INVESTIGATION OF GENE TRANSFER POTENTIAL WITH CLASSICAL HYBRIDIZATION IN *Vuralia turcica* AND IDENTIFICATION OF RHIZOBACTERIAL SPECIES CONTRIBUTING ITS DEVELOPMENT

by

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ABSTRACT

Vuralia turcica is a critically endangered endemic plant species only found in Central Anatolia region of Turkey. The most important feature of *V. turcica* is to have a gynoecium containing 2-4 fully developed carpels that distinguishes from other legumes. This dissertation comprises two studies which have not been reported to date according to a literature review. In the first study, gene transfer potential of *V. turcica* was investigated through intergeneric crosses with commercial legume plants, *Phaseolus vulgaris, Pisum sativum, Vicia faba* and *Lupinus spp.*, by the application of classical hybridization methods. In the crossing, *V. turcica* used as the paternal parent. Reciprocal crosses were also conducted with *Phaseolus vulgaris* and *Lupinus spp.* paternal parents. Histological analysis revealed pollen tube growth and extension up to ovaries in the pistils of each commercial legume variety after being pollinated with *V. turcica*. Pre-fertilization barrier was not observed in all crossed samples. To analyze whether the crossed samples were hybrid, the SSR primer used in molecular analysis was developed. Molecular analysis showed that, the plantlets obtained from the crossing of *P. vulgaris* with *V. turcica* were most likely to be pure lines. This potential finding could be important for plant breeding program for obtaining pure lines. In the second study, plant growth promoting rhizobacteria species present in *V. turcica* rhizomes were investigated. Rhizome and soil samples were obtained from the natural habitats of *V. turcica* by the workers of Nezahat Gökyiğit Botanical Garden, and bacterial isolation was conducted on the collected samples. MIS analysis, 16S rRNA and ITS sequencing results of the bacterial isolates revealed the dominance of *Bacillus megaterium* at the rhizomes of *V. turcica. B. megaterium* is often reported as a plant growth-promoting rhizobacteria species in the literature which supports its usage as a biofertilizer. It is also widely used in industrial production of secondary metabolites. The potential growth promoting effects of *B. megaterium* on *V. turcica* was discussed in detail in the second study.

Vuralia turcica BİTKİSİNDE KLASİK HİBRİDİZASYON İLE GEN AKTARIM POTANSİYELİ ARAŞTIRMASI VE YARARLI KÖK BAKTERİ TÜRLERİNİN TESPİTİ

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ÖZET

Vuralia turcica, soyu tükenme tehlikesi altında olan, Türkiye'nin İç Anadolu bölgesinde bulunan endemik bir bitkidir. *V. turcica*'yı diğer sebze bitkilerinden ayıran en önemli özelliği serbest yapıda 2-4 karpelli ovaryuma sahip olmasıdır. Bu tez, literatürde önceden rapor edilmemiş iki çalışmadan oluşmaktadır. İlk çalışmada *V. turcica* ile *Phaseolus vulgaris, Pisum sativum, Vicia faba* ve *Lupinus spp.* gibi sebze türleri arasında klasik hibridizasyon yöntemi ile gen aktarım potansiyeli araştırılmıştır. Melezlemelerde *V. turcica* baba olarak kullanılmış, *P. vulgaris* ve *Lupinus spp.* ile resiprokal çaprazlamalar yapılmıştır. Histolojik analizler, *V. turcica*'nın baba olarak kullanıldığı melezlemelerde ovaryuma kadar polen tüpü uzaması olduğunu ve ön döllenme engeli bulunmadığını göstermiştir. Elde edilen örneklerin hibritlik durumunun tespiti için SSR primeri kullanılmıştır. Moleküler analizlerde *P. vulgaris* x *V. turcica* melezlemelerinden ortaya çıkan örneklerin hibrit olmadığı ve saf hat olma ihtimali taşıdıkları görülmüştür. Bu potansiyel bulgu bitki ıslahı çalışmalarında faydalı olabilir. İkinci çalışmada, *V. turcica* köklerinde bulunan bitki gelişimini teşvik edici bakterilerin tespiti yapılmıştır. *V. turcica*'nın doğal yaşam alanlarından rizom ve toprak örnekleri Nezahat Gökyiğit Botanik Bahçesi çalışanları tarafından toplanmıştır. Örnekler üzerinden bakteri izolasyonu gerçekleştirilmiştir. İzolatların MIS analizi, 16S rRNA ve ITS sekans analizleri sonucunda *V. turcica* köklerininde *Bacillus megaterium* bakterisinin dominasyonu görülmüştür . Biyolojik gübre olarak kullanılabilen *B. megaterium*'un bitki gelişimini teşvik ettiği literatürde sıklıkla rapor edilmiştir. Endüstriyel alanda da ikincil metabolit üretiminde yaygın olarak kullanılmaktadır. *B. megaterium*'un *V. turcica* üzerindeki potansiyel bitki gelişimini teşvik edici etkileri ikinci çalışmada detaylı olarak tartışılmıştır.

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µL	Microliter
µM	Micromolar
16S rRNA	16S ribosomal RNA
ABA	Abscisic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
AHL	N-acyl-l-homoserine lactones
ATP	Adenosine triphosphate
B. megaterium	*Bacillus megaterium*
BLAST	Basic Local Alignement Search Tool
BNF	Biological Nitrogen Fixation
bp	Base Pairs
℃	Degrees Celcius
cm	Centimeter
CTAB	N-Cetyl-N, N, N-Trimethyl-Ammonium Bromide
DAP	Days After Pollination
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetraacetic Acid
FAME	Fatty acid methyl-esther
Fe	Iron
FPA	Formalin–propionic acid–alcohol
g	Grams
GA3	Giberellin
GPS	Global Positioning System
HCl	Hydrochloric acid
IAA	Indole-3-Acetic Acid
ICP-OES	Inductively coupled plasma - optical emission spectrometry
ISR	Induced Systemic Resistance
ITS	Internal Transcribed Spacer
K	Potassium
kg	Kilograms
Kn	Kinetin
L	Litres
Lupinus spp	*Lupinus* species
M	Molar
MEGA	Molecular Evolutionary Genetics Analysis
mg	Miligrams
MIS	Microbial Identification System
min	Minutes
ml	Milliliter
mM	Millimolar
Mo	Molybdenum
MS	Murishage & Skoog
N	Nitrogen
NAA	1-Naphthaleneacetic acid
NCBI	National Center for Biotechnology
ng	Nanogram

NGBG	Nezahat Gokyigit Botanical Garden
P	Phosphorus
P. sativum	*Pisum sativum*
P. vulgaris	*Phaseolus vulgaris*
PCR	Polymerase Chain Reaction
PGPR	Plant Growth Promoting Rhizobacteria
Phl	Phenazine-1-carboxylate
RNA	Ribonucleic acid
SSR	Simple Sequence Repeat
TBE	Tris/Borate/EDTA
V. faba	*Vicia faba*
V. turcica	*Vuralia turcica*
YMA	Yeast Malt Agar
Zn	Zinc

0.1 GENERAL INTRODUCTION

0.1.1 Context and Motivation

Thomas Robert Malthus proposed that food production in the world is increasing arithmetically while population and consumption of food were increasing geometrically. Technological advancements after the industrial revolution decreased the death rates but birth rates haven't changed, especially in developing countries. Thus, a population boom happened, and increasing prosperity in cities attracted more and more people. Consequently, agricultural areas were limited because of expanding cities and immigrating farmers. Hypothetically this situation would lead to a global food scarcity but scientific and technologic improvements in the area of biology and agriculture helped to meet the food demand of the increasing population (Malthus 1973; Hazell 2009; Pingali 2012).

Agricultural biotechnology applications enabled scientists to introduce new traits to mostly consumed staple crops aiming to increase their yield. The green revolution took place between 1950 and 1970, with the innovations on irrigation systems, pest and disease control methods. The most significant element of this agricultural revolution was the production of the high yielding varieties. The introduction of dwarfing genes into commercial crops prevented farmers from loosing yield due to bending of staple crops in the field which makes them impossible to harvest. Hybridization was one of the most important applications to obtain high yielding varieties (Farmer 1986). Crossing method is frequently done to produce new ornamental hybrids with high aesthetic value and also to introduce traits like heat or cold tolerance, disease or pest resistance, drought tolerance and rapid rooting into new hybrids (Hawkins et al. 2013).

Cultivar improvement depend highly on the genetic knowledge to introduce new beneficial traits. Understanding genetic mechanisms behind a useful trait are crucial for their further utilization. The subject plant of this study is *V. turcica* which is an endemic legume crop with a striking feature: its flowers contain 2-4 free carpellary ovary. The carpel is the primary element of female organ of a flower which provides space for ovules

to develop after fertilization (Tekdal et al. 2014). Genetic knowledge on *V.turcica* is too narrow , although it is a potentially valuable genetic source to offer yield increase. This study includes hybridization studies between *V.turcica* and the most consumed legume crop *Phaseolus vulgaris,* which is also known as the common bean. The possible introgression of the multicarpellary trait of *V.turcica* to a hybrid would facilitate the discovery of the genetic mechanisms behind it.

Another interesting fact about *V.turcica* is that its habitat is limited to an area in Central Anatolia in Turkey. There might be several reasons behind that, but the most significant one could be the symbiotic and mutualistic relation between *V. turcica* roots and present microflora in the habitat. Furthermore, microbial activity at roots of *V. turcica* have never been studied before. Plant Growth Promoting Rhizobacteria (PGPR) contribute to plant growth through nutrient mobilization in soil, plant growth regulator production, plant pathogen control and inhibition and toxic compound degradation (Ahemad et al. 2014). In light of those concepts, PGPR at the roots of *V. turcica* were also investigated in this study to be able to reason the endemism of this plant.

Chapter 1

INVESTIGATION OF GENE TRANSFER POTENTIAL WITH CLASSICAL HYBRIDIZATION IN *Vuralia turcica*

1.1 INTRODUCTION

Productive agricultural areas are decreasing globally in last decades. There are several human-related reasons behind this decrease like desertification, salinization, soil erosion which can be related to unsustainable land management. Yet, the most important cause of this fertile land loss is the urbanization (Nellemann 2009). People living in rural areas migrate to cities as there are more economical and social opportunities are present (Cohen 2006). As a consequence, expanding urban areas seizes the agriculturally productive lands and possible human efforts on agricultural production (Nellemann 2009). In addition to fertile land loss, worlds population is increasing exponentially; global population is expected to be over 9 billion in 2050 which means there will be a need of 70-100% more food production to provide food security (Baulcombe et al. 2009). When the narrowing agricultural lands and growing population put together, there is not much alternative solution than to produce more food from the same or even less amount of land (Godfray et al. 2010).

Studies on agricultural production rates and its relation with population predictions indicate that a global yield increase is needed to avoid possible forthcoming food scarcity. Intensive production of staple crops like maize, rice, and wheat may provide enough calories for masses to survive, but their protein content is often deficient in some essential amino acids (Tharanathan et al. 2003). Efforts on food production in developing countries prioritized cereals to provide calories to masses, but the protein availability is more significant in nutritional point of view (Godfray et al. 2010).

Legume crops carry high importance in means of food security. Protein availability for low-income families in developing countries is less than one-third of the standard requirements (Paul et al. 2011). High nutritional value of pulse crops position them as a substitute for meat in those countries where people often face protein deficiency (Tharanathan et al. 2003; Shimelis et al. 2005). Legumes like *Phaseolus vulgaris* (common bean) were usually grown to provide nourishment for the local population as it is an important source of micronutrients like iron, zinc, folic acid and thiamin (Petry et al. 2015; Broughton et al. 2003; Pennington et al. 1990; Souci et al. 1981). In food-system context, legume crops require low inputs and yield more seed protein than animal protein on a unit of land (Saxena et al. 2013).

Consideration of nutritional value and low input/output rate of legume crops makes them ideal plants for providing food for all levels of socio-economical status. Therefore they are worthy of studying for further crop improvement aiming to ensure food security. Crop improvement realizes through the transfer of genes as the genotype of a plant determines its qualitative and quantitative traits. The most basic gene transfer method that requires human effort is classical hybridization.

1.1.1 Crop Improvement Through Hybridization

Crops may contain genes that are disadvantageous for them, which decrease their fitness and survival ability. In a plant population, members may have the same deleterious genes and inbreeding in this population may result with the pairing of inferior alleles of the same genes. It has been shown that the diversification of allele combination in an organism occurs with a better state of growth and vigor when compared to the similar organism whose alleles are identical (Duvick 2001).

F1 generations resulting from the crosses between diverse parents usually have superior characteristics than their parents as increased stature, biomass, and fertility. This state is called hybrid vigor or heterosis (Birchler et al. 2006). The term heterosis was first used by George Shull in his lecture in 1914 after the verification of the phenomenon while studying on maize breeding programs (Shull 1908; Ryder et al. 2014). The characteristics of heterosis are first described by Charles Darwin before the word ‘heterosis’ became a biological term. Darwin compared the progenies of the cross and self-fertilized inbred

parents and confirmed that the F1 generation of the cross-fertilized plants was more vigorous and taller than self-pollinated plant progenies (Darwin 1876).

Hybrid plant breeding practices resulted in quantum yield jumps in vegetable, cereal and fruit crops, according to past studies (Kuznecov 1966; Alexandratos 1995; Rai and Rai 2006). Combination of parental genomes in distant hybridization increases genetic variability and creates new varieties and species (Saxena et al. 2013). Heterosis is a complex phenomenon where a lot of quantitative traits were altered. Vegetative growth rate, biomass, seed size, plant stature, metabolite accumulation, flowering time and adaptation to biotic or abiotic stress are the typical traits that are aimed to be improved to increase the yield of crops by cross-pollinating distant varieties (Baranwal et al. 2012).

Papilionoids consist 476 genera and 13860 species and they are the largest of the three subfamilies of Fabaceae. Most of the domesticated food and ornamental crops are members of the Papilionoideae subfamily and they are also known as legume plants (Gepts et al. 2005). The reproductive organs of papilionoid plants are enclosed within keel petals and this structural character limits the natural cross fertilization possibilities. This morphological favored self-pollination impedes achieving hybrid vigor in large-scale agricultural practices (Saxena et al. 1989). The subject plant of this study, *Vuralia turcica* is a legume plant with a potential ornamental and food crop value. *V. turcica* is a Turkish endemic plant and its natural habitat is restricted. Because of its papilionoid flower morphology, inbreeding is favored in the population. Reoccurrence possibility of deleterious traits in progenies increases because of the reasons above. As a result, inbreeding depression can be experienced which is defined with reduced survival and fertility of offsprings (Charlesworth et al. 2009).

1.1.2 Distant Hybridization

Distant hybridization in plants is the sexual mating of two different plants that are distantly related in a taxonomic manner. Hybrids occurring from the cross of individuals that belong to the same genus but different species is called interspecific hybrids, and progenies obtained from parents that belong to different genera are called intergeneric hybrids. Both interspecific and intergeneric crosses are done between distant relatives, but chances of obtaining progenies are lower for intergeneric crosses as the mating members are taxonomically more distant.

First agricultural societies started cultivating crops about 12000 years ago, and plant breeding practices took its place for the first time with the settling of hunter/gatherer societies (Borém et al. 2002). Plant breeding is the art and science of manipulating crop characteristics in order to produce plants that possess more suitable traits for human needs (Poehlman 2013). The main aim of the most plant breeding practices is to enhance the quality and quantity of food products that are used by humans and human herd animals. Specific outcomes are expected while breeding plants; improved taste and nutrition, biotic or abiotic stress resistance and prolonged storage time (Hartung et al. 2014).

The importance of crossing distant species is that the potential of introgression of a specific trait that is not found in a studied variety. For example, most varieties of wheat are moderately tolerant to salt stress and any varietal combination may not produce progenies with superior resistance as the levels of resistance in cultivars is limited within a narrow range (Rana 1986). To produce new wheat cultivars with enhanced salt stress, most common wheat variety, *Triticum aestivum* is crossed with *Aegilops cylindrica* that possesses better salt stress resistance traits (Farooq et al. 1995).

With the purpose of increasing genetic variability and producing new useful cultivars, plant breeders applied wide crossing. As an example to wide intergeneric hybridization, a member of *Brassica* tribe, *Crambe abyssinica,* is crossed with *Brassica* species (Youping et al. 1998). *C. abyssinica* is intriguing with its seed oil content that is mostly composed of erucic acid, an essential compound used in industry (Youping et al. 1995). A disadvantage of this crop is that it is not resistant to diseases and farmers are experiencing yield loss due to a disease that darkens its stems and seeds (Youping and Peng, 1995). Among *Brassica* species, *B. juncea* is the crop that has successfully produced a hybrid with *C. abyssinica*. The hybrid may have improved resistance as *B. juncea* contains drought and aphid tolerance (Youping and Peng, 1998).

Improving food crops for better nutrition and yield has been the main aim of many plant breeders throughout the history. To achieve this goal, numerous hybridization attempts were made between legume plants. Studying with legumes is advantageous as they do not require nitrogen fertilizers, they fix nitrogen through the symbiotic or mutualistic microorganisms that reside at the roots (Smartt 1970). Interspecific crosses have more frequently experimented than intergeneric crosses in legumes, according to a literature review. Perhaps, the difference of the possibility of success between the two

influenced researches to favor interspecific cross, as genetic differences and incompatibilities increase as the plants get taxonomically distant. Mendel (1866) reported the first distant hybridization in the genus *Phaseolus,* between *P. vulgaris* (common bean) and *P. coccineus* (runner bean). Both plants are usually self-fertilized because of the morphology of their flowers, but it is rarely possible to happen in the nature (Graham and Ranalli 1997). There are important differences in the mating systems of both species, but they are cross-fertile in some extent, especially when the common bean is the maternal parent in the cross (Singh 2001). Runner beans are widely cultivated in Europe because of its ability to grow in cold temperatures, a trait that is not equally present in other members of the genus (Evans 1980). Chances of fertilization between runner bean and common bean highly depend on the parental genotype combination (Gepts 1981). When a *P. coccineus* individual with the desired trait is detected, it has to be crossed with diverse and various *P. vulgaris* lines to determine the optimal parental combination to achieve cross-fertilization and introgression of the desired trait (Schwember et al. 2017).

P. vulgaris is crossed with *P. lunatus* (lima bean) and *P. acutifolius* (tepary bean) for the introgression of resistance genes against root rot caused by fungi *Fusarium solani* and bacterial blight caused by *Xanthomonas phaseoli* (Mok et al. 1978). In this experiment conducted by Mok et al. (1978), hybrid embryos were obtained from both species where the common bean was the maternal parent. Also, reciprocal crosses were done, and hybrid development was observed where tepary bean was the maternal parent. Reciprocal crosses are crucial in attempts of cross fertilization. A trait can be autosomal or sex-linked so that this application can give clues about the role of parental genes on a traits pattern of function (Fossella 2001).

Fertilization of distant relatives might be problematic. Incompatibility between parents can occur due to lack of genetic information in one parent to achieve pre- and post-pollination phenomena (Hogenboom 1973). Pre-fertilization barriers can be the failure of pollen germination, poor penetration of pollen through stigma and slow pollen tube growth or the arresting of pollens in gynoecium. Post-fertilization barriers can be abnormal endosperm growth resulting in embryo abortion due to lack of nutrition, hybrid sterility or lethality caused by chromosomal or genetic differences (Khush et al. 1992). As mentioned above, the common bean can be hybridized with several other *Phaseolus* species, but for further survival, hybrids are required to be cultured on synthetic media because of post-fertilization barriers (Graham and Ranalli 1997).

1.1.3 General Aspects of *Vuralia turcica*

Vuralia turcica (Uysal et al. 2014) is an endemic legume plant belongs to the subfamily Papilionoideae, and it is the only plant in Turkey that carries similar characteristics to *Thermopsis* species (Tan et al. 1983). This diploid plant contains 2n=18 chromosomes (Özdemir et al. 2008). Turkish botanists taxonomically classified and named the plant in 1983 as its previous name *Thermopsis turcica* Kit Tan, Vural & Kucukoduk (Tan et al. 1983). Among locals, *V. turcica* is called 'piyan', 'sarı meyan' or 'Eber sarisi' (Vural 2009). Other members of the genus *Thermopsis* are spread around the highlands of North America and Asia. *V. turcica* has been taken under conservation as it is classified as a critically endangered plant in Red Data Books of Turkish Plants (Davis 1965; Tan et al. 1983; Cenkci et al. 2008). The most distinguishing characteristic of *V. turcica* is the natural occurrence of 2-4 free carpels on the gynoecium (Figure 1. D, E). The plurality of the carpels can be observed in Fabaceae family, but it is rarely encountered among legume plants (Baillon 1873; Cowan 1967; Tucker 2003). Multicarpellary trait is also found in the tribe *Swartzieae* of the subfamily Papilionoideae (Paulino et al. 2013).

