A CRISPR Platform for Controlling Fusarium Dry Rot in Potato

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Introduction

Potato (*Solanum tuberosum* L.) is an annual tetraploid plant that belongs to the Solanaceae family. Potatoes rank as the third to fourth most important staple crop worldwide (1). Plant diseases contribute to 10-15% of global crop losses, with fungal pathogens accounting for a significant 70-80% of this total (2). As a vegetatively propagated crop, potatoes face various biotic stresses throughout the production season, from preharvest to postharvest. Many Fusarium species are phytopathogenic and can infect numerous horticultural crops. *Fusarium* is one such species, capable of infecting various hosts, with its primary impact being the induction of dry rot in stored potatoes (2).

Fig 1. Diseases Representation caused by various biotic stresses and their effects on plants.

Potatoes pose challenges for traditional breeding because it takes +14 years to develop a cultivar. CRISPR is advantageous because it speeds up the process and targeted changes can be made. (1).

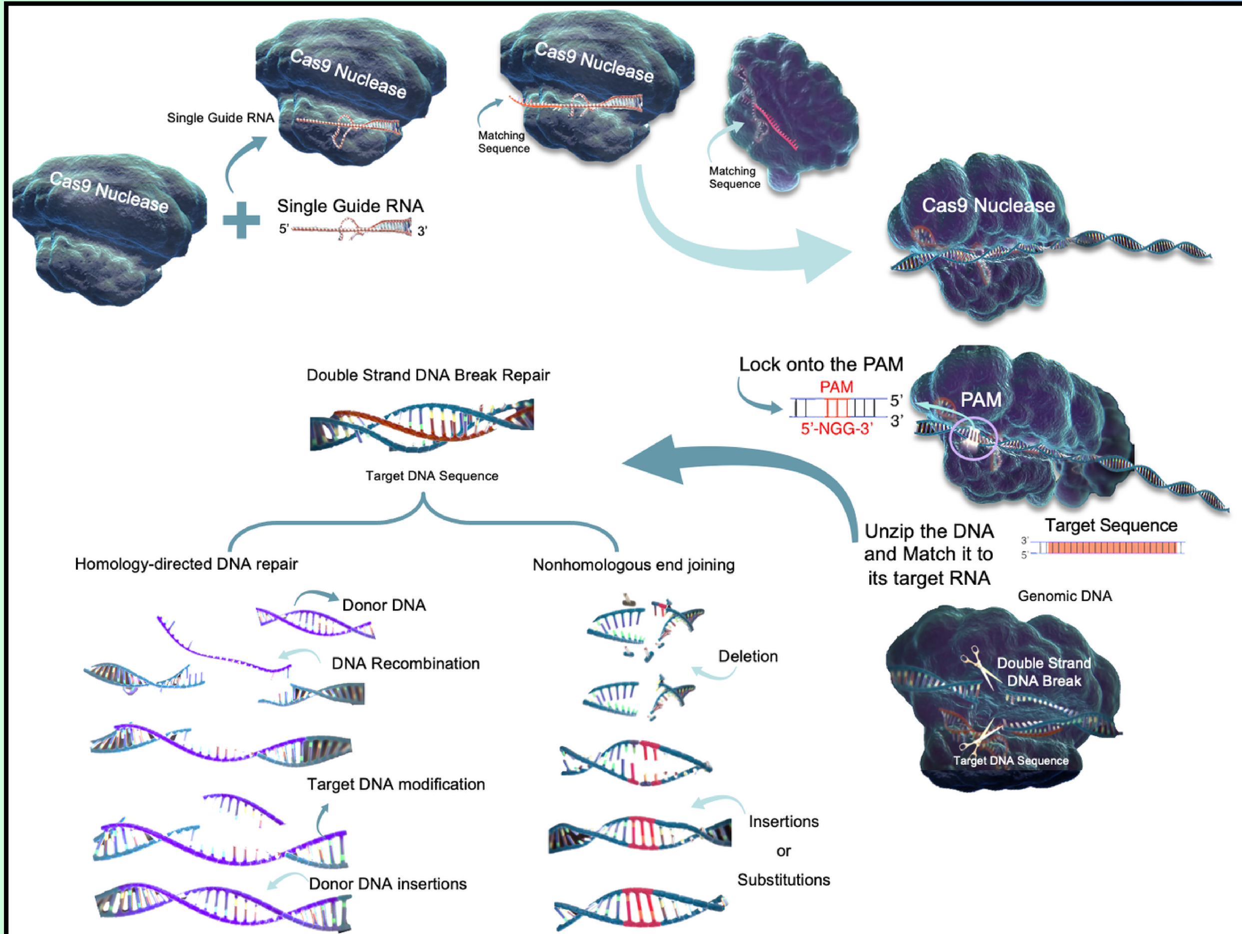

Fig 2. Targeted genome editing using CRISPR-Cas9

