The noble radish: past, present and future

Ian S. Curtis

National Institute of Agrobiological Sciences (NIAS), Department of Biotechnology, Kannondai 2-1-2, Tsukuba, Ibaraki 305-8602, Japan

Recent developments in plant biotechnology have revealed that radish can be genetically modified by a technique called 'floral-dipping'. This system has been used successfully to delay both bolting and flowering in radish by the co-suppression of the photoperiodic gene, *GIGANTEA*. Future research could use this system to improve the pharmaceutical value of the crop for global usage.

In the West, the radish is commonly seen as a small-rooted, short-season vegetable normally consumed in salads. However, in the Far East, a diverse, large-rooted radish, is widely grown. These roots are variable in shape and exhibit a great variation in color of skin and flesh. The large-rooted radish is by far the most important radish cultivated in the world. Japan produces 30 times more radishes by weight compared to the whole of Europe [1]. Such roots are eaten raw or cooked or preserved by storage, pickling, canning or drying. Nevertheless, radish is not only a vegetable crop but also an important source of medicinal compounds [2]. The development of a gene transfer system for radish would allow specific genes to be incorporated into the crop and so accelerate the production of novel germplasms compared with conventional breeding practices. The aim of this article is to illustrate the transformation system, describe how such a procedure has been used in the production of late-flowering radish and highlight recent research that has identified possible key chemical components that make the radish a highly valued pharmaceutical crop.

Development of a gene transfer system

Tissue culture studies

It is generally well known that radish is one of the most recalcitrant crop plants in culture. Early studies focusing on plant regeneration via organogenesis from seedling explants [3], embryogenic calli [4] and microspores [5] revealed a shoot regeneration frequency too low for practical usage. However, recent studies using silver nitrate and L-α-2-aminoethoxyvinylglycine (AVG) in the culture medium of hypocotyl explants of Chinese radish 'Red Coat' gave rise to a shoot regeneration frequency of 40% [6]. In spite of this improved shoot regeneration system, there is no report of transgenic radish being produced in culture.

Floral-dipping

The production of transgenic plants in culture requires careful preparation of plant cells or tissues, a method of transferring foreign DNA into these cells and a way of selecting transformed shoots. As well as being a labor-intensive procedure, regenerated plants often exhibit somaclonal variation and reductions in fertility. At present, an efficient tissue culture system does not exist for the production of transgenic radish in culture and so alternative methods must be investigated.

The first reported production of transgenic radish by a method known as 'floral-dip' [7] used the commercially important Korean cultivar 'Jin Ju Dae Pyong'. This procedure of gene transfer (Fig. 1) is currently the only system available for producing transgenic radish. From a total of 25 transgenic plants produced from this study, two groups of plants (comprising seven and five plants) revealed common gene integration patterns. It appears, therefore, that the transformation of radish by floral-dipping results in a high frequency of sibling transformants. Previous studies using the floral-dip procedure for the production of transgenic *Arabidopsis* plants often results in independent hemizygotes [8]. This suggests that the mechanism of T-DNA transfer into radish is different from the mechanism of T-DNA transfer into *Arabidopsis*. A recent study investigating the value of infiltrating seedlings and flowering plants with Agrobacteria as a means of producing transgenic plants of *Medicago truncatula* might have revealed the possible mechanism of T-DNA transfer in radish [9]. In this study, vacuum-infiltrated flowering plants produced a greater frequency of sibling transformants (77–87%) compared with the seedling infiltration method (14–33%). These researchers postulated that the targets of transformation in vacuum-infiltrated flowering plants might occur at the meristematic cells of axillary buds. This hypothesis supports our radish transformation results [7], in that, the majority of transformed seeds originated from plants dipped into a suspension of *Agrobacteria* at the stage of primary bolting, at which time, many meristematic axillary buds were present. This floral-dip procedure for radish has subsequently been used in transferring agronomically useful genes into this crop.

Production of late-flowering radish by the down-regulation of the photoperiodic-responsive gene, *GIGANTEA*

Korean ecotypes of radish are cold sensitive and therefore bolt during the fall (autumn) when ambient temperatures fall to 5–6°C. Such a phenomenon means that the production of high quality roots cannot continue into the fall. Attempts to transfer the late-flowering trait from Japanese ecotypes into commercial Korean genotypes by

Corresponding author: Ian S. Curtis (iscurtis@affrc.go.jp).

