plants, marker-assisted selection, and transformation. We have reviewed transformation only with respect to desirable traits engineered since all the other aspects of transformation have been recently covered as indicated above.

Organogenesis

Organogenesis is an indispensable tool for plant regeneration using tissue culture techniques and for plant transformation. *Brassica* species have been widely exploited for tissue culture purposes. Regeneration protocols have been developed for most of the *Brassica* species. Organogenesis has been the widely used pathway for regeneration in *Brassica* crops compared to other means of regeneration. Regeneration of plants via organogenesis has been accomplished from various tissues such as cotyledons (Sharma et al., 1990; Hachey et al., 1991; Ono et al., 1994), hypocotyls (Yang et al., 1991), peduncle segments (Eapen and George, 1997), leaves (Radke et al., 1988), thin cell layers of epidermal and subepidermal cells (Klimaszewska and Keller, 1985), roots (Xu et al., 1982), and protoplasts (Glimelius, 1984; Spangenberg et al., 1986; Kik and Zaal, 1993; Hu et al., 1999). However, hypocotyl segments remain the most desirable explants for tissue culture and have been used for most *Brassica* species because of their ability to regenerate. Numerous aspects of tissue culture conditions that affect plant regeneration are discussed below.

Genotype. Regeneration in *Brassica* is highly genotype-dependent and has been reported in several species. In *B. napus* there was a huge variation ranging from 0% to 91% in the 100 cultivars tested (Ono et al., 1994). *B. napus* cultivar GSL-1 showed better regeneration efficiency than Westar (a standard cultivar for transformation) in one study (Phogat et al., 2000). Indian cultivars were found to have better regeneration rates than Australian cultivars in *B. juncea* (Pental et al., 1990). Of the 123 genotypes of Chinese cabbage (*B. rapa*. ssp. *pekinensis*) tested, a large variation was observed in regeneration frequency, ranging from 95% to 0% (Zhang et al., 1998). Thus, genotype specificity is a limiting factor in *Brassica* tissue culture and regeneration, which severely limits the germplasm that can be manipulated or improved.

Age of the explant. In most *Brassica* species, regeneration is dependent on the age of the explant. Young explants have been shown to give better results than older explants in most *Brassica* species. Most researchers have found that explants excised from 3–4-d-old seedlings gave optimal regeneration rates. For example, 3-d-old seedlings of *B. rapa* ssp. *oleifera* yielded better regeneration than those older than 4 d (Burnett et al., 1994). In *B. napus*, 4-d-old seedling explants proved optimal, yielding a 90% regeneration rate (Ono et al., 1994). In rapid-cycling *B. rapa*, 3-d-old explants from seedlings were best for regeneration (Teo et al., 1997). In all the above cases, regeneration capacity declined when the age of the seedling was increased above 4 d. In *B. juncea* (Sharma et al., 1990) and *B. rapa* (Hachey et al., 1991), 3–5-d-old seedlings gave optimal shoot regeneration. In one instance it was found that explants as old as 2 wk resulted in good regeneration in cabbage (Jin et al., 2000).

Ethylene inhibitors. A very important adjuvant that seems to be necessary for *Brassica* regeneration is the ethylene inhibitor silver nitrate (Pental et al., 1990; Sethi et al., 1990; Williams et al., 1990; Palmer, 1992; Pua and Chi, 1993). Therefore, silver nitrate is routinely used in *Brassica* tissue culture. Other ethylene inhibitors such as silver thiosulfate (Eapen and George, 1996, 1997) and aminoethoxyvinylglycine (Chi et al., 1990; Burnett et al., 1994) have also been reported to have a positive effect on regeneration in *Brassica* species. Excluding silver nitrate in media drastically reduced regeneration frequency in *B. napus* (Phogat et al., 2000). In Chinese radish (*Raphanus sativus* var. *longipinnatus*), the combination of silver nitrate and aminoethoxyvinylglycine was found to significantly enhance shoot regeneration (Pua et al., 1996).

