Review

Contributions and application advances of japan in the development of rice genetic transformation technology systems

Aportes y avances en la aplicación de japón en el desarrollo de sistemas de tecnología de transformación genética del arroz

Contribuições e avanços na aplicação do japão no desenvolvimento de sistemas de tecnologia de transformação genética do arroz

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Abstract

Based on a systematic literature retrieval from multiple mainstream databases including Web of Science, Google Scholar and J-STAGE, this paper collected and sorted relevant research literature published from 1960 to 2024, and also supplemented information through exchanges with Japanese rice biotechnology researchers. Japan has made foundational contributions to rice genetic transformation technology, establishing key systems such as protoplast regeneration and *Agrobacterium*-mediated transformation. These advances have enabled the development of transgenic rice with improved stress resistance, enhanced quality, and nutritional fortification. Despite slow commercialization domestically, Japan's technological platforms have become essential tools for global functional genomics and molecular breeding in rice. The integration of these systems with gene editing promises innovative solutions for future food security challenges.

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Resumen

Mediante la búsqueda sistemática de literatura en las principales bases de datos como Web of Science, Google Scholar y J-STAGE, se recopilaron y clasificaron los documentos de investigación publicados entre 1960 y 2024; además, se complementó la información a través de intercambios con investigadores japoneses especializados en biotecnología del arroz. Japón ha realizado contribuciones fundamentales a la tecnología de transformación genética del arroz, estableciendo sistemas clave como la regeneración de protoplastos y la transformación mediada por *Agrobacterium*. Estos avances han permitido el desarrollo de arroz transgénico con mayor resistencia al estrés, calidad mejorada y fortificación nutricional. A pesar de una lenta comercialización a nivel nacional, las plataformas tecnológicas de Japón se han convertido en herramientas esenciales para la genómica funcional global y el mejoramiento molecular en arroz. La integración de estos sistemas con la edición genética promete soluciones innovadoras para los futuros desafíos de seguridad alimentaria.

Palabras clave: Japón, arroz, tecnología transgénica, transformación mediada por *Agrobacterium*, sistema de transformación genética.

Resumo

Com base na busca sistemática de literatura em diversas bases de dados consolidadas, incluindo Web of Science, Google Scholar e J-STAGE, foram reunidos e organizados os trabalhos de pesquisa publicados entre 1960 e 2024, e as informações foram ainda enriquecidas por meio de comunicações com pesquisadores japoneses da área de biotecnologia do arroz. O Japão realizou contribuições fundamentais para a tecnologia de transformação genética do arroz, estabelecendo sistemas-chave como a regeneração de protoplastos e a transformação mediada por *Agrobacterium*. Esses avanços permitiram o desenvolvimento de arroz transgénico com maior resistência a estresses, qualidade aprimorada e fortificação nutricional. Apesar de uma lenta comercialização doméstica, as plataformas tecnológicas do Japão tornaram-se ferramentas essenciais para a genómica funcional global e o melhoramento molecular do arroz. A integração desses sistemas com a edição genética promete soluções inovadoras para os futuros desafios de segurança alimentar.

Palavras-chave: Japão, arroz, tecnologia transgénica, transformação mediada por *Agrobacterium*, sistema de transformação genética.

Introduction

Against the backdrop of population growth, limited arable land, and climate change, conventional rice breeding methods face bottlenecks such as long cycles and limited efficiency in improving complex agronomic traits (Ahmar *et al.*, 2020). In this context, transgenic technology has become an important means to accelerate crop genetic improvement and achieve precise trait design. Internationally, transgenic crops such as maize and soybean are widely used, while transgenic research on rice, serving as both a model crop and a key food grain, holds both scientific and strategic significance.

As a leader in agricultural science and technology, Japan possesses a profound accumulation in both fundamental rice research and breeding practices (Li *et al.*, 2023). Since the mid-20th century, Japanese scientists have made pioneering breakthroughs in key rice technologies like tissue culture and genetic transformation. These advances have laid a methodological foundation for domestic biotechnology research and provided essential technological platforms for global rice functional genomics and molecular breeding (Nakagahra *et al.*, 1997).

This article reviews Japan's key contributions and the evolution of its transgenic rice research, focusing on the established genetic transformation systems and their derived applications. This review is intended to provide a reference for a deeper understanding of the development path of rice transgenic technology and its role in food security.

Methods

This review is based on a systematic retrieval and analysis of peer-reviewed journal articles published from the 1960s to 2024, sourced from databases such as Web of Science, Scopus, PubMed, Google Scholar, and J-STAGE. To supplement and corroborate the literature information, the writing of this review also referred to indirect communication with several Japanese scientists long engaged in rice biotechnology research and an analysis of viewpoints from important review authors in related fields, aiming for a more comprehensive understanding of the historical context and research challenges in technological development.