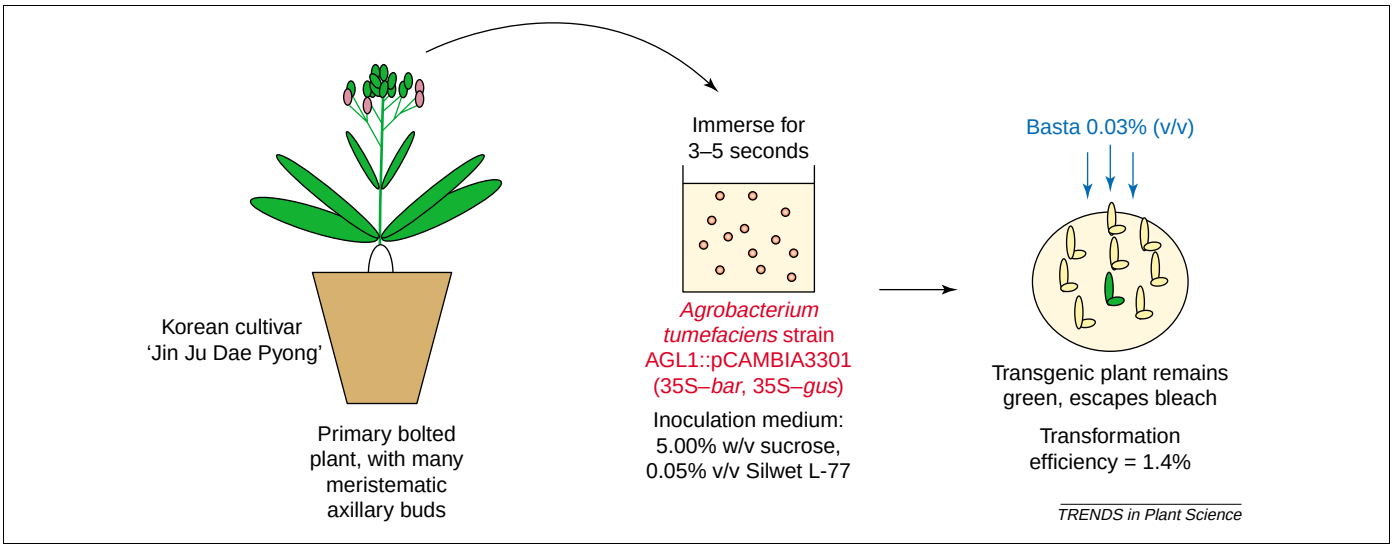

Fig. 1. Floral-dip method used for the optimal production of transgenic radish. Primary bolted plants of the Korean cultivar 'Jin Ju Dae Pyong' are dipped (3–5 seconds) into a suspension of *Agrobacterium tumefaciens* strain AGL1::pCAMBIA3301 in the presence of 5.00% (w/v) sucrose and 0.05% (v/v) Silwet L-77. Plants are returned to the glasshouse to produce seeds. After seed collection and ripening, the seeds are sown to soil. Screening for transformed plantlets is achieved by spraying with herbicide (0.03% v/v Basta).

conventional breeding produced hybrids with reduced quality that bolted during the summer months [10]. For this reason, the transfer of late-flowering genes into radish has been regarded as a major research target. *GIGANTEA* (*GI*), a gene involved in regulating photoperiodic flowering and controlling circadian rhythms was cloned by two independent research groups [11,12]. Previous studies have shown that *gi* mutants of *Arabidopsis* exhibit delayed flowering [13,14]. Because of the taxonomic closeness between *Arabidopsis* and radish, attempts to delay bolting and flowering in radish were investigated by transferring an *Arabidopsis* antisense *GI* gene fragment into the crop to down-regulate the expression of native *GI* [15]. Phenotypic studies of 11 T2 lines exhibited significant delays in bolting and flowering times compared with wild type (bolting, 17 days; anthesis, 18 days) and positive control transformants (bolting, 23 days; anthesis, 26 days). This study suggests that floral-dipping can play an important role in producing valuable radish germplasms.