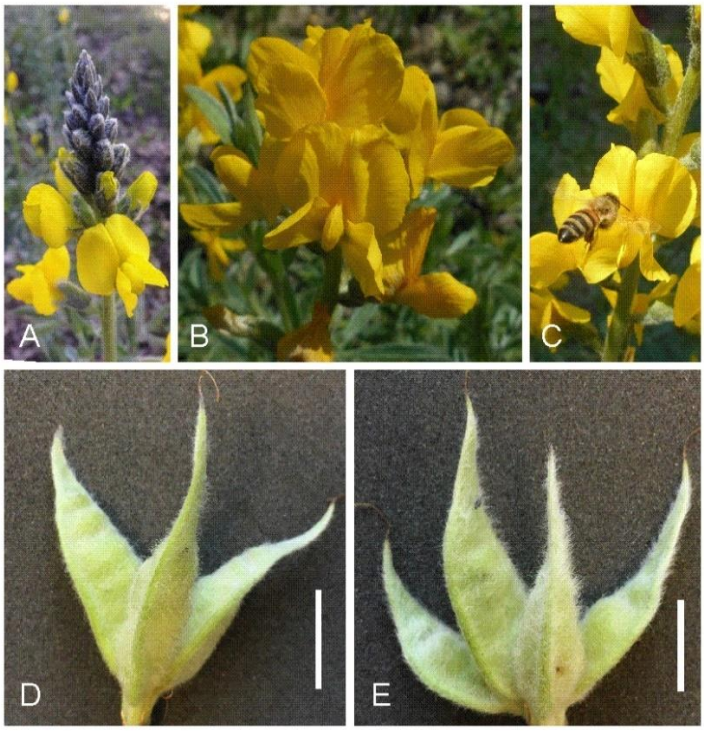

Figure 1. Morphology of flowers and fruits of *V. turcica*. (A) Racemose inflorescence. (B) Flowers at anthesis, (C) Honey bee visiting flowers, (D), Immature fruits developing from tri- (D) and tetracarpellate (E) gynoecium. Scale bars: 1 cm (Sinjushin et al. 2018)

V. turcica's multicarpellary state differs from that tribe by being the first record of uniform occurrence of morphologically independent carpels (Cenkci et al. 2009). With completely formed 2-4 pistils, *V. turcica* is distinguished from other members of the Papilionoideae subfamily, whereas the majority of the Fabaceae family contains single carpel in the gynoecium (Tekdal et al. 2014). The occurrence of single carpel is more dominant in legume plants but polymerous gynoecium formation can rarely be induced by mutations or environmental shock (Lamprecht et al. 1974; Stergios et al. 2008). Between model plant species of the subfamily Papilionoideae, polymerous gynoecia can be found among developmental mutants of *Pisum sativum* (common pea) and *Medicago truncatula* (barrel medic). Carpel polymerization of *V. turcica* is unique among legumes by its natural occurrence (Sinjushin 2014).

1.1.4 Potential Commercial Value of *Vuralia turcica*

Understanding the mechanisms behind multicarpellary trait may uncover a potential of yield increase in legume crops (Tucker 2003; Endress 2013). There is not much genetic information on multicarpellary features of *V. turcica* apart from Tekdal's work (Tekdal et al. 2017). In light of revealing the mechanisms behind the trait, it would be useful to experiment cross-fertilization with commercial legume varieties. In case of a successful introgression of the multicarpellary trait into a legume crop, its expression patterns would be more disclosed with further transcriptomic analysis.

According to the literature, the first study of cross-fertilization of *V. turcica* was carried out with *Vicia faba*, and it was shown that *V. turcica* can cross-fertilize with a legume (Tekdal et al. 2017). Post-fertilization barriers might have been an obstacle to obtaining a hybrid in that intergeneric cross, but the demonstration of crossing ability of *V. turcica* is encouraged to study its cross-fertilization with different legumes.

The fruit of a legume is called a pod, and every pollinated carpel is expected to develop into a pod. Theoretically, if the inheritance of the multicarpellary trait of *V. turcica* into a hybrid with any grain legumes in human consumption is achieved along with its expression, from one flower, 2-4 pods would be yielded instead of one. This best case scenario would result in obtaining 2-4 times more yield from the same amount of land used which may further lower the food prices by the widespread inheritance of the

trait into commercial legume varieties. In this chapter, the crossability and potential gene transfer between *V. turcica* and *P. vulgaris* was investigated. Any success on inheriting *V. turcica*'s multicarpellary trait into a hybrid resulting from a cross with a legume would be beneficial for crop improvement.

1.1.5 Aim of the Study

The aim of this study was to observe the potential of gene transfer between *V. turcica* and other legume crops through classical pollination methods.

1.2 MATERIALS AND METHOD

1.2.1 Materials

1.2.1.1 Plant Material

Plant subject plants that were used in this study are *Vuralia turcica, Phaseolus vulgaris, Lupinus spp., Vicia faba* and *Pisum sativum.* The seeds of *P. vulgaris, V. faba* and *P. sativum* were obtained from local breeders in the villages of Adana whereas that of *Lupinus sp.* were taken from the workers of NGBG. *V. turcica* plants are grown from rhizomes that are gathered from its original habitat by the workers of NGBG in late August of 2012 from the vicinity of Akşehir and Eber lakes (Figure 2).

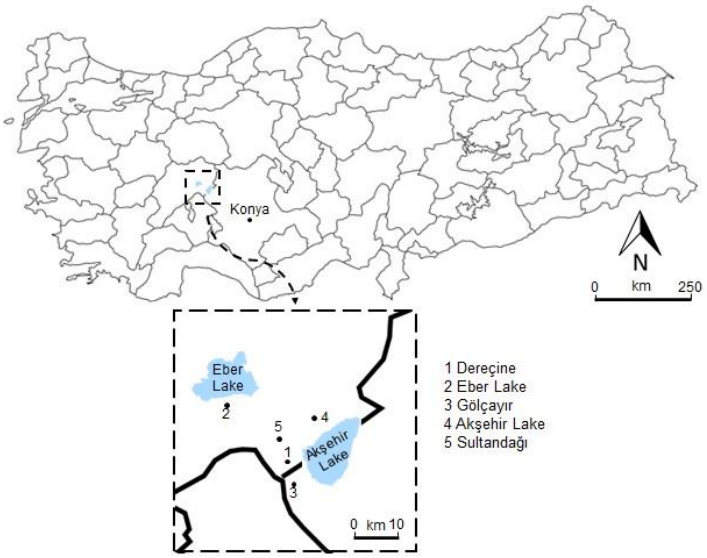

Figure 2. Natural habitat locations of the endemic plant Vuralia turcica given on the map (modified from Tekdal et al. (2018))

The main focus of this chapters study was the pollinations between common bean and *V. turcica* since the flowers of both species were obtained for crossing. *P. vulgaris* grew healthy compared to other selected species in the same environmental conditions. Also, crosses between the two species yielded with more hybrid candidates. Two different genotypes were included from *P. vulgaris* in this experiment which were Trabzon and Rize populations (Figure 3). Trabzon cultivar has a short body while Rize cultivar has a climbing habit. In order to observe the potential of gene transfer between *V. turcica* and *P. vulgaris,* the classical pollination method was conducted between the two subject species. *P. vulgaris* cultivars (2n=22) were mainly used as the maternal parent while *V. turcica* was the paternal parent.

Figure 3. (A) Trabzon cultivar and (B) Rize cultivar of common bean growing on the vegetable field of NGBG (Cultivars are indicated in the middle portion of the images).

1.2.1.2 Research Area

This study was conducted in multiple research areas like fields and greenhouse. Pollination and observation stages of the research were realized in NGBG facilities; two separate gardens were used for growing common bean and *V. turcica* separately (Figure 4). Tissue culture studies, histologic and molecular analysis were conducted in Sabanci University laboratories. Gene transfer potential of *V. turcica* was experimented on several legume crops, but the crosses were mainly focused on *P. vulgaris*. Other legumes were grown in the greenhouse at Sabanci University from the seeds.

Figure 4. (A) General view of *V. turcica* and other legume crops planted in the campus area of Sabanci University, (B) NGBG research area in which *V.turcica* and *Lupinus sp.* were planted

There is an unknown percentage of success of obtaining a hybrid in this intergeneric cross. Cross between these two species has never been tried before and as two subject plants are taxonomically distant, success chances might be low. In this manner, the more essays of the cross mean more possibility of producing a hybrid. So, as the research area, the agricultural field of Sabanci University was also used for growing legume plants (mostly common bean) for pollination studies (Figure 4). Subject legumes were germinated in Sabanci University greenhouse before the transplantation.

1.2.1.3 Equipments

Equipments used in this study are given in Appendix 1 with the manufacturer company, model and country.

1.2.1.4 Chemicals

Chemicals list used in this study are given in Appendix 2.

1.2.2 Methods

1.2.2.1 Pollination studies

Field studies related to crossing started in May 2016 and conducted until the end of June which covers the generative period of *V. turcica.* Blooming period of *V. turcica* did not coincide with *P. vulgaris* at that season. Therefore, *V. turcica* is used as the male parent while *P. vulgaris* was the maternal parent. No receptive *V. turcica* flower was available in the flowering period of *P. vulgaris*. The reciprocal cross between these species was implemented in the following season by matching their blooming period.

1.2.2.1.1 Pollen collection

Flower buds of *V. turcica* was collected in the balloon stage, which is before anthesis, and grown anthers were separated from buds without damaging. Anthers were collected on a tracing paper and incubated at room temperature under light for one night. That incubation leads bursting of anthers to release the pollens within; then pollens were collected in small tubes and saved in -80 ℃ until field work.

1.2.2.1.2 Pollen viability test

The viability of pollens is as essential as the receptivity of the gynoecium. It must be tested before crossing to be sure that pollens are functioning. In this study, pollen viability was ensured by the colorimetric test which is a simpler and faster technique than other methods like pollen germination test by omitting environmental factors like humidity, temperature, and light (Gaaliche et al. 2013).

Collected pollens were spread on glass slides by brush, then slides were stained with acetocarmine solution. For enabling the diffusion of the dye into pollens, it required resting stained slides for 5-7 minutes. Viable pollens were identified by their distinct red color while expired pollens had a ghost-like look with light red color (Figure 5). Once the pollen viability was confirmed, its stock were brought to field and used for pollination on the day that the viability is confirmed.

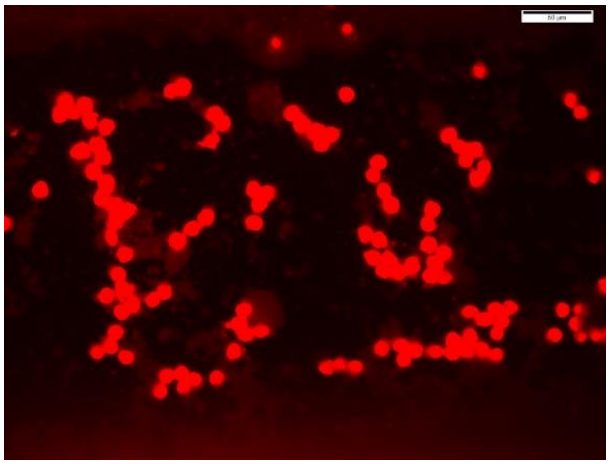

Figure 5. *Vuralia turcica* pollens analysed under a fluorescent microscope. Dots with bold red color were viable pollens. Pollen viability was checked before every pollination study (ocular measurement is 50 micrometer (µm))

1.2.2.1.3 Pollination

Pollination step can simply be described as pollinating the maternal parent's stigma only with the pollens of the donor parent. Accordingly, to ensure the cross of the interested parents, receiver flower was emasculated where its male organs were discarded before pollination. *V. turcica* flowers were collected before they were fully bloomed, which also indicates that the anthers had not dehisced yet. Collected flowers petals and sepals were removed then the anthers were separated from their stigma and spread on a tracing paper. Pollens were left under roomlight overnight for bursting.

P. vulgaris flowers at the balloon stage were chosen for pollination because their stigma was thought to be developed enough for fertilization, and their anthers had not yet burst (Figure 6. A,B). First, with a help of a forcep, sepals and petals of the bean flower were removed. Exposed reproductive organs were visually checked if the anthers were burst or not. Flowers with bursted anthers were eliminated as their stigma was pollinated with the pollens of its own flowers. Then, stamens were carefully removed, and the stigma

of *P. vulgaris* flower was pollinated with previously collected *V. turcica* pollens by using a small paint brush (Figure 6.C).

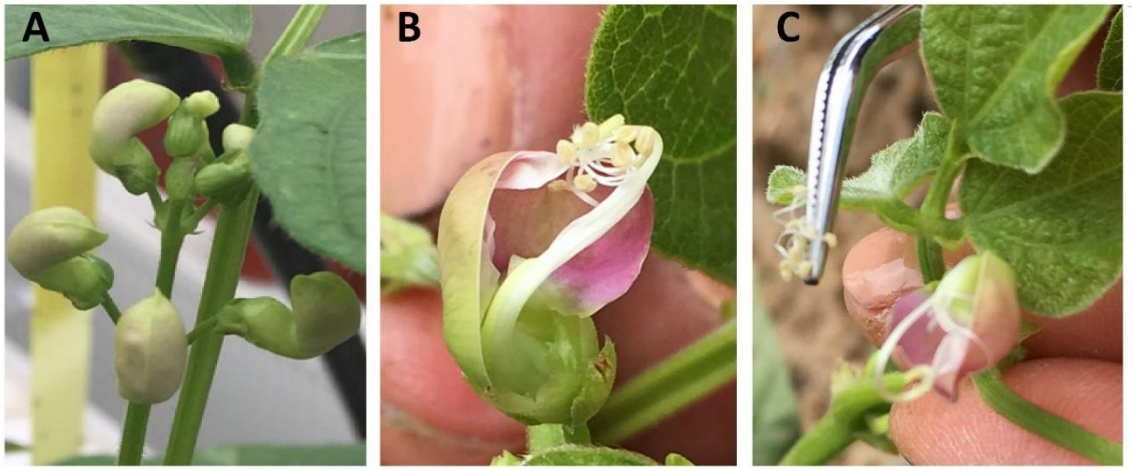

Figure 6. (A)White-purpe colored flower buds at baloon stage of *P. vulgaris* are used as maternal parent for pollination studies. (B) Exposure of reproductive organs of bean flower with unbursted anthers. (C) Removal of the male organs.

The pollinated flower was enclosed within a tracing paper bag (Figure 7) to protect it from environmental factors like rain, sunlight, and pests. Also, it is essential for avoiding foreign pollens to pollinate the stigma. Then, bags were labeled with the date of pollination. 5 days after pollination, the bags were removed to aerate the pistils and to avoid physical disturbance if there was a pod growth. Growing pods were labeled again and collected at different numbers of DAP.

Figure 7. Hand-pollinated flowers were covered with tracing paper. (A) Rize cultivar and (B) Trabzon cultivar

1.2.2.2 Tissue Culture Studies

For further investigation of the hybrid candidates, seeds and embryos that were obtained through pollination were conserved in vitro. Media with different compositions were tried for finding the optimal medium for the micropropagation of hybrid candidate embryos.

1.2.2.2.1 Growth Media

The mediums used for embryo/ovule culture were free from plant growth hormone and contained 1 mg L^{-1} NAA, 1 mg L^{-1} GA^3, 1 mg L^{-1} Kn, 1 mg L^{-1} ABA, 0.5 1 g L^{-1} casein hydrolysate, 1 g L^{-1} glutamin, and 30 mg L^{-1} sucrose. The combinations and concentrations of the media used in this study are given in Table 1. Media were tried in different stages of development of hybrid candidates as multiplication, rooting and elongation to find the optimal concentration for developmental stages.

Table 1. Concentratios and combinations of plant growth regulators used in this study

	Plant Growth Regulators (mg L^{-1})					
Medium	**NAA**	**GA3**	**Kn**	**ABA**	**Casein hydrolysate**	**Glutamin**
MS	0	0	0	0	0	0
	1	1	0	0	0	0
	0	1	1	0	0	0
	1	0	0	1	500	1
B5	0	0	0	0	0	0
	1	1	0	0	0	0
	0	1	1	0	0	0
	1	0	0	1	500	1

1.2.2.2.2 Pod surface sterilization

Collected pods resulting from pollinations were sterilized under laminar flow hood. Pods were washed in 70% ethanol for 5 minutes, then transferred into 20% bleach solution with one drop of tween20 then left there for 20 minutes. After, pods were rinsed with double distilled water 3 times to get rid of the chemicals applied before.

1.2.2.2.3 Embryo and tissue culture

Sterilized pods were cut from both ends and opened to extract the seeds. The outer membrane of the seeds was peeled and seeds were cut in half. The embryos within the seeds were transfered in the media. Seeds were also planted directly onto media without extracting the embryo.

1.2.2.3 Histological analysis preparation

Histological analysis was conducted in order to observe and confirm the travel of the pollen from stigma to ovary after pollination. Pollinated samples were collected following 2, 4, 6 ,8, and 10 days after pollination for histological analysis.

Enough bean samples were collected for each DAP counted, but because of lack of growing pea samples, just 4 DAP and 6 DAP pistils were collected for analysis. Again, for the same reason, just one 4 DAP sample of *Vicia faba* was able to collect. Collected samples were preserved in FPA-70 fixation liquid composed of 900 ml 70% ethanol, 50 ml formaldehyde and 50 ml propionic acid then stored at +4℃ until the analysis.

1.2.2.4 SSR primer development for hybrid candidates

SSR primer used in this study was developed in the plant biotechnology laboratory at Sabanci University by Dr. Dilek Tekdal and Dr. Stuart James Lucas using the methods as follows:

1.2.2.4.1 Genomic DNA isolation

DNA isolation was conducted according to the CTAB DNA isolation protocol (Dellaporta et al., 1983; Doyle 1987). Young and healthy leaves of samples were selected for this application. The chemicals that were used in this protocol were buffer (2% CTAB, 1.4 M NaCl (5 M), 0.2 M EDTA (0.5 M) pH 8.0, 0.1 M TRIS-HCl (1 M) pH 8.0), chloroform:isoamyl alcohol (24:1), Tris-EDTA (Tris 1 M pH:8, EDTA: 0.5 M pH:8), RNase A (10 mg L^{-1}) solution, isopropanol and ethyl alcohol (99%)**.** The purity of the isolated DNA's was verified by revealing the amount and quality by spectrophotometry

(NanoDrop ND-100, Wilmington, DE, USA), and then DNA unity was further confirmed by electrophoresis (ran in 2% agarose gel, stained with ethidium bromide). Isolated DNAs were then stored at - 80℃.

1.2.2.4.2 Sequencing and primer design

Genome sequence information of legumes (*Medicago truncatula, Lotus japonicus,* and *Cicer arietinum* etc.) was obtained from the NCBI website in order to compare and design microsatellite primers for SSR region amplification for *V.turcica.* To detect SSR regions in the genome, SciRoKo 3.3 (SSR Classification and Investigation by Robert Kofler) program (Kofler et al. 2007) and 'uniqueify.pl', a script coded in Perl language by Dr.Stuart James Lucas which serves to name every unique sequence in a genome was used.

Primers were designed for the sequences that covers the microsatellites by using the Primer3 program (Rozen and Skaletsky 2000). The lengths of the designed primers were 18-24 bp where the amplification products length is 200-400 bp. Melting temperature is 50-62℃, and GC content is 50%. Primers (Table 2) were produced by Sentegen company (http://www.sentegen.com/). Softwares and websites used for primer design are given in Table 3.

Table 2. Sequences of the designed primers

Ca1Mt2_2-Forward	TCGTCATTGTTTTGTTCCTCA
Ca1Mt2_2-Reverse	AGGATGACGTGTGGAATGGT

Table 3. Softwares and websites used for primer design

SOFTWARE, PROGRAM, WEB SITE	**COMPANY/WEB ADRESS**	**AIM OF USAGE**
NCBI	http://www.ncbi.nlm.nih.gov/	Genome sequencing ve Primer design
SciRoKo 3.3	http://kofler.or.at/bioinformatics/SciRoKo/	Primer design
Uniqueify.pl	Designed by Dr.Stuart James Lucas	Primer design
Primer 3	http://biotools.umassmed.edu/bioapps/primer3_www.cgi	Primer design
Thermal Cycler	BIORAD	PCR

1.2.2.4.3 SSR analysis and PCR

Genomic DNAs of hybrid candidates were used for analysing the gene transfer. PCR reactions and conditions are given in Table 4 and 5.

Table 4. A list of chemicals and their compositions that were used to prepare samples for PCR.

PCR	**Volume**	**Concentration**
Genomic DNA	x µL	5 ng
10X Taq Polymerase Buffer (+KCL; -$MgCl_2$) Fermentas: Lot: 00061586	2.5 µL	1X
dNTP mix (10 mM) Fermentas: #R0192	0.5 µL	0.2mM
25 mM $MgCl_2$ Fermentas: 00061590	2.5	2.5mM
Forward Primer (100 µM)	2 µL	0.8µM
Reverse Primer (100 µM)	2 µL	0.8µM
Taq DNA Polymerase (2.5U/µL) Fermentas: #EP0402	0.125 µL	0.125 U/µL
Betaine Sigma: 1 vial B-0300 Lot: 086K6045	6 µL	-
ddH_2O	Up to 25µL	-
Total volume	25µL	-

Table 5. Optimized thermal cycles for the designed primers PCR

95 °C 4 min	Pre-denaturation
95 °C 30 sec	Denaturation
*ºC 1 min	Annealing
72 °C 1 min	Extension
72 °C 7 min	Post-extention
+4 °C	∞
*Appropriate temperatures are used for every primer	

1.2.2.5 Agarose gel electrophoresis

10 µl of genomic DNA's were mixed with 2 µl loading dye buffer (40% saccharose, 10 mM EDTA, 25% bromophenol blue) then injected into the veils of 2% (w/v) agarose gel with 0.5X TBE (Trizma Base, Boric Acid, EDTA (Na2.EDTA.H2O) buffer and run under 100 volts electric current for 1 hour. After electrophoresis, the gel was stained with ethidium bromide (0.5 µg/ml) . Under gel visualization device (UVITEC, UVIdoc Gel Documentation System, UK), gel images were obtained and recorded. For comparison, 100 bp DNA marker was used.

1.3 RESULTS AND DISCUSSION

1.3.1 Pollinations

1.3.1.1 Studies on *Pisum sativum*

The expected result of the pollination applications is to obtain growing pods from the studied legume flowers. Subject legumes reacted in different manners against being pollinated with *V. turcica* pollens. Low number of growing *P. sativum* pods after pollination were empty, no growing embryos were observed (Figure 8). No ovule formation indicates the failure of the germination. Male and female gametes did not manage to produce a zygote. For having a better understanding of the stage where the fertilization of pea has failed, histological analysis were conducted on the pollinated pea pistils.