Other media constituents. Various media additives might increase regeneration efficiencies in *Brassica*. Methylglyoxal-bis-(guanylhydrazone) (MGBG), an inhibitor of spermidine biosynthesis, was reported to increase regeneration frequencies from 7% to 63% in *Brassica* and other genera (Sethi et al., 1990), also other researchers found a positive effect of MGBG on shoot regeneration of *B. napus* (O'Neill et al., 1996). Conversely, putrescine, a polyamine, was found to enhance shoot regeneration in Chinese radish when used with silver nitrate or aminoethoxyvinylglycine (Pua et al., 1996). However, putrescine was not effective when used alone, but was synergistic with ethylene inhibitors. Brassinolides, a relatively new class of compounds, have also been found to stimulate the production of adventitious shoots from cauliflower hypocotyl segments (Sasaki, 2002).

Somatic Embryogenesis

Somatic embryogenesis, which has been the subject of increasing research in the genus, has become one of the most desired pathways in the regeneration of plants via tissue culture because it bypasses the necessity of time-consuming and costly manipulation of individual explants, which is a problem with organogenesis. Somatic embryogenesis might also overcome difficulties with micropropagation procedures in species that are difficult to root. Though somatic embryogenesis has been used in transformation and regeneration systems in many plant species, *Brassica* crops seem to be lagging in this direction; probably because of the tractability and advanced state of organogenesis techniques in *Brassica*. Microspores or anthers have been somatic embryogenesis explants of choice in most *Brassica* species (Lichter, 1989; Sato et al., 1989; Aslam et al., 1990; Baillie et al., 1992). There have been a few reports using other explants, however. Somatic embryos have been obtained from hypocotyls (Kohlenbach et al., 1982), protoplast-derived colonies (Kranz, 1988), and immature cotyledons (Turgut et al., 1998) in *B. napus*. In Chinese cabbage, somatic embryogenesis has been induced from cotyledonary explants (Choi et al., 1996). Somatic embryos in cauliflower have been obtained by Deane et al. (1997) and Leroy et al. (2000) using hypocotyl explants. Somatic embryogenesis has been reported in rapid-cycling *B. napus* from hypocotyl explants using MS (Murashige and Skoog, 1962) basal medium with low pH (3.5–5) (Koh and Loh, 2000).

As for organogenesis, genotype is a very important factor in the embryogenic frequency of most *Brassica* species. Only one of three different Chinese cabbage cultivars ('Top Salad') yielded somatic embryos (Choi et al., 1996). Genotype effects have been shown to be important in *B. rapa* (Baillie et al., 1992), *B. carinata* (Barro and Martin, 1999), and *B. napus* (Chuong et al., 1988).

Anther/Microspore Culture and Doubled Haploids

One of the most exciting developments in biotechnology has been the production of haploid and doubled haploid plants. Haploids and doubled haploids have been produced in *Brassica* species using anther culture or isolated microspores, thus providing a tool for the rapid production of homozygous lines for the production of hybrid seeds. This technology serves as a valuable tool in breeding self-incompatible, outcrossing lines, which are typical in vegetable *Brassica*. Various techniques for microspore/anther culture have been developed for *Brassica* species (Palmer et al., 1996b). Somatic embryos and plantlets were produced in *B. napus* as early as 1977 (Keller and Armstrong, 1977) and this technology has been exploited for various purposes since that time. However, most genotypes respond better to isolated microspore culture, and these explants give higher embryo yield than anther culture (Cao et al., 1995). Microspore and anther culture are being employed as breeding tools to improve vegetable *Brassica* such as *B. oleracea* (Keller and Armstrong, 1981, 1983; Chiang et al., 1985; Ockendon, 1985; Arnison et al., 1990; Dias and Martins, 1999) and pakchoi (*B. rapa* ssp. *chinensis*) (Cao et al., 1994).

An array of studies has been conducted using microspore culture. Microspore-derived embryos of *B. napus* have been used to investigate biochemical pathways and screen metabolic products (Wiberg et al., 1991). Microspore-derived embryos have been used to study the amount of glucosinolate content (Iqbal et al., 1995) leading to the selection of desirable *B. napus* lines. Gene linkage and interactions have been studied in *B. campestris* using haploid plants (Guo and Pulli, 1996). Microspore-derived somatic embryos have been used for the production of VLCMFAs (very long-chain monounsaturated fatty acids) (Qi et al., 1998). Microspores have also proven to be viable transformation targets for the production of transgenic *B. napus* plants (Fukuoka et al., 1998; Nehlin et al., 2000).

