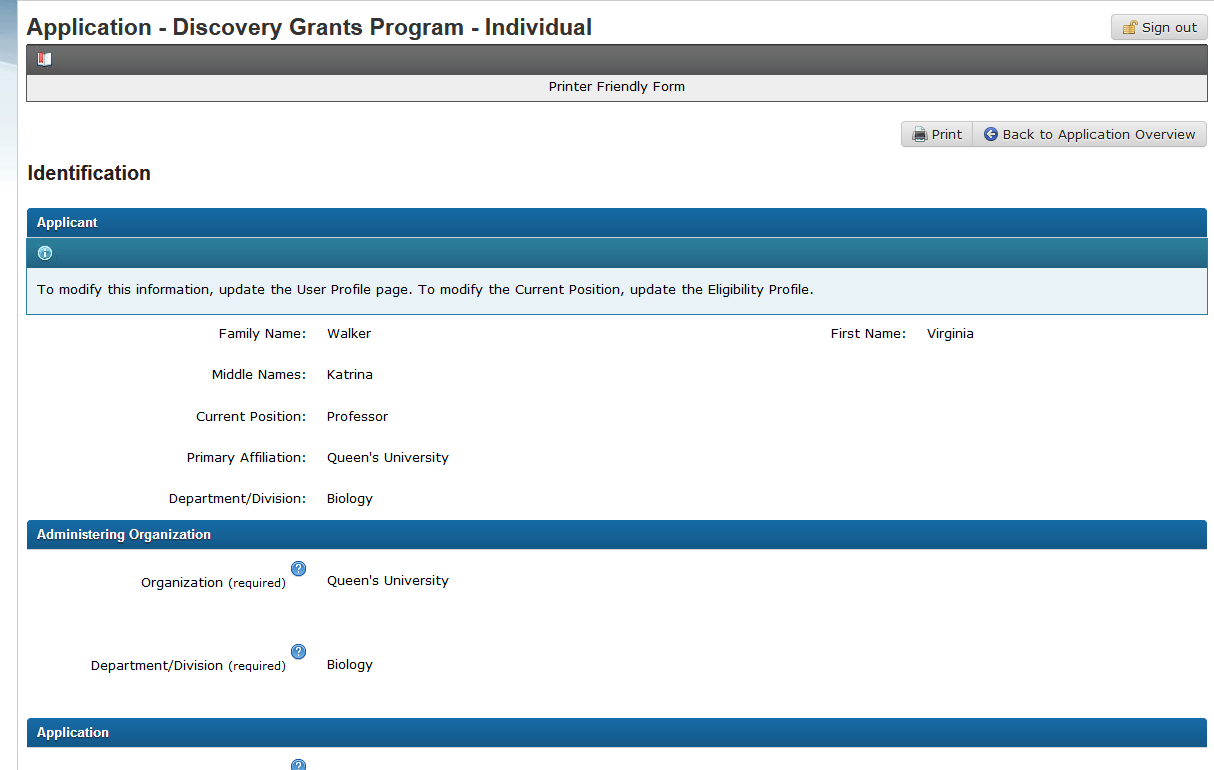
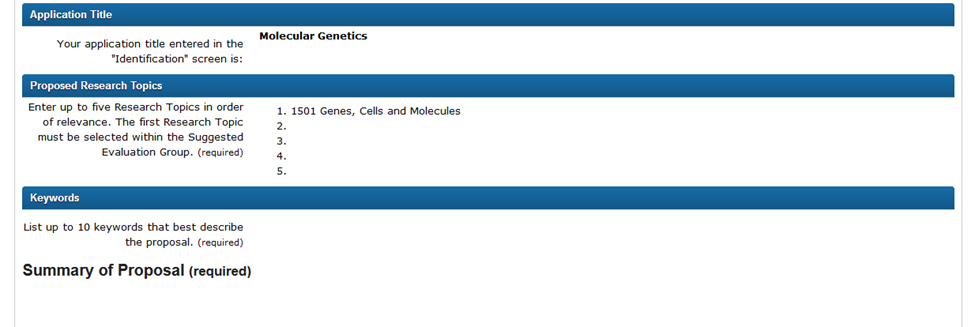
BIOLOGY 441 NSERC-style Notice of Intent (NOI)

