**Introduction**

For many years upon their discovery, ‘jumping genes’ were dismissed as nothing more than ‘junk DNA’ that contributed little to the genome. Today, it is well established in scientific literature that jumping genes play a crucial role in the genomic repertoire of many species. As the name suggests, a jumping gene, also known as a transposon or a transposable element, is a gene sequence that can change its position within a genome thereby altering the cell’s genetic identity. Some transposable elements are able to mediate their own propagation, at times being referred to as ‘selfish’ genetics elements due to the fact that they can prioritize their own transmission at the expense of other genes. Thus, they do not always act cohesively with the rest of the genome.

Nonetheless, many of these elements have coevolved to be important constituents of host genomes. Increasing evidence suggests that these mechanisms contribute to genetic variability and aid in survival in stressful environmental conditions (Richter & Ronald, 2000). Transposition within the genome often results in the duplication of genetic material, allowing for novel uses of the genetic segments by means of their integration into new positions within the genome. The interaction of genome structure, organization and function with these transposable elements can facilitate rapid adaptation within a population, especially if said population lacks genetic diversity which selection can act on (Stapley et al. 2015). Thus, these elements can act as drivers of genome evolution in many eukaryotes. This can be particularly useful in times of abrupt environmental change when survival of the population depends on a rapid phenotypic response to the stressors. Species that are constantly subjected to such stressors are likely to employ transposable elements to facilitate a rapid genomic response.

There are at least two main types of transposable elements that contribute to genomic function: Class I and Class II transposons. Class I transposable elements or retrotransposons, generally function via reverse transcription of an RNA intermediate. The transcribed DNA is then inserted into the target site. Class II elements of DNA transposons are more common; they function by encoding proteins necessary for the insertion and excision of a transposon, thus catalyzing its movement to another part of the genome. This class of TEs are characterized by the presence of terminal inverted repeats that flank their 5’ and 3’ ends.

Such incorporations of transposons are frequently observed in plant genomes, whereby TEs can contribute to the amplification of gene families, as well as the facilitation of gene fusions (Krasileva, 2019). Transposable elements thus function in a manner comparable to that of the plant’s DNA repair machinery, with these two mechanisms often acting synergistically (Krasileva, 2019). Epigenetic modifications have been shown to regulate the function of TEs within the plant genome, with various epigenetic modifications contributing to control of TE transcription and transposition with a plant’s genome (Underwood et al., 2017). Notably, RNA interference (RNAi) leads to the accumulation of small interfering RNAs (siRNAs) at the 5’ and 3’ repeats of transposable element segments, which in turn affects DNA cytosine methylation, thus contributing to the regulation of these segments (Underwood et al., 2017). On the other hand, stress-induced changes in epigenetic status of TE activity may facilitate TEs to propagate specific stress response elements to host genes which permits phenotypic plasticity and adaptation to stress (Negi et al., 2016). The ability of transposons to increase genetic diversity, coupled with the ability of the genome to regulate and at times repress TE activity, results in a balance that is key in maintaining diversity that acts to enhance the plant’s survival in changing environments.

Activity of transposable elements is often lineage specific, preferentially affecting specific gene families such as disease resistance genes (Krasileva, 2019). In plants, defense-activating compounds can move cell-to-cell and systematically through the plant’s vascular system. However, unlike higher level organisms, plants do not have circulating immune cells, so most cell types exhibit a relatively broad immune response. This means that while qualitative differences in plant disease resistance can be observed when comparing multiple specimens, these mechanisms are normally discussed in quantitative terms, as this is more relevant to the way in which plant disease resistance genes function. This necessitates the regulations of these immune responses. It has long been hypothesized that transposable elements play a role in this, whereby they reconstruct genomes in response to pathogen infection.