RNA interference (RNAi) has evolved into a crucial regulatory mechanism in plant research, offering invaluable insights into gene regulation in response to abiotic and biotic stress (5). RNAi is triggered by double-stranded RNA (dsRNA), which can be exposed to the target pathogen through the exogenous application of dsRNA complementary to a pathogen (6). The beta-tubulin families are the major constituents and serve as the primary components that form the microtubule tubulin fibers that are responsible for chromosome separation during cell-splitting and cell structure support (7). Therefore, tubulin genes could be effective candidate genes for an RNAi-based control strategy. The most widely used approach for defining gene function is to reduce or completely disrupt its normal expression. RNAi and CRISPR-based technologies have offered a practical resource to use this fantastic array of powerful tools and to contrast these technologies to disrupt gene expression in many organisms

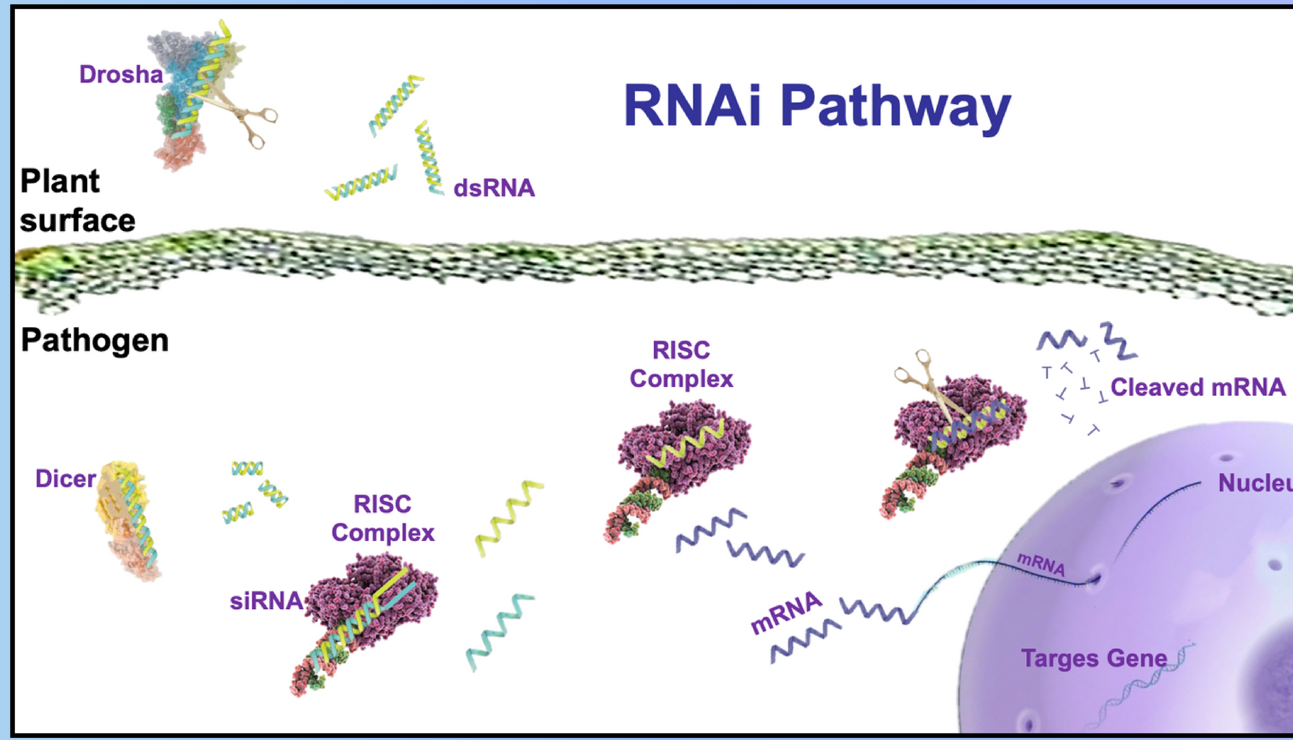

Fig 3. RNAi Pathway

RNAi and CRISPR-based technologies have offered a practical resource to use this fantastic array of powerful tools and to contrast these technologies to disrupt gene expression in many organisms (3). This research focuses on the endeavors, applications, and prospects of CRISPR/Cas-based approaches in potatoes with the potential to introduce fusarium resistance into potato cultivars based on RNAi and exogenous dsRNA application research. The current study established a systematic platform for producing potato cultivars resistant to Fusarium species.

