

**DEVELOPMENT OF FUNCTIONAL MUTANTS,
CLONING AND EXPRESSIONAL ANALYSIS OF
RESISTANCE GENE ANALOGS (RGAs) AND
TRANSCRIPTION FACTOR (TF) GENES IN
FRENCH BEAN (*Phaseolus vulgaris* L.)**

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**COLLEGE OF HORTICULTURE, BENGALURU- 560 065
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BAGALKOT
JUNE, 2016**

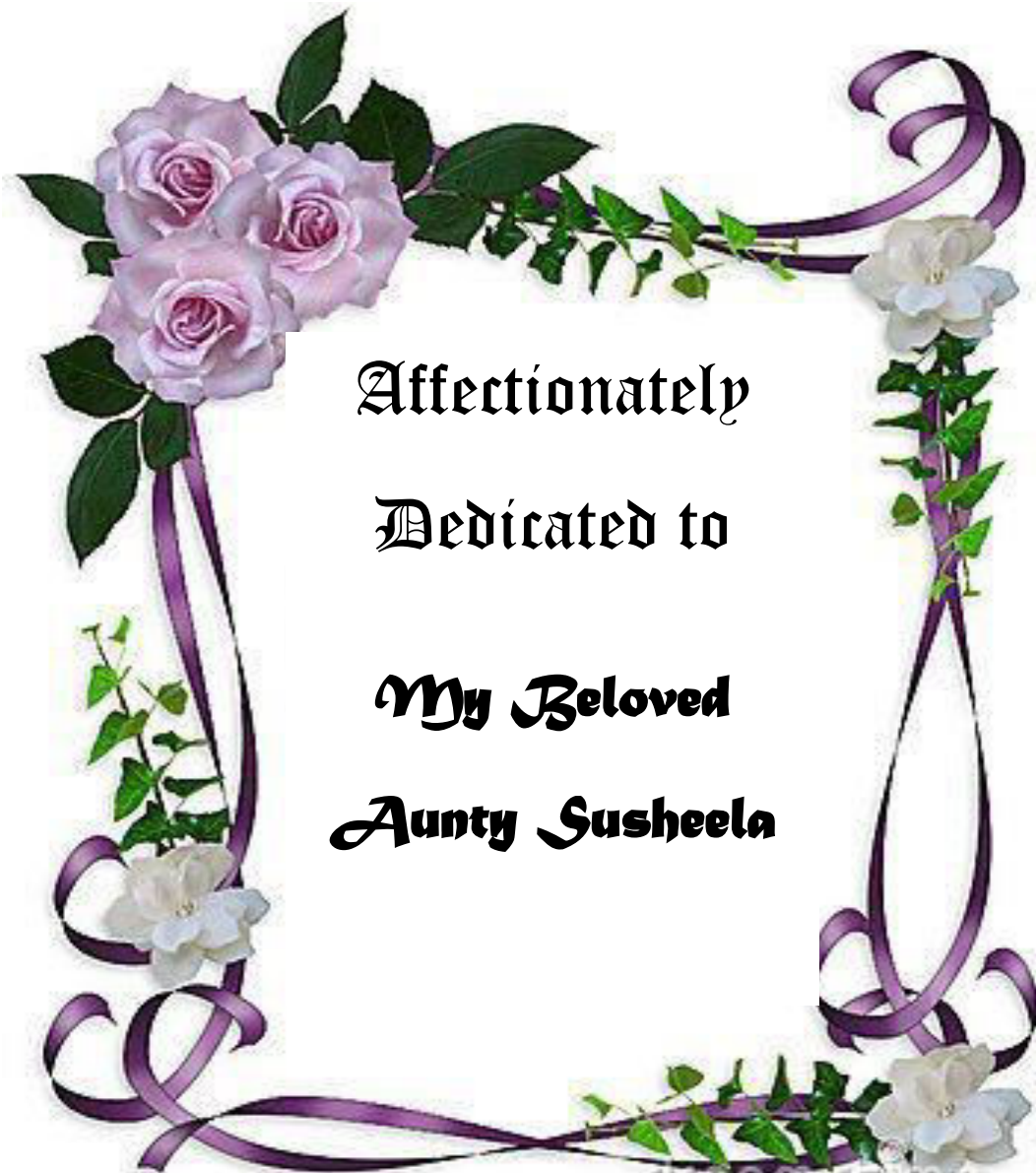
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*Thesis submitted to the
University of Horticultural Sciences, Bagalkot
in partial fulfillment of the requirements for the
Degree of*

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In
Horticulture

By
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**COLLEGE OF HORTICULTURE, BENGALURU- 560 065
UNIVERSITY OF HORTICULTURAL SCIENCES
BAGALKOT
JUNE, 2016**



Affectionately
Dedicated to
My Beloved
Aunty Susheela

**COLLEGE OF HORTICULTURE, BENGALURU
UNIVERSITY OF HORTICULTURAL SCIENCES, BAGALKOT**

CERTIFICATE

This is to certify that the thesis entitled " **DEVELOPMENT OF FUNCTIONAL MUTANTS, CLONING AND EXPRESSIONAL ANALYSIS OF RESISTANCE GENE ANALOGS AND TRANSCRIPTION FACTORS IN FRENCH BEAN (*Phaseolus vulgaris* L.)**" submitted by **Ms. DIVYA, B.** for the degree of **DOCTOR OF PHILOSOPHY** in **HORTICULTURE**, to the University of Horticultural Sciences, Bagalkot is a record of research work done by her during the period of her study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar titles.

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*Bengaluru
June, 2016*

(DIVYA, B.)

**DEVELOPMENT OF FUNCTIONAL MUTANTS, CLONING,
CHARACTERISATION AND EXPRESSONAL ANALYSIS OF
SELECTED RESISTANCE GENE ANALOGS (RGAs) AND
TRANSCRIPTION FACTOR (TF) GENES IN FRENCH BEAN
(*Phaseolus vulgaris* L.)**

DIVYA B.

ABSTRACT

The present study was undertaken to create spectral variability and to develop functional mutants using Ethyl Methyl Sulphonate (EMS) in the background of the cv. Arka Anoop of french bean. The EMS at 0.25% was found to be the LD₅₀. A set of 1500 plants in M₁ and similar population in plant-to-row manner in M₂ generation was studied for spectral variability. A wide range of variability for both qualitative and quantitative traits including pole type, thin, thick, dark green and pale green colour and variegated leaved, pink flowered and curved pod mutants in varying frequencies was recorded. A set of ten mutant entries from each mutant category constituting 100 mutant lines was evaluated in M₃ generation. Cloning and sequence analysis of 95 RGA clones revealed 85% - 99% similarity to known *R* genes and recorded P-Loop (13 clones), kinase (4 clones) and TIR (4 clones) in the NBS-LLR domains that fell into six distinct phylogenetic groups. The *in silico* genome wide identification of Transcription Factors (TFs) lead to the identification of 58 TF families with maximum homology in french bean. A set of 20 TF genes and 10 RGAs was tested for expression in manifesting the rust and the *MYMV* diseases. Nine RGAs and AP2-2, AP2-3, AP2-4, AP2-5, B3-1, B3-2, B3-3, BHLH-1, BHLH-2, BHLH-3, BHLH-4, BHLH-5, BZIP-1, BZIP-2 and BZIP-3 TFs found upregulated in rust resistance. COHFBRGA-3, 4, 5 and 7, AP2-3, AP2-5, B3-2, B3-3, BHLH-4, BZIP-1 and BZIP-2 genes down-regulated and COHFBRGA-2, 25, 27 and 38 genes of french bean were up-regulated in *MYMV* resistance manifestation compared to control.

