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An Overview of Biotechnological Approaches for Crop Plant Improvement

Siddra Ijaz, Iqrar Ahmad Rana
Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture Faisalabad, Faisalabad, Faisalabad, 38040, Pakistan
Corresponding author: drsiddraijaz@uaf.edu.pk; Authors

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Abstract Conventional crop breeding is being restricted due to the use of only closely related species and it takes many generations to initiate significant improvement. Biotechnology is getting a great interest as basic techniques for tailoring crop plants as per specific requirement. It allows researchers to shorten this time and this imitate the evolutionary process but in highly selective way. As a biotechnological approach, genetic manipulation strategy is something like tearing out a page of the instruction manual for one organism and gluing it into the instruction manual of another organism. Around the globe, this emerging science is playing an imperative role to help agricultural productivity. In this review, biotechnological approaches that are commonly used for the improvement of crop plants are highlighted and briefly described.

Keywords Biotechnological approach; Crop improvement; Conventional crop breeding

Introduction

The expected crash of performance of world trade organization, industrial liberalization and free market economy has formed extraordinary risk for various countries to protract even at low economic profile. Therefore it may not be matter-of-fact to rely on industrial sector for economic boost and expansion. Under this state of affairs it becomes indispensable to strengthen other possible assets like agriculture. Agriculture is one of the promising sectors which offers marvelous potential for sustainable growth and can play a key role in helping to sanctuary the economy of the country. Furthermore, a threatening increase in the world population and limited food supply strained and anxious biologists to introduce and advance agriculture management and modern technologies along with conventional practices to attain highest possible crop productivity.

With the development of plant molecular biology, genetic transformation has become one of the innermost issues in molecular breeding (Vasil, 1994). Lack of a stout and vigorous regeneration system to regenerate transformed plants at a satisfactory rate is still the key factor which seriously restricts the enhancement of crops through genetic transformation (Popelka and Altpeter, 2003). Establishment of putative regeneration system, target genome, a candidate gene, and a vector to carry the gene, modification of foreign DNA to enhance its expression, transformed cell identification and characterization of aspirant plants at the molecular levels are the pre-requisite for genetic transformation (Sharma et al., 2000).

Methods for site specific integration in nuclear genome of plants have not been developed but *in situ* introduction of small mutation in known gene has been described by Zhu et al (1999) and Beetham et al (1999). Biological and physical parameters optimization may increases the effectiveness of these processes (Jefferson, 1987). Stable transformation efficiency and increased transient expression can be attained by particles bombardment (Finer et al., 1992) of target tissue treated with osmoticum (Vain et al., 1993).

The intricacy in the development of gene transfer methods may be due to the deficiency in various cellular responses essential for transformation (Potrykus, 1985). Genetic engineering offers an additional source of disparity through which breeders can develop new

resistant varieties and introduce the genes which confer resistance.

Plant Tissue culture studies

Plant tissue culture techniques have become a powerful tool for studying and solving basic and applied problems in plant biotechnology (Villalobos, 1987). From the last three decades micropropagation and other *in vitro* techniques have routinely used in horticulture and agriculture for rapid mass multiplication of crop plants (Dodds, 1991; Das et al., 1996). Effectual exploitation of biotechnological approach such as somaclonal variation, somatic hybridization and genetic transformation, rely on proficient and unswerving regeneration systems.

A tissue culture system provides considerable quantities of highly regenerable target tissue. Numerous protocols for somatic embryogenesis and organogenesis from callus have been established. But a swift callus induction has been achieved from immature leaves and immature inflorescences. Minimal genetic changes, has been noticed in regeneration through axillary buds even though it is utilized for plant multiplication (Hendre et al., 1983; Taylor and Dukic, 1993). Indirect embryogenic have been induced by going through callus or undifferentiated mass of cells. This has been done by taking leaf or floral parts as starting material also called ex-plant (Bower and Birch, 1992; Gallo-Meagher and Irvine, 1993; Snyman et al., 1996; Ingelbrecht et al., 1999).

Callus cultures establishment and maintenance is a labor demanding and the regenerated plants are ready for green house planting in at least 36 weeks (Bower et al., 1996). From cell, tissue and organ cultures, production of somatic embryo-like structures may happen either directly or indirectly (Reinert et al., 1977 and Warren and Fowler, 1977). Physical separation of the globular, heart and torpedo stages of embryogenesis has been achieved through somatic embryogenesis, using glass beads to screen the cultures. Somatic or asexual embryogenesis is the production of embryo-like structures from somatic cells, a process which can occur directly from an explant or indirectly via a callus stage. The resulting somatic embryos are independent bipolar structures that can develop and germinate to form plants in a manner analogous to their zygotic counterparts (Ammirato, 1987). As described by many workers (Ho and Vasil, 1983; Ammirato, 1987), the embryogenic areas were compact, nodular and white and comprised relatively small, thin-walled, richly cytoplasmic, basophilic cells with prominent nuclei, whereas the friable and yellow non-embryogenic calli consisted of large, thick walled, highly vacuolated and irregularly-shaped cells. Indirect somatic embryogenesis occurs when the explant is exposed to an auxin, which causes the formation of callus from which plantlets can be regenerated (Ho and Vasil, 1983).