Materials and Methods

Sterilized potato shoots were cultivated on a medium consisting of Murashige and Skoog (MS) salts and vitamins, 30 g/L sucrose, and 6 g/L plant agar, pH 5.75 (9). The shoots were subcultured every two weeks and were kept in a growth chamber at 25 °C under light provided by cool-white fluorescent lamps (photosynthetic photon flux = 60 umol/m2/s) with a 16 h photoperiod.

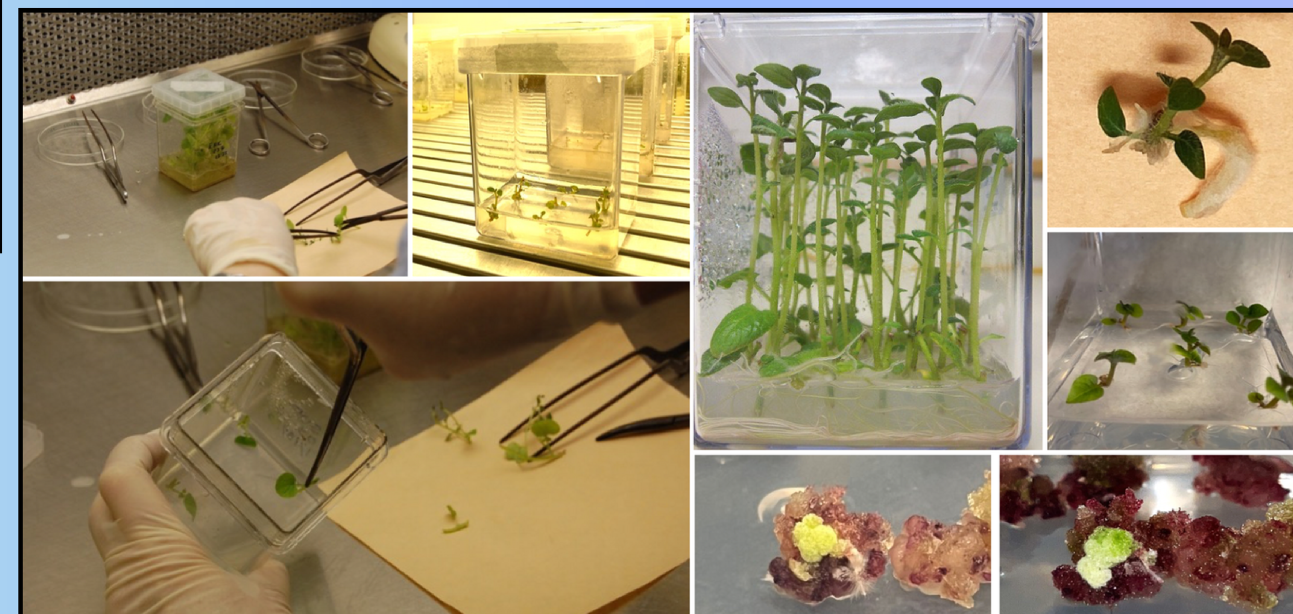

Fig 4. Tissue Culture and Preparation of Materials for Protoplasts

Leaf strips were incubated in a cell wall digestion solution for different durations and protoplasts were purified using a 20% sucrose solution. Protoplasts were cultured in Richard medium until they developed into microcalli.