June, 2016

Signature of the Major Advisor
(B. FAKRUDIN)

ತಿಂಗಳ ಹುರುಳಿಯ ಕ್ರಿಯಾತ್ಮಕ ಪರಿವರ್ತಿತಗಳ ಅಭಿವೃದ್ಧಿ, ಪ್ರತಿರೋಧಕ ವಂಶವಾಹಿ ಮತ್ತು ಪ್ರತಿಲಿಪಿ ಅಂಶಗಳ ನಿರ್ಲಿಂಗ ಸಂತಾನೋತ್ಪತ್ತಿ, ಚಿತ್ರಣ ಮತ್ತು ಹಾವಭಾವಗಳ ವಿಶ್ಲೇಷಣೆ

ದಿವ್ಯ ಬಿ.

ಸಾರಾಂಶ

ಪ್ರಸ್ತುತ ಅಧ್ಯಯನದಲ್ಲಿ ಈಥೈಲ್ ಮೀಥೈಲ್ ಸಲ್ಫೋನೇಟ್ (ಇಒS) ಮುಖೇನ ತಿಂಗಳ ಹುರುಳಿಯ ತಳಿ ಅರ್ಕ ಅನುಪ್ಪನಲ್ಲಿ ವೈವಿಧ್ಯತೆಯನ್ನು ಉಂಟುಮಾಡಲಾಗಿದೆ ಹಾಗೂ 0.25% ಇಒS ಪ್ರಮಾಣವನ್ನು ಇದರ ಐಆ50 ಎಂದು ಗುರುತಿಸಲಾಗಿದೆ. ಒ1 ಪೀಳಿಗೆಯಲ್ಲಿ 1500 ಗಿಡಗಳನ್ನು ಮತ್ತು ಈ ಎಲ್ಲಾ ಗಿಡಗಳನ್ನು ಒ2 ಪೀಳಿಗೆಯಲ್ಲಿ ಗಿಡ-ಸಾಲು ರೀತಿಯಲ್ಲಿ ಬೆಳೆಸಿ ಪರಿವರ್ತಿತಗಳ ಪುನರಾವರ್ತಿತ ದರದ ಏರಿಳಿತವನ್ನು ಮೌಲ್ಯಮಾಪನ ಮಾಡಿದಾಗ ಗುಣಾತ್ಮಕ ಮತ್ತು ಪರಿಣಾತ್ಮಕ ಗುಣಗಳಲ್ಲಿ ವ್ಯಾಪಕ ಏರಿಳಿತ ದಾಖಲಾಗಿದೆ ಹಾಗೂ ಗುಲಾಬಿ ಬಣ್ಣದ ಹೂವು, ತೆಳು, ಮಂದ, ಕಡು ಹಸಿರು, ಮಸುಕಾದ ಹಸಿರು, ಮಿಶ್ರ ವರ್ಣದ ಎಲೆ, ಬಾಗಿದ ಮತ್ತು ನಾರುರಹಿತ ಕಾಯಿಗಳನ್ನು ಹೊಂದಿರುವ ಪರಿವರ್ತಿತ ಗಿಡಗಳನ್ನು ಗುರುತಿಸಲಾಗಿದೆ. ಪ್ರತಿ ರೂಪಾಂತರಿತ ವರ್ಗದಲ್ಲಿ ಹತ್ತು ರೂಪಾಂತರಿತ ನಮೂದುಗಳನ್ನು ಆಯ್ಕೆ ಮಾಡಿ, ಒಟ್ಟಾರೆ 1000 ಗಿಡಗಳನ್ನು ಒ3 ಪೀಳಿಗೆಯಲ್ಲಿ ಪರೀಕ್ಷಿಸಲಾಯಿತು. ನಿರ್ಲಿಂಗ ಸಂತಾನೋತ್ಪತ್ತಿಯಿಂದ ಪಡೆದ 95 ವಸಾಹತುಗಳನ್ನು ಅನುಕ್ರಮ ವಿಶ್ಲೇಷಣೆಗೆ ಗುರಿಪಡಿಸಿದಾಗ ಆರ್-ವಂಶವಾಹಿಗಳೊಂದಿಗೆ 85-95% ಹೋಲಿಕೆ ಕಂಡುಬಂದಿದ್ದು. ಎನ್‌ಬಿ ಎಸ್- ಎಲ್‌ಎಲ್‌ಆರ್ (ಓS-ಐಐಐ) ಜಾಗದಲ್ಲಿ ಕೈನೇಸ್ (13 ವಸಾಹತುಗಳ), ಪಿ-ಲೂಪ್ (4 ವಸಾಹತುಗಳ) ಮತ್ತು ಟಿಐಆರ್ (4 ವಸಾಹತುಗಳ) ಇರುವಿಕೆ ದೃಢಪಟ್ಟಿದೆ ಮತ್ತು ಇವುಗಳನ್ನು ಆರು ಬೇರೆ ಜಾತಿವಿಕಸನೀಯ ಗುಂಪುಗಳಾಗಿ ವಿಂಗಡಿಸಲಾಗಿದೆ. ಇನ್-ಸಿಲಿಕೊ ವಂಶವಾಹಿಯಿಂದ ಪ್ರತಿಲಿಪಿ ಅಂಶಗಳನ್ನು ತಿಂಗಳ ಹುರುಳಿಯಲ್ಲಿ ಗುರುತಿಸಿದಾಗ 58 ಪ್ರತಿಲಿಪಿ ಕುಟುಂಬಗಳು ಗೋಚರಿಸಿವೆ. ಡೋಮೈನ್‌ಗಳನ್ನು Pಜಿಚಿm ನಲ್ಲಿ ಪತ್ತೆಹಚ್ಚಿದ್ದು, 8 ಕುಟುಂಬಗಳಲ್ಲಿ ಪ್ರತಿ ಲಿಪಿ ವಂಶವಾಹಿ ಕುಟುಂಬಗಳ ಡೋಮೈನ್ ಇರುವಿಕೆಯು ದೃಢಪಟ್ಟಿದೆ. ಗುಣಧರ್ಮಗಳನ್ನು ಊಹಿಸಿ 20 ಪ್ರತಿ ಲಿಪಿ ವಂಶವಾಹಿ ಮತ್ತು 10 ಪ್ರತಿರೋಧ ವಂಶವಾಹಿಗಳನ್ನು ತುಕ್ಕು ಮತ್ತು ಹೆಸರುಕಾಳಿನ ಹಳದಿ ಮೊಸಾಯಿಕ್ ನಂಜಾಣು ರೋಗಗಳಿಗೆ ಕ್ಯೂಆರ್‌ಟಿ-ಪಿಸಿಆರ್ (qಬಿಖಿ Pಅಖಿ) ಮೂಲಕ ಹಾವಭಾವಗಳ ವಿಶ್ಲೇಷಣೆಯನ್ನು ಪರೀಕ್ಷಿಸಿದಾಗ 9 ಪ್ರತಿರೋಧ ವಂಶವಾಹಿಗಳು (ಖಲೂಒs) ಮತ್ತು 15 ಪ್ರತಿಲಿಪಿ ವಂಶವಾಹಿಗಳು (ಖಿಈs: ಒP2-2, 3, 4, 5, ಂ3-1, 2, 3, ಂಊಐಊಊ-1, 2, 3, 4, 5, ಂZIP-1, 2 ಮತ್ತು 3) ತುಕ್ಕು ನಿರೋಧಕ ತಳಿಯಲ್ಲಿ ಮೇಲ್ಮುಖವಾಗಿ ನಿಯಂತ್ರಿಸಲ್ಪಟ್ಟಿತ್ತು. ಹೆಸರುಕಾಳಿನ ಹಳದಿ ಮೊಸಾಯಿಕ್ ನಂಜಾಣು ರೋಗಕ್ಕೆ ಸಸಪ್ಪಿಬಲ್ (susಫೀರಿಣಿಹಿಟೇ) ತಳಿಯಲ್ಲಿ ಅಔಊಊಃಖಲೂಒ-3, 4, 5 ಮತ್ತು 7, ಒP2-3 ಮತ್ತು 5, ಂ3-2 ಮತ್ತು 3, ಂಊಐಊಊ-4, ಂZIP-1 ಮತ್ತು ಂZIP-2 ಜೀನುಗಳು ಕೆಳನಿಯಂತ್ರಿಸಲ್ಪಟ್ಟಿವೆ. ಅಔಊಊಃಖಲೂಒ-2, 25, 27 ಮತ್ತು 38 ಜೀನುಗಳು ಮೇಲ್ಮುಖವಾಗಿ ನಿಯಂತ್ರಿಸಲ್ಪಟ್ಟಿವೆ.