Discussion

Callus Induction and Plant Regeneration

The theoretical foundation of plant tissue culture comes from the concept of "totipotency," first proposed by Gottlieb Haberlandt in the early 20th century. Building on this concept, Hiroshi Niizeki and Kiyoshi Oono achieved the first successful rice anther culture in 1968, producing haploid plants and marking the beginning of rice tissue culture research (Niizeki & Oono, 1968). This milestone subsequently enabled the development of doubled haploid (DH) production through anther culture. In the 1970s, rapid optimization of anther culture conditions ensued, including cold pretreatment of materials before/after heading, control of donor plant physiological status, adjustment of sugar concentration and hormone ratios (e.g., 2,4-D, cytokinins), and selection of culture medium systems. Genotype dependency was clarified (Japanese *japonica* rice generally responds more readily than *indica* rice) (Komamine, 2003). Anther culture entered a trial stage in breeding for Japanese *japonica* materials, forming an early methodological basis for rapid DH line fixation. During 1978-1980, Japanese research groups systematically established an embryogenic callus induction and plant regeneration system using immature embryos (specifically the scutellum) as explants, promoting the routine regeneration of plants from somatic starting materials (Radi & Maeda, 1987). Ozawa and Komamine (1989) conducted early and systematic histological and physiological studies on somatic embryogenesis in rice, providing theoretical and technical support for high-frequency regeneration.

Concurrently, explorations into protoplast culture and regeneration were undertaken, systematically evaluating the manifestation and utilization potential of "somaclonal variation" in rice (e.g., obtaining resistance or quality trait variations) (Sun *et al.*, 1991; Zong-Xiu *et al.*, 1983). In the 1990s, stable and efficient regeneration systems based on embryogenic calli induced from mature/immature seeds were established, paving the way for gene function research.

Plant regeneration from protoplasts and its role in rice genetic transformation

The first successful regeneration of whole plants from rice protoplasts marked a significant breakthrough in protoplast culture for cereal crops. Fujimura *et al.* (1985) were the first to regenerate complete plants from protoplasts isolated from rice suspension cells, demonstrating that establishing embryogenic suspension cell lines with strong regenerative capacity was key to achieving protoplast regeneration. Subsequently, Yamada *et al.* (1986) and Toriyama *et al.* (1988) also achieved plant regeneration from protoplasts in several Japanese rice cultivars, further confirming the applicability of this technique across different genotypes. To improve regeneration efficiency, Kyozuka *et al.* (1987) developed a novel nurse culture method. This method combined agarose bead culture with actively growing nurse cells, achieving a colony formation frequency of up to 10 % from protoplasts and a plant regeneration frequency of 17 %-50 %, regenerating numerous transplantable plants via somatic embryogenesis. The establishment of this technique transformed rice protoplast culture from a laboratory exploration into a stable, reproducible operational system, laying a solid foundation for subsequent genetic manipulation.

With the maturation of the protoplast regeneration system, Japanese researchers quickly applied it to the direct introduction of foreign genes, pioneering rice transgenic technology. Uchimiya *et al.* (1986) first used the polyethylene glycol (PEG) method to introduce a plasmid carrying a kanamycin resistance gene into rice protoplasts and obtained transgenic calli via antibiotic selection, with Southern blotting confirming foreign gene integration. Shortly after, Toriyama *et al.* (1988) successfully regenerated fertile transgenic rice plants from protoplasts into which a kanamycin resistance gene had been introduced via electroporation, accomplishing the full process from gene introduction to whole plant regeneration. Thereafter, Shimamoto *et al.* (1989) further optimized electroporation conditions, co-introducing a hygromycin phosphotransferase (*hph*) gene and a β-glucuronidase (*GUS*) reporter gene into protoplasts, obtaining transgenic rice stably expressing the foreign genes, and confirming stable inheritance through progeny analysis. Collectively, these studies demonstrated that obtaining transgenic plants via direct gene introduction into protoplasts had become a stable technique for rice genetic transformation.

