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Short Communication

Pollen Grain Mediated Gene Transfer for Efficient Plant Transformation

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Plant transformation has become an integral part of crop improvement programs, for breeding new varieties of crops with higher yields and resilience to different stress conditions [1]. However, there are several challenges that have to be overcome to create genetically modified (GM) crops. The most challenging part is the recovery of a transgenic event, which depends on our ability to regenerate a new plant from a single transformed cell and killing off all the non-transformed cells surrounding it [2]. Most of existing transformation methods rely on a sophisticated tissue culture based protocol (organogenesis or somatic embryogenesis) for regeneration of the transgenic plant, that can be long, complicated and labor intensive [3,4]. Plant cells from different species and even cultivars of the same species respond very differently in the standard tissue culture media, making the regeneration procedure challenging [3]. Development of alternate, and simple transformation protocols can eliminate the need for tissue culture and regeneration. As of now, the model weed plant, *Arabidopsis* is the only plant species for which a rapid transformation method called 'floral dip' exists [5]. Most plant species have a closed gynoecium, while *Arabidopsis* flowers have an open gynoecium that allows, the soil bacterium *Agrobacterium tumefaciens*, to infect the ovules deliver the recombinant DNA into the egg [6].

Sexual reproduction in flowering plants begins after deposition of pollen grains containing the male gametes, onto the receptive surface of the 'female' stigmatic tissue. Following this, the pollen grain hydrates, germinates, and produces a pollen tube that penetrates the stigma and grows directionally towards the ovule to deliver the sperms for double fertilization [7]. Since long time, pollen grains have been proposed as an alternate for successful delivery of the recombinant DNA into the egg during pollination and fertilization [8]. Exogenously transformed pollen can be used for pollination of the stigma (female part of a flower) and transgenic seeds can be directly generated without the need for tissue culture or regeneration. Although pollen transformation seems attractive, limited success has been achieved in transforming pollen though physical methods such as electroporation, bombardment and sonication or methods like *Agrobacterium* infection and pollen tube mediated transfection [9-11].

Recently, Zhao., *et al*. reported a new technology that can be used for efficient pollen transformation, which is applicable for a wide range of plant species [12]. The new approach called pollen magnetofection, utilizes magnetic nanoparticles as DNA carriers into the pollen and also utilizes the peculiar morphological feature of the pollen grain called aperture [12]. The DNA-coated particles are moved into living cells by applying a magnetic field. Apertures (5 to 10 µm in size) are structures that are exit sites for pollen tube during pollen germination and they represent the most promising entry point for introducing foreign DNA into pollen. Zhao., *et al.* first used this technique to introduce DNA constructs that contained a reporter gene into cotton pollen. The transient expression of the reporter gene was observed at a higher frequency than reported from an earlier work. The transformation frequencies reported by the authors were in the range of 5 to 10% (ratio of the number of transgenic plants to the number of pollinated flowers), which is much higher that previously observed [13]. The authors also demonstrated the effectiveness of this method in four other plant species with larger pollen apertures, including the crop plants like chili pepper and pumpkin, which are difficult to transform [13].

Pollen magnetofection has the potential to revolutionize agriculture and transgenic research in plants. It will simplify the generation of transgenic plants in many plant species, which are already transformable by existing methods. Also, it will enable transformation of recalcitrant plant species for which no transformation protocols currently exist. The only limitation of this method is that, it cannot be used for genetic transformation of the two DNA-containing cell organelles of plants: chloroplasts and mitochondria that are maternally inherited and therefore not present in pollen [3,14]. Nevertheless, this method is a powerful technology that can be used for nuclear DNA transformation many recalcitrant plant species.

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