ಜೂನ್, 2016

ಪ್ರಧಾನ ಮಾರ್ಗದರ್ಶಕರು

(ಬಿ. ಫಕ್ರುದ್ದೀನ್)

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LIST OF ABBRIVATIONS

Sl. No.	Abbreviations	Description
1	%	Per cent
2	°C	Degree Celsius
3	µg	Microgram
4	µl	Microlitre
5	<i>APS</i>	ATP sulfurylase
6	<i>ARF</i>	Auxin Response Factor
7	BLAST	Basic local aligenment search tool
8	bp	Base pair
9	cm	Centimeter
10	CTAB	Cetyl Trimethyl Ammonium Bromide
11	CV	Coefficient of variability
12	<i>DCL</i>	Dicer-like protein
13	DEPC	Diethyl Pyrocarbonate
14	DNA	Deoxyribo Nucleic Acid
15	dNTP	Deoxy Nucleotide Tri Phosphate
16	EDTA	Ethylene Diamine Tetra Acetic acid
17	<i>EIF-4A</i>	Translational initiation factor eIF 4A
18	EMS	Ethyl Methane Sulphonate
19	<i>et al</i>	Et allii (and others)
20	EtBr	Ethidium Bromide
21	Fig.	Figure

22	g	Gram
23	GA	Genetic Advance
24	GAM	Genetic Advance as per cent Mean
25	GCV	Genotypic coefficient of Variability
26	h^2	Heritability in broad sense
27	ha	Hectare
28	<i>LAC</i>	Laccase
29	LD	Lethal Dose
30	LNA	Locked Nucleic Acid
31	<i>MADS</i>	MADS-box transcription factors
32	Max.	Maximum
33	Mg	Magnesium
34	min	Minute
35	Min.	Minimum
36	miRNA	microRNA
37	ml	Millilitre
38	mM	Millimeter
39	MT	Metric tones
40	nm	Nanometer
41	PCR	Polymerase Chain Reaction
42	PCR	Polymerase chain reaction
43	PCV	Phenotypic Coefficient of Variability
44	PIs	Plant Introductions
45	pM	Picometer

46	<i>PPR</i>	Pentatricopeptide repeat
47	RAPD	Random Amplified Polymorphic DNA
48	<i>RNAase</i>	Ribonucleases
49	rpm	Revolutions Per Minute
50	S.Ed±	Standard Error of Difference
51	S.Em±	Standard Error of Mean
52	T ₁₀ E ₁	Tris EDTA
53	TAE	Tri Acetate EDTA
54	TFs	Transcription factor
55	UAS	University of Agricultural Sciences
56	<i>UBC24</i>	Ubiquitin conjugating enzyme
57	UHS	University of Horticultural Sciences
58	UV	Ultra Violet
59	<i>viz.</i>	As follows

I. INTRODUCTION

French bean, *Phaseolus vulgaris* L. ($2n = 22$) is a member of the family Fabaceae. It is an important legume vegetable grown for its tender green pods either for fresh consumption or for processing as canned, frozen or freeze dried product. It is a nutritive vegetable which supplies protein (1.8 g), calcium (132 mg), thiamin (0.08 mg), riboflavin (0.06 mg) and vitamin C (24 mg) per 100 g of edible pods. Its pods can be used to strengthen diuretic, flushing of toxins from the body and for the treatment of diabetics (Prajapati, 2003). It is also referred as common bean, pulse or rajma type where dried seeds are consumed as food.

It is native of new world, principally Central and South America (Kaplan, 1981) with small genome 633 Mbp. It is originated from wild species *Phaseolus aborigineus* L. and domesticated in Mexico, Peru and Colombia about 8000 years ago (Arumuganatham and Earle, 1991). It is self pollinated crop. Plants may be dwarf bush or pole type, depending upon growth habit (Vanderplank, 1963). Leaves are trifoliate; flowers are diadelphous (9+1) in condition. Flowers may be white, purple or pale yellow in colour. Pods are round or oval or flat on cross section with or without fiber.

As per the FAO estimates, In world, french bean is grown over an area of 1.53 million ha with annual production of 20.74 million MT and the productivity of 13.5 t/ha. In India, its cultivation is in 0.22 million ha with production of 0.62 million MT and productivity of 2.8 t/ha (Anon., 2015). It is extensively grown in Himachal Pradesh, Punjab, Haryana, Uttaranchal, Bihar, Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu. In Karnataka, it is cultivated in an area of 11986 ha with annual production of 138137 tones and productivity of 11.53 t/ha (Anon., 2015).

Being a self pollinated crop, in french bean the genetic variability is rather limited. Moreover, interspecific and inter varietal incompatibilities pose problems in crossing and getting desirable recombination. Thus, it has been difficult to develop improved varieties by exploiting the limited genetic variability available through selection and recombination breeding. The discovery, that X-ray induced mutations in the fruit fly (Muller, 1927) and in barley (Stadler, 1928), initiated a new field – induced mutagenesis, which was soon taken up by many plant breeders and geneticists to investigate the use of radiation induced mutations for modifying plant traits.

Later, a number of chemical mutagens were also identified for this purpose. Use of mutations is a valuable supplementary approach to plant breeding. It creates variability, qualitative and quantitative, required for selection. Mutations are very helpful particularly when it is desired to improve one or two easily identifiable characters in an otherwise well adapted variety. A desired mutation can be recovered in a homozygous stage in M_2 or M_3 generation compared with F_6 or F_7 generation in case of hybridization. Further, a single mutant can be exploited easily to develop a new variety through pedigree selection in self-pollinated crops, as there is no inbreeding depression in these crops. As in hybridization, in mutation breeding too, it is imperative to know the nature and magnitude of components of induced genetic variation to choose appropriate breeding methodology for maximum exploitation of induced variability. Also, it is usually desirable to take the best available genotype lacking in one or two traits, which could be improved through mutagenesis.

Biotic stresses such as pest and pathogen have detrimental effects on plant growth and development and play a major role in determining the distribution of plant species across environments. Biotic stress, like pathogens have gained further importance in recent times due to changing global climatic regimes. Crops are affected by biotic stress throughout the life cycle, with the greatest losses being observed when stress occurs in the development phase just before and after flowering (Lopez *et al.*, 2003). Depending upon an extent of stress, plants try to acclimatize to the changing environmental conditions. Therefore, under these conditions, the

adaptive mechanisms should be directed to this objective. The closure of stomata limits entry of pathogen, the initiation of a series of physiological processes. In addition to external stress signals, a variety of internal signals such as hormones and solutes modify plant cell growth and development. A cascade of complex events involving several interacting components is required for initial recognition of signal and subsequent transduction of these signals to the physiological response is triggered. The cascade of events is called signal transductions, which normally act through second messengers that can trigger the molecular events leading to physiological response, often by modifying the gene expression pattern (Trewavas, 1997; Kaur and Gupta, 2005). Studies on molecular responses of plants to various types of stresses indicate that different types of stresses provide the cells with different signaling information. The multiplicity of the information generated by different types of stresses makes the response of plants complex and hence also the stress signaling pathways.

The products of stress-inducible genes include the enzymes responsible for the synthesis of various antioxidants, proteins, chaperones and detoxification enzymes and another group of gene products involved in gene expression and signal transduction pathways includes transcription factors, protein kinases and enzymes of metabolic pathways. Further, a thorough analysis of available information indicates that there are interconnection between biotic and abiotic signal transduction pathways (Yamaguchi-Shinozaki and Shinozaki, 2006).