As with the other tissue culture techniques already mentioned, *Brassica* microspore culture is also genotype-dependent (Palmer et al., 1996a). For example, only 30% of *B. juncea* genotypes tested for microspore culture were responsive (Hiramatsu et al., 1995). Genotype-dependent effects were also observed in *B. carinata* (Barro and Martin, 1999). Hence, there is still a need to extend this technology to non-responsive cultivars before it becomes a universally efficient tool in breeding.

Once haploid plants are produced, doubled haploids can be obtained by either colchicine application on haploid plants or by the process of spontaneous doubling of chromosomes. In *Brassica* species, spontaneous doubling depends on genotype, microspore stage, and culture conditions. Low-temperature treatment has especially facilitated the production of doubled haploids (Keller and Armstrong, 1978; Charne et al., 1988; Chen and Beversdorf, 1992). *B. juncea* doubled haploids have been produced by culturing microspores and treating the haploid plants with colchicine (Lionneton et al., 2001). Spontaneous doubled haploids have been reported in *B. napus* cv. Topas (XuHan et al., 1999), and doubled haploids from colchicine applications have been reported by Murphy and Scarth (1998). In *B. napus* (Zhao et al., 1996), colchicine simultaneously induced embryogenesis in microspores and doubled the ploidy level. Doubled haploids obtained using microspores are preferred over the anther culture doubled haploids especially in the vegetable (*B. oleracea*) species, since ploidy levels are mixed in anther culture-derived doubled haploids as compared with the microspore-derived doubled haploids, which were found to be mostly diploid (Wang et al., 1999). Because the most popular broccoli (*B. oleracea* var. *italica*) cultivars and breeding lines used in breeding programs are F_1 hybrids, microspore-derived doubled haploids are helpful in crop improvement. Recently, breeders have used doubled haploids to produce homozygous lines in relatively short periods.

Somatic Cell Fusion

Protoplast fusion allows the creation of hybrid and cybrid combinations of species that are sexually incompatible, thus facilitating the transfer of genes from a related, but sexually incompatible species, to another without genetic transformation. This technology has allowed not only intrageneric hybridizations, but the production of intergeneric hybrids and cybrids as well. Various desirable traits from the parents have been transferred to the hybrids and cybrids using this technology.

One success of protoplast fusion has been the production of disease-resistant hybrids. Somatic hybrids that are resistant to bacterial soft rot have been produced by the fusion of *B. rapa* and *B. oleracea* protoplasts (Ren et al., 2000). Somatic hybrids that were resistant to *Leptosphaeria maculans* were produced by fusing protoplasts of *B. napus* and *Sinapis arvensis*, which is a wild relative of *B. napus* (Hu et al., 2002), that were fully fertile. Interspecific hybrids have been produced by fusing mesophyll protoplasts of *B. juncea* and *B. spinescens* (Kirti et al., 1991b). The hybrids had morphological features and chromosomes of both parents, but had sterile pollen. Novel rapid-cycling *B. napus* lines have been produced by protoplast fusion between rapid-cycling *B. oleracea* and *B. rapa*, and their seeds possessed novel fatty acid compositions (Hansen and Earle, 1994). Somatic hybridization between *B. napus* and the metal hyperaccumulator *Thlaspi caerulescens* produced hybrids that tolerated high levels of zinc (Brewer et al., 1999). Intergeneric somatic hybrids were produced by fusing mesophyll protoplasts of *Trachystoma ballii* and *B. juncea* (Kirti et al., 1992). The hybrids were intermediate between the parents in morphology. Even though hybrids had sterile pollen, backcrosses with *B. juncea* yielded viable seeds. Somatic hybrids between *B. napus* and *Lesquerella fendleri* have been produced by fusing mesophyll protoplasts (Skarzhinskaya et al., 1996). Protoplast fusion between *B. oleracea* and *Moricandia nitens*, a C3–C4 photosynthesis intermediate wild species, resulted in the production of intergeneric hybrids that expressed a gas-exchange character that was intermediate between the two parents (Yan et al., 1999).