Specifically, transposable elements have been shown to cause disease resistance inactivation and diversification, contributing to plants’ defense strategies against pathogens (Richter & Ronald, 2000). A study done by Hayashi and Yoshida (2009), provides evidence of such TE activity; authors found data suggesting that transposon-mediated transcriptional activation may play a role in functionalizing ‘dormant’ resistance genes in the plant genome, reiterating the role of TEs in regulating resistance genes. Additional diversification of resistance genes can further be prompted by these mechanisms, as genes themselves can serve as templates for variation that can arise from non-allelic homologous recombination (Krasileva, 2019). While such effects have been shown, there is currently little evidence for the generation of new specificity at resistance gene loci as a direct result of the insertion of a transposable element into the genome.

In a study by Song et al. (1998), the authors investigated the rice disease resistance gene *Xa21*. This resistance gene encodes a receptor-like kinase (RLK), a transmembrane protein with an amino-terminal extracellular domain and a carboxyl-terminal intracellular kinase domain (Shiu & Bleeker, 2001). RLKs are a large superfamily of proteins involved in an array of plant responses ranging from development and growth to response to pathogens. Song et al. (1998) compared the genomic sequences of seven family members of the *Xa21* gene, as it is part of a multigene family. They found that most of these members can be mapped to a single locus on chromosome 11 that is linked to at least 9 major resistance genes, as well as one quantitative trait locus for resistance (Song et al., 1998).

In mapping these genes, authors identified fifteen transposon-like elements in the 5’ and 3’ flanking regions and introns of rice disease resistance gene *Xa21* (Song et al., 1998). These sequences were designated as transposons based on possession of terminal inverted repeats indicative of TEs, duplication of target sequence sites, or similarity in sequence and structure of known transposons. Thirteen of these TEs were found to insert into noncoding regions, whereas two elements insert into presumed coding regions. These two elements, termed *Truncator* and *Retrofit* insert into regions of the *Xa21*gene referred to as and E and D, respectively (Song et al., 1998). The arrangement of these TE-like segments within the genome results in duplication of target sequences and disruption of the open reading frames of these two members (Song et al., 1998). Specifically, *Truncator* and *Retrofit* are presumed to contribute to the diversification of resistance genes by causing premature truncation of the receptor kinases of the *Xa21* gene by means of insertion into the N terminus of the kinase domain (Song et al., 1998). This would in turn result in a receptor-like molecule that is structurally similar to other known resistance gene products, which presumably would produce resistance. These findings provide sufficient evidence that transposable elements play a role in conferring disease resistance within this particular disease resistance gene family.

**Objectives**

These findings can be applied as a study system whereby rice plant variants with known resistance to a pathogen encoded by the *Xa21* gene can be studied for the generation of new specificity at resistance gene loci in a sister species lacking resistance to that given pathogen, or perhaps transgenic breeds of the plant that have been made to be nonresistant. The gene *Xa21* is known to confer resistance to bacterial blight caused by the pathogen *Xanthomonas oryzae pv. oryzae*, specifically in the *indica* Rice Cultivar LT2(Nguyen, 2018)*.* An additional study investigating the relationship between the rice *Xa21* gene and resistance to bacterial blight found that the *Xa21*gene primed critically important genes and signaling pathways which enhanced resistance against the bacterial infection (Peng, 2015). Incorporation of the specific transposable element-like regions known to contribute to *Xa21* disease resistance into the genomes of such plants can shed light on the emergence of novel specificity, and thus resistance by means of the insertion of TEs. Furthermore, in incorporating these elements independently of one another, followed by incorporating them conjointly into nonresistant transgenic breeds, this can shed light on possible additivity of resistance that can be obtained by the incorporation of more than one resistance-associated TE.

Bacterial blight is a devastating rice disease that has a strong impact on rice agriculture, necessitating investigation into mechanisms that can possibly enhance plant resistance to this particular pathogen. The aim of this study is to provide insight into mechanisms of resistance against this pathogen in the *indica* rice plant (specifically in the context of transposable elements) in turn allowing for enhanced plant resistance protocols in the farming/agricultural sector.