Many defense responses are initiated by resistance gene/genes, providing a mechanism by which the plant can recognize a pathogen and execute a defense response against it. Plant resistance (R) genes are thought to be one of the components of the genetic resistance mechanism in plants (Flor, 1956). In recent years, many disease resistance genes have been cloned from diverse plant species (Baker *et al.*, 1997). Five main classes of R genes have been defined according to the structural characteristics of their predicted protein products (Dangl and Jones, 2001) and majority of them are of nucleotide binding site-Leucine Rich Repeat (NBS-LRR) type.

Conserved motifs in NBS domain have been used to isolate Resistance Gene Analogs (RGAs) from many crop species (Collins *et al.*, 1998). As a result, a sizable collection of PCR-derived NBS-LRR sequences from diverse plant species are now available. Many of these sequences have been located to chromosomal regions containing major R-genes as well as quantitative trait loci (QTLs). Thus, conserved domains serve as signatures to isolate the full length versions of resistance gene homologues in crop plants. NBS-LRR homologues encode structurally related proteins, suggesting that they function in common signal transduction pathways, even though they confer resistance to a wide variety of pathogen types. The few functionally defined R-genes and the much larger repertoire of resistance gene homologues available in the databases provide a facile system to study the interaction between plants and pathogens.

Transcription factors (TFs) are master-control proteins in all living cells. They often exhibit sequence specific DNA binding and are capable of activating or repressing transcription of multiple target genes. In this way, they control or influence many biological processes, including cell cycle progression, metabolism, growth and development and responses to external environments. Alteration in the expression of TF genes normally results in dramatic changes to a plant (Latchman, 2003; Broun, 2004). In recent times knowledge of genomic resources representing the gene regulatory networks and pathways along with their patterns of expression is available in model systems such as *Arabidopsis thaliana* and to some extent in rice and maize, which can serve as reference resource to explore the gene networks and specific pathways associated with response of crop plants to biotic stresses.

Development of plant organs is determined by differential gene expression which can be regulated at different levels. The major mechanism of differential gene expression is transcriptional regulation which is controlled by transcription factors that bind to DNA *cis*-elements

(Lee and Young, 2000). Identification and functional characterization of TFs at transcripts and whole genome level using computational approaches are the preliminary step in understanding the mechanism of TFs expression and regulation (He *et al.*, 2010). The rapid expansion in the next generation sequencing (NGS) technologies, a large volume of data in genome and transcriptome level has been developed and it will facilitates identification of putative TF in other genomes through computational studies (Riechmann *et al.*, 2000; Martínez-Bueno *et al.*, 2004;). The availability of TF families resource at genomic level in model plant such as *Arabidopsis thaliana* can serve as reference source to explore the TFs associated with biological processes in unknown TF families in genomes. In legume plants, however, genome-wide computational identification of TFs reported in few genomes. But, no TFs characterization has been done in french bean till-to-date.

Keeping these in view, the present investigation on “development of functional mutants, cloning and expressional analysis of resistance gene analogs and transcription factors in french bean (*Phaseolus vulgaris* L.)” has been undertaken with the following objectives.

1. Prospecting the induced mutations (EMS) to create spectral variability in targeted loci for the development and characterization of functional mutants population in french bean.
2. Genome-wide identification, cloning and characterization of selected resistant gene analogs (RGAs) and transcription factor (TF) genes in french bean.
3. Assessing the expressional dynamics of selected *R*-genes and transcription factors in manifestation of rust and *MYMV* diseases.

II. REVIEW OF LITERATURE

Mutations are recognized since the beginning of this century as one of the driving forces of evolution, besides selection and hybridization (Micke *et al.*, 1990). First observation about artificial induction of genetic changes dates back to the beginning of 20th century (Gager, 1908), but proper proof of such induced changes came in the late twenties with the discovery of X-rays induced mutations in the fruitfly, *Drosophila melanogaster* (Muller, 1927) and barley (Stadler, 1928).

Use of mutagenesis strictly for plant breeding initiated in Germany (Freisleben and Lein, 1942) and it began to flourish in several countries by 1950, using mainly gamma rays or neutrons. However, a certain disappointment about the detrimental effects of ionizing radiations during 1960's led to the identification and use of chemical mutagens by the researchers (Auerbach, 1961). The Indian Agricultural Research Institute (IARI) has been a pioneer Institution for research on induced mutations in the country since 1957, and has released many mutant varieties of crops and ornamentals. Various researchers (Jinks, 1954, Hayman, 1954, 1958; Scossiroli, 1962, Palenzona, 1965; Lawrence, 1965 and Scossiroli *et al.*, 1966) used different biometrical analysis methods to demonstrate that the increase of phenotypic variation following irradiation in generations, particularly in self pollinated plants, is mainly due to an increase in genetic components and it may be accounted for by the effect of mutations on the genetic factors influencing quantitative characters.

Yonezawa (1979) proposed a method for predicting the additive dominance components induced variations, which are predictable up to M3 generation, as the M3 is regarded as the best generation to start selection for breeding. This method overcomes the shortcomings of the methods earlier proposed by Aastveit and Gaul (1967) and Virk *et al.* (1978) which requires a single generation of population with hierarchical pedigree system.

In other side, plants cannot move to escape environmental challenges like biotic and abiotic stresses. Biotic stresses results from a battery of potential pathogens: fungi, bacteria, nematodes and insects intercept photosynthate produced by the plants and viruses use replication machinery at the host's expense.

Among such diseases, rust caused by *Uromyces phaseoli* Reben Wint. and MYMV are major destructive diseases in beans which causes severe yield loss. The pathogens have many physiological races which pose severe problem in bean growing regions of the world. Owing to their severity, many plant breeders and pathologists attempted to find the remedies for the constraints. Considerable research has been undertaken in order to study the nature, mechanism of resistance and understand the environment-host- pathogen interaction and to identify the most effective control measure for the diseases. Use of chemicals to control diseases causes environmental pollution and also makes its cultivation costly.

Plants have evolved sophisticated mechanisms to perceive such interactions, and to translate that perception into an adaptive response (Dangl *et al.*, 2001). The plants responds to infection using a two branched innate immune system. The first branch recognizes and responds to molecules common to many classes of microbes, including non-pathogens. The second response to the pathogen virulence factors either directly or through their effects on host targets. The latter includes the R-genes and TFs mediated resistance. Therefore, growing cultivars with inherent resistance to major diseases is the most cost-effective and environment friendly method of protecting crop plants from diseases. With the advancement in molecular techniques and approaches, *in silico* genome identification of TFs, cloning specific genes has become easy and genes for specific traits have been deployed in selected crop plants. In this endeavor, *in silico* gene identification, cloning and characterization of plant transcription factors and disease resistance (R) genes have become a major long-term strategy in several crop plants worldwide.

Therefore, the available literature relating to the induced mutation, long-term strategy for disease resistance especially cloning, characterization and expressional analysis of resistance gene analogs and transcription factors in response to rust and MYMV disease manifestation in french bean are reviewed here under following heads.

2.1 Mutation

2.1.1 Mutagenic treatment and handling of crop

Mohan *et al.* (1980) suggested 0.035-0.045 M EMS treatment for 7.5 hours as the most optimum for inducing mutations in beans. Cheah and Lim (1982) suggested that 30 kR gamma irradiation should be used for mutation breeding in this crop, while Manrique *et al.* (1988) suggested 5 and 10 kR doses as most effective ones giving 50 per cent survival of M₁ plants. Highest mutation frequency was observed following gamma irradiation at 8-12 kR (Dekov and Radkov, 1982). Cheah and Lim (1982) also recommended a suitable procedure for handling of M₁ and M₂ generations. According to them, M₁ seed should be harvested in such a way that single seed bulk population is used for M₂ screening and irradiated seeds should be planted in the field at double the normal planting density of the control.