Another important use for protoplast fusion is the production of male-sterile lines. Male-sterile, cold-tolerant *B. napus* somatic hybrids were produced by fusing an Ogura male-sterile cold-sensitive cauliflower inbred (*B. oleracea* var. *botrytis* inbred NY7642A) and a cold-tolerant, fertile canola (*B. rapa* cv. Candle) (Heath and Earle, 1996). Male-sterile cybrids have also been produced by the fusion of protoplasts of *B. napus* and *B. tournefortii* (Liu Clarke et al., 1999). A cytoplasmic male-sterile cybrid of *Brassica oleracea* was produced by the transfer of the sterile 'Anand' cytoplasm (originally from a wild species of *B. tournefortii*) from *B. rapa* to *B. oleracea* (Cardi and Earle, 1997). Cold-tolerant cytoplasmic male-sterile (CMS) cabbage (*B. oleracea* ssp. *capitata*) was produced by the fusion of leaf protoplasts from fertile cabbage and a cold-tolerant Ogura male-sterile broccoli (Sigareva and Earle, 1997). An interesting application of protoplast fusion is the

combination of male-sterile and fertility restoration systems, which would be amenable for the production of heterotic hybrids. This technology was applied in *B. juncea* by protoplast fusion with *Moricandia arvensis* with the fertility restoration function of this male-sterile *B. juncea* by introgression (Prakash et al., 1998). However, these CMS lines were chlorotic. Protoplast fusion of chlorotic male-sterile *B. juncea* with green male-sterile *B. juncea* resulted in green male-sterile plants (Kirti et al., 1998). Protoplast fusion between *A. thaliana* and *B. napus* has resulted in the production of asymmetric hybrids that included three male-sterile hybrids (Yamagishi et al., 2002). The male-sterile plants would be excellent candidates for the study of genes involved in CMS. Because the *Arabidopsis thaliana* genome has been sequenced, *A. thaliana* and *Brassica* species somatic hybrids and cybrids should help unravel genomic functions in several crops in the family.

Somaclonal Variation

Genetic variation is very important in crop improvement and forms the basis of development of new varieties. Somaclonal variation is a valuable tool in plant breeding wherein variation in tissue culture-regenerated plants from somatic cells can be used in the development of crops with novel traits. By applying selection pressure during tissue culture it is also possible to develop somaclones resistant to biotic and abiotic stresses (Jain, 2001). Somaclonal variation has been associated with changes in chromosome number and structure, point mutations and DNA methylation (Brown et al., 1993). Somaclonal variation has been observed in cauliflower plants propagated from adventitious root meristems (Grout and Crisp, 1980) and anther culture-derived doubled haploids of *Brassica napus* (Wenzel et al., 1977; Hoffmann et al., 1982). In anther culture-derived plants of *B. juncea* var. Rai-5, variation in agronomic characters, oil content and fatty acid composition were observed (George and Rao, 1983). Yellow-seeded variants were observed in the progeny of the plants regenerated from cotyledonary explants of *Brassica juncea* cv. TM-4 (George et al., 1987). Somaclonal variants in the R_1 generation were selected from Indian mustard (*B. juncea* cv. Prakash) plants developed via shoots induced from cotyledonary callus (Jain et al., 1989). These Indian mustard plants displayed large variation in all the characters evaluated. Some of the plants also showed significantly higher yield and other improved agriculturally important characteristics compared with controls. Somaclonal variation has led to the selection of a dwarf mutant and true breeding lines in the R_2 generation. Somaclones of *B. juncea* producing high yields and resistant to shattering have been selected and commercially released (Katiyar and Chopra, 1995). Selection pressure *in vitro* yielded salt-tolerant somaclones of *B. juncea* (Jain et al., 1990; Kirti et al., 1991a).

Brassica Molecular Markers and Breeding

Nearly all modern plant breeding relies on molecular markers and they have myriad uses. The advent of various molecular markers has made it possible to assess genetic variability, identify genotypes, perform phylogenetic analysis, establish the true-to-type nature of clonally propagated or micropropagated plants, as well as to devise conservation strategies and perform marker-assisted selection and breeding. Molecular markers are being used to assess the genetic fidelity of *in vitro*- developed *Brassica* crops.

