



TABLE OF CONTENTS

APPROVAL PAGE	I
STATEMENT OF NON-PLAGIARISM.....	II
PREFACE.....	III
TABLE OF CONTENTS.....	IV
LIST OF TABLES.....	VII
LIST OF FIGURES.....	VIII
ABSTRACT.....	IX
CHAPTER I INTRODUCTION.....	1
1.1 Background.....	1
1.2 Problem Statement.....	2
1.3 Objective.....	3
1.4 Benefit.....	3
CHAPTER II LITERATURE REVIEW, BACKGROUND THEORY, AND HYPHESIS.....	4
2.1 Literature Review.....	4
2.1.1 Type III Secretion System (T3SS).....	4
2.1.2 Nodulation mechanism.....	6
2.1.3 Transposon mutagenesis.....	8
2.1.4 <i>Bradyrhizobium elkanii</i> USDA61.....	8
2.1.5 <i>Lupinus pilosus</i>	9
2.1.6 Mutants construction.....	10
2.1.7 Obtaining primers.....	11
2.2 Background Theory.....	12
2.3 Hypothesis.....	13
CHAPTER III METHODOLOGY.....	14
3.1 Location.....	14
3.2 Tools and Materials.....	14
3.3 Experiment Procedure.....	14
3.3.1 Bacteria preparation.....	15
3.3.2 Plant preparation.....	16
3.3.3 Inoculation test with <i>B.elkanii</i> USDA61 WT, Tn5, and BERhcJ mutant...	16



3.3.4 Symbiotic phenotype analysis.....	17
3.3.5 Identification of Tn5 insertion.....	17
CHAPTER IV RESULT AND DISCUSSION.....	20
4.1 Plant Phenotype Analysis.....	20
4.1.1 Leaf color analysis.....	20
4.1.2 Plant biomass analysis.....	22
4.1.3 Nodule phenotype analysis.....	24
4.2 Nitrogen Fixing Activity Analysis.....	27
4.3 Tn5 Insertion Analysis.....	28
4.4 Discussion.....	29
4.4.1 Phenotypes of <i>L. pilosus</i> and its relation with nitrogen fixing activity under symbiosis with <i>B. elkanii</i> USDA61 T3SS.....	29
4.4.2 Tn5 insertion in <i>B. elkanii</i> USDA61 mutant samples.....	35
CHAPTER V CONCLUSION AND SUGGESTION.....	37
5.1 Conclusion.....	37
5.2 Suggestion.....	37
REFERENCE.....	39
LIST OF ATTACHMENT.....	47
Attachment 1. B&D Nitrogen-free Nutrient (D.W 10L).....	47
Attachment 2. AG Media Composition (1L).....	48
Attachment 3. Spectrophotometry Result for each Bacteria Samples.....	48
Attachment 4. Inoculation Formula Example.....	48
Attachment 5. Agronomical Data.....	49
Attachment 6. Acetylene Reduction Assay Result.....	51
Attachment 7. Comparison of Plant, Stem, Root, and Nodule Weight.....	53
Attachment 8. Anova Test of Acetylene Reduction Assay (ARA).....	54
Attachment 9. Tn5 DNA Extraction.....	54
Attachment 10. DNA Post Digestion and Precipitation.....	54
Attachment 11. Nodulation Phenotype.....	55
Attachment 12. Leaf Color Analysis.....	56
Attachment 13. Isolate for Bacteria Inoculation.....	57
1. Nodulation Phenotype Analysis.....	58



2. Acetylene Reduction Assay.....	58
3. DNA Extraction and Tn5 Detection via PCR.....	59