Milkov (1989) obtained the highest survival with 0.2 per cent EMS and 6 kR gamma rays, while the combined treatment resulted in the greatest number of morphological mutants in M₂. Inverse relationship between germination percentage and mutagen concentration or dose was observed when *Phaseolus vulgaris* was tested for sensitivity to different doses of gamma rays and chemical mutagens (Budennyi and Naumov, 1994). Gamma radiation appeared highly effective. It has been observed by number of researchers that physiological/ biological damage caused by gamma rays depends upon its dose (Yankulov *et al.*, 1980; Ciftci *et al.*, 1994 a, b; Singh and Verma, 1998 and Dimova *et al.*, 2000).

Sena *et al.* (1991) reported that highest mutation frequencies were induced with 24 kR gamma rays while Kalonji- Myubi *et al.* (1993) found 240-280 Gy gamma ray dose to be most suitable in variety Rubona 5, on the basis of the epicotyl length. Vieira *et al.* (1995) observed that gamma irradiation at 20 and 25 kRad generated the greatest variation for several morphological characteristics from M₁ to M₃ in French bean cv. Ouro, and Carneiro *et al.* (1995) obtained the highest number of mutants with 0.125 per cent EMS treatment in the same cultivar.

However, Svetleva and Mekhandzheiv (1999) reported that combined application of gamma rays and EMS on bean seeds increased their effectiveness and mutation frequency than if they were applied alone. Dimova *et al.* (2000) reported a significant negative correlation ($\gamma = -0.069$ to -0.95) between increasing doses of radiation and traits characterizing productivity.

2.1.2 Chlorophyll and morphological mutations

Yankulov *et al.* (1980) found a direct correlation between mutagen dose and the percentage of chlorophyll mutants in the M₂. Barbosa *et al.* (1988) treated the germinating seeds with 0.0625, 0.125, 0.25 and 0.5 per cent ethyl- methane sulphonate (EMS) solutions and observed that the frequency of chlorophyll deficiency mutants' albina, xantha and viridis increased with increasing concentrations upto 0.25 per cent with viridis the most frequent and albina the least frequent for all EMS concentrations.

Gautam *et al.* (1998) also found a progressive increase in mutation frequency of chlorophyll and viable mutations with the increase in gamma rays and EMS dose. Ronning *et al.* (1991) investigated factors involved in chlorophyll breakdown during foliar senescence in a non-yellowing mutant of *Phaseolus vulgaris*. They concluded that there might be a difference in the

lipid environment in such a mutant than that of normal chloroplasts. Barbosa and Sena (1992), while scoring for chlorophyll mutations in the gamma irradiated M_2 , found that the M_1 plants were derived from about 2-9 initial embryo cells but the data were not sufficient to indicate whether the mutated sectors were randomly distributed on the M_1 plants or not. Sena and Barbosa (1992) reported chlorophyll and morphological induced mutants by gamma rays in common bean. Four types of leaf mutants were reported to be controlled by a single recessive gene in each case by Bassett (1992).

Sena *et al.* (1991) obtained mutants with white flowers, different seed coat etc. with gamma irradiation. Tulmann *et al.* (1994) selected two mutants with determinate habit in M_2 after treating the seeds of indeterminate variety Carioca with 32 kR gamma rays and these ripened 9-days earlier than the control. Guner and Myers (2001) characterized a single stemmed mutant in common bean with branching nearly absent and designated this trait as topiary and assigned gene symbol 'top'.

2.1.3 Polygenic variability

Swarup and Gill (1968) treated French bean seeds with X rays and analysed their means and variances in the following generations. They isolated several families with higher pod and seed number per plant, and higher 100-seed weight in the M_4 generation. Tulmann Neto *et al.* (1983) stated that M_2 and M_3 populations of gamma irradiated or EMS treated seeds displayed a greater variability in several attributes as compared to untreated controls, and selection was more effective in the progenies of determinate than of indeterminate varieties in producing higher yielding, more uniform and more rust resistant lines.

Cheah (1988) reported increased variability in yield components by treatment with gamma rays at 30kR while Svetleva and Dimova (1992) did not find any significant differences from control in yield related characters in M_2 . They found more marked effects of the mutagen in M_1 itself. Svetleva and Petkova (1992) stated that combined treatment with gamma rays and N-ethyl-N-nitrosourea prolonged the maturity period of the M_1 but not the M_2 generation. Tulmann Neto *et al.* (1994) obtained a mutant with increased basal height (by a factor of 1.7) in M_2 after treatment with a 24 kR gamma ray dose, while Nandi (1995) obtained two mutant lines, one of which had significantly greater pod length, diameter, and weight than the untreated parent, and other with significantly more number of pods per plant and a 47 per cent higher green yield per plant. High genotypic coefficient of variation in green pod yield per plant along with high genetic advance and heritability estimates was also observed (Nandi *et al.*, 1996)

Dimova *et al.* (1997) found that seed weight per plant was indirectly and positively correlated with plant height, number of fruiting branches, number of pods per plant and number of seeds per plant in mutant lines and the control. Data from M_1 of gamma irradiated and EMS treated seeds on six yield components revealed high levels of genetic variance, heritability and genetic advance in most of the traits (Ignacimuthu and Babu, 1990). Jai Dev and Gupta (1997) recorded high heritability estimates for most of the traits of gamma irradiated varieties and concluded that selection within lines would be more effective than between lines and should be practiced from the M_3 onwards. Further, Jai Dev and Gupta (1998) observed significant, positive, additive and dominance genic effects for almost all the traits in all the doses in Jawala. However, they obtained varying effects for different traits in different doses in HPR-35.

Nandi *et al.* (1998a) obtained significantly high yielding mutant lines in 30kR gamma irradiated populations. Nandi *et al.* (1998b) also studied the genetic divergence and grouped the advanced mutants in four clusters. Cluster I had the maximum number of mutants and recorded highest number of pods per plant and green pod yield per plant whereas, cluster IV had mutants for length, girth and weight of pods and also 100- seed weight. Dimova *et al.* (2000) observed that the traits *viz.*, pod length, number of fruiting branches and height of the first pod set were

relatively more stable to increasing doses of gamma rays as compared to plant height, number of pods per plant and number of seeds per plant.

2.1.4 Isolation of useful mutants

Reddy (1982) obtained some mutants which were resistant to drought, *Xanthomonas phaseoli* and *Colletotrichum lindemuthianum* and developed a variety which excelled the well adapted variety by in yield, protein content and had better cooking quality. Allavena *et al.* (1985) obtained two new dwarf cultivars 'Montalbano' and 'Mogano' resistant to bean common mosaic virus (BCMV) after treating the seeds of line P-106 and P-224 with ethyl-methane sulfonate while Tulmann Neto *et al.* (1983) selected some plants in EMS treated M₂ generation of 'Carioca' and M₃ of 'Roxo 749' which were less susceptible to *Xanthomonas phaseoli* than their parent. Similarly mutant groups resistant to mixture of *Uromyces phaseoli* were also obtained (Fadl, 1988; Ribeiro *et al.*, 1993). Manrique *et al.* (1988) isolated two plants with different growth habits and two with changed flower colour than that of the parent in M₆ generation. Similarly, a bushy mutant which was 5 to 14 days earlier than the indeterminate parent variety in M₂ (Tulmann Neto *et al.*, 1994).

Jai Dev and Gupta (1997) selected some lines in M₁-M₃ of gamma irradiated cv. Canadian Wonder for resistance to anthracnose, angular leaf spot and rust. These lines when advanced, gave higher seed yield (11 per cent) and 100-seed weight and matured earlier than non-irradiated controls in M₅ and M₆ generations. Myers and Bassett (1993) obtained two unifoliate mutants and found them to be under single recessive gene control. Rep'ev *et al.* (1991) selected desirable mutants for different traits i.e., either they were early maturing, or with high seed yield, or with good nodulation or were bearing pods on upper branches. Svetleva *et al.* (1999) selected new lines which were of considerable value for breeding. They further reported that the number of pods per plant exhibited the highest direct effect on marketable yield. On the other hand, Stoyanova *et al.* (1999) obtained three stabilized mutant bean lines of cv. Dubrudzhanski- 2 with improved biochemical (crude protein) and technological (seed coat) qualities which were significantly different from their parental cultivar. Few M₂ progenies of 5, 10 and 15kR gamma irradiated and 0.02, 0.03 and 0.04M EMS treated 'Contender' and 'Him 1' varieties of french bean were found moderately resistant to floury leaf spot and bacterial blight pathogens (Ashok Kumar and Kaushal, 2003).