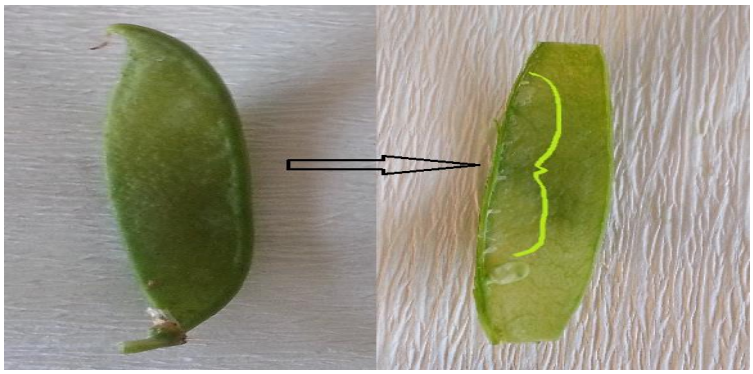

Figure 8. No embryo formation was observed in pea pods resulting from *P. sativum* x *V. turcica* cross. Left, cloed pod, right opened pod. The yellow bracket covers the area where the ovules were supposed to develop.

1.3.1.2 Studies on *Lupinus spp*.

Only 3 pods were managed to develop between the *Lupinus* flowers that were pollinated. Most of the studied flowers were abscissed before growing into the pod stage. Development of flowers stopped and flower death started 10 days after the pollination (Figure 9.B). Failed to develop *Lupinus* flowers have a dry look and a yellowish color, and all of them had similar sizes when their growth was stopped.

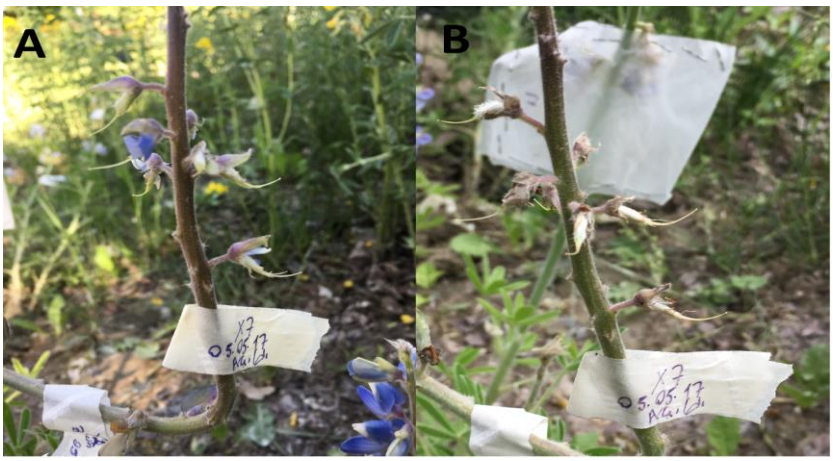

Figure 9. Hand pollinated maternal flowers of *Lupinus spp*. (A) 6 days after pollination, (B) 10 days after pollination

Aiming to prove the fragility of *Lupinus* flowers, one flower at the balloon stage is selfed. Pollinated pistil managed to form into a pod but no further growth is observed (Figure 10).

Figure 10. Selfed *Lupinus* flower growth with incomplete development

The growth of the pod stopped 10 days after the pollination, but the pod survived and remained on the plant 1 week more than the other *Lupinus* flowers pollinated with *V. turcica*. The pod was left on the plant expecting a further growth for enabling tissue culture studies, but the limited growth resulted with the death of the sample. Low survival rate of the pollinated flowers might be related to the scars resulting from the removal of petals and emasculation. Plants release phenolic acids from their wounds as a defence mechanism against pathogens and those compounds can also be harmful to its tissues (Savatin et al. 2014; Mbaveng et al. 2014). In this case, there is a possibility that small *Lupinus* flowers could not bear the deteriorating effects of the released defensive compounds after the mechanical stress caused by pollination. Another reason of flower lethality might be the early exposure of the pistils to the environmental conditions as heat, wind, UV light and pest. In light of these results, it could be suggested that *Lupinus* flowers might favor self pollination and it was observed that any outer mechanical intervention leads necrosis and result with fall the of the flower from the plant body.

1.3.1.3 Studies on *Phaseolus vulgaris*

Most positive pollination results were taken from the common bean flowers. Pod growth after pollination was more frequently observed in bean maternal parents than other pollinated legume flowers, so it was possible to obtain enough amount of samples for histologic analysis and tissue culture experiments (Figure 11). As the gene transfer possibility of *V.turcica* was investigated through classical pollination in this research,

productive results of the crosses between *P. vulgaris* and *V. turcica* oriented the focus of this study on common bean rather than other legume subjects.

Figure 11. Growing *P. vulgaris* pods after pollination with *V. turcica* pollens. (A) 14 DAP, (B) 8 DAP

1.3.1.4 Studies on *V. turcica*

For a deeper investigation of the gene transfer potential between *V. turcica* and commercial legume species, reciprocal crosses were done. In the Spring season of 2016, *V. turcica* bloomed earlier than other legumes, therefore no commercial legume pollens were available for the reciprocal cross. One year later, legumes were planted earlier than the previous year and their blooming period was coincided with *V. turcica.* In 2017 spring, bean and *Lupinus* plants were available for crossing studies with *V. turcica.* Hereby, reciprocal crosses were done in 2017 Spring. Reciprocal cross between *V. turcica* (maternal parent) and *Lupinus spp.* (paternal parent) were not productive like the previous cross. Pollinated *V. turcica* flowers started to lose their healthy look and their abscission started 7 days after pollination. For example, in Figure 12, 7 DAP flowers that were pollinated on 4 March 2017 look healthy, but on the day when the 10 DAP flowers photos were taken, all the flowers pollinated on 4th March were abscissed (Figure 12). The reciprocal cross where *P. vulgaris* was the paternal parent was also failed. There were no flowers but one at 7 DAP, and that flower was almost abscissed (Figure 13). Hereby, it is logical to assume that there might be pre-fertilization barriers when *V.turcica* was used as maternal parent that leads flower death after pollination.

Figure 12. Reciprocal cross between *V.turcica* (♀) x *Lupinus spp.* (♂). Left, 7 DAP flowers, right, 10 DAP flowers.

Figure 13. Reciprocal cross between *V. turcica* (♀) x *P. vulgaris* (♂). Photo taken at 7 DAP

The reason of failure in this reciprocal cross might be similar to *Lupinus* case, where flowers were abscissed resulting from tissue injury. In order to explain this situation more clearly, *V. turcica* flowers were selfed. From selfed *V. turcica* flowers, pod growth was observed without any abnormality (Figure 14). Flowers could not survive more than 7 days when pollinated with a foreign pollen, but selfed flowers yielded pods. Thus, it is reasonable to consider that *V. turcica* flowers are not fragile as *Lupinus* flowers and they simply reject foreign pollen. Failure of reciprocal crosses is probably related to pre-fertilization barriers.

Figure 14. Pods growing from selfed *V. turcica* flowers at 12 DAP

1.3.2 Histological Analysis

Specific interactions between pollen and pistil is the main arbiter of the success of sexual plant reproduction (Madureira et al. 2012). For this reason, it is crucial to observe the events that realize in the gynoecium after the pollination. Visual confirmation of the travel of the pollen from stigma to ovarium is required to ensure the success of pollination. Pollinated pea flowers did not develop any ovules, *Lupinus* flower growth were arrested a while after the pollination and only bean flowers managed to produce ovules. Crosses with faba beans were also implemented and enough amount of samples was obtained for histologic analysis. To obtain further information about the pollen-pistil interactions in these intergeneric crosses, crossed pistils were collected at different days after pollination

and analysed under a fluorescent microscope. Samples of pea, bean and faba showed pollen tube growth at 4 DAP. When the ovarium of the pollinated *P. vulgaris* samples at 5 DAP were analysed under fluorescent microscope, the travel of the male gametophyte to ovarium is observed (Figure 15).

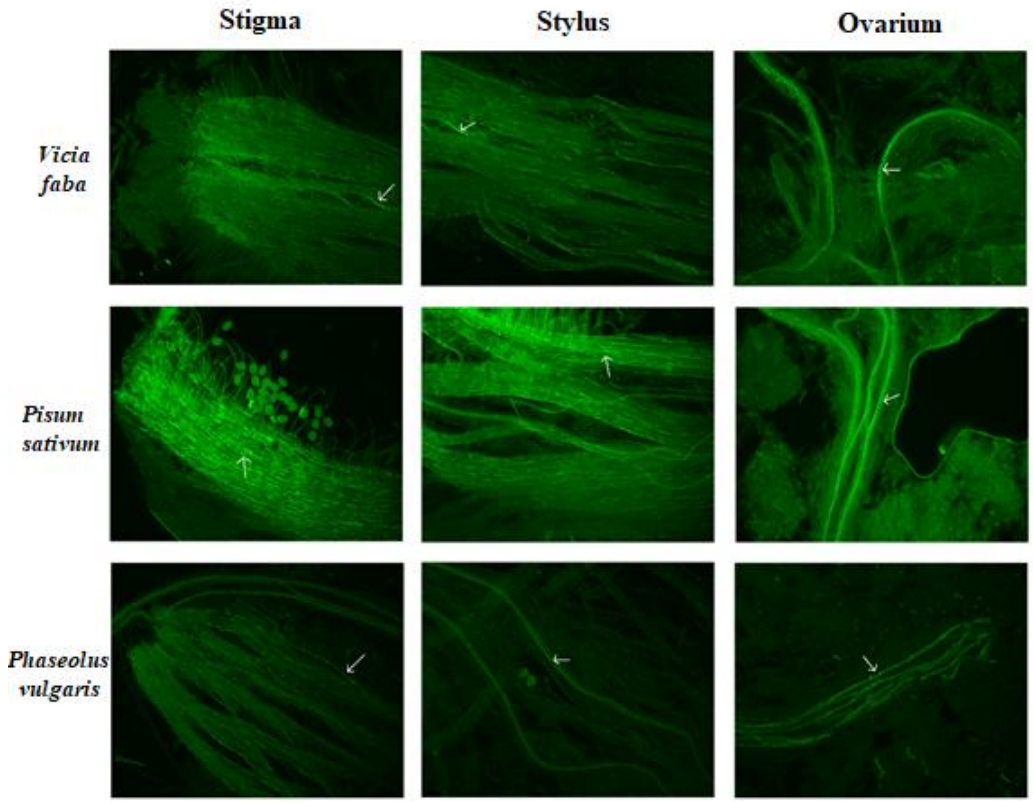

Figure 15. Histologic analysis of pollinated faba, pea and bean pistils. White arrows show pollen tubes. (Magnification: 6.3x ; Filter: UMVIBA3; Dichronic: 505; Emitter: 510-550; Exciter: 460-495)

Also, male gametophyte-ovule contiguity was detected (Figure 16). Thus, the pollination is succesful, but further analysis is required to confirm the fertilization and if the fertilization were happened between the desired parents. Grown pea pods after the pollination did non contain any embryo. Even if the fertilization occured, embryo abortion might have happened at early stages.

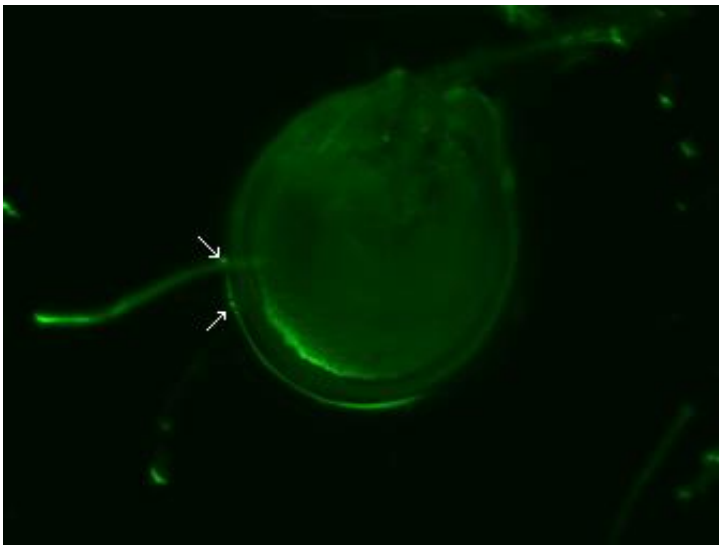

Figure 16. The contact between *P. vulgaris* ovule and *V. turcica* gametophyte in the ovarium. Gametophytes are indicated with white arrows. (Magnification: 12.6x ; Filter: UMVIBA3; Dichronic: 505; Emitter: 510-550; Exciter: 460-495)

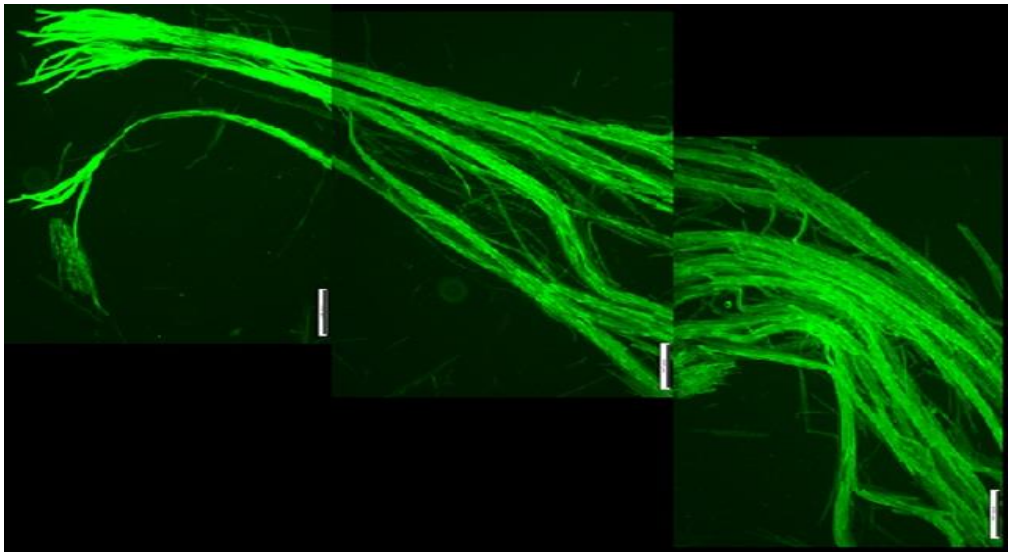

Figure 17. Unified sections of pollen tube images extending through stigma and stylus to ovarium of *Lupinus* pistil after pollination. Pollen tubes are visible from stigma to ovarium. (Magnification: 6.3x ; Filter: UMVIBA3; Dichronic: 505;Emitter: 510-550; Exciter: 460-495)

Another positive result was taken from the crosses between *Lupinus* flowers and *V. turcica* pollens. Their mating resulted with pollen germination at the stigma and elongation of the pollen tube through the stylus until ovarium (Figure 17). When the ovarium of studied *Lupinus* flowers was analysed, contact between gametophyte and ovule was observed (Figure 18).

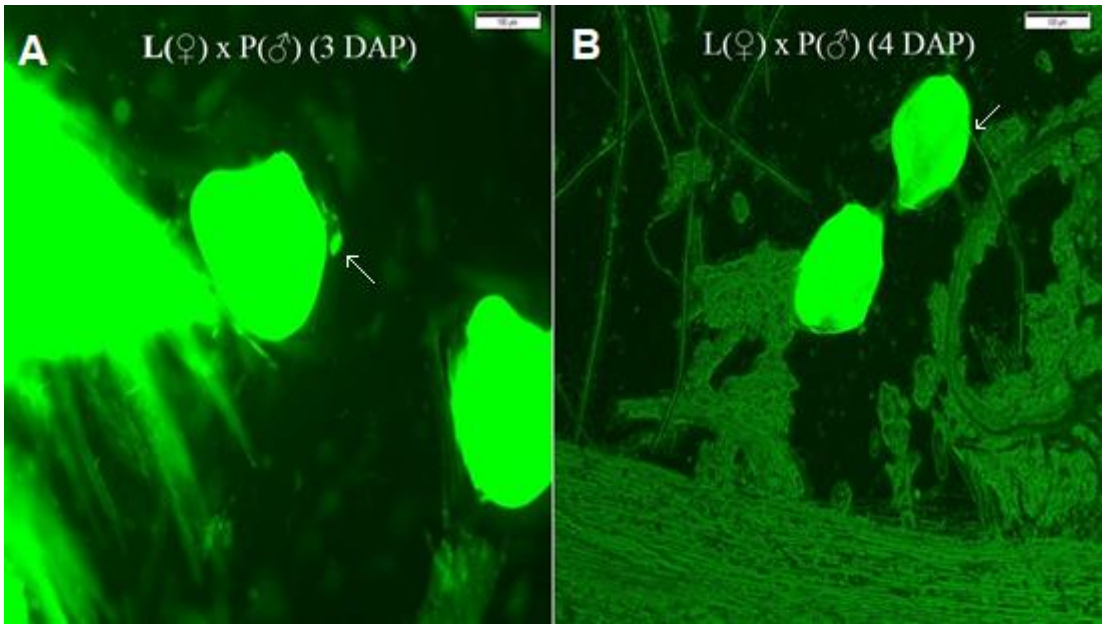

Figure 18. (A) Contact of *Lupinus* ovules with gametophyte, (B) male gametophyte fusing with the ovule. White arrows indicate the male gametophyte. (Magnification: 12.6x ; Filter: UMVIBA3; Dichronic: 505;Emitter: 510-550; Exciter: 460-495)

Apart from *Lupinus* and *P. vulgaris,* there is not enough information to confirm gametophyte-ovule contact in studied pea and faba pistils because of shortage of collected samples for histologic analysis.

The data obtained from histologic analyses show that there were no pre-fertilization barriers between maternal parents, *P. vulgaris* and *Lupinus spp.* and paternal parent *V. turcica*. Despite the taxonomic distance, pollen germination, pollen tube growth and male reproductive cell travel through ovule is realized in maternal parents gynoecia. The failure of development of the ovules after the fertilization could be related with post-fertilization barriers. Embryo mortality at the initial stages could be the reason of the sample loss after pollination. Another possible reason of this failure might be the mechanical damage given to the flowers in the pollination applications.

1.3.3 Tissue Culture Studies

Hybridization studies require tissue culture applications for several reasons. Post-fertilization barriers mentioned in the introduction part consists the main reasons of transferring hybrid candidates in vitro. Mating of distal parents may cause disharmony between parental genomes in new generations which may further lead to embryo mortality, endosperm breakdown or seed inviability (Stalker 1980). In most distal crosses, fertilization realizes but embryo abortion occurs prior to maturation (Tekdal et al. 2017). Even if the ovules or seeds are grown, they most probably fail to germinate or give rise to weak seedlings which have a low survival rate (Agnihotri 1993). To overcome post-fertilization barriers, hybrid embryo could be planted on another endosperm, embryos or ovules could be cultured in vitro, or organogenesis (somatic or not) could be realized from callus that is derived from hybrid embryos (Monnier 1990; Raghavan 1986).

In light of the outcomes of the hybridization studies present in the literature, the first appearing *P. vulgaris* pods after pollination were gathered from the research fields and brought to the lab. Then, embryo rescue was immediately done to avoid sample loss. Collected pods were sterilized according to the protocol present in the methods part and the embryos were extracted from the ovules then transferred in vitro mediums. Unlike *P. sativum,* once crossed with *V. turcica, P. vulgaris* was able to produce seeds (Figure 19).

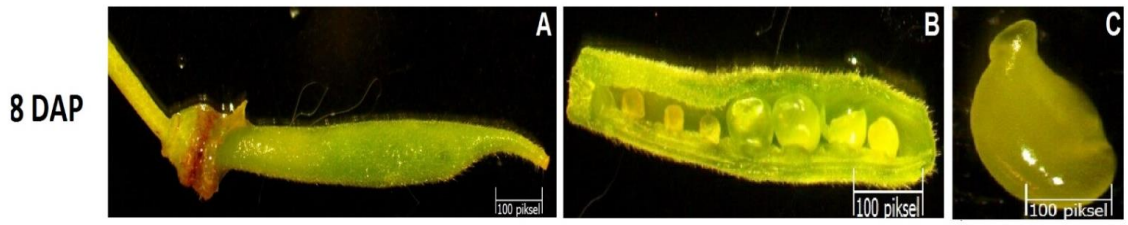

Figure 19. (A) Image of 8 DAP pod, (B) developed ovules, (C) ovule-isolated embryo

Seed inviability was rarely observed in this cross. From the crossed *Lupinus spp.* flowers, only 3 pods were obtained, but the seeds did not germinate in vitro. Beans maternal parents used in this cross were the most productive flowers between the plants used as maternal parent in this research. Several growing bean pods were left on the plants aiming to observe their further growth. It was observed that initiated bean pods after pollination were able to survive until fully ripening and so it was revealed that cross between *P. vulgaris* and *V. turcica* did not strictly require embryo abortion where *P. vulgaris* was the maternal parent (Figure 20 and 21).

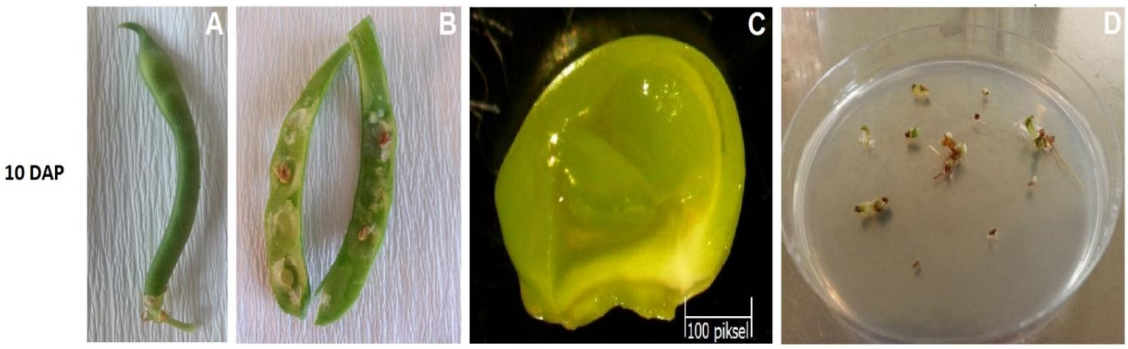

Figure 20. (A) 10 DAP pod, (B) ovules, (C) isolated embryo from ovules, (D) in vitro development of isolated embryos after 1 month

Figure 21. (A) 12 DAP pod, (B) ovules, (C) isolated embryo from ovules, (D) in vitro development of isolated embryos after 1 month

Between the mediums described in the methods part, best propagation of hybrid candidates is observed in MS medium added with NAA (1 mg L^{-1}), ABA (1 mg L^{-1}), Casein hydrolysate (0.5 g L^{-1}) and glutamine (1 g L^{-1}). Isolated embryos of the first collected pods were micropropagated in this medium (Figure 20.D, Figure 21.D). D column of the Figure 22 made us suggest that when samples get more mature in vivo, they show better succes after in in vitro development. Better root growth, larger plantlet body and higher chlorophyll content referring to the color differences were observed in hybrid candidates with higher DAP. Propagated plant tissues induced root growth without any abnormality when they were transferred into MS added with IBA (1mg/ L^{-1}) medium. However, subcultures of the same medium show incomplete growth (Figure 23.B). Hybrid candidates were able to regenerate indirectly in 2, 4-D medium by initiating callus (Figure 24).