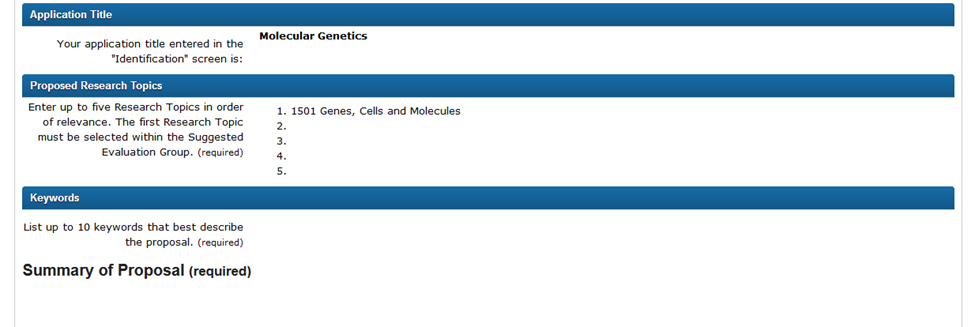


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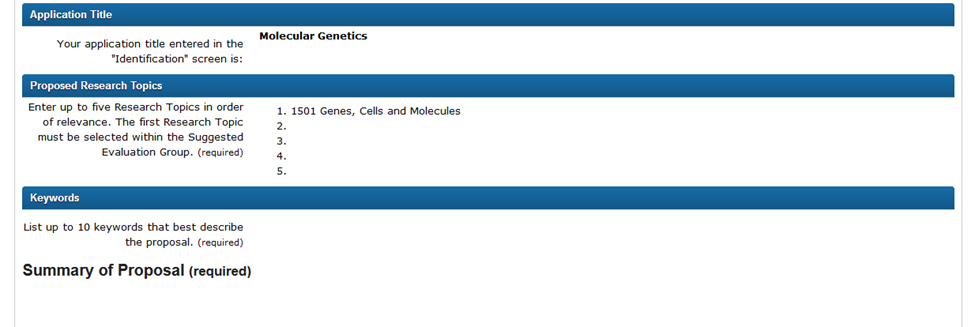


**Proposal Title**: Incorporation of transposable elements involved in Xa21 R-gene in non-resistant mutants of *indica* rice cultivar L2

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 **Keywords:** Transposable elements, plant, disease, resistance, Xa21, CRISPR, specificity

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A transposable element, also known as a jumping gene, is a gene sequence that can change its position within a genome, altering the cell’s genetic identity. Transposition within the genome often results in the duplication of the same genetic material. While these elements are ‘selfish’ in the sense that they prioritize their own transmission at the expense of other genes, many 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). Thus, they can act as drivers of genome evolution in many eukaryotes. This is especially relevant in species that are constantly being subjected to external stresses that require them to respond relatively quickly to ensure their survival.

There are at least two types of transposable elements that contribute to this mechanism: Class I and Class II transposons. Class I transposable elements or retrotransposons, generally function via reverse transcription, amplifying themselves in host genomes. Class II elements or DNA transposons encode proteins that are necessary for the insertion and excision of a transposon, thus catalyzing its movement to another part of the genome.

An application of such incorporations of transposable elements can be seen in plants, whereby they contribute to the amplification of gene families as well as the facilitation of new gene fusions, often acting in synergy with the plant’s DNA repair machinery (Krasileva, 2019). These events work to maintain genetic diversity within the genome, thus acting 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, it has long been hypothesized that these elements play a role in the reconstruction of genomes in response to pathogen infection. Specifically, transposable elements have been shown to cause resistance gene inactivation and diversification, contributing to plants’ defense strategies against pathogens (Richter & Ronald, 2000). In addition to this, once duplicated by these mechanisms, genes themselves can serve templates for additional 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 result of the insertion of a transposable element into the genome.

In a study by Song et al. (1998), the authors studied the rice disease resistance gene *Xa21*. By comparing the genomic sequences of seven family members, seventeen transposon-like elements were identified in the 5’ and 3’ flanking regions and introns of rice disease resistance gene *Xa21* (Song et al.,1998). This suggests that transposable elements play a role in conferring disease resistance within this particular disease resistance gene. This can be used 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 with no resistance to that given pathogen.

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)*.* Thus, this can be used as the main study system, whereby a similar strain of rice cultivar that lacks resistance to the bacterial blight, but contains the Xa21 loci can be considered. Incorporation of the specific transposable element-like regions known to contribute to the *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. This can be done achieved by isolating the transposable regions in the resistant plant and inserting them into the non-resistant species using CRISP-mediated targeting of the *Xa21*gene. One would then test the plant for resistance to bacterial blight and if such resistance is gained by means of the incorporation of TEs, it can be said that there is new specificity at that particular loci. If no such species can be found, the *indica* Rice Cultivar LT2 itself can be manipulated and the seventeen transposon-like elements previously identified in the 5’ and 3’ flanking regions and introns, can be spliced out of the genome completely, creating a group of controls that are wildtype for the gene and an experimental group lacking the TEs. Upon the excision of these elements from wildtype *indica* Rice Cultivar LT2, these will be tested for resistance against the pathogen to make sure that removal of the TEs conferred a lack of resistance in a causal manner.

I hypothesize that incorporation of known transposable-like elements in the *Xa21*gene of a nonresistant sister species of *indica* Rice Cultivar LT2 or a mutant version 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.* 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 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.

Transposable elements (TEs), while can be seen as a ‘selfish’ evolutionary by-product, have important modern applications in genomics. Due to the fact that these elements have become fabricated in eukaryotic genomes impacting the way in which the genomes behave, it is important to understand the impact of specific TE insertions into the genome, and whether this can induce the affect that one would hypothesize. Transposons are especially useful where regulatory mechanisms are relevant, thus impacting the study of adaptation, disease management and much more. Such crucial implications on modern medicine and science as a whole, warrants further analysis of transposable elements and the mechanisms by which they function.

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