Molecular markers for genetic fidelity of in vitro *plants*. As we have just discussed, somaclonal variation can occur in plants regenerated from tissue culture, and it is a major constraint in the clonal propagation of elite cultivars or clones where genetic variation is undesirable. Several markers have been used to assess the genetic fidelity of *in vitro*-grown plants such as isozymes, restriction fragment length polymorphism (RFLP), and random amplified polymorphic DNA (RAPD). However, these markers are suboptimal for genetic identification, giving way to improved PCR fingerprinting technology. In *B. oleracea* var. *botrytis*, Leroy et al. (2000) have used inter-simple sequence repeat (ISSR) markers to analyze the genetic stability of somatic embryos derived from hypocotyls. They did not find any polymorphism between different regenerants. However, in cauliflower callus, out of the 224 calluses analyzed, six exhibited original patterns, and in one of these PCR patterns differed at four polymorphic loci. The most frequent primer used for detection of polymorphisms was $(CAA)_5$ (Leroy and Leon, 2000; Leroy et al., 2001). An ISSR marker homologous to a gene involved in cellular proliferation was also characterized, and effects of modifications of this gene on callogenesis were also studied (Leroy et al., 2001). ISSR markers are very efficient markers, since they require low DNA quantities, generate a high number of bands, and are highly reproducible. ISSR marker fingerprinting seems to be a promising tool in analyzing somaclonal variation in cauliflower that could be applied to the other *Brassica* species as well.

Quantitative trait loci, marker-assisted selection, and genomics. The development of novel markers and genetic mapping of the *Brassica* genome has opened up new avenues in marker-assisted breeding programs. Various markers such as RAPD, RFLP, amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) analyses have been used in marker-assisted selection of various *Brassica* crops. Marker-assisted selection offers great potential for improved efficiency and effectiveness in selection of plant genotypes with the desired combinations of traits. This approach relies upon chromosomal linkages between a molecular marker and the characteristic and gene(s) to be selected.

Using various markers, genes affecting quantitative trait loci (QTL) have been mapped in several *Brassica* species. QTL are controlled by several genes and the phenotype observed is the combined effect of all the alleles at all the loci, influenced by environmental conditions, which can then be untangled with certain statistical determination. QTL are being extensively used in identifying the genes responsible for quantitative variation of different traits in *Brassica*. The use of QTL could help in addressing queries concerning the evolutionary and ecological significance of variation as well.

The development of markers and identification of QTL controlling various traits such as oil content (Badani et al., 2002; Mahmood et al., 2003), disease resistance (Grandclément and Thomas, 1996; Pilet et al., 1998; Manzanares-Dauleux et al., 2000; Somers et al., 2002; Zhao and Meng, 2003), flowering time (Ferreira et al., 1995; Teutonico and Osborn, 1995; Camargo and Osborn, 1996), and fertility restoration (Hansen et al., 1997; Jean et al., 1997) are positive steps toward marker-assisted breeding of *Brassica*. High-density maps will facilitate increased utility of marker-assisted breeding in *Brassica*.

One obvious and important tool that will benefit the improvement of *Brassica* crops probably more than any other plant group is the tremendous early and thorough genomic information of the most

scientifically popular member of the *Brassicaceae*: *Arabidopsis thaliana*. Since *Brassica* species are more closely related to *Arabidopsis* than any other economically important plant group, the sequencing of the *Arabidopsis* genome and the synteny between them should accelerate *Brassica* genomics. Genomic work in *Brassica* is being facilitated by an international consortium (http://www.brassica.info) and the integration, annotation, and public data interfacing will be done by The Institute for Genomic Research (TIGR), Munich Information Center for Protein Sequences (MIPS) and the National Institute of Agricultural Biotechnology (NIAB). Genome sequencing is aimed at completion by 2007, which should greatly benefit *Brassica* breeding in the coming years.

Genetic Transformation

Transformation systems have been developed in almost all the economically important species of *Brassica* such as *B. juncea* (Barfield and Pua, 1991), *B. napus* (Moloney et al., 1989), *B. rapa* (Radke et al., 1992), *B. oleracea* (De Block et al., 1989), *B. nigra* (Gupta et al., 1993), and *B. carinata* (Narasimhulu et al., 1992). Various methods used for *Brassica* transformation and the factors affecting transformation efficiencies have been reviewed by Poulsen (1996). *Agrobacterium tumefaciens*-mediated transformation is most widely used for *Brassica* and it is generally quite efficient and practical for most species in the genus. However, there is still a need for developing efficient transformation methods to overcome genotype dependency. There have been recent reports on increasing transformation efficiencies in *Brassica* such as broccoli (Henzi et al., 2000) and canola (Cardoza and Stewart, 2003), which are two of the most important *Brassica* crops.

