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Recent genetic transformation and gene editing technology used in cucurbit crops

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DESCRIPTION

Cucurbits are a large group of horticultural crops grown worldwide and contain many species of great economic and nutritional value. Melons such as melons, pumpkins, watermelons and cucumbers are very popular with consumers. Pumpkin is also used as a model material for research on plant growth and quality improvement because it is easy to change shape and rich in flavor components. In recent decades, traditional genetic breeding techniques have played an important role in breeding selection for high yielding, high quality squash varieties. However, the genetic diversity and low mutation rates of these crops limit breeding of complex genetic traits and hinder innovation. With the development of modern biotechnologies, innovations through transgenes is and gene editing have become a key focus in the development of horticultural crops, leading to new breeding approaches such as de novo dosing, rapid breeding, haploid breeding and gene deligation. I'm here. In particular, the recent development of transgenic and gene editing techniques for several pumpkin species has greatly facilitated the study of the molecular mechanisms underlying gene function in these plants, yielding pumpkin plants with economically desirable traits. It should provide new ways to grow.

Gene transformation is a genetic engineering assisted technique that involves cloning, delivery, integration, and expression of targeted genes, as well as regeneration, screening, and identification of transgene positive plants. Vector delivery is the first step in transformation by methods such as protoplast transfection, *Agrobacterium* mediated transformation, and particle bombardment. *Agrobacterium* mediated transformation is currently the most widely used genetic transformation method due to its high efficiency. *Agrobacterium* mediated transformation involves the stable integration of the T-DNA from the Ti-plasmid containing the target gene into the plant chromosomal DNA by infection. The transformation process depends on the efficiency of the plant regeneration system. Established methods such as organ culture, somatic embryogenesis, separate culture, and protoplast culture have made great progress in *in vitro* culture of pumpkin. The most common transformation method for pumpkin crops uses cotyledons as explants for direct or indirect plant regeneration, with indirect regeneration mainly used for watermelons and direct regeneration for cucumbers and melons will be used. In recent years, the maturation of transgenic technology has enabled the widespread application of gene editing techniques in many plants. In gene editing, engineered endonucleases identify specific DNA sequences or guide RNAs through their DNA binding domains and precisely and efficiently cleave target DNA sequences to edit specific DNA sequences. Researchers have developed a number of specific gene editing systems, including classes of nucleases that specify targets through protein DNA interactions such as mega nucleases, zinc finger nucleases and transcription activator like effector nucleases. Another class of nucleases contains clustered, regularly spaced short palindromic repeats that identify targets by RNA-DNA base pairing. This technique is considered to be the optimal method to obtain genome edited plants because of its low cost, high flexibility, and high reliability. Gene editing techniques have been applied to pumpkins relatively recently, with

particular success in cucumbers, watermelons, melons and pumpkins.

Transformation systems have been established for some pumpkins, but these systems have the problems of low gene transformation efficiency and immature technology still has a gap. Researchers tried to improve the efficiency of genetic transformation of pumpkin plants by various methods and optimized the genetic transformation steps to improve the transformation efficiency of multiple plant cultivars. Additionally, researchers have used the technology to achieve gene knockouts and base editing in pumpkins and the genes responsible for many important agronomic traits have been studied. Here, we summarize the methods used to improve the efficiency of squash genetic transformation and the application of gene editing techniques to them. Method for improving gene transformation efficiency in crops.

***Agrobacterium* mediated transformation:** This method of using cotyledons as explants is now the main technique used for many pumpkin crops. The efficiency of genetic transformation is affected by many factors including plant regeneration efficiency, infection method, *Agrobacterium* strain, hormones. Researchers have recently optimized screening method and use of exogenous plant the transformation process based on previous studies, including genotypic screening, *Agrobacterium* infection methods, and application of morphogen genes and diversification of screening markers thereby improving transformation efficiency.

Seed germination: Sterilized seeds were sown on germination medium and cultured for 24-72 hours until the cotyledons were swollen and the hypocotyls expanded to approximately 5-7 mm (various plant standards).

Explant preparation: The cotyledon of the seed was divided into two parts along the gap and 2/3 of the adaxial end was taken as the explant. Physical treatments such as sonication, micro brushing and vacuum infiltration were applied to aid infection according to experimental requirements.

Co-culture: Transformation vectors carrying morphogen genes were transformed into Agrobacteria and co-cultivated with explants for 3-4 days.

Shot playback: The co-cultured explants were placed on an appropriate induction medium to induce germination.