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Electrofusion and Regeneration of Protoplast in Potato

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Abstract – Somatic hybrids obtained directly after fusion contains all organelles from the cytoplasms of both parents. Somatic hybridization via protoplast isolation, electrofusion and regeneration is a useful tool to transfer polygenic traits such as late blight resistance in a single step. It enables a development of tetraploid somatic hybrid between diploid wild species and dihaploid of common potato. As a result, tetraploid somatic hybrids may be utilized in conventional breeding for late blight resistance and improvement of other traits.

Keywords: Protoplast, fusion, Plants

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INTRODUCTION

Protoplast fusion and subsequent in vitro plant regeneration, leading to somatic hybridization, offer opportunities for transferring entire genomes from one plant into another, regardless of the inter specific crossing barriers. In contrast to techniques for plant transformation that are aimed at single-gene transfer, protoplast fusion is needed when polygenic traits are concerned, as is frequently encountered in the genetics of higher plants. Several Solanaceous species, including potato, have been used with greater success than other higher plant species in somatic hybridization because they are more responsive to the protoplast regeneration process. There are two commonly used procedures to induce cell fusion, namely polyethylene glycol (PEG)-induced protoplast fusion and protoplast electro fusion. These procedures have been the subject of several reviews indicating that electro fusion is generally more efficient (1-5). Electro fusion is superior to PEG-induced protoplast fusion in the following aspects:

1. Simplicity of the fusion process;
2. Less toxicity and less physical damage to the protoplasts;
3. Large fusion volume allowing more protoplasts to be treated; and
4. Fine control of the fusion process with the availability of commercial electro fusion equipment.

PLANTS FOR PROTOPLAST ISOLATION

Use in vitro grown plants initiated from nodal cuttings of greenhouse or field-grown plants or from seeds for protoplast preparation. Nodal cuttings with 1 or 2 axillary buds are surface-sterilized by immersion in 20% (v/v) commercial bleach for 10 min (seeds are disinfected in 50 % [v/v] commercial bleach for 10 min), then rinsed in sterilized distilled water three times. Bleached ends of the cuttings are excised prior to inserting the lower end of the tissue into the propagation medium.

SOMATIC HYBRIDIZATION IN POTATO IMPROVEMENT

Gene transfer is the basis for almost all crop improvement including potato. Conventionally, this is achieved through sexual hybridization; this rather limits the range of species from which gene flow can occur into a crop species. Wild species have contributed remarkably to the success of latter, they allowed the crops to retain their commercial status. As a result plant breeders have sought to utilize an increasing number of wild species as a source of valuable genes ranging from disease resistance to grain yield, and produce quality. But many sources of useful genes cannot be included in crop improvement programme primarily because of sexual incompatibilities. Genetic transformation, a focused and direct gene transfer approach require identification, isolation and cloning of the concerned genes. Further it is expensive and technically most exacting, although it may represent the ultimate strategy. However, some characters of interest may be govern by two or more and yet unknown genes; transfer of such characters through genetic transformation may pose many difficulties. Finally transfer of cytoplasmic organells, viz., chloroplast and

mitochondria may often be desired objectives; this, however is not possible through genetic transformation, while it can readily achieved by somatic hybridization. Trait is particularly attractive to breeders to widen the potato genetic base, but the barrier between the cultivated potato and the many wild species has proved a difficult task, even when unconventional crossing methods are used (Orczyk et al. 2003).

PROCEDURES

The protoplast fusion by electric field requires sufficient amount of suitable plant material for protoplast isolation and their culture after electro fusion for obtaining plant regeneration. The procedure involves the following stages.

- In vitro culture of donor plants
- Protoplast isolation from leaf meshophyll tissues
- Verification of protoplast viability and protoplast fusion
- Protoplast fusion by electric field
- Regeneration and culture fusion products
- Characterization of putative somatic hybrids

APPLICATIONS

Symmetric protoplast fusion approaches involving diploid Solanum species in combination with dihaploid S. tuberosum have been essentially used to develop tetraploid somatic hybrids potato having desirable introgression from wild relatives. In the current years, application of this technology has been observed widely for the production of multiple resistant somatic hybrids. Interspecific potato somatic hybrid between commercial cultivars of potato S. tuberosum Agave and Delikat and wild diploid species S. cardiophyllum (1 EBN) has been produced for resistances to Colorado potato beetle, foliage blight and PVY (Thieme et al. 2010). In addition, somatic hybrids between a diploid potato clone DG 81-68 susceptible to P. infestans and a resistant diploid tuber-bearing species Solanum x michoacanum were generated (Szczerbakowa et al. 2010). Polzerová et al (2011) have developed interspecific somatic hybrids between wild diploid species S. pinnatisectum (1 EBN) and S. tuberosum for the late blight resistance in potato. Following the successful production, somatic hybrids have been applied in the potato breeding for the development of advance progenies for transferring the resistance trait. For example, Thieme et al. (2008) have developed novel somatic hybrids and their fertile BC1 progenies having resistances to late blight, Colorado potato beetle and PVY from a diploid wild species S. tarnii into common potato.

CONCLUSION:

In this paper we found that Somatic hybridization allows move of cytoplasmic organelle in a single generation and offer only one of its kind opportunities for combining mitochondria of one species and chloroplast of another species in a single hybrid. This ability may permit development of characteristics certain cytoplasmic male sterile line, which may lead to their commercial exploitation.

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