2.2 Cloning and characterization of RGAs

2.2.1 Resistance (R) genes and Resistant Gene Analogues (RGAs): Biology and Importance

Resistance (R) genes are thought to be the major components of plants defense against a variety of pathogens. Considerable knowledge has accumulated on the biochemical and genetic basis of disease resistance, while the use of resistant cultivars is the most valuable strategy to control diseases (Hammond-Kosack and Jones, 1996; Michelmore, 1995; Crute and Pink, 1996). In many plant-pathogen interactions, incompatibility (resistance to disease) is controlled by the interaction of plant encoded R-genes and their corresponding pathogen-encoded avirulence genes (Avr-genes) of matched specificity. The "gene-for-gene" model predicts that the pathogen Avr-gene products act as elicitors and are directly or indirectly recognized by specific receptors, the products of plant R-genes. Specific interaction between an elicitor and its receptors trigger a signal transduction pathway that is thought to induce a strong resistance response by activating an array of host defense mechanisms (Flor, 1971; Lamb, 1996; Gabriel and Rolfe, 1990).

The R-genes constitutively expressed in a plant form a part of the refined surveillance and recognition system that detects the presence of a pathogen by interacting with its elicitor

molecules during infection. The hallmark of most *R-Avr* gene interactions is the activation of the hypersensitive response (HR), in which cells surrounding the sites of attempted pathogen ingress undergo immediate death to prevent further colonization. HR can be phenotypically diverse, but it usually appears as very small necrotic flecks within hours of contact with the pathogen (Dangl *et al.*, 1996, Dasgupta *et al.*, 2003). Other features of the R-gene-mediated response include the induced synthesis of antimicrobial metabolites (often referred to as phytoalexins), pathogenesis-related (PR) proteins and enzymes harmful to invading pathogens, reinforcement of the plant cell wall, generation of toxic amounts of reactive oxygen species, and the initiation of a distinct signal transduction pathway known as a systemic acquired resistance (SAR) (Bent, 1996; Ryals *et al.*, 1996 and Metraux, 2001).

Specific recognition of multiple pathogens could necessitate the activity of numerous R-genes. Alternatively, the guard hypothesis proposes that resistance proteins guard plant targets against pathogen effector proteins. In this scenario, the pathogen products act as virulence factors to enhance the susceptibility of the host plant in the absence of specific recognition (Van-der Biezen and Jones, 1998a; Dangl and Jones, 2001).

R genes have been shown to encode two broad categories of Leucine Rich Repeat (LRR) proteins that can be distinguished by protein domain structure and site of pathogen perception (Jones and Takemoto, 2004). The NBS-LRR-containing R proteins mediate the recognition of an intracellular pathogen-derived signal. Thus far, NBS-LRR proteins have been shown to function in resistance signaling only in response to pathogen. The second category of R proteins is inserted in the plasma membrane and minimally consists of an extracellular LRR domain and a transmembrane (TM) domain (Jones and Takemoto, 2004).

Many studies suggested that there were three main relationships between RGA and resistance genes. First, RGA is part of R gene or pseudo gene. In Arabidopsis, a sort of RGA was tightly linked with *Rpp5* and was proved to be part of *Rpp5* (Kanazin *et al.*, 1996). Second, RGA was tightly linked with R gene. For instance, RGA were found to be tightly linked with different located R genes in rice (Noir *et al.*, 2001). Third, RGA is unrelated to target gene. For example, some RGA did not link with located R-genes in lettuce (Gaspero and Cipriani 2002).

Sequences with unknown function, but encoding these conserved domains have been defined as Resistant Gene Analogues (RGAs) or Resistance Gene Candidates (RGCs). In other way, resistant gene analogues are potential gene fragments that contain structural motifs, such as the NBS and LRR regions that are common to most cloned *R* gens. Resistance Gene Analogs (RGAs) are the amplified products got through the PCR amplification of genomic DNA using primers designed from the conserved sequences of known disease resistance genes (Khan, 2009). They may function in resistance against similar or different pathogens, or other function(s) unrelated to resistance. The conserved motifs within plant NBS domains make it possible to use degenerate primers and PCR to isolate RGAs or resistant gene like (RGLs) sequences. RGAs have the potential to serve as closely linked markers for marker assisted breeding or even as resistant gene candidates (Taylor *et al.*, 1993).

2.2.2 Nature and types of disease resistance genes

During the past decade, many R-genes have been cloned from different plant species for resistance to a wide spectrum of pathogens, including bacteria, viruses and fungi. The predicted protein products (R-proteins) of most of these R-genes, however, share some common sequence motifs typically involved in signal transduction and protein-protein interaction (Micheltore, 1996; Hammond-Kosack and Jones, 1997; Martin *et al.*, 2003). Based on their deduced amino acid sequence and the combination of protein structural domains, R-genes have been classified into eight major classes.

Class-I has only one member, the tomato *Pto* gene, whose product carries a serine/threonine kinase (STK) catalytic region similar to the cytoplasmic domain of Brassica self-incompatibility gene *SRK*, the mammalian signaling factor Raf, the *Drosophila* pelle kinase, and the human IRAK kinase (Cao *et al.*, 1996; Shelton and Wasserman, 1993; Stein *et al.*, 1996). *Pto* governs resistance to the bacterial speck pathogen *Pseudomonas syringae* pv. tomato, expressing the corresponding avirulence gene *avr Pto*.

After the intracellular recognition of the *avr Pto* product, *Pto* is activated and involved in signal transduction *via* self and specific phosphorylation of additional protein substrates that leads to disease resistance responses (Martin *et al.*, 1993; Bogdanove, 2002).

The second class includes majority of the R-genes, whose encoded proteins carry a region of Leucine-Rich Repeats (LRRs) and a putative Nucleotide Binding Site (NBS). NBS-LRR proteins have two distinct N-terminal domain structures, on the basis of which they have been categorized into two subgroups. Genes belonging to the first subgroup have a domain referred to as Toll and Interleukin Receptor (TIR) because of its similarity to the *Drosophila* Toll and the mammalian Interleukin-1 receptor. While, second subgroup genes possess leucinezipper (LZ) or coiled-coil sequence (CC) region, referred to as LZ-NBS-LRR genes (Bent, 1996; Hammond-Kosack and Jones, 1997; Hulbert *et al.*, 2001). TIR-NBS-LRR genes have not been recognized in grasses in spite of the fact that this class is predominant among the NBS-LRR genes of *Arabidopsis* (Ellis *et al.*, 2000).

The R-genes belonging to class II include; *Arabidopsis* *RPS2*, *RPM1* genes conferring resistance to the bacterial blight pathogen *Pseudomonas syringae* and *RPP5* conferring resistance to the fungus, *Peronospora parasitica* (Bent *et al.*, 1994; Mindrinos *et al.*, 1994; Grant *et al.*, 1995; Parker *et al.*, 1997), the tobacco *N* gene conferring resistance to tobacco mosaic virus (*TMV*) (Whitham *et al.*, 1994), the *L6* and *M* genes conferring resistance to flax rust caused by *Melampsora lini* (Lawrence *et al.*, 1995; Anderson *et al.*, 1997), the *I2C-1* locus conferring resistance to race 2 of tomato vascular wilt fungus, *Fusarium oxysporum* sp *lycopersici* (Ori *et al.*, 1997) and the *Xa1* gene conferring resistance to the bacterial blight pathogen *Xanthomonas oryzae* in rice (Yoshimura *et al.*, 1998). *Prf* genes of tomato, which confer resistance to *Pseudomonas syringae* pv *tomato* (Salmeron *et al.*, 1996), nematode resistance gene *Rx1* in potato (Van der Vossen *et al.*, 2000), and the *Cre-3* nematode resistance locus of wheat also belong to NBS-LRR category of sequences (Lagudah *et al.*, 1997).