Transformation has improved *Brassica* species for many traits, but the most prominent trait has been for herbicide resistance (HR) and HR canola is the fourth most planted transgenic crop in the world. In Canada, in 2003, 3.15 million hectares of transgenic canola were planted, about two-thirds of the total canola cultivation in that country. Canola tolerant to herbicides such as imidazoline, glufosinate, and glyphosate is now available commercially in the USA and Canada. Other examples of herbicide resistance include glufosinate resistance in broccoli (Waterer et al., 2000) and *B. rapa* (Qing et al., 2000), sulfonylurea resistance in *B. napus* (Blackshaw et al., 1994), and bromoxynil resistance in *B. napus* (Zhong et al., 1997).

Oil quality improvement has been another target for *Brassica* transformation. *Brassica* oil is in great global demand and technology is available to custom-tune fatty acid profiles in seeds. We shall briefly examine some prominent examples. Silencing the endogenous oleate desaturase increased oleic acid levels in *B. napus* and *B. juncea* (Stoutjesdijk et al., 2000). Canola with high γ-linolenic acid was produced by transformation of δ12-desaturase genes from the fungus *Mortierella alpina* (Liu et al., 2001). By expressing Garm FatA1, an acyl-acyl carrier protein (ACP) thioesterase isolated from *Garcinia mangostana* in canola, Hawkins and Kridl (1998) have been able to produce canola with elevated levels of stearate. By site-directed mutagenesis, Facciotti et al. (1999) have engineered the Garm FatA1 gene for increased activity and have been able to generate seven mutants that conferred a 13-fold increase in enzyme activity towards ACP *in vitro*. The seed-specific expression of the mutant gene resulted in transgenic plants that accumulated 55–58% more stearate than plants expressing the wild-type gene. By crossing transgenic bay thioesterase (BTE) canola with transgenic canola expressing a coconut lyphosphatidic acid acyltransferase (CLP) cDNA, plants containing both transgenes (BTE and CLP plants) were produced. Seed oil from these lines showed an increase in laurate at sn-2 position (Knutzon et al., 1999).

In addition to oil improvements, *Brassica* transformation can convert a crop to biochemical factories for the production of pharmacological and industrial products, such as the biodegradable polymer poly(β-hydroxybutyrate) (PHB). Oilseed *Brassica* species are ideal candidates for the commercial production of PHB since acetyl-CoA, the substrate required for the first step of PHB biosynthesis, is prevalent during oilseed fatty acid biosynthesis. Three genes (*phbA* or *bktB*, *phbB* and *phbC*) that are required for the production of the biopolymer were engineered in *B. napus* and the polymer was produced in the leucoplasts (Houmiel et al., 1999). *B. carinata* was used in the production of a blood anticoagulant protein, hirudin (Chaudhary et al., 1998). An oleosin–hirudin fusion protein was engineered using *Agrobacterium*. The advantage of using *Brassica* oilseeds for the production of biologically active components fused with proteins such as hirudin is the ease of purification of the protein by floatation centrifugation. *B. napus* has also been used in the production of carotenoids (Shewmaker et al., 1999), which act as antioxidants in the human body.

Insect resistance is a target trait since there are increasing pest problems in *Brassica* crops. Since *Brassica* crops are susceptible to the diamondback moth, a good approach to control it is to overproduce a *Bacillus thuringiensis* endotoxin crystal protein such as *Bt Cry1A (c)*. This gene has been introduced in *B. napus* (Stewart et al., 1996; Halfhill et al., 2001), Chinese *B. napus* cultivars (Li et al., 1999), rutabaga (Li et al., 1995), cabbage (Metz et al., 1995a), broccoli (Metz et al., 1995b; Cao et al., 1999), and Chinese cabbage (Cho et al., 2001).

Salt-tolerant *Brassica* plants have been developed by over-expressing AtNHX1, a vacuolar Na^+/H^+ antiporter from *Arabidopsis thaliana* (Zhang et al., 2001). The engineering of the bacterial *codA* gene has enhanced the salt- and cold-tolerance level in *B. juncea* (Prasad et al., 2000). The development of salt-tolerant plants could help extend canola cultivation in saline soils and several *Brassica* species could be useful for wasteland reclamation where the salt content is lethal for most crops.

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*, it was possible to obtain male-sterile plants by introducing the *barnase* gene with tapetum-specific promoters (Jagannath et al., 2001). The fertility of the male-sterile line was restored by crossing it with a *barstar*-containing transgenic line (Jagannath et al., 2002). This male sterility/fertility restorer system has tremendous potential in hybrid breeding.