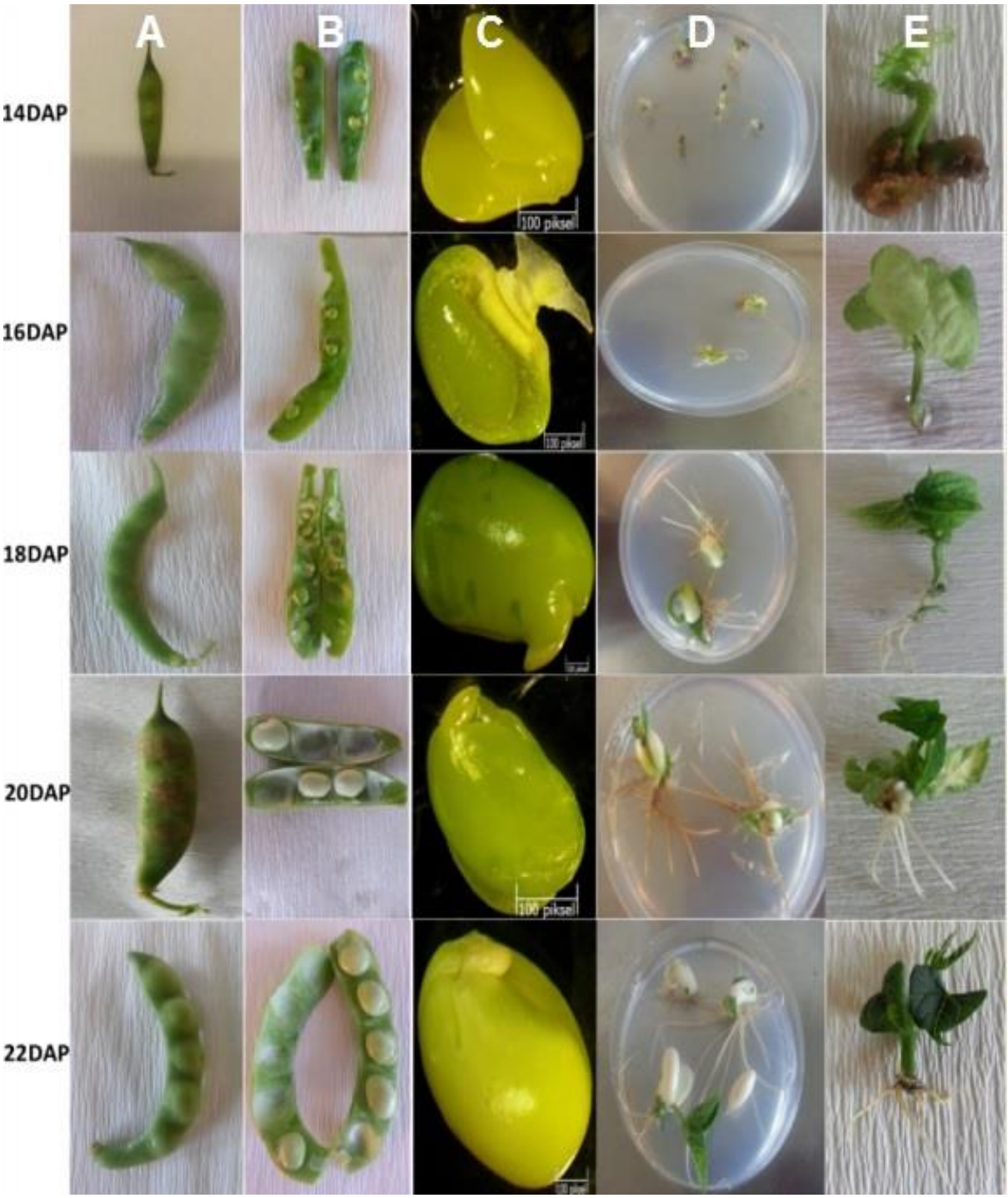

Figure 22. Images of pods (A), ovules (B), embryos (C), in-vitro micropropagation after 1 month, 2 months old in-vitro grown plantlets

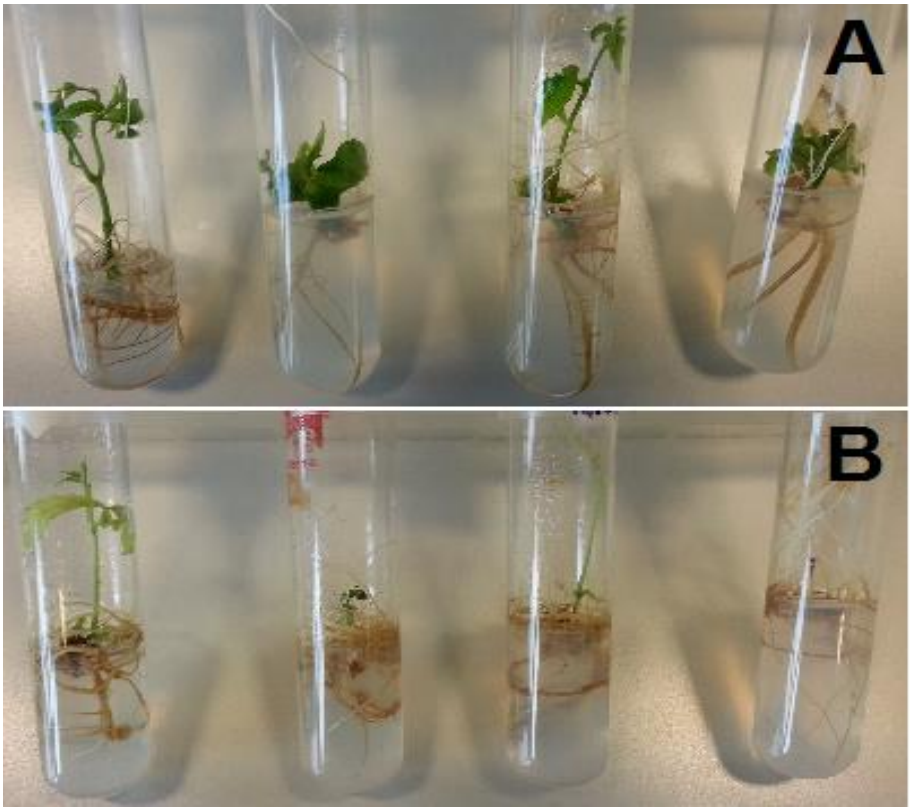

Figure 23. In vitro propagation of hybrid candidates. (A) First plants cultured in vitro, (B) sub-cultured plants. Mediums contain MS added with IBA(1 mg L^{-1}).

Figure 24. Indirect plant regeneration in 2, 4-D containing medium (1 mg L^{-1}). (A) 8 DAP, (B) 10 DAP, (C) 12 DAP

1.3.4 Molecular Studies

Between the designed primers, healthiest results were taken from the Primer 4. The size difference of the PCR products had to be distinguished in order to observe bands specific to maternal and paternal parent. Thus, polymorphic primers were chosen for a clear identification of parental genes which would differ in size when run in agarose gel. All hybrid candidates had similar bands with the *P. vulgaris*, none of them had matching bands with *V. turcica* (Figure 25). Stutter bands can be observed in wells of *P. vulgaris* and hybrid candidates, which is probably due to slipping of the DNA polymerase during microsattellite amplification (Hosseinzadeh-Colagar et al. 2016).

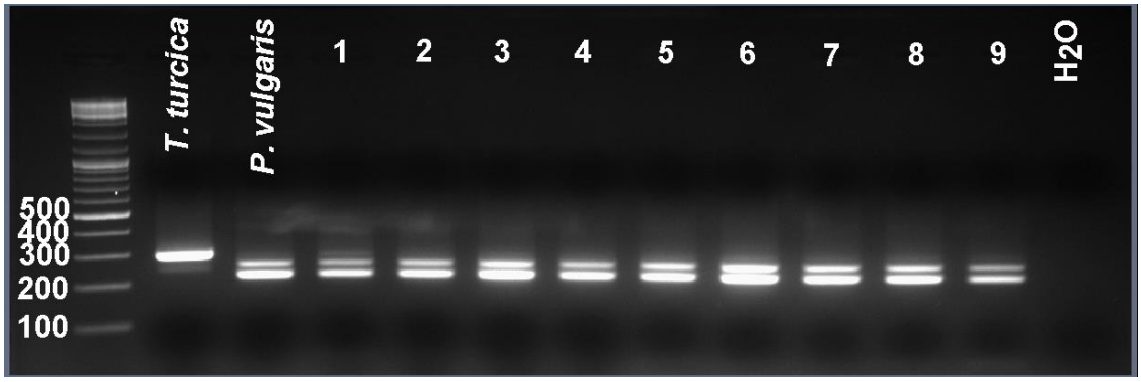

Figure 25. Agarose gel electrophoresis results of the hybrid candidates (1 to 9) with *P. vulgaris* maternal band and *V. turcica* paternal band

According to the molecular analysis of the hybrid candidates, no candidate possessed paternal *V. turcica* genes. Maternal specific genes were present in all samples. This result indicated several possibilities. All samples could have been selfed while pollinating or all hybrid candidates were double haploids. Double haploid production in distant mating is frequently seen and it is a classic technique to obtain pure lines of plants which are fully homozygous (Campbell et al. 2000). After the fertilization, the paternal chromosome set is eliminated by the organism and by doubling the present maternal chromosomes, a double haploid generation could be produced (Croser et al. 2006). Diploidity of the hybrid candidates were confirmed by conducting flow cytometry on the samples in Namık Kemal University (APPENDIX 9).

1.4 CONCLUSION

Findings obtained from the histological analysis of this study indicates that there were no pre-fertilization barriers between *V. turcica* and the subject legumes that were used as maternal parent. Pollen germination and pollen tube growth from stylus to ovarium realized in maternal parents of *P. sativum, V. faba, P. vulgaris* and *Lupinus sp*. when pollinated with *V. turcica*. *P. sativum* yielded empty pods without embryo development after the crossing. Embryos obtained from the crossing of *V. faba* x *V. turcica* required embryo rescue as it was previously reported by Tekdal et al. (2017).

Ovule fertilization was detected in the ovaries of *Lupinus sp.* and *P. vulgaris* when they pollinated with *V. turcica*'s pollen. However, embryo development failed in the crossing of *Lupinus sp.* x *V. turcica*. It was also observed that *Lupinus sp.* flowers were fragile and they favor self pollination. According to the pollination treatments, outer interventions arrests the flower development of *Lupinus sp.* a while after pollination.

Among the tested legumes in this study, only *P. vulgaris* yielded healthy pods and shown embryo development when pollinated with *V. turcica* pollens. Pods resulting from the cross were able to fully develop, thus no embryo rescue was required. The most successful in vitro growth of the hybrid candidates obtained from *P. vulgaris* x *V. turcica* cross was observed in MS medium added with NAA (1 mg L^{-1}), ABA (1 mg L^{-1}), Casein hydrolysate (0.5 g L^{-1}) and glutamine (1 g L^{-1}). Molecular analysis of the hybrid candidates shown that no *V. turcica* genes were available in their genome. As a future work, homozygosity of the hybrid candidates is going to be analysed expecting to reveal their doubled haploidy. Proving the doubled haploidy of those candidates resulting from the cross would be beneficial as *V. turcica* pollens could be used to produce pure lines of *P. vulgaris* in plant breeding programs.

Chapter 2

IDENTIFICATION OF RHIZOBACTERIAL SPECIES CONTRIBUTING DEVELOPMENT OF *Vuralia turcica*

2.1 INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are responsible for a wide range of biotic activities in the soil ecosystem which enhances the nutrient turnover in the soil (Ahemad et al. 2009). PGPR's contribution to plant growth and survival occur through nutrient mobilization in soil, plant growth regulator production, plant pathogen control and inhibition, toxic compound degradation (Ahemad and Kibret 2014). Bacteria situated around plant roots are further sophisticated in solubilizing and mobilizing plant-beneficial compounds than bacteria available in bulk soils (Hayat et al. 2010). Various abilities of the rhizobacteria in recycling soil nutrients makes them indispensable for a fertile soil (Glick 2012). In purpose of achieving more sustainable agricultural solutions in integrated plant nutrient management, rhizobactera is widely being researched for its traits like heavy metal detoxification, pesticide degradation, salinity tolerance, plant growth hormone secretion/induction, nitrogen fixation, phosphate solubilization and pathogen control (Ahemad and Kibret 2014).

Plant roots most evident tasks are facilitating nutrient and water uptake and providing mechanical support. Apart from that, plant roots synthesize and secrete compounds that attracts actively metabolising microbial populations (Walker et al. 2003). Released exudate composition is determined by the species of plants and microbes (Kang et al. 2010). This secretion alters the physical and chemical properties of the soil, thereby regulates microbial community around the rhizosphere (Dakora and Phillips 2002). Regulation of this community also includes the repelling feature of the exudates against certain microorganisms (Ahemad et al. 2014). While inhibiting the growth of competing plant species, exudates promote symbiotic interactions of roots and microorganisms in

the rhizosphere which is mutually beneficial (Nardi et al. 2000). Small percentage of the secretions from the roots are consumed by neighbor microorganisms as carbon and nitrogen source and some microbial secretions are absorbed by the roots for plant growth (Kang et al. 2010). 5-21% of the photosynthetically fixed carbons in plants are translocated to the rhizosphere by exudation (Marschner 2011).

PGPR's are distinguished from other microorganisms residing in the soil by their ability to promote plant growth, proficiency to colonize on the rhizosphere and ability to survive and compete with other microorganisms until revealing its plant growth promoting traits (Kloepper 1994). They can be classified according to their functions: phytostimulators which promote plant growth, biofertilizers to increase plant available nutrient concentration, rhizoremediators to degrade organic wastes in soil and biopesticides to control microbial and fungal pathogens (Antoun and Prévost 2005). PGPR's can be distinguished into two classes regarding to their preference of location to anchor and colonize: one is extracellular PGPR's which colonize in the rhizosphere or in unoccupied spaces between root cortex cells (for example *Agrobacterium* (Bhattacharyya and Jha 2012)), the other is intracellular PGPR, where rhizobia exist in the nodular structures fused with the root cells (Figueiredo et al. 2010). Rhizobacteria is often reported to be gram-negative bacteria (Bhattacharyya and Jha 2012).

2.1.1 PGPR's Role in Plant Growth

Mutualistic relationship between plants and PGPR's have several beneficial outcomes for plant growth like nitrogen fixation, phytohormone production, phosphorus solubilization and increasing available nutrients by siderophore production (Glick et al. 2007). Secondary metabolites that PGPR's secrete to their environment may facilitate nutrient uptake of plants as those metabolites convert soil minerals into available forms for plant roots to absorb.

2.1.1.1 Siderophores

Siderophores are low molecular weight compounds with high affinity towards iron molecules. They can be found inside or outside of organisms as secretion products. The most distinguishing ability of siderophores is to chelate iron, but they can also form stable

complexes with other metals like Al, Cd, Cu, Pb and Zn (Neubauer et al. 2000). With the ability of forming complexes with the elements described, bacterial siderophores reduces the stress on plants caused by heavy metal toxification (Ahemad et al. 2014). There are various siderophores with different molecular composition and mechanism of action in chelating heavy metals, mostly beneficial for plants by increasing the nutrient availability in the environment.

In the soil, iron commonly forms insoluble hydroxides and oxyhydroxides which are unlikely for plants to absorb and metabolize (Rajkumar et al. 2010). Many living organisms like pathogenic/non-pathogenic bacteria, plants, mammalian cells and marine organisms uptake iron via its chelation by siderophores (Beneduzi et al. 2012). Once secreted, siderophores solubilize iron present in the environment and forms a ferric-siderophore complex that can further mobilize towards the cell surface by diffusion and taken up by active transport as the complex is recognized (Boukhalfa and Crumbliss 2002; Andrews et al. 2003). With a transport mechanism linking the inner and outer membranes of the root cells of plants, iron ion in the Fe^{3+}-siderophore complex is reduced to Fe^{2+}, then released into the cytosol. The siderophore in this event is destroyed or recycled for further use (Neilands 1995; Rajkumar et al. 2010). There are various mechanisms for plants to assimilate iron from bacterial siderophores; uptake of Fe ions by a ligand exchange reaction, direct absorption of ferric siderophore complex, or chelation and release of the Fe ion into the cell (Schmidt 1999).

Ferric-pyoverdine complex resulting from pyoverdine secretion of *Pseudomonas fluorescens*, is available for *Arabidopsis thaliana* and it is observed that this mutualism results with increased plant iron content and better growth (Vansuyt et al. 2007). PGPR-secreted siderophores are beneficial to plants only if the ferric siderophore complex is recognized by the plant roots (Beneduzi et al. 2012). PGPR's ability to produce siderophores provide them competitive advantages against other microorganisms. Microorganisms around rhizosphere compete for carbon sources like root exudates. Bacteria with greater ability to solubilize iron into bioavailable forms for the host plant are the most advantageous in this competition as they reach to plant derived carbon source facilitates (Haas and Défago 2005; Crowley 2006). PGPR's producing pyoverdin, a potent siderophore, are able to overgrow other bacteria and fungi which secretes less potent siderophores in the iron deficient environment (Kloepper et al. 1980).

2.1.1.2 Nitrogen fixation:

Nitrogen is an essential element for plants for growth and development. Proteins are composed of aminoacids and they structurally require nitrogen. Its absence in a field would directly affect crops as plants require it for vital biochemical process. In the nature, the main source of nitrogen for plants is the organic debris resulting from dead animals and plants, but presence of bioavailable nitrogen is a problem. Approximately, 78% of the air is composed of nitrogen, but it is not bioavailable for plants to metabolise. This unavailable nitrogen is converted into bioavailable forms by biological nitrogen fixation (BNF) by soil bacteria, where the nitrogen is changed into ammonia by diverse mechanisms that involve nitrogenase enzyme (Kim and Rees 1994). Two thirds of the globally utilized nitrogen by crops is fixed through BNF while other one third is synthesized industrially by Haber-Bosch method (Rubio and Ludden 2008).

Industrially manufactured nitrogen fertilizers may pose economic problems and it could be harmful for the environment. Even if there is an unlimited supply of nitrogen in the air, 6 times more energy is required to produce 1 kg of N fertilizer than to produce the same amount of phosphorus (P) or potassium (K) fertilizers (Da Silva et al. 1978). Also, at least half of the chemical N fertilizer applied to field is lost through denitrification, ammonia volatilization and leaching which is economically damaging the farmer and pollutes the environment. As a result of denitrification, nitrous oxide, a greenhouse gas, is released into the air, then disrupts the ozone layer (Ladha et al. 1997). N fertilizers lost by leaching mixes into underground waters and it can accumulate in lakes and causes eutrophication, which ends life in lake ecosystems by hypoxia (Conley et al. 2009). Utilization of soil bacteria for BNF do not pose the environmental hazards as chemical N fertilizers and it is economically beneficial (Ladha et al. 1997).

In exchange of the carbon secreted as plant root exudates, diazotrophic microorganisms provide a bioavailable form of N to plants (Glick 1995). Also, several free living bacteria located around rhizosphere are able to fix atmospheric N for legumes (Drinkwater et al. 1998). Soil living bacteria fixes around 180x10^6 tons of N for plants annually and 83% of it is a result of symbiotic relations between bacteria and plant roots while the rest is provided by free living bacteria (Sylvia et al. 2005). Free living N fixing bacteria as *Azoarcus, Acetobacter diazotrophicus, Azotobacter, Azospirillum etc.* are capable of fixing low amounts of N, and it is not enough to meet the N needs of the host

plant. In legumes, *Rhizobium,* and in non-leguminous trees, *Frankia* are symbiotic bacteria that are capable of providing satisfying amounts of N for the host plant (Glick 2012; Ashraf et al. 2013). N fixing members of the *Rhizobiaceae* family infects most of the leguminous plants roots and establish a symbiotic relationship (Ahemad et al. 2014). Complex mechanisms are involved in the establishment of this symbiotic relationship where rhizobia colonize within the root cells and forms nodules as a result of this colonization (Giordano and Hirsch 2004).

The N fixing ability of the soil bacteria comes from a two component metalloenzyme mechanism that consists of dinitrogenase reductase, an iron protein, and dinitrogenase, an enzyme which depends on a metal cofactor to function. With a high reducing ability, dinitrogenase reductase provides electrons while dinitrogenase utilizes those electrons for reducing N2 to NH3 (Dean 1992). The metal cofactor of the nitrogenase enzyme determines the N fixing system between identified systems which are the Mo-nitrogenase, V-nitrogenase and Fe-nitrogenase. Mo-dinitrogenase system is found in all diazotrophes and it is the most coincided N fixing system between other BNF systems. The structure of N fixing mechanisms can differ among different bacterial genera (Bishop and Joerger 1990; Ahemad et al. 2014).