The R-gene products of the first two classes have no physical association with the cell membrane and they are thought to be localized in the cytoplasm. The intracellular localization of these R-proteins have implications in understanding the interception of pathogen *Avr*-gene products, as well as the mechanism used to deliver such products into the plant cell at the beginning of pathogenesis (Hammond-Kosack and Jones, 1997; Hulbert *et al.*, 2001).

Class III of R-proteins, typified by the tomato *Cf*- glycoproteins lack NBS but possess a transmembrane anchor domain and large extracellular LRRs. This group includes the gene families *Cf-9*, *Cf-2*, *Cf-4* and *Cf-5*, which confer race-specific resistance to the leaf mold pathogen *Cladosporium fulvum* by recognizing the *avr*-gene products secreted by the fungus in intercellular spaces (Jones *et al.*, 1994; Dixon *et al.*, 1996).

Class IV consists of only the rice *Xa21* gene that governs resistance to bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* (Song *et al.*, 1995). *Xa21* product contains an extracellular LRR, a transmembrane domain and a cytoplasmic serine/threonine kinase.

The architecture of *Xa21* protein suggests that its LRR domain recognizes extracellular pathogen *avr*-gene products; whereas its intracellular protein-kinase is involved in signal

transduction to trigger plant resistance mechanisms by phosphorylation mediated activation (Hulbert *et al.*, 2001).

The fifth class of resistance genes contain the putative extracellular LRRs, along with a PEST (Pro-Glu-Ser-Thr) domain for protein degradation (found only in Ve2, and not Ve1), and short proteins motifs (ECS) that might target the protein for receptor mediated endocytosis (e.g. tomato Ve1 and Ve2 genes). However, these Ve1 and Ve2 proteins have recently been proposed as PAMP receptors (Thomma *et al.*, 2011).

The sixth major class of resistance genes which contains a membrane protein domain (TrD), fused to a putative coiled coil domain (CC) (Wang *et al.*, 2009). eg. The Arabidopsis RPW8 protein.

The seventh major class of resistance genes includes the Arabidopsis RRS1-R gene conferring resistance to the bacterial phytopathogen *Ralstonia solanacearum*, and it is a new member of the TIReNBS- LRR R protein class. RRS1-R has a C-terminal extension with a putative nuclear localization signal (NLS) and a WRKY domain (Deslandes *et al.*, 2003). The WRKY domain is a 60 amino acid region that is defined by the conserved amino acid sequence WRKYGQK at its N terminal end, together with a novel zinc-finger-like motif.

The eighth major class of resistance genes includes the enzymatic R-genes which contain neither LRR nor NBS groups. For example the maize Hm1 gene which provides protection against southern corn leaf blight caused by the fungal pathogen *Cochliobolus carbonum* (Johal and Briggs, 1992). The different classes of R genes are summarized in Table.

2.2.3 R protein motifs and their potential functions

2.2.3.1 Nucleotide Binding Site (NBS): Genes that encode an NBS-containing sequence are common in plant genomes. For example, the superfamily of GTP-binding proteins is prevalent in plants, including signal transducing proteins such as the small GTP-binding proteins (Bourne *et al.*, 1991; Downward, 1990) and the G-protein family (Kaziro *et al.*, 1991). The presence of conserved NBS domain in some R-gene products suggests their possible role in activation of a kinase or as G-protein in signal transduction (Hammond Kosack and Jones, 1997). The NBS domains found in R-proteins are most similar to those found in RAS proteins, adenylate kinase and ATP synthase sub units (Hamm and Gilchrist, 1996; Traut, 1994). The NBS protein sequences can be assigned to separate sub-groups on the conserved motifs within the larger domain, leading to change in sequences among many species (Gaspero and Cipriani, 2002). Mutagenesis of amino acids known to be required for NBS function destroys the biological activity of such R proteins (Salmeron *et al.*, 1996).

2.2.3.2 Leucine Rich Repeats (LRR): Leucine rich repeats (LRRs) consist of imperfect amino acid repeat segments of leucine or hydrophobic residues at regular intervals and can also contain regularly spaced prolines and asparagines that fold into solvent exposed strand turn structures in a manner that would generate less constrained flexible domain, stabilized by hydrogen bonds between the sheets. This domain is thought to be involved in ligand binding and pathogen recognition (Jones and Jones, 1997). Mutant alleles compromising resistance to specific pathogen are shown to reside in the LRR region of NBS-LRR gene products (Bent *et al.*, 1994; Gassmann *et al.*, 1999; Grant *et al.*, 1995; Mindrinis *et al.*, 1994; Warren *et al.*, 1998). Direct evidence for interaction of LRR domain with avirulence factor is based on the finding that single amino acid differences in the LRR domain distinguished susceptible and resistant alleles of rice against *Magnaporthe grisea* (Bryan *et al.*, 2000).

LRR domains are hypothesized to be the most important determinants in the specificity of pathogen recognition. This is confirmed by high levels of allelic polymorphism found among the encoding genes, and the presence of LRR's solvent-exposed residues in many other plant

proteins, not related to resistance or defense responses but required for protein-protein communication. Additional experiments such as domain swapping; yeast-two hybrid and invitro binding analysis have also supported this hypothesis (Ellis and Jones, 1998; Hulbert *et al.*, 2001).

Table : The major classes of cloned plant disease resistance genes

Class	Gene	Plant	Pathogen	Infection Type/ Organ Attacked	Predicted Features of R protein*	References
1	<i>Hm1</i>	Maize	<i>Helminthosporium maydis</i> (race 1)	Fungal necrotroph/leaf	Detoxifying enzyme HC toxin reductase	Slot and Knogge (2002)
2	<i>Asc-1</i>	Tomato	<i>Alternaria alternata</i> f.sp. <i>Lycopersici</i> (AAL toxin)	Fungal necrotroph/leaf	TM helix-LAG1 motif	Brandwagt <i>et al.</i> (2000)
3A	<i>Pto</i>	Tomato	<i>Pseudomonas syringae</i> pv. tomato (<i>avrPto</i>)	Extracellular bacteria/leaf Intracellular serine/threonine protein	Kinase	Slot and Knogge (2002)
3B	<i>PSB1</i>	Arabidopsis	<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i> (<i>avrPphB</i>)	Extracellular bacteria/leaf	Different subfamily	Swiderski and Innes (2001)
4A	<i>RPS2</i>	Arabidopsis	<i>Pseudomonas syringae</i> pv. <i>maculicola</i> (<i>avrRpt2</i>)	Extracellular bacteria/leaf	CC-NB-LRR Intracellular protein	Slot and Knogge (2002)
	<i>Mla1/ Mla6</i>	Barley	<i>Blumeria graminis</i> f.sp. <i>hordei</i> (resp. race 1, race 6)	Biotrophic intracellular fungus with haustoria/leaf		Zhou <i>et al.</i> (2001)
	<i>R1</i>	Potato	<i>Phytophthora infestans</i> (race 1)	Biotrophic intracellular Oomycete with haustoria/leaf and tuber		Ballvora <i>et al.</i> (2002)
	<i>RPP8</i>	Arabidopsis	<i>Peronospora parasitica</i>	Biotrophic intracellular Oomycete with haustoria/leaf		Slot and Knogge (2002)
4B	<i>N</i>	Tobacco	Mosaic virus	Intracellular virus/leaf and phloem	TIR-NB-LRR Intracellular protein	Slot and Knogge (2002)