Rice genetic transformation using *Agrobacterium*

Although transgenic technology had been established in rice via direct gene delivery methods into protoplasts (e.g., PEG, electroporation), this approach had significant limitations: many rice cultivars (especially *indica*) were difficult to culture as protoplasts, regeneration capacity varied greatly among cultivars, and regenerated plants often exhibited high deformity rates and difficulty in obtaining normal fertile plants; furthermore, protoplast culture technology was complex, time-consuming, and required highly skilled operation. The gene gun method (particle bombardment) partly circumvented the difficulties of protoplast culture but still suffered from issues such as high copy number integration and frequent DNA rearrangements. Concurrently, due to differences in monocot cell wall structure and phenolic compound secretion, the natural infection capability of *Agrobacterium tumefaciens* towards them was very weak, leading to the long-held belief that *Agrobacterium*-mediated gene transfer was difficult to achieve in rice. In 1994, Japanese scientists first broke through this bottleneck. Using embryogenic calli (primarily derived from immature embryo scutella) as recipients, they successfully obtained a large number of morphologically normal, fertile transgenic rice plants by optimizing *Agrobacterium* strains, co-culture conditions, and selection strategies, achieving transformation efficiencies comparable to those in dicot plants (Hiei *et al*., 1994). This study first proved that *Agrobacterium* could efficiently transfer T-DNA into rice cells with stable integration, expression, and inheritance, establishing a milestone technological foundation for rice genetic transformation.

Building upon the breakthrough by Hiei *et al.* (2008), Japanese teams further promoted vector and transformation system optimization. Komari *et al.* (2006) developed the super-binary vector, which incorporated additional virulence genes (e.g., virB, virG from pTiBo542) on the basis of standard binary vectors, significantly enhancing T-DNA transfer efficiency, particularly showing high transformation efficiency for difficult-to-transform *japonica* cultivars (e.g., 'Koshihikari') and some *indica* materials. Subsequently, Hiei and Komari (2008) systematically summarized an efficient transformation protocol using calli induced from immature or mature embryos as recipients. This protocol, through heat shock and centrifugation pretreatment, addition of suitable phenolic inducers (e.g., acetosyringone), and optimized selection procedures, achieved high-efficiency transformation (50 %-90 %) for various genotypes, including *japonica* and *indica* (e.g., 'Kasalath'). This standardized protocol became a core technological platform for global rice functional genomics research and genetic improvement.

Agrobacterium-mediated transformation became the mainstream method, replacing direct delivery methods (Wakita *et al*., 1998), due to its distinct advantages: Direct delivery methods (e.g., gene gun) often lead to complex integration of foreign genes as multiple copies, rearrangements, or fragments, prone to causing gene silencing or unstable expression. In contrast, *Agrobacterium*-mediated transformation typically results in low-copy (1-2), precise T-DNA integration, favoring stable transgene expression and inheritance. The *Agrobacterium* method does not require expensive specialized equipment (e.g., gene gun) and can leverage extensive experience in vector construction and strain manipulation accumulated in dicots, presenting a relatively lower technical barrier. Using embryogenic calli as recipients avoids the genotype dependency and regeneration difficulties associated with protoplast culture, making it particularly suitable for *indica* cultivars that are difficult to regenerate from protoplasts.

Diversified systems for rice genetic transformation

Following the establishment of the *Agrobacterium*-mediated transformation system pioneered by Japanese teams as the core platform for global rice functional genomics and molecular breeding, its technical procedures gradually became standardized and scaled up in the 2010s. This efficient and stable regeneration system, particularly protocols based on easily regenerable model *japonica* cultivars like 'Nipponbare' and 'Kitaake', provided the indispensable gene delivery and plant regeneration steps for the efficient implementation of CRISPR/Cas gene editing technologies in rice (Sukegawa *et al.*, 2023). Japanese researchers continuously optimized culture medium hormone ratios, light/temperature conditions, and osmotic regulation, effectively shortening culture cycles, reducing somaclonal variation, and improving the uniformity of regenerated plants (Ozawa, 2012). To overcome the bottleneck of difficult regeneration in some *indica* and local cultivars, emerging strategies such as introducing developmental regulator genes (e.g., *BBM*, *WUS*) or their transient expression systems were employed to directly enhance the regenerative capacity of recipient cells, further expanding the genotype applicability of the *Agrobacterium* method (Chen *et al.*, 2022).

Despite the widespread adoption of *Agrobacterium*-mediated transformation, research into simpler and less expensive transformation methods is still ongoing. Among these, silicon carbide whisker (SCW)-mediated transformation offers a unique pathway. SCWs are fine needle-like crystals (Karasek *et al.*, 1989). The principle involves vortex-mixing plasmid DNA, SCWs, and plant cells (e.g., embryogenic tissues), where the whiskers create micro-wounds on cell surfaces, facilitating direct DNA uptake (Asad & Arshad, 2011). This method has been successfully applied in crops like maize. Notably, Japanese research teams have successfully achieved SCW-mediated rice genetic transformation using the same mature embryo-derived scutellum tissues as recipients commonly used in gene gun methods (Matsushita *et al.*, 1999). The greatest advantage of this method is that it does not require expensive specialized gene delivery equipment (e.g., gene gun) nor complex protoplast or callus culture systems; its operation is extremely simple. It is regarded as one of the most promising simplified transformation schemes, particularly suitable for application in developing countries with limited laboratory resources.