Every symbiotic or free living bacteria capable of BNF, contain *nif* genes whose product are the nitrogenase enzymes and proteins related to the N fixing mechanism (Kim and Rees 1994). Those genes are involved in Fe protein activation, Fe-Mo cofactor production, electron donation, structural formation and regulation of the related genes involved in the synthesis and functioning of the enzyme (Glick 2012). Once symbiosis is established between the host plant and the rhizobacteria, low concentrations of oxygen are required in the environment for the activation of the *nif* genes. This activation is regulated by *fix* genes which are commonly found in both symbiotic and free living N fixing bacteria (Dean 1992; Kim and Rees 1994).

2.1.1.3 Phosphorus Solubilization

The most important nutrient that plants require after nitrogen is phosphorus (P). It is available in both organic and inorganic complexes and its deficiency can easily limit the growth and development of plant (Khan et al. 2009). Phosphorus has a structural role in various vital cellular components like DNA, RNA, cellular membranes and ATP. In

the most of the soil, P element is widely found, but plants can only acquire it when it is present in bioavailable forms such as monobasic and diabasic forms (Bhattacharyya and Jha, 2012). P deficiency in plants is generally caused because of the presence of the mineral as insoluble forms in the soil (Ahemad et al. 2014). To meet the P needs of the crops, P fertilizers are commonly used in agricultural practices, but plants absorb low amounts of the applied fertilizers as most of the fertilizer is rapidly converted into unavailable forms to plants (McKenzie and Roberts 1990).

P fertilizers can be environmentally hazardous and may cause an economic pressure on farmers when it is regularly applied. Similar to nitrogen fertilizers case, by leaching, phosphate minerals can mix into underground waters, then acumulate in water beds which further causes eutrophication (Daniel et al. 1998). To overcome or minimize those negative effects, microorganisms that are able to convert P in the soil to plant-available forms can be a substitute for chemical P fertilizers (Khan et al. 2007). In soils with P-deficiency, microorganisms which could also be referred as PGPR, are able to supply available forms of P to plants (Zaidi et al. 2009). Bacteria with phosphate solubilizing ability are widely found in genera like *Azotobacter, Bacillus, Flavobacterium, Pseudomonas, Rhizobium* and *Serrata* (Bhattacharyya and Jha, 2012).

2.1.1.4 Phytohormone production

Various bacteria residing in soil have the ability to produce plant hormones like auxins, ethylene and researchers also encountered soil bacteria that provide gibberellins and cytokinins to plants which induces shoot development (Van Loon 2007). PGPR infected plants generally show better growth and lateral root development, these findings are related to the phytohormone production ability of the rhizobacteria (Ashraf et al. 2013)

80% of the rhizobacteria residing at the roots of various crops are releasing auxins as secondary metabolites (Patten and Glick 1996). Mostly encountered auxin is Indole-3-Acetic Acid (IAA) that occurs naturally in plants and several bacteria (Simon and Petrášek 2011). Plants endogenous pool of IAA can be altered by the absorption of bacterial secreted IAA and this change interferes with various plant developmental processes (Glick 2012). IAA has diverse roles in plant growth and it is also required in defensive pathways. This multifunctionality comes from the complexity of biosynthetic

and signalling pathways of IAA (Santner et al. 2009). According to the review of (Ahemad et al. 2014), the most distinguishing effects of IAA in plants are cell division, extension and differentiation; xylem and root development; stimulation of seed and tuber germination; initiation of lateral and adventitious root formation; vegetative growth regulation; controlling responses to light, gravity and florescence; triggering pigment formation for photosynthesis and biosynthesis of various metabolites; regulation of defence responses against stressful conditions. It is observed that bacterial IAA mostly helps plant by stimulating root growth where root surface, length and area is increased. Consequently, plants have a better access to soil nutrients (Ahemad et al. 2014). Positive effects of bacterial IAA on plants have further benefits for the rhizobacteria too. As bacterial IAA enhances vascular bundle formation and cell division and differentiation in plants, there would be better nutrient transport to root nodules from the plant body (Glick 2012). Plant cell walls of the roots can be loosened by rhizobacterial IAA, this loosening increases the amount of the plant exudates that rhizobacteria use as a carbon source (Glick 2012). As a result, nodule formation is facilitated.

Another important plant hormone provided exogenously by rhizobacteria to plants is ethylene (Van Loon 2007). Generally, ethylene is known to inhibit plant growth and stimulate ripening but when it is provided in low concentrations, it promotes growth in various plant species (Pierik et al. 2006; Ashraf et al. 2013). At moderate levels, root and shoot elongation is inhibited and in elevated levels senescence and organ abscission is induced (Abeles et al. 1992).

1-aminocyclopropane-1-carboxylic acid (ACC) is an important compound in the ethylene level regulation in PGPR plant relations as it is the precursor of ethylene. ACC is present in the root exudates and rhizobacteria that contains ACC deaminase enzymes can break this compound down to its further utilization as carbon source. When rhizobacteria with mentioned ability is present in the roots of a plant, ACC does not accumulate at the rhizosphere, so it is not re-absorbed by the plant and root growth inhibition due to high level of ethylene is prevented (Glick 2005; Van Loon 2007; Ashraf et al. 2013).

2.1.1.5 Host plant defence

PGPR can directly promote plant growth by increasing nutrient availability, but there are also indirect mechanisms that can contribute to a healthy development. Plant associated bacteria could be referred as biocontrol agents as several of them reduce the incidence or severity of the pathogen infection (Beattie 2007). As an antagonistic activity towards pathogens, PGPR are able to synthesize and secrete hydrolytic enzymes as proteases, lipases, glucanases and chitinases which can disrupt pathogenic organisms cellular structures (Neeraja et al. 2010; Maksimov et al. 2011). Suitable colonization niches at the root surface gives PGPR an advantage to populate faster than the pathogens, consequently pathogens fail at nutrient competition so their infection rates decrease (Kamilova et al. 2005).

Pathogenic antagonism of PGPR is most commonly related to their ability to produce antibiotics (Glick et al. 2007). Antibiotics are low-molecular weight organic compounds that can be disruptive towards the growth or metabolic activities of foreign microorganisms (Duffy et al. 2003). There are 6 antibiotics with revealed mechanisms and commonly produced by PGPR in microbial antagonism: diffusable antibiotics as phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides and volatile antibiotic, hydrogen cyanide (Haas and Défago, 2005). Action mechanisms of those antibiotics include the inhibition of pathogen cell wall synthesis and protein synthesis disruption by inhibiting the formation of the initiation complexes on the small ribosomal subunit (Maksimov et al. 2011). For example, 2, 4-diacetylphloroglucinol, an antibiotic produced by pseudomonads can disrupt the membranes and the zoospores of the *Pythium spp.* which most species are pathogenic to plants (de Souza et al. 2003).

Protection that PGPR provides against pathogens and biotic stress factors also includes indirect mechanisms where the host plant is encouraged to defend itself. With the help of PGPR's, plant defence mechanisms are induced to suppress the diseases that pathogens or biotic vectors cause. This defence mechanism is called 'Induced Systemic Resistance'(ISR), a state where enhanced defensive responses are activated by the host plant when properly stimulated (Van Loon et al. 1998). ISR responses require Jasminoic acid and ethylene in their pathways as a signalling molecule, and it is proven that ISR is not activated in the mutants that are deficient in both compounds separately (Pieterse et al. 1998; Knoester et al. 1999)**.** Defence mechanisms related to ISR are coordinated by

the accumulation of those compounds and their exogenous application is enough for the induction of defensive responses (Ryals et al. 1996). The most encountered ISR triggering bacterial genera are *Pseudomonas* and *Bacillus* (Pieterse et al. 2000). Bacterial compounds as flagella, salicylic acid, siderophores, cyclic lipopeptides, the signal molecule AHL's, Phl antifungal factor, acetoin and 2, 3-butanediol are able to initiate signalling mechanisms of ISR (Lugtenberg and Kamilova 2009). It is not rational to generalize the **s**ignal transduction mechanisms of ISR as pathways imply much complexity and differ according to the specific relation between PGPR strain and the plant species (Van Loon and Bakker 2005).

2.1.2 Aim of the Study

In this study, microorganisms presented both at the rhizomes and the soil from the natural habitat of *V. turcica* were researched and PGPR strains in the bacterial flora were identified.

2.2 MATERIALS AND METHODS

2.2.1 Materials

From the natural habitat of *V. turcica*, 6 different locations were chosen and their coordinates were identified with GPS. During the blooming period of the plant (April 2017), rhizomes of the plants were collected from the selected locations, then taken into sterile cups containing double distilled water (Figure 26). Soil samples were also collected from the depth where rhizomes were present (~30 cm) and taken into clean plastic bags. All samples were labeled according to the selected location that they were taken from. This sample collection from the natural habitat is done by NGBG workers.

All rhizome and soil samples were stored at +4 ℃ and immediately brought to Sabanci University. The location information of the soil samples was given in Table 6.

Table 6. GPS information of the extraction points of the collected soil and rhizome samples

No	Samples	X (North)	Y (East)	Altitude (m)	Region	Depth (cm)
1	Location 1	38° 46´ 987´´	31° 34´ 559´	980	Gölçayır	0-30
2	Location 2	38° 28´ 10.5´´	31° 21´ 04.4´´	996	Gölçayır	0-30
3	Location 3	38° 28´ 17.328´´	31° 20´ 52.468´´	976	Akşehir	0-30
4	Location 4	38° 30´ 36.702´´	31° 17´ 56.702´´	966	Dereçine	0-30
5	Location 5	38° 36.42´3864´´	31° 08´ 55.968´´	960	Eber	0-30
6	Location 6	38° 32´43.2168´´	31° 16´ 54.4728´´	956	Sultandağı	0-30

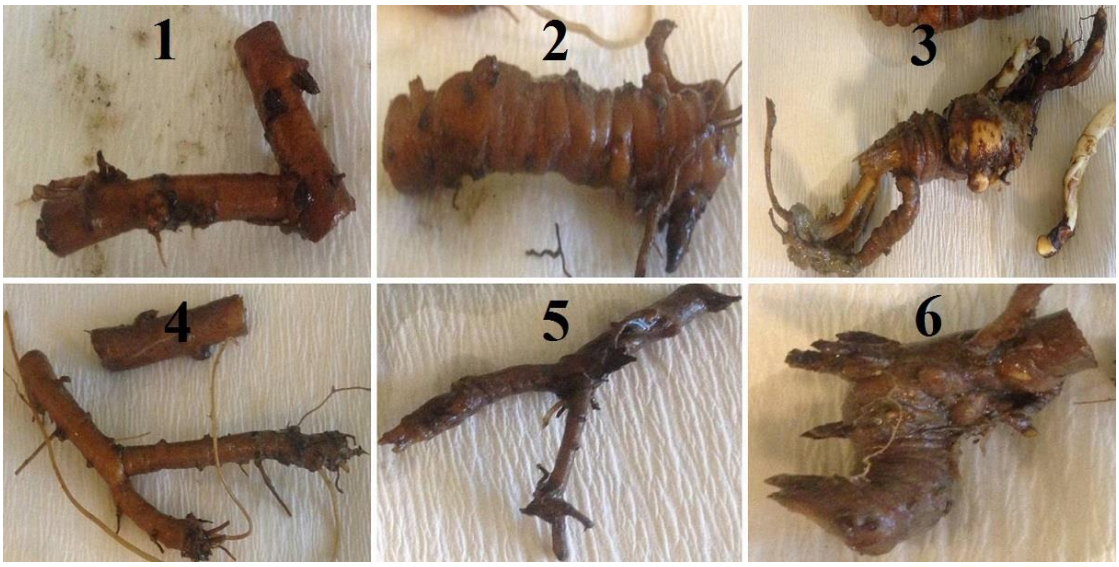

Figure 26. Images of the rhizomes extracted from the selected locations

2.2.2 Methods

The mineral element content of the collected soil and rhizome samples was analysed according to the methods described in Tekdal et al. (2018). This step of the research was conducted in Sabanci University Plant Physiology laboratory (Figure 27).

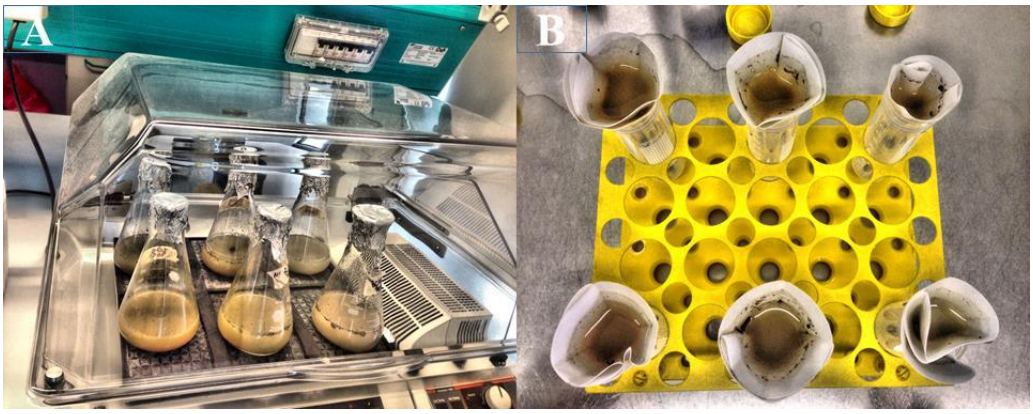

Figure 27. Soil samples prepared for mineral nutrient analysis. (A) Soil solutions in shaker, (B) filtering the soil solution

2.2.2.1 Plant Growth Promoting Rhizobacteria Isolation

Bacterial isolation was done from the collected soil and rhizome samples. Pinches were taken from random parts of the rhizomes and grinded in mortars with the addition of 10 ml liquid YMA medium. Liquid medium contains no agar and its composition is given in Table 7. The diluted rhizome-liquid YMA medium was streak on solid mediums. Same process were done on randomly taken soil samples weighing 2 g.

2.2.2.1.1 Pre-selection of bacteria with selective mediums

Bacterial growth in YMA medium was done first for further isolation of PGPR strains. For pre-selection, YMA medium containing either Congo red or Bromothymol blue were used. Gram staining of the isolates were also done. Medium content was given in Table 7.

Table 7. YMA medium composition

Medium Composition	***1 L***
K_2HPO_4	0.5 g
$MgSO_4$	0.2 g
NaCl	0.1 g
Mannitol	10 g
Yeast extract	0.4 g
Agarose	15 g
pH: 6.8-7.0	

While preparing Congo red YMA, 1/400 Congo red solution was sterilized, 10 ml of it then was added into 1L YMA medium. For YMA with Bromothymol blue, ½ Bromothymol solution was prepared and sterilized, then 5 ml of the solution was added to 1L YMA medium.

2.2.2.2 Molecular Analysis of the isolates

With the help of selective mediums, bacteria were isolated according to their color and morphology. Characterization of the bacterial genus of the isolates were done by 16S rRNA sequence analysis and their species were identified with Internal Transcribed Spacer (ITS) sequence analysis. Primers used for both processes were selected from the published studies and shown in Table 8. Sequencing for 16S rRNA and ITS processes were done by 'BM Laboratory Systems' company (https://www.bmlabosis.com/).

Table 8. Primers used for 16S rRNA and ITS gene sequence analysis

Name	Direction	Sequence (5'-3')-	Scanned Region
D1-F	Forward	AGAGTTTGATCCTGGCTCAG	16S
D1-R	Reverse	AAGGAGGTGATCCAGCC	16S
FGPS1490-72	Forward	TGCGGCTGGATCCCCTCCTT	ITS
FGPL132-38	Reverse	CCGGGTTTCCCCATTCGG	ITS

2.2.2.2.1 Phylogenetic analysis

Phylogenetic analysis of the isolated bacteria was done by analysing the revealed sequences belonging to 16S rRNA and ITS regions present in the genome. Softwares used for this analysis and related links were given in Table 9**.**

Table 9. Programs and websites used for phylogenetic analysis of the 16S rRNA and ITS sequences of the isolates

Software, Program	Company, Website	Purpose of use
NCBI BLAST	http://blast.ncbi.nlm.nih.gov/Blast.cgi	Sequence scanning
Clustal W2	https://www.ebi.ac.uk/Tools/msa/clustalw2/	Sequence analysis
HIV sequence database	https://www.hiv.lanl.gov/content/sequence/FORMAT_CONVERSION/form.html	Converting txt extentions to FASTA format
MEGA 6	https://www.megasoftware.net/	Phylogenetic analysis
EMBOSS Needle	http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html	Sequence analysis
Reverse complement	https://www.bioinformatics.org/sms/rev_comp.html	Sequence analysis

2.2.2.2.2 MIS Analysis

Identification of the bacterial isolates were made by Yeditepe University Microbiology Laboratory by using gas chromatography (6890N GC, Agilent Technologies INC., USA) and MIS software (Sherlock 6.0 MIDI, Inc., Newark, DE, 2005). Then the resulting data was compared with the commercial database (RTSBA6). Fatty acid methyl esther (FAME) groups were identified by MIS software. The unit with the highest score between the diagnosis results was considered as the absolute result.

2.3 RESULTS

2.3.1 Determination of pH, Salt Content and Nutrient Elements of the Soil Samples

As soil samples of specific locations were brought to the lab, some part of them were immediately taken for bacterial isolation. Remaining samples were prepared for salt, pH and nutrient element analysis. pH and salt contents of the collected samples were given in Table 10. ICP-OES analysis was done to reveal the nutrient elements of the soil samples and the results were given in Table 11.

Table 10. Salt and pH levels of the researched soil samples

Depth (cm)	Sample	Region	pH	Salt (µs/cm)
0-30	1	Gölçayır	7.57	309
0-30	2	Gölçayır	7.50	3.18
0-30	3	Akşehir	7.98	1654
0-30	4	Dereçine	8.05	512
0-30	5	Eber	8.15	1354
0-30	6	Sultandağı	8.20	396

Table 11. The mineral element content of the soil samples taken from selected locations

Location	B	Mo	Cd	Cu	Fe	Mn	Ni	Zn	Ca	K	Mg	N	C
	mg kg^{-1}								%				
1	0.77	0.02	0.02	2.52	12.29	0.02	0.02	0.02	345	15	70	0.41	6.59
2	4.22	0.05	0.01	1.43	8.71	0.05	0.05	0.05	616	24	92	0.37	7.99
3	2.36	0.03	0.01	2.46	9.03	0.03	0.03	0.03	697	35	135	0.16	4.27
4	0.54	0.01	0.02	3.99	7.79	0.01	0.01	0.01	253	12	53	0.06	4.07
5	1.45	0.05	0.01	5.00	34.32	0.05	0.05	0.05	35	2	8	0.28	5.56
6	0.64	0.04	0.01	1.89	12.12	0.04	0.04	0.04	7038	10	1517	0.42	7.68

2.3.2 Determination of Nutrient Elements of the Rhizome Samples

The nutrient element content of the collected rhizomes was revealed with ICP-OES analysis and given in Table 12.

Table 12. The mineral nutrient content of the rhizome samples

Sample	Location	B	Mo	Cd	Cu	Fe	Mn	Ni	Zn	Ca	K	Mg	N	C
		mg kg^{-1}								%				
Rhizome 1	1	34	0.33	0.03	22	429	14	1.01	43	0.60	1.14	0.23	2.53	45.89
Rhizome 2	2	28	1.11	0.04	11	404	16	0.52	30	0.33	0.72	0.37	1.29	47.39
Rhizome 3	3	16	0.03	0.05	11	604	22	0.74	15	0.52	0.24	0.34	1.29	47.08
Rhizome 4	4	27	0.49	0.04	15	380	52	0.67	33	0.38	0.53	0.18	1.72	46.72
Rhizome 5	5	14	0.07	0.03	8	379	12	0.62	12	0.22	0.82	0.58	1.01	47.14
Rhizome 6	6	36	0.46	0.03	9	379	23	0.65	32	0.59	1.06	0.56	2.08	47.30

2.3.3 PGPR Isolation

2.3.3.1 Pre-selection of the bacteria isolated from *V. turcica* rhizomes

Bacterial isolation was succeeded from the rhizome samples taken from location 1, 3 and 6. Pre-selective studies resulted with 4 isolates from location 1, 2 isolates from location 3 and 6.

The results of the reaction that isolates gave to Congo red, Bromothymol blue and gram-staining are shown in Table 13. Images of isolates in mediums containing Congro red and Bromothymol blue were given in Figure 28 and gram-staining results can be seen in Figure 29.

