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(54) **Title:** AN IMPROVED MUTAGENESIS METHOD USING POLYETHYLENE GLYCOL MEDIATED INTRODUCTION OF MUTAGENIC NUCLEOBASES INTO PLANT PROTOPLASTS

(57) **Abstract:** Method for targeted alteration of a duplex acceptor DNA sequence in a plant cell protoplast, comprising combining the duplex acceptor DNA sequence with a donor mutagenic nucleobase, wherein the duplex acceptor DNA sequence contains a first DNA sequence and a second DNA sequence which is the complement of the first DNA sequence and wherein the donor mutagenic nucleobase comprises at least one mismatch with respect to the duplex acceptor DNA sequence to be altered, preferably with respect to the first DNA sequence, wherein the method further comprises a step of introducing the donor mutagenic nucleobase into the cell protoplasts using polyethylene glycol (PEG) mediated transformation and the use of PEG protoplast transformation for enhancing the rate of targeted mutagenesis.