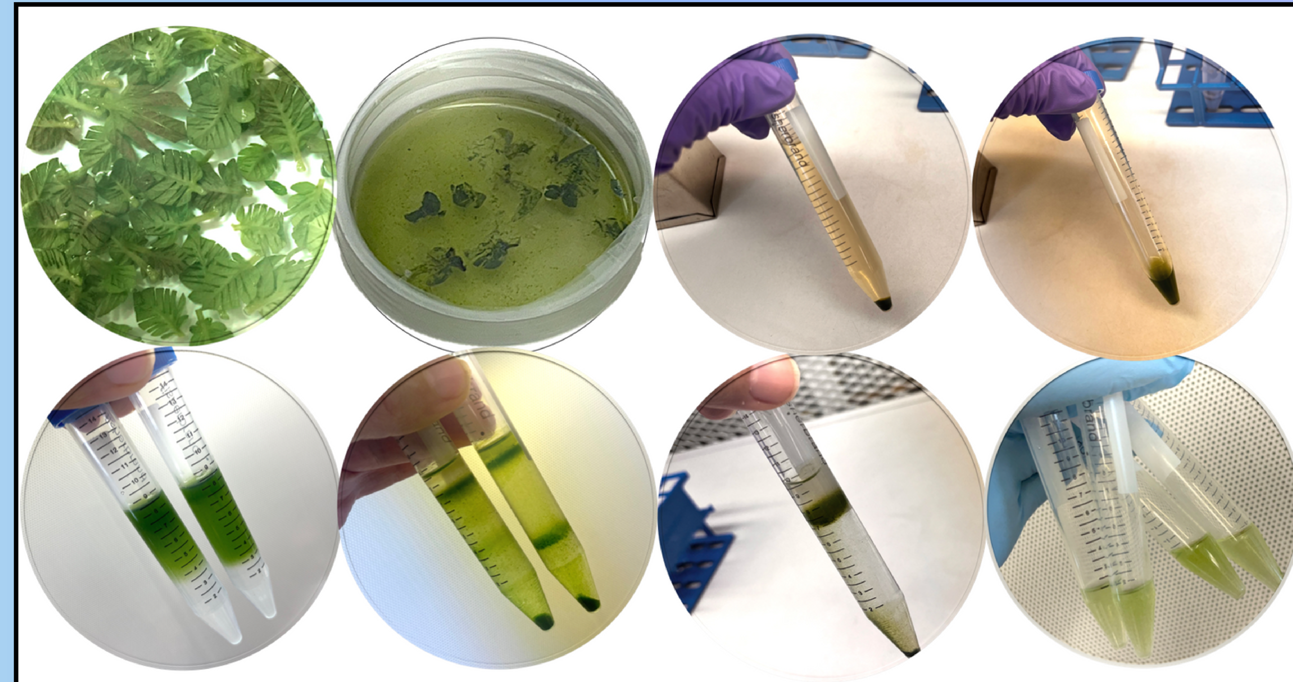

Fig 5. Protoplast Extraction and Purification

Results

A tissue culture technique with MS medium and 16.65 µmol/L of 2,4-D was effective for callus induction. Shoot regeneration was achieved on MS medium with 11.10 µmol/L of 2,4-D, 11.10 µmol/L BAP, and 0.55 µmol/L NAA. A combination of 2.77 µmol/L GA3, 16.65 µmol/L BAP, 0.55 µmol/L NAA, and 1.39 µmol/L KIN in MS medium gave the most effective rooting of in vitro shoots.

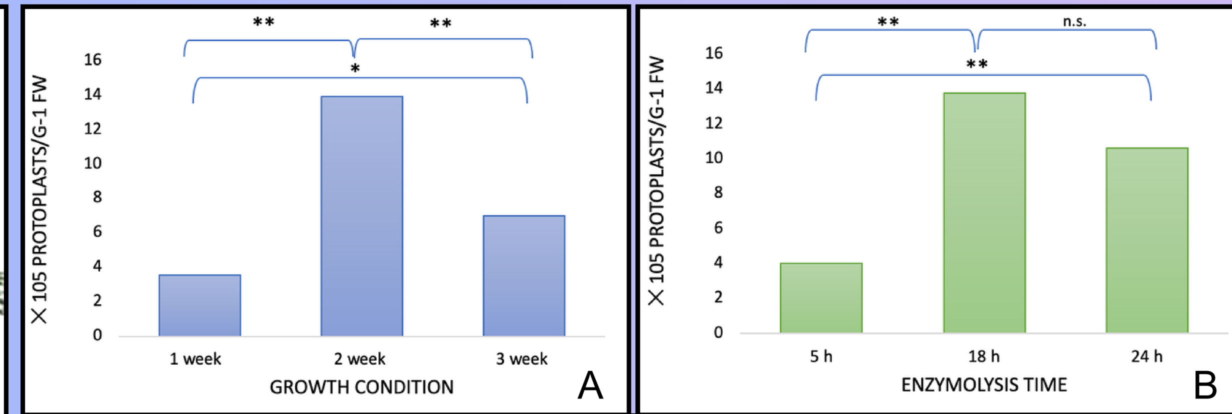

Fig 6. Effects of the physiological state of donor plant material on the extraction efficiency of protoplasts (A) Comparison of extraction efficiencies according to the enzymolysis time (B). *p*-values were obtained using Student's t-test.*, $p < 0.01$; **, $p < 0.0001$; n.s., $p > 0.05$ for three samples with three replicates.