Table 13. Reactions of isolates to Congo red, Bromothymol blue and Gram-staining

Location	No	Strain Code	Gram-staining	Congo red	Bromothymol blue
1	1	Vt1N1	Positive-Bacillus	Red-Black	White-Basic
	2	Vt1N2	Positive -Bacillus	Red-Black	White-Basic
	3	Vt1N3	Positive -Bacillus	Red	Yellow-Acidic
	4	Vt1N4	Positive -Bacillus	Red-Black	Yellow-Acidic
3	1	Vt3N1	Positive -Bacillus	Red-Black	Yellow-Basic
	2	Vt3N2	Positive -Bacillus	Red-Black	Yellow-Basic
6	1	Vt6N1	Positive -Bacillus	Red-Black	Yellow-Basic
	2	Vt6N2	Positive -Bacillus	Red-Black	Yellow-Basic

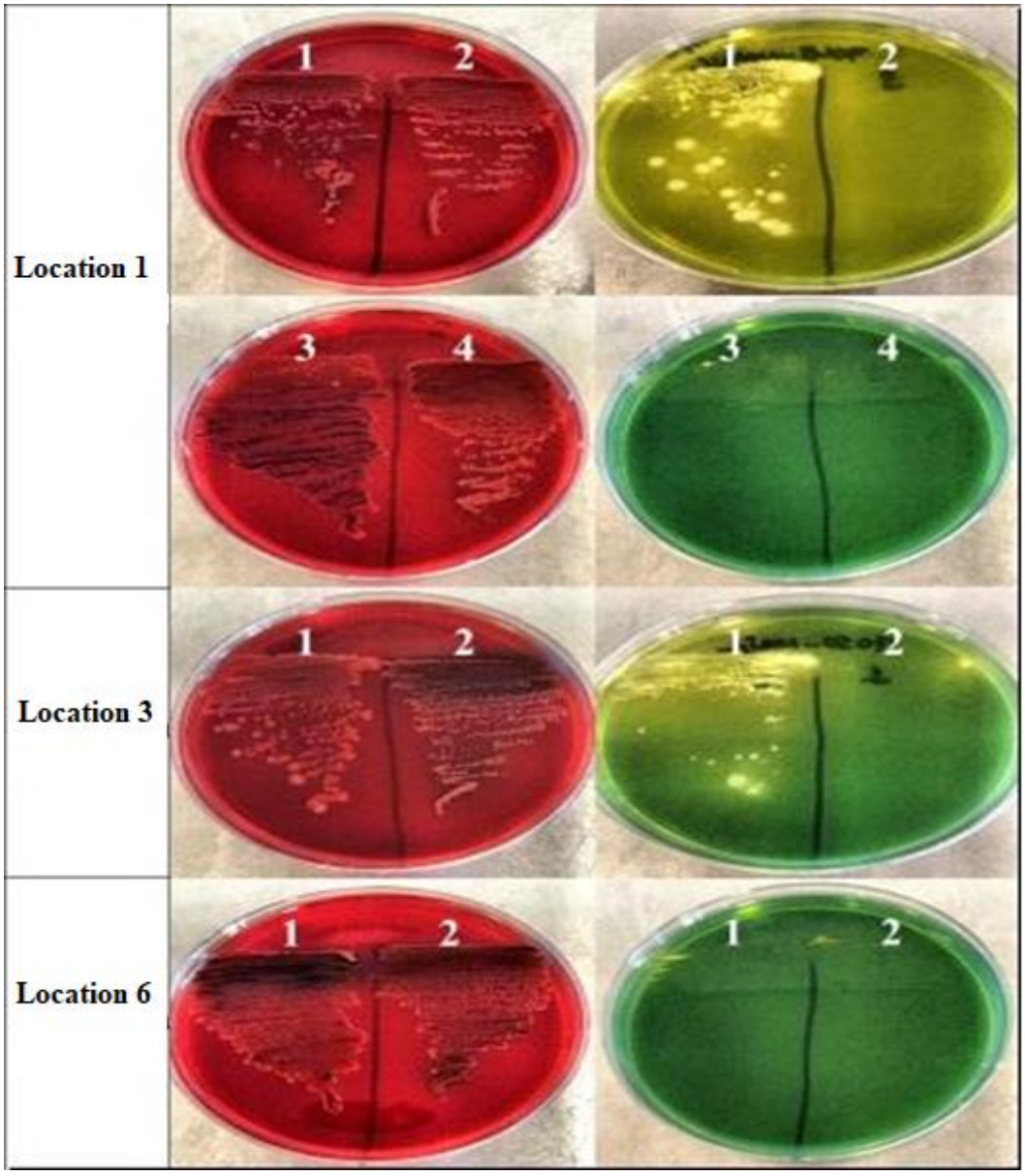

Figure 28. Status of isolated bacteria from the rhizomes collected from various habitats of *V. turcica* in the medium containing Congo red (left) or Bromothymol blue (right)

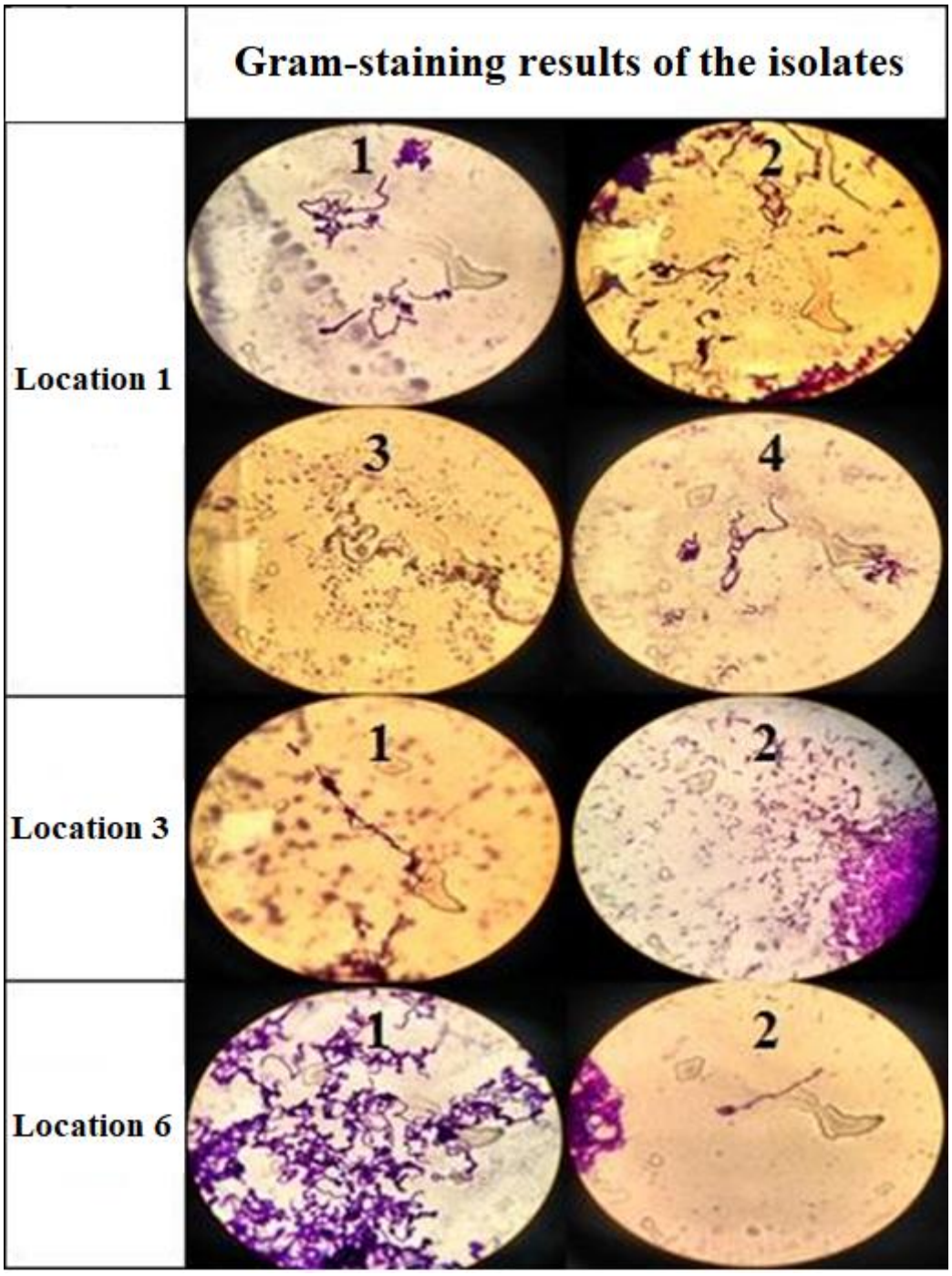

Figure 29. Gram-staining results of rhizome-isolated bacteria

2.3.3.2 Pre-selection of the bacteria isolated from the soil samples collected from the various habitats of *V. turcica*

6 isolates were obtained from each soil sample of location 2, 3 and 6; 2 isolates were obtained from the sample of location 4. No bacteria were isolated from the samples of location 1 and 5. Isolates responses to Congo red, Bromothymol blue and gram-staining were given in Table 14.

Table 14. List of reactions of isolates to Congo red, Bromothymol blue and Gram-staining

Location	Strain Code	Gram-staining	Congo red	Bromothymol blue
2	Vt2S1	Negative-Bacillus	Transparent-Viscous	Orange-Basic
	Vt2S2	Negative-Bacillus	Transparent-Viscous	Orange-Basic
	Vt2S3	Negative-Coccus	Transparent-Viscous	Orange-Basic
	Vt2S4	Negative-Coccus	Red	Orange-Basic
	Vt2S5	Negative-Coccus	Black	Fungus-Acidic
	Vt2S6	Positive-Coccus	Orange	Yellow-Orange-Basic
3	Vt3S1	Negative-Bacillus	Red	Yellow-Orange-Acidic
	Vt3S2	Negative-Coccus	Red	Yellow-Orange-Basic
	Vt3S3	Negative- Coccus	no growth	Yellow-Basic
	Vt3S4	Negative- Coccus	Red	Green-Basic
	Vt3S5	Negative- Coccus	Red-Black	Yellow-Basic
	Vt3S6	Negative- Coccus	Yellow	Yellow-Acidic
4	Vt4S1	Positive- Coccus	Red	Yellow-Green-Basic
	Vt4S2	Negative- Coccus	Red-Black	Green-Acidic
6	Vt6S1	Negative-Bacillus	Red	Yellow-Acidic
	Vt6S2	Negative- Coccus	Red	Yellow-Orange-Basic
	Vt6S3	Negative-Bacillus	no growth	Yellow-Orange-Basic
	Vt6S4	Negative- Coccus	Red-Black	Yellow-Orange-Acidic
	Vt6S5	Negative-Bacillus	Red	Yellow-Orange-Acidic
	Vt6S6	Negative-Bacillus	Red	Yellow-Orange-Acidic

2.3.4 Phylogenetic Analysis

2.3.4.1 Phylogenetic analysis of rhizome-isolated bacteria

16S rRNA and ITS analysis results of the bacteria isolated from rhizomes were given in Figure 30 and Figure 31 respectively.

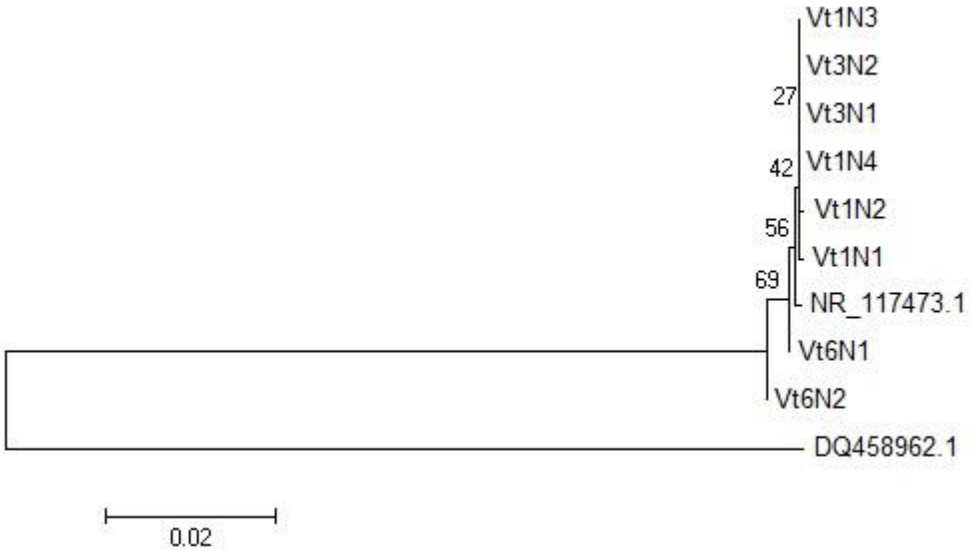

Figure 30. Phylogenetic relations of isolates and 2 other species (NR_117473.1 (*Bacillus megaterium*) and DQ458962.1 (*Agrobacterium tumefaciens*)) depending on 16S rRNA sequence similarities

Due to the ressemblance of 16S rRNA sequences of NR_117473.1 and DQ458962.1, they were chosen as outer group. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 0.18599723 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei and Kumar 2000) and are in the units of the number of base differences per site. The analysis involved 10 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 1326 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

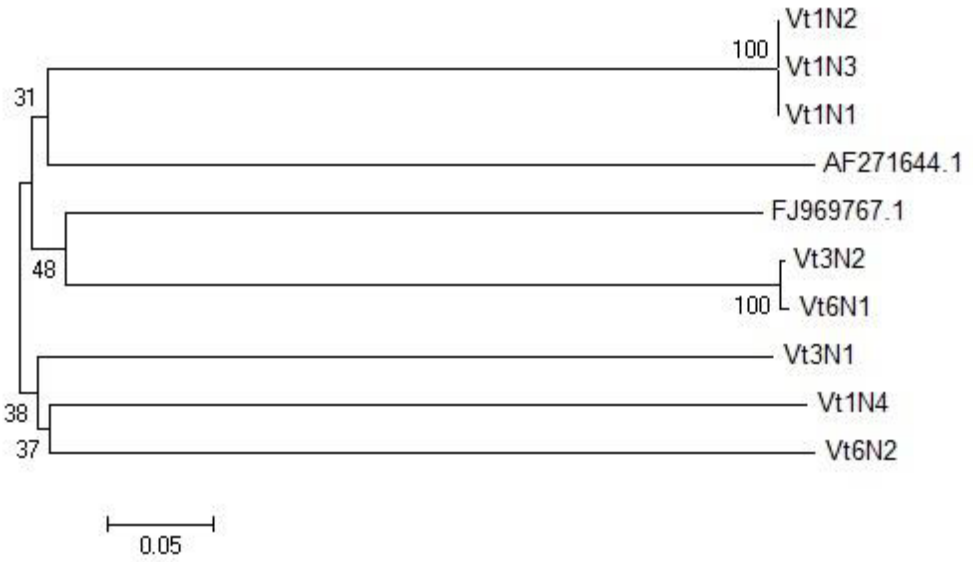

Figure 31. Phylogenetic relations of isolates and 2 other species (FJ969767.1 (*Bacillus megaterium*) and AF271644.1 (*Agrobacterium tumefaciens*)) depending on their ITS sequence similarities

Due to the ressemblance of ITS sequences of FJ969767.1 and AF271644.1.1, they were chosen as outer group. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 2.52614583 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei and Kumar 2000) and are in the units of the number of base differences per site. The analysis involved 10 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 300 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

2.3.4.2 Phylogenetic analysis of soil-isolated bacteria

16S rRNA and ITS analysis results of the bacteria isolated from the soil samples were given in Figure 32 and Figure 33 respectively.

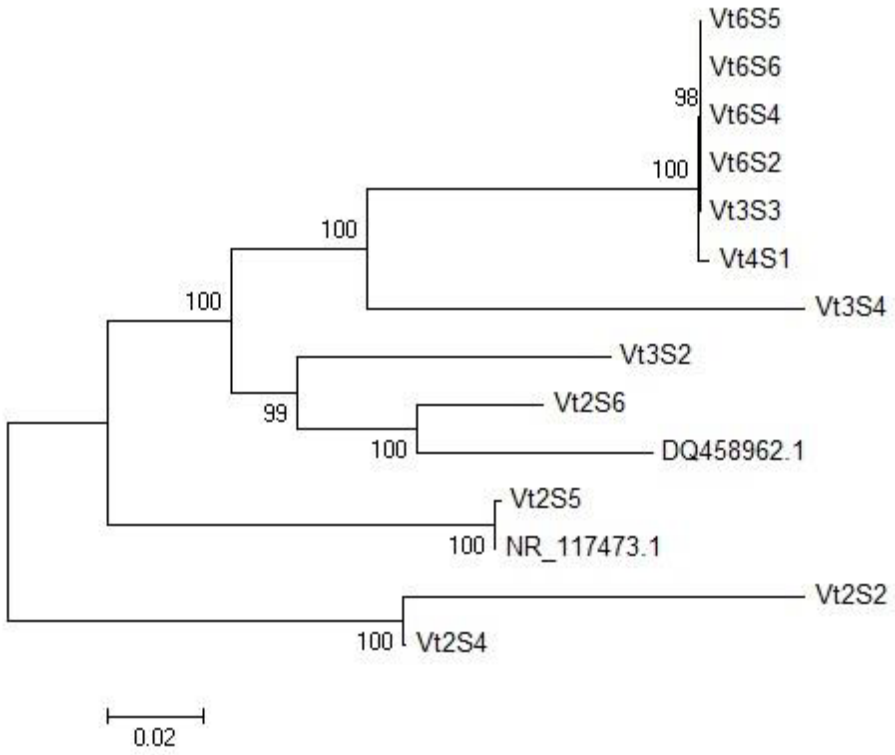

Figure 32. Phylogenetic relations of isolates and 2 other species (DQ458962.1 (*Agrobacterium tumefaciens*) and NR_117473.1 (*Bacillus megaterium*)) depending on their 16S rRNA sequence similarities

DQ458962.1 and NR_117473.1 were chosen as outer group. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 0.67431967 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei and Kumar 2000) and are in the units of the number of base differences per site. The analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 1354 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

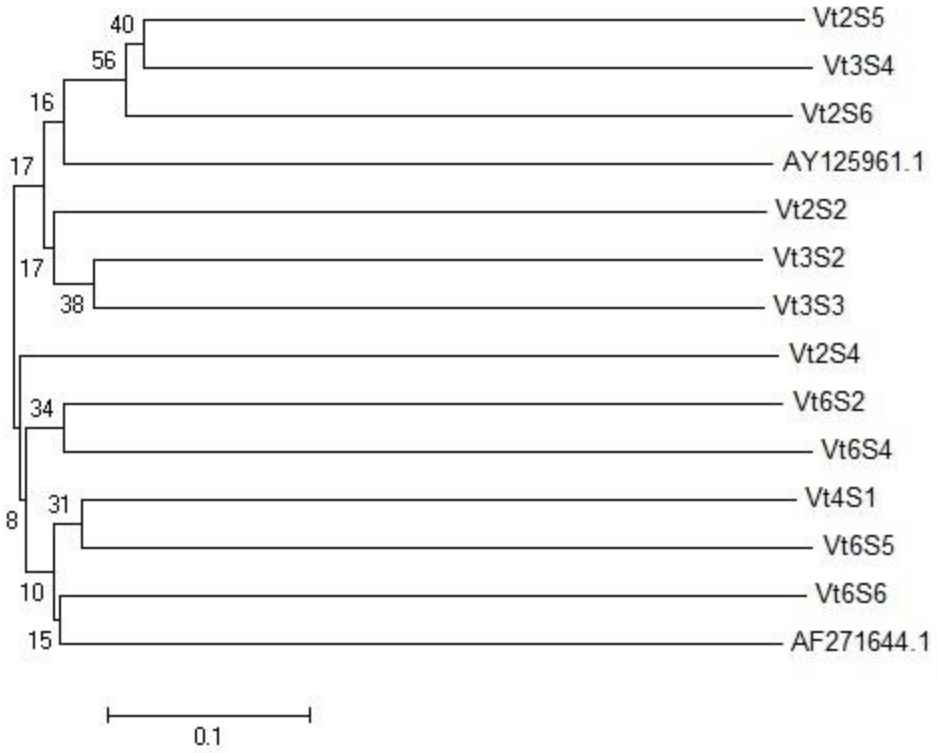

Figure 33. Phylogenetic relations of isolates and 2 other species (AY125961.1 (*Bacillus megaterium*) and AF271644.1 (*Agrobacterium tumefaciens*)) depending on their ITS sequence similarities

AY125961.1 ve AF271644.1 were chosen as outer group due o their ITS sequence ressemblance. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 5.03315923 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei and Kumar 2000) and are in the units of the number of base differences per site. The analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 178 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

2.3.5 MIS Results

Bacterial identity of the isolates were tried to be revealed by analysing their FAME groups with MIS. Results of rhizome-isolates and soil-isolates were given in Table 15 and Table 16, respectively.

Table 15. MIS results of the bacteria isolated from rhizomes

Location	Strain Code	MIS result
1	Vt1N1	*Bacillus megaterium*-GC subgroup B (0.469)
	Vt1N2	*Bacillus megaterium*-GC subgroup A (0.855)
	Vt1N3	*Bacillus megaterium*-GC subgroup A (0.851)
	Vt1N4	*Bacillus megaterium*-GC subgroup A (0.755)
3	Vt3N1	*Bacillus megaterium*-GC subgroup A (0.729)
	Vt3N2	*Brevibacillus parabrevis*-(0.598)
6	Vt6N1	*Bacillus megaterium*-GC subgroup A (0.729)
	Vt6N2	*Bacillus megaterium*-GC subgroup A (0.834)

Table 16. MIS results of the bacteria isolated from soil samples

Location	Strain Code	MIS result
2	Vt2S1	*Virgibacillus pantothenticus* (0.666);
	Vt2S2	*Kocuria rhizophila* (0.694)
	Vt2S3	*Ochrobactrum anthropi* (Achromobacter Vd) (0.856)
	Vt2S4	*Kocuria kristinae*-GC subgroup A (0.692)
	Vt2S5	*Bacillus megaterium*-GC subgroup A (0.805)
	Vt2S6	*Virgibacillus pantothenticus* (0.675)
3	Vt3S1	*Bacillus megaterium*-GC subgroup A (0.782)
	Vt3S2	*Bacillus megaterium*-GC subgroup A (0.792)
	Vt3S3	*Pseudomonas syringae*-syringae (0.380)
	Vt3S4	No result
	Vt3S5	No result
	Vt3S6	*Neisseria cinerea*-GC subgroup B (0.612)
4	Vt4S1	*Stenotrophomonas maltophilia* (0.305)
	Vt4S2	*Stenotrophomonas maltophilia* (0.374)
6	Vt6S1	No result
	Vt6S2	*Kocuria erythromyxa* (Deinococcus) (0.374)
	Vt6S3	*Stenotrophomonas maltophilia* (0.376)
	Vt6S4	*Bacillus viscosus* (0.496)
	Vt6S5	*Pseudomonas huttiensis* (0.605)
	Vt6S6	*Pseudomonas huttiensis* (0.552)

2.4 DISCUSSION

Bacterial identification applications such as MIS analysis, ITS and 16S rRNA sequence analysis were done for bacterial identification of the isolates. In total, around 30 isolates were obtained from both rhizome and soil samples. 18 of the soil isolates and 8 rhizome isolates were analysed by MIS technology. Then the isolates were sequenced to enable the comparison of the 16S rRNA and ITS sequences of the isolates with the present ones in the web database. 12 bacterial samples were sequenced between the soil isolates and 8 isolates were sequenced from the rhizomes. Obtained sequences were searched in https://ncbi.nlm.nih.gov website using the BLAST and the results with the highest percentages of identity and coverage were given in Appendix 3-6**.** The narrow library that is used in the MIS analysis (1000 elements), made this study to rely more on the sequence analysis done by using the BLAST. Some of the isolates particular 16S rRNA and ITS sequences had different bacterial matches when analysed. The results that have possible plant growth promoting activities on *V. turcica* will be handled more in this discussion.