**Hypotheses**

I hypothesize that incorporation of known transposable-like elements *Truncator* and *Retrofit* in the *Xa21*gene in a nonresistant sister species of *indica* Rice Cultivar LT2 or a mutant line of the plant lacking resistance, would generate new specificity within the genome that would encode resistance for the pathogen at hand, *Xanthomonas oryzae pv. oryzae.* I further hypothesize that the addition of these two elements conjointly would provide increased resistance against the bacterial pathogen that causes bacterial blight in this plant.This hypothesis would likely be supported as there is sufficient evidence currently to suggest that transposable elements and resistance interact in a causal manner within the plant genome. Since transposable elements are ‘jumping genes’ in nature, their incorporation in such a study system is more likely to induce new specificity at a given loci by means of structural change in the genome.

**Proposed Research**

The proposed experiment will isolate the transposable elements *Retrofit* and *Truncator* found in the coding regions of the *Xa21*gene to test for potential resistance to the rice disease bacterial blight, when inserted into nonresistant transgenic lines of *indica* Rice Cultivar LT2. These transposable-like elements are two of fifteen found in the *Xa21* gene but are likely to contribute most to resistance, as they induce transcriptional changes in resistance due to their being in coding regions within the gene; they will thus be the focus of this study. Their targeting will be done by means of CRISPR-mediated targeting of these regions, first to isolate the specific elements to be extracted and later to insert said elements into the transgenic non-resistant LT2 plants.

To begin, the transgenic *indica* Rice Cultivar LT2 plants will be produced, creating the experimental population for this study. Firstly, a resistant LT2 plant bearing a non-mutated *Xa21*gene will be used as a control that showcases the extent of resistance that the wildtype gene provides. This will be used to contrast phenotypes of nonresistance in the transgenic plants. Although it has been established in scientific literature that the *Xa21* gene confers resistance to the bacterial blight pathogen, this will be further substantiated using CRISPR-mediated targeting. Specifically, the *Xa21* gene will be ‘knocked-out’ in another wildtype LT2 plant, serving as a means of casually linking resistance to the *Xa21* gene.

Four other transgenic mutant plants will be produced, each lacking both the *Retrofit* and *Truncator* elements to serve as the experimental lines for the experiment. This will be achieved by a specific CRISPR technique described in a study by Bennet-Baker and Mueller (2017) whereby CRISPR enzymology is used to target and clone short single copy genomic sequences. Specifically, this will be done by designing pairs of single guide RNAs to flanking DNA sequences that are specific to the elements being targeted, in this case *Retrofit* and *Truncator*; these sequences are followed by the protospacer-adjacent motif (PAM). This CRISMR technology, referring to the CRISPR-mediated isolation of specific megabase-sized regions of the genome, will be used to release the intact DNA segment by means of CRISPR digestions and isolate it via pulsed-field gel electrophoresis (PFGE), as is done in the Bennet-Baker and Mueller study (2017). Results of this study demonstrate that the CRISPR-Cas 9 system serves as a sufficient tool for gene target and precise editing in plants (Bennet-Baker and Mueller, 2017). These plants will then be tested for resistance upon extraction of these elements; details of how this will be carried out follows in the methods below. It is anticipated that as these components of the genome are extracted the plant will lose resistance to the bacterial blight pathogen.

Upon isolating the DNA segments, the samples obtained will then be amplified using Polymerase Chain Reaction (PCR) in order to make several copies of the elements. To do this, complimentary primers will be generated and the PCR reaction will be allowed to run for an adequate number of cycles. This will be done for both segments of the DNA elements that are isolated, with these elements serving as materials in the next components of the experiment.