The effects of the physiological status of the plant material on the extraction efficiency were analyzed. The extraction efficiencies of protoplasts from the leaves of the 1-week-old, 2-week-old, and 3-week-old node culture in vitro-grown plants were recorded (Figure 6A). The 2-week-old node culture material exhibited the best results with an extraction efficiency of 13.91×10^5 protoplasts/g FW. To optimize the enzymolysis time, leaves were digested for 5, 18, and 24 hours. For the 5 and 24 h, the viability of the protoplasts decreased compared to that at 18 h, the latter of which was determined to be the best enzymolysis time (Figure 6B).

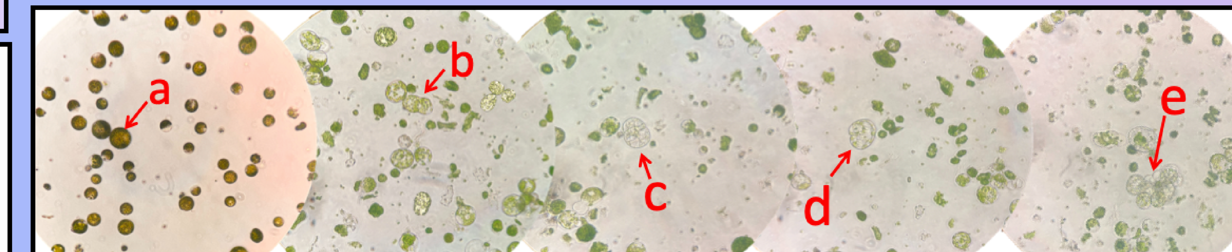

Fig 7. Establishment of Conditions for Microcallus Induction (a) Purified individual protoplasts (b) First division (c and d) Second division (e) Multiple divisions.

Isolated protoplasts were incubated for 6 weeks in Richard medium to develop medically (Figure 7a–f). It was found that obtaining appropriate space and the concentration of the protoplasts were important factors. A polyethylene glycol (PEG) mediated transfection method will introduce CRISPR constructs into the protoplasts of potatoes.

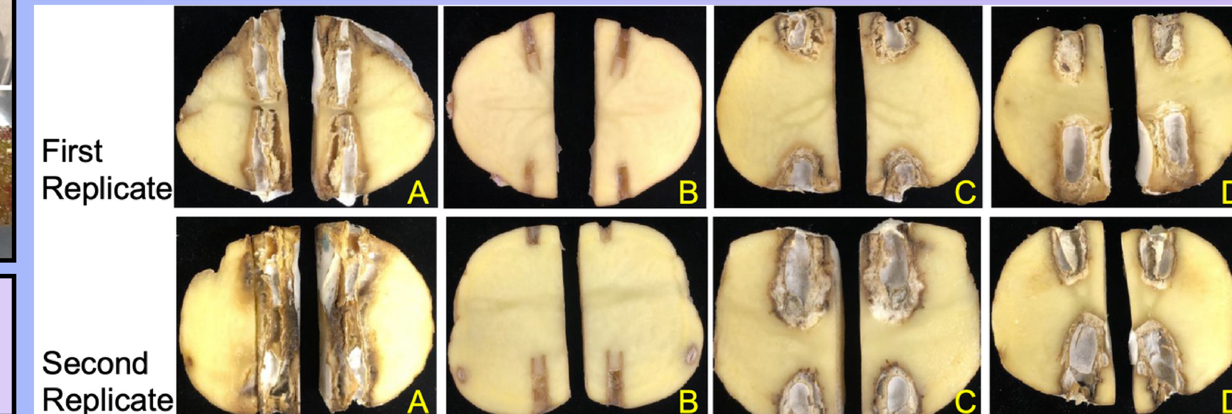

Fig 8. Fs inoculation Experiment in Yukon yellow Potato tuber after 7 days. First replicate: ((A) cube inoculated alone, (B) PDA alone, (C) cube immersed in bacteria DE3 with beta-tubulin, (D) cube immersed in bacteria HT115 with 400bpbetatubulin). Second replicate: ((A) cube inoculated alone, (B) PDA alone, (C) cube immersed in bacteria DE3 with beta-tubulin, (D) cube immersed in bacteria HT115 with 400bpbetatubulin.

The entire beta-tubulin and a 400 bp portion of the gene were used to make dsRNA duplexes. The duplexes were expressed in two strains of Ecoli. Experiments were completed and replicated twice and dry rot symptom expression was observed at 7- and 14 days post-inoculation. In Yukon Gold, it appears we get a 50% reduction in symptom development at 7 and 14 dpi. The results of the current investigation provide a reliable protocol for producing potato plants that overcome pressures from Fusarium species.

Acknowledgments

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