Future perspectives

Recent studies have identified two important medicinal compounds in radish – peroxidase and isothiocyanates. Hyperlipidemia, a condition associated with excess fat in the blood, is the main cause of coronary heart diseases. Radish roots are a rich source of peroxidase, an oxidoreductase, which can scavenge harmful free radicals. Clinical trials using hyperlipidemia mice fed with different purities of peroxidase from radish showed a significant reduction of cholesterol and triglyceride in their blood [16]. In a separate study, the concentration of total isothiocyanates (a group of chemicals capable of inducing antimicrobial, antimutagenic and anticarcinogenic activities) was sevenfold higher in grated roots compared with diced roots measured 30 min after cooking [17]. In addition, a correlation between the potency of antimutagenicity (as determined by a UV-induced mutation assay of *E. coli* B/r WP2) and the amount of 4-(methylthio)-3-butenyl isothiocyanate (MTBITC; the major isothiocyanate in radish roots) in *n*-hexane extracts was also observed. The development of a gene transfer system in radish enables agronomically important traits to be transferred and is also a means of manipulating the identified endogenous medicinally valued chemicals for the benefit of the world.

Acknowledgements

I thank Hong Gil Nam (Pohang University of Science and Technology, South Korea) for inviting me to conduct the pioneering research on the genetic engineering of radish. This research was largely supported by the Korean Federation of Science and Technology Societies (KOFST). Finally, I thank Yugi Kamiya (Plant Science Center, RIKEN, Wako-shi, Japan) for assistance.

References

1 Crisp, P. (1995) Radish. In *Evolution of Crop Plants*, 2nd edn, (Smartt, J. and Simmonds, N.W., eds) pp. 86–89, Longman Group UK, Harlow, UK
2 Watt, J.M. and Breyer-Brandwijk, M.G. (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd edn, E. & S. Livingstone, Edinburgh and London, UK
3 Matsubara, S. and Hegazi, H.H. (1990) Plant regeneration from hypocotyl callus of radish. *HortScience* 25, 1286–1288
4 Jeong, W.J. *et al.* (1995) Somatic embryogenesis and plant regeneration in tissue cultures of radish (*Raphanus sativus* L.). *Plant Cell Rep.* 14, 648–651
5 Takahata, Y. *et al.* (1996) Microspore culture of radish (*Raphanus sativus* L.): influence of genotype and culture conditions on embryogenesis. *Plant Cell Rep.* 16, 163–166
6 Pua, E-C. *et al.* (1996) Synergistic effect of ethylene inhibitors and putrescine on shoot regeneration from hypocotyl explants of Chinese radish (*Raphanus sativus* L. var. *longipinnatus* Bailey) *in vitro*. *Plant Cell Rep.* 15, 685–690
7 Curtis, I.S. and Nam, H.G. (2001) Transgenic radish (*Raphanus sativus* L. *longipinnatus* Bailey) by floral-dip method – plant development and surfactant are important in optimizing transformation efficiency. *Transgenic Res.* 10, 363–371
8 Clough, S.J. and Bent, A.F. (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* 16, 735–743
9 Trieu, A.T. *et al.* (2000) Transformation of *Medicago truncatula* via infiltration of seedlings or flowering plants with *Agrobacterium*. *Plant J.* 22, 531–541

10 Lee, S-S. (1987) Bolting in radish. In *Improved Vegetable Production in Asia* (Series no. 36) (Asian and Pacific Council, eds), pp. 60–70, Food & Fertilizer Technology Center, Taipei, Republic of China
11 Park, D.H. *et al.* (1999) Control of circadian rhythms and photoperiodic flowering by the *Arabidopsis GIGANTEA* gene. *Science* 285, 1579–1582
12 Fowler, S. *et al.* (1999) *GIGANTEA*: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. *EMBO J.* 18, 4679–4688
13 Redei, G.P. (1962) Supervital mutants of *Arabidopsis*. *Genetics* 47, 443–460
14 Koornneeff, M. *et al.* (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 229, 57–66
15 Curtis, I.S. *et al.* (2002) Expression of an antisense *GIGANTEA* (*GI*) gene fragment in transgenic radish causes delayed bolting and flowering. *Transgenic Res.* 11, 249–256
16 Wang, L. *et al.* (2002) Effects of peroxidase on hyperlipidemia in mice. *J. Agric. Food Chem.* 50, 868–870
17 Nakamura, Y. *et al.* (2001) 4-(Methylthio)-3-butenyl isothiocyanate, a principal antimutagen in daikon (*Raphanus sativus*; Japanese white radish). *J. Agric. Food Chem.* 49, 5755–5760

