RESEARCH ARTICLE

Plant Growth Promoting Rhizobacteria (PGPRs) Alter Plant Host Somatic Mutation Frequencies

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Abstract

Plant growth promoting rhizobacteria (PGPRs) are a group of soil bacteria which can induce positive growth in plants by different mechanisms. This work intends to find the effect of PGPRs on two classes of somatic mutations in the host, frame shift mutation (FSM) and somatic homologous recombination (SHR) and, compare the same with that of a pathogen. Somatic mutations in plants are important as they are an adaptation strategy to overcome stressful conditions and also get passed on to the next generations. The mutation detector *Arabidopsis thaliana* lines carrying a non-functional β-glucuronidase gene (*GUS*) were used to score the mutation events. One day-old mutant seedlings were co-cultivated with the PGPRs (*Rhizobium leguminosarum* and *Pseudomonas fluorescens*) and the pathogenic strain (*P. syringae*) for two different post-infection durations (4 h and 48 h). A reversion of the mutated *GUS* to its functional form resulted in blue spots in the host plant. Based on the number of blue spots seen, the mutation frequencies were estimated. An increase in FSM was observed in plants co-cultivated with *R. leguminosarum* for 4 h as well as 48 h. *R. leguminosarum* suppressed SHR frequency 4 h-post infection, which significantly increased at 48 h. In contrast, *P. fluorescens* infection lead to a temporal suppression of FSM and induction of SHR at 4 h. Subsequently, the SHR rates reduced significantly, i.e. lower than the uninfected controls at 48 h. The pathogenic strain *P. syringae* temporally increased FSM in plants and also enhanced SHR rates in plants 4 h post-infection, which also subsequently reduced 48 h post infection. To the best of our knowledge, there are no other reports comparing the effect of PGPRs on host somatic mutation rates.

Key words: *Arabidopsis*, Frame-shift mutation, Host somatic mutation frequencies, Plant growth promoting rhizobacteria (PGPR), Somatic homologous recombination.

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Introduction

Plants, being sedentary in lifestyle, often have to face extremities of various abiotic and biotic factors leading to stressful conditions. As a result, several mechanisms have been developed which allow them to cope up with various stresses (Hauser *et al*., 2011; Kranner *et al*., 2010; Tuba and Lichtenthaler, 2007). Genomic instability that results in flexibility is one among such adaptation strategies. Somatic mutation events are a major cause of this genome instability (Puchta *et al*., 1994). In case of plants, somatic mutations are of great importance as plants do not have a pre-determined germline and the reproductive structures are derived from somatic cells in due course of their development (Kovalchuk *et al*., 2000). Such mutations include frame-shift mutation (FSM) , point mutation and somatic homologous recombination (SHR).

Studies have already been done on the effect of different stresses on the somatic mutation frequency. Different abiotic stresses have been known to show heritable alterations in the frequency of all three major kinds of somatic mutations (Rahavi *et al*., 2011). Previous studies on the effect of biotic stress using some plant pathogens resulted in high rates of recombination, transposition and double strand breaks (Kathiria *et al*., 2010) while different strains of *Agrobacterium tumefaciens* resulted in suppression of various classes of somatic mutations (Shah *et al*., 2015).

This study is oriented to check the rates of somatic mutation frequency when plants are infected by two plant growth promoting rhizobacteria (PGPRs), *Rhizobium leguminosarum*, a close relative of *Agrobacterium* and, *Pseudomonas fluorescens,* which are widely used biocontrol agents. PGPRs are a group of bacteria which can induce a positive growth in plants by both direct and indirect means like nitrogen fixation, solubilisation of nutrients, production of growth regulators, competitive exclusion of pathogens or removal of phytotoxic substances, stimulation of mycorrhizal development etc. (Lugtenberg and Kamilova, 2009; Bashan and de-Bashan, 2010). Mutation frequency was determined for another set of plants also that were infected with *P. syringae*, a known plant pathogen to compare the effects with.

We analysed the frequencies of two different classes of somatic mutations-SHR and FSM in this study. SHR is the intra-chromosomal recombination that occurs in somatic cells. FSM are characterised by insertion or deletion of nucleotides resulting in a shift in the reading frame. Mutation frequency rates were scored using *Arabidopsis* mutation detector line plants that harbour mutated *GUS* construct that is capable of revealing mutation frequency during *GUS* histochemical staining due to reversion of mutations. The line R2L1 was used in scoring SHR frequency while the line G10 was used to score FSM rates. *Arabidopsis* seedlings were co-cultivated with the bacteria for two time intervals, 4hours and 48 hours to study the dynamics of mutations.

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Materials and Methods

Arabidopsis mutation detector lines

Line G10 having a microsatellite insertion (stretch of 10 Gs) within the *GUS* ORF was used to score FSMs (Fig. 1A). SHRs were scored