According to both MIS and sequencing results, *Bacillus megaterium* is the most dominantly colonized bacteria around the rhizomes and it is also present in the soil where the rhizomes were taken from (Table 14-15) (Appendix 3-6). This study is first to report plant growth promoting rhizobacteria presence in the natural habitat and the roots of the endemic plant *V. turcica*. Plant growth promoting activity between *B. megaterium* and host plants were reported many times in the literature and its possible growth promoting effects on *V. turcica* would be interesting to discuss on (López-Bucio et al. 2007; Liang et al. 2011; Chakraborty et al. 2012). The main focus of this discussion will be on the suggestions about the dominance and plant growth promoting effects of these bacteria on this endemic plant with the support of the literature information.

Mineral analysis of rhizomes and soil samples were shown significant differences. Levels of B, Cu, Fe, Zn and C minerals were high in rhizomes but in soil samples, those elements were available in poor amounts (Table 10-11). This difference is most probably related with the plant growth promoting abilities of *B. megaterium* that was found in the rhizospheres of *V. turcica* in the bacterial isolation and identification studies. *B. megaterium* is known with its usefulness in phytoremediation of heavy-metal

contaminated soils and it has been reported that this bacterium is able to enhance the desorption of B from the soil and increase its accumulation in plants (Esringü et al. 2014). Also, *B. megaterium* is able to remove the copper from the environment and to store it internally (Hohapatra et al. 1993). Thus, it is suggested that high levels of B and Cu present in the rhizome samples is related to the activity of *B. megaterium*.

B. megaterium is a free-living bacterium like other PGPR species and it was endophytically found in *V. turcica* rhizomes (Persello-Cartieaux et al. 2003). The rhizome-carved samples were shown to host this bacterium. *B. megaterium* were previously found endophytically in legume plants and promote plant growth (Khalifa and Almalki 2015). No significant nodules were found on the rhizomes, no symbiotic relation between *V. turcica* and other microorganisms were detected in this study. This endophytic presence of this bacterium might have protective effects on the rhizomes against invasive pathogens.

Like most of the PGPR's, *B. megaterium* produces and secretes siderophores (Cornelis and Andrews 2010). It is observed that siderophore secretion by this bacterial species is enhanced in iron-deficient conditions (Arceneaux and Byers 1980). Those siderophores chelate ferric ions in the environment and plants are able to acquire Fe ions in that siderophore-iron complex. After the intracellular release of the iron in the root cells, the siderophore is then released to the rhizosphere and recycled (Santos et al. 2014). This siderophore-dependent iron availability increasing ability of *B. megaterium* could be the explanation of the high levels of iron in the rhizomes of *V. turcica* while it is relatively low in soil samples. Decreased amounts of Fe in the soil might have triggered Fe-chelating siderophore production of *B. megaterium* present in the rhizospheres of *V. turcica* rhizomes and provided plant-available Fe. It is also reported in the literature that *B. megaterium* produces siderophores that solubilizes Zn for plant uptake (Kucey et al. 1989). In this study, high levels of rhizome-Zn in a relatively deficient soil could be interpreted with Zn-chelating activity of *B. megaterium*. Elevated levels of B, Cu, Fe, Zn elements in the rhizomes were tried to be explained by the information on *B. megaterium* present in the literature, but specific interactions between *V. turcica* and *B. megaterium* must further be researched to reveal the molecular mechanisms behind this increase of nutrient availability.

Various plants with *B. megaterium* root-inoculation showed significant growth with an increase of total plant N and N-fixing activities compared to control plants (Elkoca et al. 2007). *B. megaterium* has been found to contain nitrogenase iron protein (NifH) genes which encode important enzymes taking a role in N-fixing mechanisms and those genes are widely used as marker genes to identify N-fixing bacteria (Ding et al. 2005; Gaby and Buckley 2012). In light of this information, it is assumed that *B. megaterium* has growth promoting effects on *V. turcica* plants by fixing or helping to fixation of N. This also might be the explanation of the healthy growth and N content of the plant in its natural habitat where the soil is not enough nutritious.

Chlorophyll content of a plant is an indicator of the levels of N-fixation activity and N content of a plant (Kumawat et al. 2000). *B. megaterium* might positively affect the photosynthetic capacity of a plant by stimulating shoot growth and increasing chlorophyll content by N-fixing, thus the plant would produce more photosynthates to secrete from the roots (Elkoca et al. 2007). Root exudates are utilized as carbon source by rhizobacteria (Vacheron et al. 2013). Including various mono and disaccharides, *B. megaterium* can use more than 62 different carbon sources singularly for energy production, which makes this species versatile and facilitates its colonization (Stülke and Hillen 2000; Vary et al. 2007). Bacterial isolation results of this study showed that the bacteria found in the soil were not present on the rhizomes. Bacterial flora at *V. turcica* rhizomes were dominated with *B. megaterium* colonization. Its ability to metabolize different root exudates as carbon source might have put this species one step forward than other bacteria while competing for colonization. More chance to find nutrition can result with better colonization. As this bacteria enhances the photosynthetic activity by increasing the N content of the plant, higher amounts of photosynthates would be produced and utilized by the bacteria as carbon source. This mutualistic loop might be one of the reasons why the microflora on the *V. turcica* rhizomes is dominated by *B. megaterium*.

It has been mentioned in the introduction part of this chapter that PGPR species possess the ability to produce phytohormones that regulate or induce plant growth. By secretion of the plant growth-promoting substances as auxins and cytokinins, species belonging to genus *Bacillus* can directly influence root architecture and shoot development (Persello-Cartieaux et al. 2001; Arkhipova et al. 2005). The ability of *B.*

megaterium to produce plant hormones like auxins, gibberellins, cytokinins and abscisic acid has been shown in the literature (Karadeniz et al. 2006). With cytokine signalling, shoot growth and robust root development are stimulated in plants by *B. megaterium* (Ortíz-Castro et al. 2008). It has been reported that this bacterial species also enhances the lateral root formation, root hair length and root growth with an auxin/ethylene independent way where the root surface area is increased (López-Bucio et al. 2007). In light of the phytohormone production and plant growth promoting abilities of *B. megaterium*, there is a great chance that this bacteria promotes growth in *V. turcica* plants through hormone signalling pathways. In order to prove this suggestion, hormone levels, types and specific interactions, including signalling pathways between the bacterium and the plant must further be revealed.

Literature research on indirect growth promoting abilities of *B. megaterium* is done to support our interpretations on the relation of *B. megaterium* with endemism of *V. turcica*. Indirect growth promoting mechanisms of this bacterium involves antibiotic production, induced systemic resistance activation, lytic enzyme secretion and siderophore production (Ngoma et al. 2012). Siderophores that *Bacillus spp.* produce, not only provide iron to the host plant, but also decrease the availability of the metal ions for pathogens around the rhizosphere (Mathiyazhagan et al. 2004). High affinity between siderophores and iron ions causes deprivation of this element around the rhizosphere in, hence pathogens were suppressed due to iron deficiency (Kloepper et al. 1980). This suppression puts *Bacillus spp.* one step forward in means of nutrient competition and colonization which provides further protection to plant (Labuschagne et al. 2010). *B. megaterium* residing at the rhizomes of *V. turcica* might have a protective effect on its host with a possible siderophore release. The Fe composition difference between the rhizomes and the soil that they have grown led us to interpret that *B. megaterium* decreases the available Fe even more for pathogens and promote the plant growth indirectly by decreasing pathogen occurrence. The nature of the siderophores that rhizome-isolated *B. megaterium* release and their interactions with the host plant must further be researched to reveal the plant-protective mechanisms.

Indirect growth promoting ability of *B. megaterium* consists antibiotic production. It has been previously shown that this bacteria is capable of producing antibiotics such as bacimethrin, cytidines, oxetanocin, iturin, bacillomycin, zwittermycin A, surfactin and

other fungitoxins (Malanicheva et al. 2012; Uthandi and Sivakumaar 2013). *B. megaterium* might contribute to *V. turcica* growth indirectly with this antimicrobial activity and this might explain the colonial dominance of this bacteria at *V. turcica* rhizomes. With potentially secreted antibiotics, *B. megaterium* might limit the growth of other microorganisms and increase its colonization rate on the rhizomes. To make certain assumptions on this subject, a further research is required, which reveals the antibiotics that *B. megaterium* produce at the natural habitat of *V. turcica*. The presence of this bacterium might be crucial for *V. turcica* survivability. As this plants natural habitat is restricted to an area, it is possible that lack of *B. megaterium* in the soils might be the reason why *V. turcica* is not encountered anywhere else.

Induction of ISR in plants is one of the indirect growth promoting application of PGPR as mentioned in the introductory part of this chapter. ISR in plants is characterized by the increased activity of several enzymes such as phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, chitinase and also with higher levels of accumulated phenolics (Siddiqui 2005). It has been shown that *B. megaterium* application on tea plants leads to accumulation of phenolic compounds in the leaves, a compound reported to be related with plant resistance against various stresses (Chakraborty et al. 2012). Turmeric has rhizomic root structure like *V. turcica* and turmeric plants have shown an increase in all mentioned compounds related to ISR and prevention from fungal infections when *B. megaterium* were present at their rhizomes (Uthandi and Sivakumaar 2013). Expression levels of superoxide dismutase and catalase together with the previously mentioned ISR related enzymes, observed to be higher in *B. megaterium* inoculated cucumber plants in means of defence against fungal infection (Liang et al. 2011).

Various bacteria were isolated from the soil samples including *B. megaterium*. *Rhodococcus erythropolis*, *Xanthomonas albilineans*, *Lysobacter enzymogenes*, and *Stenotrophomonas rhizophila* were the significant bacterial species identified from the soil samples (APPENDIX 5). *R. erythropolis* were reported to show PGPR effects in cold temperatures (Trivedi et al. 2007). *X. albilineans* were shown to have pathogenic effects on sugarcane plants (Zhang et al. 2017). This bacterium might be pathogenic or non-pathogenic to *V. turcica* but even if it is pathogenic, it might have been suppressed by the antimicrobial activity of *B. megaterium*. *L. enzymogenes* was distinguished with its antifungal activity through lytic enzyme secretion (Jochum et al. 2006). Maybe together

with *B. megaterium*, this bacterium might be playing an important role in the survival of *V. turcica* in its natural habitat. *S. rhizophila* were found to promote plant growth in salty soils (Egamberdieva et al. 2016). The soil where *V. turcica* naturally occurs was found to be salty (Table 10). It is reasonable to assume that this bacterium protect *V. turcica* against salt stress in its natural habitat.

2.5 CONCLUSION

Bacillus megaterium is a renowned PGPR species that promotes plant growth through P-solubilization, N-fixation, increasing mineral uptake and antimicrobial activity (López-Bucio et al. 2007). Identification study of beneficial rhizobacterial species promoting *V. turcica* growth in this study has shown that there is a strong dominance of *B. megaterium* at the rhizomes of *V. turcica*. This bacterium was also found in the soil where *V. turcica* naturally occurs. This study is the first report on PGPRs identification in *V. turcica*. By being influenced from the previous reports on this bacterial species in the literature, we strongly suggest that *B. megaterium* has an important role in the growth and development of *V. turcica* plants in their natural habitat.

The finding of this study might be a clue for further explanations on the endemism of this plant and also it could give a lead to various future researches. The first research that is going to be done in light of the findings of this study must reveal the growth dependence of *V. turcica* on *B. megaterium* by comparing the growth of non-infected and infected *V. turcica* plants with *B. megaterium*. To introduce new insights to the endemism of *V. turcica*, plant growth promotion related molecular interactions between the plant and the PGPR must be researched. Revealing specific antibiotics, siderophores and enzymes behind this mutualism and finding out the pathways from which the growth promotion is exhibited would be beneficial. More consistent explanations could be done on this endemism by taking together the environmental features of *V. turcica*'s natural habitat and specific plant growth promoting mechanisms that were induced by *B. megaterium* on *V. turcica*.

B. megaterium has been an important bacteria used in industrial production of enzymes, vitamins, fungicides and viral inhibitors (Vary et al. 2007). Because of its plant growth promoting traits, it could be used as a biofertilizer (Patel et al. 2016). Increasing knowledge on the molecular interactions between this bacterial species and *V. turcica* could be agriculturally and industrially productive through the secondary metabolite production ability and the potential usage as a biofertilizer of *B. megaterium*.

B. megaterium is diversly found in the soils worldwide (Vary et al. 2007). It is also found in the natural habitat of *V. turcica*, but *V. turcica* is only found in a restricted area in Central Anatolia (Tekdal et al. 2018). This bacterium probably play crucial roles in the survival of this plant but the most important factor behind this survival is probably the collective work of the identified beneficial bacterial species in the soil samples.

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APPENDIX

APPENDIX 1: List of equipments used in this study

Equipment	Company, Model, Country
Autoclave	Hirayama, Hiclave HV-110, Japan
Balance	Sartorius, BP221S, Germany - Schimadzu, Libror EB-3200 HU, Japan
Centrifuge	Eppendorf, 5415D, Germany - Hitachi, Sorvall RC5C Plus, USA
Deepfreeze -80°C	Forma, Thermo ElectronCorp., USA - -20°C, Bosch, Turkey
Distilled Water Millipore	Elix-S, France
Electrophoresis Apparatus	Biogen Inc., USA
Gel Documentation	Biorad, UV-Transilluminator 2000, USA
Heater	ThermomixerComfort, Eppendorf, Germany
Ice Machine	Scotsman Inc., AF20, USA
Incubator	Memmert, Modell 300, Germany
Laminar Flow	Kendro Lab. Prod., Heraeus, HeraSafe HS12, Germany
Liquid Nitrogen Tank	Taylor-Wharton,3000RS, USA
Magnetic Stirrer	ARE Heating Magnetic Stirrer, Italy
Microliter Pipettes	Gilson, Pipetman, France
Microscope	Olympus CK40, Japan Olympus CH20, Japan Olympus IX70, Japan Zeiss Confocal LSM710, German
Microwave Oven	Bosch, Turkey

pH meter	WTW, pH540 GLP MultiCal, Germany
Power Supply	Biorad, PowerPac 300, USA
Refrigerator	Bosch, Turkey
Shaker Incubator	New Brunswick Sci., Innova 4330, USA
Thermocycler	Eppendorf, Mastercycler Gradient, Germany

APPENDIX 2: List of chemicals used in this study

Chemicals	Company, Country
Agarose	peQLab, Germany
Boric Acid	Molekula, UK
Bromophenol Blue	Sigma, Germany
Distilled water	Millipore, France
DNA Gel Loading Solution	5X Quality Biological, Inc, USA
EDTA	Applichem, Germany
Ethanol	Riedel-de Haen, Germany
Ethidium Bromide	Sigma, Germany
Hydrochloric Acid	Merck, Germany
Isopropanol	Riedel-de Haén, Germany
Liquid nitrogen	Karbogaz, Turkey
Methanol	Riedel-de Haen, Germany
Monoclonal Anti-HA Antibody	Sigma, Germany
Phenol-Chloroform-Isoamyl alcohol	Amersco, USA
RNase A	Roche, Germany
Tris Hydrochloride	Amresco, USA

APPENDIX 3: ITS sequence results of rhizome-isolates

Strain Code	Sequence 5'-3'	NCBI Accession ID	Species/Strain
Vt1N1	TTGCGGTTGGATCCCCTCCTTTCTAAGGATTTTTACATGACGTACGTTTTGACACTTTGTTCAGTTTTGAGAGTTCAATCTCTCAATTATAGAAAGCACACTACTTTCTTCTTATTTGAT AAGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATT CGCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTT	CP001983.1	*Bacillus megaterium* QM B1551
Vt1N2	TTGCGGCTGGATCCCCTCCTTTCTAAGGATTTTTACATGACGTACGTTTTGACACTTTGTTCAGTTTTGAGAGTTCAATCTCTCAATTATAGAAAGCACACTACTTTCTTCTTATTTGAT AAGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATT CGCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTT CCGAATGGGGAAACCCGGA	CP001983.1	*Bacillus megaterium* QM B1551
Vt1N3	TTGCGGTTGGATCCCCTCCTTTCTAAGGATTTTTACATGACGTACGTTTTGACACTTTGTTCAGTTTTGAGAGTTCAATCTCTCAATTATAGAAAGCACACTACTTTCTTCTTATTTGAT AAGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATT CGCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTT CCGAATGGGGAAACCCGG	CP001983.1	*Bacillus megaterium* QM B1551
Vt1N4	TTGTTCAGTTTTGAGAGTTCAATCTCTCAATTATAGAAAGCACACTACTTTCTTCTTATCAAATAAGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAG GAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTCGCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGA TGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTTCCGAATGGGGAAACCCG	CP018874.1	*Bacillus megaterium* JX285
Vt3N1	CCCTCCTTTCTAAGGATTTTTACATGACGTACGTTTTGACACTTTGTTCAGTTTTGAGAGTTCAATCTCTCAATTATAGAAAGCACACTACTTTCTTCTTATCTGATAAGAAGAATTTTG GTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTCGCTAATGGTTAA GTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTTCCGAAT	CP001983.1	*Bacillus megaterium* QM B1551
Vt3N2	TGCGGCTGGGTCCCCTCCTTTCTAAGGATTTTTACATGACGTACGTTTTGACACTTTGTTCAGTTTTGAGAGTTCAATCTCTCAATTATAGAAAGCACACTACTTTCTTCTTATTTAATA AGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTC GCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTTC CGAATGG	CP001983.1	*Bacillus megaterium* QM B1551
Vt6N1	TGCGGCTGGGTCCCCTCCTTTCTAAGGATTTTTACATGACGTACGTTTTGACACTTTGTTCAGTTTTGAGAGTTCAATCTCTCAATTATAGAAAGCACACTACTTTCTTCTTATCAAATA AGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTC GCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTTC CGAATGG	CP001983.1	*Bacillus megaterium* QM B1551
Vt6N2	TCCCCTCCTTTCTAAGGATTTTTACATGACGTACGTTTTGACACTTTGTTCAGTTTTGAGAGTTCAATCTCTCAATTATAGAAAGCACACTACTTTCTTCTTATCTAATAAGAAGAATTT TGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTCGCTAATGGTT AAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTTCCGAAT	CP001983.1	*Bacillus megaterium* QM B1551

APPENDIX 4: 16S rRNA sequence results of rhizome-isolates

Strain Code	Sequence 5'-3'	NCBI Accession ID	Species/Strain
Vt1N1	GCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGGAGATG ATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACAC TGGGACTGAGACACGGAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGT ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTG AAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGC GAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCG CCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAA GCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGAT GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA TGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGC CTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAA	KY616832.1	*Bacillus megaterium* DSW-CAP-1
Vt1N2	GCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGGAGATG ATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACAC TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGAGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCT GTTGTTAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCG GAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAAGCGGAAT TCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCC TGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAG GAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGG GACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAATTGCCAGCATTTAGTTGGGCACTCTAAGGT GACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAA GCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTAC ACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGTAAGTC	CP026736.1	*Bacillus megaterium* YC4-R4
Vt1N3	TATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGA AAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGA CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGT TAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATT ATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAAGCGGAATTCCAC GTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA GTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATT GACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAG AGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTG CCGGTGACAAACCGGAGGAAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCA ATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACAC CGCCCGTCACACCACGAGAGTTTGTAACACC	CP026736.1	*Bacillus megaterium* YC4-R4

Vt1N4	ACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCGGCTAT CACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGA CTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTA CGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGC GCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATG CGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG ATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACA AGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCA TGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGA GGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAATCCCATAAAACCATTC TCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGA GAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGT	CP026736.1	*Bacillus megaterium* YC4-R4
Vt3N1	TGCAGTCGAGCGAACTGATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCG GATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATA GCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAG TGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGG AACTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAA GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGG GAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAA CTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGT TGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGG TACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGC CGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGAG	MH142578.1	*Bacillus megaterium* NSE1
Vt3N2	CGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTC GGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGG CCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAA CAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTA AAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGT GAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG TAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCC CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGT GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAA ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAATCCCATAAAA CCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACA CCACGAGAAGTTTGTAACACCCGAAGTCG	CP026736.1	*Bacillus megaterium* YC4-R4
Vt6N1	TGCAGTCGAGCGAACTGATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCG GATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATA GCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAG TGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGC GCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATG CGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG ATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACA AGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCA TGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGA GGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAATCCCATAAAACCATTC TCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGA GAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGTAAGGAGCTAGCCGCCTAAGGTGGGACAGATGAT	CP026736.1	*Bacillus megaterium* YC4-R4

Vt6N2	AGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT ACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTG AGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGG GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTAC GGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAG AGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAG CATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAA GGGCTGCAAGACCGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGTAAAGGAGCTAGCCGCCTAAGGGTGGGACAGATGATTG	CP026736.1	*Bacillus megaterium* YC4-R4