Organogenesis is complicated process involving cellular, molecular and tissue level change in the metabolism. The unorganized mass of cells differentiates into shoots by undergoing modifications in the metabolic reaction etc. It is necessary to study the metabolic change by investigating glucose utilization pattern. However there are no reports on C14 glucose uptakes studies in 3 organogenetic stages. During short term feeding the highest glucose activity is observed in callus stage and it declines as the tissue dedifferentiates into shoots. Similar pattern is observed during long term exposure. It indicates that C14 glucose utilization pattern depends on the organogenetic stage and its requirements are higher at the initial callus stage than in the completely regenerative shoots.

Genetic transformation of crop plants via Agrobacterium

Agrobacterium mediated gene delivery method for genetic transformation of plants is ineffective in monocotyledonous crops, because of host range specificity of *Agrobacterium* which is a bacterium of dicotyledonous plants (Weising et al., 1988). Several alternative approaches have been developed for monocot transformation e.g. electroporation (Fromm et al., 1986), silicon carbide fiber (Keappler et al., 1990), polyethylene glycol (lorz et al., 1985) microinjection (Crossway et al., 1986) and gene gun delivery system (Klein et al., 1987). In dicots, gene transfer through Agrobacterium is proficient than gene gun delivery

system but in monocots, transformation via *Agrobacterium* is limited (Elliot et al., 1998). In monocots for gene transfer via Agrobacterium different successful attempts have been made recently such as in rice (Park et al., 1996), and banana (May et al., 1995).

Agrobacterium present quite a lot of merits, such as technical simplicity, nominal genome rearrangements in transformants, low copy number and the capacity to transfer long stretches of DNA. In maize plants, *bar* gene expressions at a high degree was reported (Gordon-Kamm et al., 1990) and have integration of approximately 20 copies of intact gene. Gene transfer methods intricate in Poaceace and this may be due to the lack of various cellular responses which are essential for transformation (Potrykus, 1985). Protoplast regeneration and *Agrobacterium* host range were the major bottlenecks for the production of transgenic in many crops and this problem has been solved by micr-oprojectile mediated gene delivery system (Rathus and Birch, 1992).

Genetic transformation of crop plants via Gene bombardment

Microprojectile mediated genetic transformation open up the ways which have blocked by the problem of *Agrobacterium* host range and protoplast regeneration for production of transgenic plants. A boost up in transient expression frequency has been observed in some species and tissue types by bombarding target tissue twice (Wang et al., 1988) but a reduction in frequencies was observed in others (Kartha et al., 1989; Reggiardio et al., 1991). This showed condition for gene gun method should be optimized for each type of tissue. The first report of transgenic production was published by Bower and Birch (1992) and described the applicability of micro-projectile, gene delivery system for transformation of grass family in which embry-ogenic callus could easily be established. To avoid, inhibition in expression of introduced gene, a low copy number is desirable but complex integration patterns are commonly found in Microprojectile-mediated trans-formation.

A low copy number of the introduced gene is enviable for practical genetic engineering to avoid probable tribulations of co-suppression of expression caused by multiple gene copies (Smith et al., 1990). Transfor-mation efficiency and frequency may be affected by bombardment distance (Taylor and Vasil, 1991) and velocity of the micro-projectile. These factors may damage the plant tissue physically (Gambley, 1993). Gene delivery methods have been employed in scutellar tissues of maize, wheat and rice (Napoli et al., 1990; Smith et al., 1990; Fromm et al., 1990; Gordon-Kamm et al., 1990).

For micro-projectile mediated transformation embryo-genic callus cultures are ideal targets because regenerable cells are not extremely secured, and can be arranged to occupy most of the target area. After bombardment of scutellar tissue of immature embryos (Christou et al., 1991) regeneration of transgenic plants is a beautiful approach for cereals, which are propagated by relatively large seeds.

Conclusions

Plant biotechnology presents considerable improvement in almost every area of crop production with possible profit for farmers, industries and consumers. The growth in the world population, their demands for food and clear consumer preference for environmentally sustainable agriculture will extend biotechnology's role in food production. Successful applications of various biotechnological tools offer great promise to crop plants in future.

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