Advances in the introduction of desired novel traits have led to the development of potentially commercially important *Brassica* oil crops and vegetables. Table 1 gives a list of various genes and traits engineered in *Brassica* in recent years. Work prior to 1995 has been reviewed by Poulsen (1996).

Conclusions and Future Prospects

Science and technologies in plant cellular and molecular biology offer tremendous potential for plant improvement. Tissue culture,

TABLE 1

LIST OF GENES OF IMPORTANCE RECENTLY INTRODUCED IN *BRASSICA* CROPS AND THEIR FUNCTIONS

Species	Gene introduced	Function	Reference
Brassica napus L.	*crsI-1*	Sulfonylurea resistance	Blackshaw et al., 1994
Brassica napus L.	*Bxn*	Bromoxynil resistance	Zhong et al., 1997
Brassica napus L.	δ12-desaturase	Production of high γ-linolenic acid	Liu et al., 2001
Brassica napus L.	*Garm FatA1*	Increase in enzyme activity towards acyl-acyl carrier protein (ACP)	Facciotti et al., 1999
Brassica napus L.	*phbA.phbB.phbBc* or *bktB.phbB.phbC*	Production of poly(β-hydroxybutyrate) (PHB)	Houmiel et al., 1999
Brassica napus L.	*CrtB*	Increase in carotenoid production	Shewmaker et al., 1999
Brassica napus L.	Truncated synthetic *Bt Cry1A (c)*	Resistance to diamondback moth and cabbage looper	Stewart et al., 1996; Halfhill et al., 2001
B. rapa ssp. *pekinensis*	Synthetic *Bt Cry1c*	Resistance to diamondback moth	Cho et al., 2001
B. rapa (syn. *B. campestris*) ssp. *parachinensis*	Synthetic *Bt Cry1Ab, Cry1Ac*	Resistance to diamondback moth	Xiang et al., 2000
Brassica carinata	OBHIRT (oleosin–hirudin) fusion protein	Production of hirudin	Chaudhary et al., 1998
Rutabaga (*B. napobrassica*)	*Bt Cry1A (c)*	Resistance to cabbage caterpillar (*Pieris rapas*)	Li et al., 1995
Broccoli (*B. oleracea* L. var. *italica*)	*Bt Cry1A (c)*	Resistance to diamondback moth	Metz et al., 1995b; Cao et al., 1999
Brassica oleracea var. *capitata* (cabbage)	*Bt Cry1Ab3*	Resistance to diamondback moth larvae	Jin et al., 2000
Brassica juncea	Bacterial *CodA*	Enhanced salt and cold tolerance	Prasad et al., 2000
Brassica juncea	*Barnase*	Male sterility	Jagannath et al., 2001

somatic cell fusion, somaclonal variation, marker-assisted breeding, and transformation together can be of use in the development of plants with novel desirable traits. Mapping and sequencing *Brassica* genomes will facilitate the isolation of specific genes to aid in crop improvement and help us better understand the basic biology of this fascinating genus.

Many genetic improvements with the highest impact have come from genetic transformation. Even though these developments are at least partially the result of the increased efficiency in many of the tissue culture technologies reviewed here, perhaps the most promising technology on the horizon for *Brassica* is *in planta* transformation, which would, paradoxically, negate the need for tissue culture and much of the information reviewed in this paper. *In planta* transformation has been accomplished most extensively in *Arabidopsis thaliana*, in which flowers are immersed in *Agrobacterium tumefaciens* cultures, where the target of transformation is ovules (reviewed in Pelletier and Bechtold, 2003). Subsequent to *in planta* transformation, seeds are harvested and then transformants selected. To date, the only crops to be transformed by this method have been pakchoi (*B. rapa* ssp. *chinensis*) (Liu et al., 1996; Qing et al., 2000), radish (*Raphanus sativus* L. *longipinnatus* Bailey) (Curtis and Nam, 2001), and most importantly, canola-quality *B. napus* (Wang et al., 2003) – all in the *Brassicaceae*. We predict these successes are bell-wethers of universal *in planta* transformation. The great benefits of this technology include negating genotype specificity, the labor and specialized facilities needed for tissue culture, and increased speed to transgenic plants.

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