APPENDIX 5: ITS sequence results of soil-isolates

Strain Code	Sequence 5'-3'	NCBI Accession ID	Species/Strain
Vt2S2	AAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTCGCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAAGCTTTGATCCGGAGATTTCCGAATGGGGAAACCCG	CP018874.1	*Bacillus megaterium* JX285
Vt2S4	TTCCCGTATGTGGAACGGTGGTTGCTCATGGGTGGACGCTGACAAACATTCCTTTTCGTTACTGCCAGCCTCAATGCTGGTGGGAGAACAAATTATATCGGCATACTGTTGGGTCCTGAGAGAACACGCGAGTGTTTCCTCGAAGGAAGTAACGACAATGTTCAGGCGCGGTCATACCGCAAGAATTTTTTTCTTGTTGGTGTCTGCTTGGAGCCTGAGTGTTGTGTGTTGTTTGAGAACTGCACAGTGGACGCGAGCATCTTTGTTGTAAGTAATGAAGAGCGTACGGTGGATGCCTTGGCACCAGGAGCCGATGAAGGACGTAGGAGGCTGCGA	CP007255.1	*Rhodococcus erythropolis* R138
Vt2S5	GCACACTACTTTCTTCTTATCTAATAAGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTCGCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTTCCGAATGGGGAAACCCGG	CP018874.1	*Bacillus megaterium* JX285
Vt2S6	CACACTACTTTCTTCTTATCTAATAAGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTCGCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTTCCGAATGGGGAAACCCGG	CP018874.1	*Bacillus megaterium* JX285
Vt3S2	TCAATTATAGAAAGCACACTACTTTCTTCTTATCTAATAAGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTCGCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTTCCGAATGGGGAAACCCGG	CP018874.1	*Bacillus megaterium* JX285
Vt3S3	CTCTCAATTATAGAAAGCACACTACTTTCTTCTTATCAAATAAGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTCGCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATATGCTTCGGGGAGCTGTAAGTGAGCTTTGATCCGGAGATTTCCGAATGGGGAAACCCG	CP018874.1	*Bacillus megaterium* JX285
Vt3S4	CCTCCTTTCTAAGGATTTTTACATGACGTACGTTTTGACACTTTGTTCAGTTTTGAGAGTTCAATCTCTCAATTATAGAAAGCACACTACTTTCTTCTTATCTAATAAGAAGAATTTTGGTTGCGATTGTTCTTTGAAAACTAGATAACAGTAATTGCTGAGGAAAAGTGAAACTTTTCTTTAATCAAATCAATAAATAACACAACATTATGTTGTACCATTTATTCGCTAATGGTTAAGTTAGAAAGGGCGCACGGTGAATGCCTTGGCACTAGGAGCCGATGAAGGACGGGACTAACACCGATA	CP001983.1	*Bacillus megaterium* QM B1551
Vt4S1	TAATTGCCTGTCAGGCGTCCGCACAAGTGACCTGCATCCAGGAGTTCCACGGCTAGCGCCGGGGGACCGACCCCTTTTGGGGCCATAGCTCAGCTGGGAGAGCACCTGCTTTGCAAGCAGGGGGTCGTCGGTTCGATCCCGACTGGCTCCACCACGCTGGGGAAGACATTGGGTCTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGCCGGTGGTTCGAGTCCTCCCAGACCCACCATTCCTGGATGTCGATTGCGCACACATAAAGATTTGAATCGGATTGGCATCGTGGCCGGTCCGTGTTCTTTGAAAACCAGGGACGTAGCGAGCGTTTGAGACGAATATCCAGACGTGTCGTAGAGGNTAAGGCGGGGCCCTAGAGGCCCTACATTTGAAGTTGTCGTATCTATTCGCGACGAATGATCCCGAGGCGACTTGGGGTTATATGGTCAAGCGAATAAGCGCACACGGTGGATGCCTTGGCGGTCAGAGGCGATGAAGGACGTGGCAGCCTGCGAAAAGTGTCGGGGAGCTGGCAACAAGCTTTGATCCGGCAATGTCCGAATGGGGAAACCCGG	AY940629.1	*Xanthomonas albilineans* IBSBF 1374

Vt6S2	TTGCGGCTGGATCCCCTCCTTTAGAGACTAAAGACAGCTAATTGCCTGTCAGGCGTCCGCACAAGTGACCTGCATTCAGAGATTCCGGCCTAGCGCCGGGATTCGACCCGCGCTGTTCAAAAC AGAACATCGGGGCCATAGCTCAGCTGGGAGAGCACCTGCTTTGCAAGCAGGGGGTCGTCGGTTCGATCCCGACTGGCTCCACCACTGGGATGAAGACACATTGGGTCTGTAGCTCAGGTGGTT AGAGCGCACCCCTGATAAGGGTGAGGCCGGTGGTTCGAGTCCTCCCAGACCCACCATTCTCTGGATGTCGATTGCGCACACATATCAAGATTTGAAGCGAACTGGCGCTGAAGCCGGTATCGC GTTCTTTGAAAACCGGGACGTAGCGAGCGTTTGAGACGAATGTCCAGACGTGTCGTAGAGGCTAAGGCGAGATGTCGAGAGACATCTTTATTGAAGTTGTAGTCGTTGTGTTCGCGACGACAT TAACTCCGAGGCGACTTGGGGTTATATGGTCAAGCGAATAAGCGCACACGGTGGATGCCTTGGCGGTCAGAGGCGATGAAGGACGTGGCAGCCTGCGAAAAGTGTCGGGGAGCTGGCAACAAG CTTTGATCCGGCAATGTCCGAATGGGGAAACCCGG	CP013140.1	*Lysobacter enzymogenes* C3
Vt6S4	TTGCGGCTGGATCCCCTCCTTTTGAGCAAAGACAGCATCGTCCTGTCGGGCGTCTTCACAAGTAACCTGCATTCAGAGTTTCACGTCGGCCTGGCCGGCGTGGATAGTCCCGTATATGGGGCC TTAGCTCAGCTGGGAGAGCACCTGCTTTGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCACAGGCCGCGAGTTTATCGACGCGGTCGCAATGAGCTGAACGGACATTGGGTCT GTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTAGTTCGAGTCTACCCAGACCCACCACTCTGAATGATCAGCGCATACAAAGAATTTATTACGGATCGGCATTGTGGCC GGTACGTGTTCTTTTAAAACTTGTGACGTAGCGAGCGTTTTGAGATTCTATCTTAGACGTGTCGTGAGGCTAAGGCGGAAGACTTAAATGTCTTCTTATTAATTGAGTCGTTATATTTCGTAT CTGGGCTTTGTACCCCCAGGTCATATATATAACCCGAGGCAACTTGCGGTTATATGGTCAAGCGAATAAGCGCACACGGTGGATGCCTTGGCGGTCAGAGGCGATGAAGGACGTGGCAGCCTG CGAAAA	CP007597.1	*Stenotrophomonas rhizophila* DSM14405
Vt6S5	GTAACCTGCATTCAGAGTTTCACGTCGGCCTGGCCGGCGTGGATAGTCCCGTATATGGGGCCTTAGCTCAGCTGGGAGAGCACCTGCTTTGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAG GCTCCACCACAGGCCGCGAGTTTATCGACGCGGTCGCAATGAGCTGAACGGACATTGGGTCTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTAGTTCGAGTCTACCC AGACCCACCACTCTGAATGATCAGCGCATACAAAGAATTTATTACGGATCGGCATTGTGGCCGGTACGTGTTCTTTTAAAACTTGTGACGTAGCGAGCGTTTTGAGATTCTATCTTAGACGTG TCGTGAGGCTAAGGCGGAAGACTTAAATGTCTTCTTATTAATTGAGTCGTTATATTTCGTATCTGGGCTTTGTACCCCCAGGTCATATATATAACCCGAGGCAACTTGCGGTTATATGGTCAA GCGAATAAGCGCACACGGTGGATGCCTTGGCGGTCAGAGGCGATGAAGGACGTGGCAGCCTGCGAAAAGTATCGGGGAGCTGGCAACAAGCTTTGATCCGGTAATGTCCGAATGGGGAAACCC GGA	CP007597.1	*Stenotrophomonas rhizophila* DSM14405
Vt6S6	CCTGCATTCAGAGTTTCACGTCGGCCTGGCCGGCGTGGATAGTCCCGTATATGGGGCCTTAGCTCAGCTGGGAGAGCACCTGCTTTGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTC CACCACAGGCCGCGAGTTTATCGACGCGGTCGCAATGAGCTGAACGGACATTGGGTCTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTAGTTCGAGTCTACCCAGAC CCACCACTCTGAATGATCAGCGCATACAAAGAATTTATTACGGATCGGCATTGTGGCCGGTACGTGTTCTTTTAAAACTTGTGACGTAGCGAGCGTTTTGAGATTCTATCTTAGACGTGTCGT GAGGCTAAGGCGGAAGACTTAAATGTCTTCTTATTAATTGAGTCGTTATATTTCGTATCTGGGCTTTGTACCCCCAGGTCATATATATAACCCGAGGCAACTTGCGGTTATATGGTCAAGCGA ATAAGCGCACACGGTGGATGCCTTGGCGGTCAGAGGCGATGAAGGACGTGGCAGC	CP007597.1	*Stenotrophomonas rhizophila* DSM14405

APPENDIX 6: 16S rRNA sequence results of soil-isolates

Strain Code	Sequence 5'-3'	NCBI Accession ID	Species/Strain
Vt2S2	GGTGCTTGCACTGGGGGATTAGTGGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCCTGACTCTGGGATAAGCCCGGGAAACTGGGTCTAATACCGGATATGACTTCCTGCCGCATGGTGGGT GGTTGAAAGATTTATCGGTGGGGGATGGACTCGCGGCCTATCAGCTTGATGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCTACTGGGACTGAGACG CGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGTAGGGAAGAAGC GAAAGTGACGGTACCTGCAGTAAGTGCGCCGGCTAGCTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGATTTATTGGGCGTAGAGAGCTCGTATGCGGTTTGTCGCGTC TGCCGTGAAAGTCCAAGGCTCAACCTTGGATCTGCGTGGGTACGGGCTCCTACGAGTGATGTACGGGAGACTGGAATTCCTGGTGTAGCGGTGAATGCGCAGATATTCCGAGGAACACCGATGGC GAAGGCAGTCTCTGGGATTTACTGGCGCTGAGAGCGAAAGTTGGTAACACCCGAAGCCGATGGCCTAACCACCTTGTGTGGGGGGAGTCGTCGAAGG	NR_136480.2	*Arthrobacter endophyticus* EGI 6500322
Vt2S4	TGCAAGTCGAACGATGAACCCGGTGCTTGCACTGGGGGATTAGTGGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCCTGACTCTGGGATAAGCCCGGGAAACTGGGTCTAATACCGGATATG ACTTCCTGCCGCATGGTGGGTGGTTGAAAGATTTATCGGTGGGGGATGGACTCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGAC CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAA CCTCTTTCAGTAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGATTTATTGGGCGTAAAGAG CTCGTAGGCGGTTTGTCGCGTCTGCCGTGAAAGTCCAAGGCTCAACCTTGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCA GATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCATTTACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACT AGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCAT GCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGTGCCAGACCGCCTCAGAGATGGGGTTTCCCTTCGGGGCTGGTTCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCCATGTTGCCAGCACGTAATGGTGGGGACTCATGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATC ATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCGGTACAATGGGTTGCGATACTGTGAGGTGGAGCTAATCCCTAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGAC CCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAAAGTTGGTAACACCCGAAGCCGATGGCCTAACC ACCTTGTGT	NR_136480.2	*Arthrobacter endophyticus* EGI 6500322
Vt2S5	CTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGGAGATGAT TGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACT GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGA AGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAA AGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATG CGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACACGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAG TGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAG CATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCT CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCA AATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACT CGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAAGTCGGTGGAGT AACCGTAA	CP026736.1	*Bacillus megaterium* YC4-R4
Vt2S6	GGGGAGTGGCAGACGGGTGAGTAACGCGTGGGAATCTACCCTTTTCTACGGGATAGCTCCGGGAAACTGGAATTAATACCGTATAAGCCCTTCGGGGGAAAGATTTATCGGGAAAGGATGAGCCC GCGTTGGATTAGCTAGTTGGTGAGGTAACGGCCCACCAAGGCGACGATCCATAGCTGGTCTAAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGG GGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCAGTGGGGACGATGATGACGGTACCCACAGAAGAAGCCCCGGCTAA CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGATATTTAAGTCGGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCC TTCGATACTGGGTATCTTGAGTGTGGTAGAGGTGAGTGGAATTGCGAGTGTAGAGGTGAAATTCGTAGATATTCGCAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCACAACTGACGCTGAG GTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGAAGCTAGCCGTCGGGGGATTTATCTCTCGGTGGCGCAGTTAACGCATTAAGCTTCCCGCCTGG GGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCTCTTGACATGCCTCGACGGTATC CGGAGACGGATGCCTTCCTTCGGGACGAGTGCACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCATTC AGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGCGGTGACAGTGGGCAGCCA ACCAGCGATGGTGAGCTAATCCCAAAAAGCCGTCTCAGTTCGGATTGCACTCTGCAACTCGGGTGCATGAAGTTGGAATCGCTAGTAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGG CCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTCTACCCGAAGGCGTTGCGCTAACTCGCAAGAGAGGC	NR_152083.1	*Aurantimonas endophytica* EGI 6500337

Vt3S2	TCCTTCGGGGTTTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCTTGGGTTCGGGATAACAGTTGGAAACGACTGCTAATACCGGATGATGGCTTCGGCCCAAAGATTTATCGCCCAGG GATGAGCCCGCGTAGGATTAGGTAGTTGGTGGGGTAAAGGCCCACCAAGCCGACGATCCTTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG CAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTTACCCGGGAAGATAGTGACTGTACCGGGAGAATAAGC TCCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGAGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCTTTGTAAGTAAGAGGTGAAAGCCTGGAGCTCAACTCC AGAACTGCCTTTTAGACTGCATCGCTTGAATCCAGGAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGGCTCACTGGACTGGTATT GACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCGGGGCTCTTAGAGTTTCGGTGGCGCAGCTAACGCATTAAGTT ATCCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAGAGGAATTGACGGGGGCCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCGTTTGACATGTCC GGACGATTTCGGGAGACCGATCTCTTCCCTTCGGGGACTGGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGACTTTAG TTACCATCATTAAGTTGGGTACTCTAGAGTAACCGCCGGTGATAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGCGCTGGGCTACACACGTGCTACAATGGCAGGTACA GTGGGCAGCAATCCCGCGAGGGTGAGCTAATCTCCAAAACCTGTCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGGCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAAT ACGTTCCCAGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGATTCACCCGAAGGCGTTGCGCTAACTCGCAAGAGAGGCAGGCGACCAC	KP191980.1	*Sphingomonas asaccharolytica* BGSLP2
Vt3S3	TTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTATTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTG CGCTAATAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCT ACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTAGATTAATA CTCTGCAATTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGT TAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGTCGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACA CCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAGCCTT GAGCTCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA ACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA GTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGC CAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATC GCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAACC	NR_152710.1	*Pseudomonas turukhanskensis* IB1.1
Vt3S4	CAGCACAGTGGTAGCAATACCATGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACCTTTTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACGACCTTAGGGTG AAAGCGGAGGACCTTCGGGCTTCGCGCGGATAGATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGGCCACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGG AACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTG GGAAAGAAAAGCAGTCGGTTAATACCCGATTGTTCTGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAACAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGC GTAAAGCGTGCGTATGTGGTTGTTTAAGTCCGTTGTGAAAGCCCTGGGCTGGCGGAATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGGGAGGAACATCAACCTGGGATTGCAGTGGATACTG GGCAACTAGAGTGTGGTAGAGGATCTGTGGCGAAGGCGGCCATCTGGACCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGAT GCGAACTGGATGTTGGGTGCAACTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTG GAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGC TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACG TCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTACTACAATGGTGAGGACAGAGGGCTGCAAGCTCGCGAGAGTAAGCCAATCCCAGAAAACCTCATCTCAGTCCGGATTGGAGTCTGCA ACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTTGCACCAGAAGCAGGTAG CTTAACCTTCGGGAGGGCGC	MH000694.1	*Xanthomonas translucens* Tal22
Vt4S1	TGCAGTCGAGCGGATGAAGGAGCTTGCTCCTTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTATTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTA CGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTAATAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCA CACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTT TAAGTTGGGAGGAAGGGTTGTAGATTAATACTCTGCAATTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTA CTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGTCGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAG CGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT AAACGATGTCGACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAA GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCAGAAAATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTC GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGG ATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAG TCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGGAAGT AGCTAGTCTAACCTTCGGGGAGGGACGGTTACCACGGTGTGGATTCATGACT	NR_152710.1	*Pseudomonas turukhanskensis* IB1.1

Vt6S2	AAGGAGCTTGCTCCTTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTATTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGG ACCTTCGGGCCTTGCGCTAATAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACA CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGG GTTGTAGATTAATACTCTGCAATTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCG CGTAGGTGGTTCGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGTCGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGA TATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAG CCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG TTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATC ATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGC GTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAACCTTCG GGAGGACGGTTACCACGGTGTGA	NR_152710.1	*Pseudomonas turukhanskensis* IB1.1
Vt6S4	AAGGAGCTTGCTCCTTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTATTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGG ACCTTCGGGCCTTGCGCTAATAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACA CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGG GTTGTAGATTAATACTCTGCAATTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCG CGTAGGTGGTTCGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGTCGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGA TATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAG CCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG TTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATC ATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGC GTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAAGTAGCTAGTCTAAC	NR_152710.1	*Pseudomonas turukhanskensis* IB1.1
Vt6S5	CGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTATTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTAATAG ATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC AGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTAGATTAATACTCTGCAAT TTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGA TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGTCGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAGCCTTGAGCTCTTA GTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGA ACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAA CGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTAC ACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAAT CGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGGAAGTAGCTAGTCT	NR_152710.1	*Pseudomonas turukhanskensis* IB1.1
Vt6S6	AGCTTGCTCCTTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTATTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCT TCGGGCCTTGCGCTAATAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGT CCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTG TAGATTAATACTCTGCAATTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTA GGTGGTTCGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGTCGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATA GGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGT TGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA ATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT GTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGG CCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGA AGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAACCTTCGGGAG GACGG	NR_152710.1	*Pseudomonas turukhanskensis* IB1.1

APPENDIX 7: Table of pollination studies

Legume	**# of pollinated flowers**	**# of obtained pods**	**# of cultured samples**
Bean	135	62	22
Faba	35	2	0
Lupine	312	0	0
Pea	19	0	0
V. turcica	20	0	0

APPENDIX 8: Bacterial identification results of the isolated bacteria

Vt in the sample code indicates *Vuralia turcica*. First number coming after indicates the location that the sample was taken from. The letter N signifies the rhizome while the letter S signifies the soil.

Samples	MIS result	16SrRNA result	ITS result
Vt1N1	*Bacillus megaterium*-GC subgroup B (0.469)	*Bacillus megaterium* DSW-CAP-1	*Bacillus megaterium* QM B1551
Vt1N2	*Bacillus megaterium*-GC subgroup A (0.855)	*Bacillus megaterium* YC4-R4	*Bacillus megaterium* QM B1551
Vt1N3	*Bacillus megaterium*-GC subgroup A (0.851)	*Bacillus megaterium* YC4-R4	*Bacillus megaterium* QM B1551
Vt1N4	*Bacillus megaterium*-GC subgroup A (0.755)	*Bacillus megaterium* YC4-R4	*Bacillus megaterium* JX285
Vt3N1	*Bacillus megaterium*-GC subgroup A (0.729)	*Bacillus megaterium* NSE1	*Bacillus megaterium* QM B1551
Vt3N2	*Brevibacillus parabrevis*-(0.598)	*Bacillus megaterium* YC4-R4	*Bacillus megaterium* QM B1551
Vt6N1	*Bacillus megaterium*-GC subgroup A (0.729)	*Bacillus megaterium* YC4-R4	*Bacillus megaterium* QM B1551
Vt6N2	*Bacillus megaterium*-GC subgroup A (0.834)	*Bacillus megaterium* YC4-R4	*Bacillus megaterium* QM B1551
Vt2S1	*Virgibacillus pantothenticus* (0.666);	No result	No result

Vt2S2	*Kocuria rhizophila* (0.694)	*Arthrobacter endophyticus* EGI 6500322	*Bacillus megaterium* JX285
Vt2S3	*Ochrobactrum anthropi* (Achromobacter Vd) (0.856)	No result	No result
Vt2S4	*Kocuria kristinae*-GC subgroup A (0.692)	*Arthrobacter endophyticus* EGI 6500322	*Rhodococcus erythropolis* R138
Vt2S5	*Bacillus megaterium*-GC subgroup A (0.805)	*Bacillus megaterium* YC4-R4	*Bacillus megaterium* JX285
Vt2S6	*Virgibacillus pantothenticus* (0.675)	*Aurantimonas endophytica* EGI 6500337	*Bacillus megaterium* JX285
Vt3S1	*Bacillus megaterium*-GC subgroup A (0.782)	No result	No result
Vt3S2	*Bacillus megaterium*-GC subgroup A (0.792)	*Sphingomonas asaccharolytica* BGSLP2	*Bacillus megaterium* JX285
Vt3S3	*Pseudomonas syringae*-syringae (0.380)	*Pseudomonas turukhanskensis* IB1.1	*Bacillus megaterium* JX285
Vt3S4	No result	*Xanthomonas translucens* Tal22	*Bacillus megaterium* QM B1551
Vt3S5	No result	No result	No result
Vt3S6	*Neisseria cinerea*-GC subgroup B (0.612)	No result	No result
Vt4S1	*Stenotrophomonas maltophilia* (0.305)	*Pseudomonas turukhanskensis* IB1.1	*Xanthomonas albilineans* IBSBF 1374

Vt4S2	*Stenotrophomonas maltophilia* (0.374)	No result	No result
Vt6S1	No result	No result	No result
Vt6S2	*Kocuria erythromyxa* (Deinococcus) (0.374)	*Pseudomonas turukhanskensis* IB1.1	*Lysobacter enzymogenes* C3
Vt6S3	*Stenotrophomonas maltophilia* (0.376)	No result	No result
Vt6S4	*Bacillus viscosus* (0.496)	*Pseudomonas turukhanskensis* IB1.1	*Stenotrophomonas rhizophila* DSM14405
Vt6S5	*Pseudomonas huttiensis* (0.605)	*Pseudomonas turukhanskensis* IB1.1	*Stenotrophomonas rhizophila* DSM14405
Vt6S6	*Pseudomonas huttiensis* (0.552)	*Pseudomonas turukhanskensis* IB1.1	*Stenotrophomonas rhizophila* DSM14405

APPENDIX 9: Flow cytometry result of the hybrid candidates