The two elements *Retrofit* and *Truncator* will now be inserted back into the genomes of the experimental transgenic plants in a tactical fashion. Firstly, *Retrofit* will be introduced back into the transgenic plant lacking both elements once again using CRISPR-mediated insertion into target gene segments. This will be carried out as described above. The transgenic plant will then be inoculated with the bacterial blight pathogen and tested for resistance. A similar protocol will be carried out for the *Truncator* element in another transgenic plant. Finally, both elements will be introduced into the third transgenic plant. In this case, the plant is expected to have similar resistance to the wildtype type bearing the non-mutated *Xa21* gene. That said, the transgenic plants with only one copy of the transposable-like elements (either *Retrofit* or *Truncator*) are expected to have an intermediate level of resistance. This is due to the fact that additive effects can come into play in the context of resistance, whereby having two elements carrying out resistance can enhance the resistance phenotype. In their study regarding bacterial blight in the indica Rice Cultivar LT2, authors noted that higher levels of bacterial blight resistance are observed in lines carrying more genes, and that this can be attributed to interaction/additive effect between the resistance genes (Nguyen, 2018). In a manner similar to this, increased resistance will likely be observed as both elements are introduced into the non-resistant LT2 transgenic plant.

Measuring disease response will be done by inoculating each experimental transgenic plant (excluding the controls) with the *Xanthomonas oryzae pv. oryzae* pathogen known to contribute to bacterial blight in the rice plant. The pathogen will be isolated from a bacterial blight infected sample on Potato Sucrose Agar medium, as described in a study by Yasmin et al. (2017). Plants will be inoculated by spraying each experimental plant with a suspension of the bacterial pathogen prepared in distilled autoclaved water. To ensure that all other factors are constant, control treatments will be sprayed with distilled autoclaved water. The disease response will be evaluated 18 days after inoculation by measuring the lesion lengths and will be scored as high resistance (lesion length < 8 cm), moderate resistance (lesion length 8-12 cm), and susceptible (lesion length > 12 cm). These scores are based on previous study of bacterial blight infection in the *indica* Rice Cultivar LT2 plant (Nguyen, 2018).

While it is likely that these methods will lead to insight regarding the study question at hand, it is often difficult to account for the way in which experimental protocols will play out when experiments are carried out. In saying this, it is important to anticipate the use of other methods to reach the experimental conclusions that are being sought out, especially when proposing novel research. Other strategies could be used to achieve similar protocols. For example, transposable elements have been shown to be mobilized in several studies using a variety of stressors such as heat or UV radiation (Guerreiro, 2012). A study system can be designed around this where the transposable elements of a Rice Cultivar plant bearing the *Xa21* gene, specifically with a known resistance to the pathogen *Xanthomonas oryzae pv. oryzae*, can be induced with such stressors. The specific transposon-like elements can then be isolated and tagged using established methods that are more likely to contribute to expected outcomes. From there, experimental protocols can be carried out as detailed above.

**Research Significance and Impact**

Rice is one of the most vastly consumed foods in the world, and is a staple for much of the world’s populations. Bacterial blight of rice, caused by the pathogen *Xanthomonas oryzae pv. oryzae* has long been reported in the agricultural harvest of rice. The significance of rice as a crop necessitates scientific interference to adopt strategies that aim to combat disease and find means of increasing crop resistance to the pathogens that infect the plant. While considerable research has been done on bacterial blight and its causal agent, further applications of modern genomic techniques must be established to enhance crop yield and ensure sustainable farming practices. This study aims to contribute to the already existing body of research regarding bacterial blight and the Xa21 gene in hopes of enhancing our understanding of the pathogen and the ways in which resistance can be induced in the rice plant.

In addition to the agricultural applications, there remains a ‘gap in the literature’ regarding aspects of transposable elements, as they are quite unpredictable in nature. As transposable elements play a large role in determining disease states and can contribute to crucial regulatory mechanisms within the genome, understanding the methods of action of these elements is detrimental to the ability to better current therapeutics for conditions that these elements may bring on. Genomic technologies have enabled us to understand the basic mechanisms under which TEs operate, but further study is necessary to bridge the gap that remains.

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