1360-1385/03/$ - see front matter
doi:10.1016/S1360-1385(03)00127-4

RNA silencing bridging the gaps in wheat extracts

Olivier Voinnet

Institut de Biologie Moléculaire des Plantes du CNRS 12, Rue du Général Zimmer 67084 Strasbourg, Cedex, France

In plants, RNA silencing plays important roles in antiviral defence, genome integrity and development. This process involves nucleotide sequence-specific interactions that are mediated by small RNA molecules of 21–25 nucleotides. Although the core biochemical reactions of RNA silencing have been well characterized in animals, such information was crucially missing in plants. Recent work now addresses this question and reveals an overall similarity between the plant and animal RNA-silencing pathways, as well as some intriguing plant-specific aspects.

Eukayotic cells have developed a powerful system to wipe out foreign nucleic acids such as transposons and viruses. Several manifestations of this defence, collectively referred to as 'RNA silencing', are triggered by double-stranded RNA (dsRNA), a replication intermediate of many viruses. Experimentally, this molecule can be produced through transcription of inverted repeat transgenes or delivered directly into cells. In *Drosophila*, an Rnase III-like enzyme named Dicer cleaves the dsRNA into 21 nucleotide-long RNA duplexes, the 'short interfering' RNAs (siRNAs) [1,2]. The siRNA is then incorporated into a multi-subunit endonuclease, the RNA-induced silencing complex (RISC), and so ensures, upon base-pairing, that it specifically cleaves RNA sharing sequence identity with the dsRNA [3]. In terms of defence, the logic behind this two-step degradation process is impeccable because it not only targets the initially unwelcome dsRNA but also any potential sibling viral molecules, thanks to the activity of RISC. Small RNAs correlating with RNA silencing were originally discovered in plants [4] whose genomes encode several Dicer-like proteins [5]. Thus, the reactions characterized in *Drosophila* were also likely to be the core of the RNA-silencing mechanism in plants. However, there was no biochemical proof for Dicer or RISC activities in plants, nor was there a direct indication that the plant small RNAs were bona fide siRNAs. This has now been established in recent work by Guiliang Tang and colleagues from Phillip Zamore's group [6].

Separate Dicer activities generate two species of siRNAs in plants

Tang *et al.* [6] used an *in vitro* silencing assay based on wheat germ extracts, in which they incubated labelled dsRNA. The dsRNA became rapidly processed into a discrete species of small RNAs, with no intermediate products, a reaction characteristic of the activity of purified Dicer from *Drosophila* or humans [1,7]. As for animal siRNAs [1,8], the occurrence of the small RNAs depended on the presence of ATP in the wheat germ extract. Moreover, RNase protection indicated that the plant small RNAs, just like their animal counterparts, have a double-stranded body with 2 nt-long 3′ overhangs, the hallmarks of Dicer cleavage products [9]. Plant siRNAs arose as two distinct species of 25 nt and 21 nt in the wheat extract (Fig. 1), which confirmed earlier *in vivo* observations made in tobacco and *Arabidopsis* [10]. By contrast, incubation of labelled dsRNA in fly embryo extracts led exclusively to 21 nt-long siRNAs. Because Dicer is encoded by a single gene in *Drosophila,* whereas at least four homologues are found in the rice and *Arabidopsis* genomes [5], Tang *et al.* reasoned that the two siRNA classes in plants were probably produced by two distinct Dicers. They tested the hypothesis by adding 25 nt-long, cold siRNA duplexes as competitor molecules in the extract. Synthesis of 25 nt-long siRNA from labelled dsRNA was strongly inhibited by this treatment, whereas the production of 21 nt species remained unaffected. The simplest explanation for this contrasting sensitivity to competitor siRNAs is that distinct Dicer-like enzymes generate each class of siRNA [6].

More dsRNA makes more siRNAs

Historically, plant scientists' interest in RNA silencing originated from observations made in transgenic plants

Corresponding author: Olivier Voinnet (olivier.voinnet@ibmp-ulp.u-strasbg.fr).