Application directions and representative genes in transgenic rice

In the early 1990s, rice transgenic technology began transitioning from methodological exploration to practical application, and for the first time, exogenous genes with clear practical value were introduced into rice, marking the entry of rice genetic engineering breeding into a substantial stage. Subsequently, research utilizing this technology for rice improvement expanded extensively across multiple directions, evolving from initial focus on enhancing resistance to comprehensively improving quality, strengthening environmental adaptability, and endowing rice with novel functions.

Resistance improvement targeting major production challenges

Early applications focused on addressing key pest, disease, and weed problems. Researchers successfully introduced the rice stripe virus coat protein gene into rice, developing resistant materials, with some lines completing environmental safety assessments and entering general farmland cultivation (Satoh *et al.*, 2010). Concurrently, the development of insect-resistant rice by introducing *Bacillus thuringiensis* (Bt) endotoxin genes was also achieved (Fujimoto *et al.*, 1993). Regarding herbicide resistance, research was not limited to single herbicides but explored introducing genes with broad-spectrum detoxification potential to achieve wider field adaptability (Kawahigashi *et al.*, 2003).

Quality optimization for consumer demand

To directly improve rice grain quality, genetic engineering was applied to regulate key components. Using antisense technology to suppress the expression of the rice *Waxy* gene aimed to reduce amylose content, breeding high-gluten rice suitable for specific dietary needs (Isshiki *et al.*, 1998). Similarly, suppressing *storage protein glutelin* genes via antisense technology could reduce grain protein content, aiming to breed raw material rice more suitable for brewing (Takaiwa *et al.*, 1999; Washida *et al.*, 1999). Addressing food safety, transgenic rice with suppressed expression of the major rice allergen 16kD albumin via antisense genes was developed, and related low-allergen rice has entered the practical application testing stage (Nakase *et al.*, 1997).

Integrated strategies for enhanced abiotic stress tolerance

To cope with complex environmental stresses, research shifted towards traits involving intricate physiological pathways. For example, genetically engineering enhanced synthesis of glycine betaine not only significantly improved rice salt tolerance but also synergistically enhanced adaptation to multiple stresses including drought, low temperature, and high temperature (Hayashi & Murata, 1998). Furthermore, attempts were made to improve temperature adaptation in rice by altering membrane system-related properties such as fatty acid composition (Matsumura *et al.*, 2002; Takeuchi *et al.*, 2001).

Yield and physiological function optimization for the future

To further explore yield potential and address global climate change, research delved into core physiological processes like photosynthesis. By introducing various genes related to plant defense responses, their effects on enhancing pest and disease resistance were evaluated (Sharoni *et al.*, 2011; Yamaguchi *et al.*, 2009). Simultaneously, genetic modification research aiming to increase yield and optimize photosynthetic capacity to adapt to global warming was also conducted (Matsuoka *et al.*, 2001; Suzuki *et al.*, 2000).

Innovative exploration for novel functions in rice

Going beyond the scope of traditional breeding, genetic engineering was employed to endow rice with entirely new production functions. Examples include breeding rice capable of producing soybean protein (Katsube *et al.*, 1999), iron-fortified rice with high iron content (Masuda *et al.*, 2013), and functional rice containing specific immunity-enhancing substances (Suzuki *et al.*, 2003).

In summary, genetic engineering technology has enabled a leap in rice breeding objectives—from single resistance to comprehensive superior traits, from environmental adaptation to the creation of novel functions. Rice combining high environmental resilience, strong pest/disease resistance, and enhanced nutritional value holds promise for achieving stable high yields under adverse conditions, offering more possibilities for addressing future food security challenges. As more novel genes with application potential are isolated and characterized, and evaluated using mature transformation systems, the value of genetic engineering in rice breeding will continue to grow.

Conclusion

Japan's research in rice genetic transformation has evolved from foundational methods to a versatile technological system, with *Agrobacterium*-mediated transformation at its core. This platform supports global efforts in trait improvement, including resistance, quality, and nutrition. Although regulatory and public perception barriers limit local commercialization, Japan's innovations provide critical support for next-generation breeding techniques. Moving forward, combining established transformation systems with gene editing and synthetic biology will enable more precise and multifunctional rice improvement, contributing significantly to global food and nutrition security.

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