Class	Gene	Plant	Pathogen	Infection Type/ Organ Attacked	Predicted Features of R protein*	References
4C	<i>RPP4</i>	Arabidopsis	<i>Peronospora parasitica</i>	Biotrophic intracellular Oomycete with haustoria/leaf	NB-LRR Intracellular protein	Kawchuk <i>et al.</i> (2001)
	<i>Bs2</i>	Pepper	<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> (<i>avrBs2</i>)	Extracellular bacteria/leaf		Slot and Knogge (2002)
	<i>Dm3</i>	Lettuce Bremia	Lactuca	Biotrophic intracellular Oomycete with haustoria/leaf		Shen <i>et al.</i> (2002)
4D	<i>RRS-1</i>	Arabidopsis	<i>Ralstonia solanacearum</i> (race 1)	Extracellular bacteria/leaf	TIR-NB-LRRNLSWRKY	Deslandes <i>et al.</i> (2002)
4E	<i>Pi-ta</i>	Rice	<i>Cladosporium fulvum</i> (<i>Avr9</i>)	Biotrophic extracellular fungus without haustoria/leaf	eLRR-TM-sCT Extracellular protein with single membrane-spanning region and short cytoplasmic C terminus	Slot and Knogge, 2002
5A	<i>Cf-9</i>	Tomato	<i>Cladosporium fulvum</i> (<i>Avr9</i>)	Biotrophic extracellular fungus without haustoria/leaf	eLRR-TM-sCT Extracellular protein with single membrane-spanning region and short cytoplasmic C terminus	Slot and Knogge (2002)
5B	<i>Ve1</i> <i>Ve2</i>	Tomato	<i>Verticillium albo-atrum</i>	Extracellular vascular wilt fungus without haustoria/root and stem	CC-eLRR-TM-ECS; eLRR-TM-PEST-ECS	Kawchuk <i>et al.</i> (2001)
6	<i>Xa-21</i>	Rice	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> (all races)	Extracellular bacteria/leaf	eLRR-TM-kinase	Slot and Knogge (2002)
	<i>FLS2</i>	Arabidopsis	Multiple bacteria (flagellin)	Extracellular bacteria/leaf		Slot and Knogge (2002)

Class	Gene	Plant	Pathogen	Infection Type/ Organ Attacked	Predicted Features of R protein*	References
7	<i>RPW8.1</i>	Arabidopsis	Multiple powdery mildew species	Biotrophic intracellular fungus with haustoria/leaf	Small, probable membrane protein with CC domain	Xiao <i>et al.</i> (2001)
8	<i>Rpg1</i>	Barley	<i>Puccinia graminis</i> f.sp. <i>tritici</i>	Biotrophic intracellular fungus with haustoria/stem	Receptor kinase-like protein with 2 tandem kinase domains	Brueggeman <i>et al.</i> (2002)

*ECS= endocytosis signal; LAG1= longevity assurance gene; LR= leucine-rich domain; PEST= Pro-Glu-Ser-Thr; sCT= single cytoplasmic tail; TM= transmembrane (Hammond-Kosack and Parker, 2003).

2.2.3.3 Protein Kinase (PK): The protein kinase motif is about 1 kb, coding for a peptide of about 300 amino acids (Shivaramakrishnan and Seetharam, 2001), across organisms. Protein kinases characteristically exhibit a modular construction, with a conserved catalytic domain for binding specific receptor ligand. They are classified into two sub groups depending on their ability to phosphorylate serine/ threonine or tyrosine (Hanks *et al.*, 1988; Martin *et al.*, 1993; Song *et al.*, 1995).

2.2.3.4 Toll and Interleukin receptor (TIR): Some of the well defined NBS-LRR containing R genes; *N*, *L6*, *M*, *RPP1*, *RPP2*, *RPS2* and *RPP5* contain a large N-terminal domain called Toll/ Interleukin / Receptor (TIR) domain, which has some similarity to cytoplasmic signaling domain of the *Drosophila* Toll protein, the mammalian interleukin receptor (IL-1R), and a family of mammalian Toll-like receptors, one of which participate in recognition and response to Lipopolysachrides (LPS). Toll, IL-1R, and the mammalian Toll homologs contribute to the immune response against pathogens (Medzhitov *et al.*, 1997). The presence of TIR domain in several plant R-proteins suggests signaling role for this domain but not in ligand binding (Ellis and Jones, 1998). In a study of the alleles of *L*-locus of flax it was shown that alleles with differing specificities were found to possess changes only in their TIR domains (Ellis *et al.*, 1999). Thus, it appears that the TIR domain plays a role in signal transduction or pathogen recognition.

2.2.3.5 Leucine Zipper (LZ): Leucine zipper region found in *RPM1*, *RPS2* and *Prf* genes potentially facilitates homo-dimerization of the proteins themselves or hetero-dimerization with other proteins (Landschulz *et al.*, 1988). R-proteins themselves may exist as monomers before pathogen challenge and then undergo dimerization upon activation. Alternatively, they could exist initially as dimers or multimers that dissociate upon activation (Bent, 1996). Computer data-base searches with LZ regions revealed very high similarity to the coiled-coil regions in myosins and paramyosin proteins (Bent *et al.*, 1994; Landschulz *et al.*, 1988).

The direct interaction between the R-gene product of *Pto* and the *Avr*-gene product of *Avr Pto* was demonstrated unequivocally using a yeast two-hybrid system (Schofield *et al.*, 1996; Tang *et al.*, 1996). The serine/threonine kinase activity possessed by *Pto* and *Xa-21* clearly facilitated downstream signaling. *Pto* kinase may have a role in transcription factor activation. For many a gene mediated resistance, the addition of either kinase or phosphatase inhibitors are known to significantly block the induction of rapid defense responses (Doke and Ohashi, 1988).

There are many independent signal cascades like ion channels, protein kinase and transcription factors, which may also be involved in resistance mechanism. In many cases, truncated forms of the R-gene transcripts have been obtained and it has been postulated that they may have the regulating activity of the full-length R-genes (Parker *et al.*, 1997).

2.2.4 Strategies for R-gene cloning

A number of approaches have been followed to clone and characterize specific genes depending on the extent of information available (Johal and Briggs, 1992). The two most common methods used for gene isolation are positional or map based cloning and transposon tagging. A third and popular method of isolation of R-gene is by PCR approach using degenerate primers to the conserved domains. The other strategies involved are, gene prediction from databases of plant genome sequencing initiatives, subtractive hybridization and shotgun complementation.

The original R-genes were isolated, despite their unknown structure and function by either positional cloning such as the tomato *Pto* gene (Martin *et al.*, 1993a) or by transposon tagging like in the case of maize *Hm1* gene (Johal and Briggs, 1992). Transposon tagging or insertional inactivation involves the use of transposable elements to prevent expression of well characterized and selectable phenotype. While, the isolation of genes through map based approach requires precise location of the gene on a sufficiently saturated genetic map (Rommens *et al.*, 1989). It involves identification of markers that are genetically linked to a target locus, physical and genetic mapping to determine distance, chromosome walking from the marker by

identification of overlapping clones and complementation (Shivaramakrishnan and Seetharam, 2001). These two approaches led to the first wave of R-gene cloning; *Arabidopsis* *RPS2* and *RPM1* (Bent *et al.*, 1994; Grant *et al.*, 1995), tomato *Cf-9*, *Cf-2* and *Prf* (Jones *et al.*, 1994; Dixon *et al.*, 1996; Salmeron *et al.*, 1996), tobacco *N* (Whitham *et al.*, 1994), flax *L6* (Lawrence *et al.*, 1995) and rice *Xa21* (Song *et al.*, 1995). Surprisingly, the sequences of these R-genes, after being translated to peptides, showed a remarkable similarity to each other, but no resemblance to the original *Pto* or *Hm1*. Based on their deduced peptide sequences and predicted protein structures, the new R-genes were classified as TIR and non TIR-NBS-LRR genes.

The NBS-LRR classification of these R-genes enhanced the detection of additional candidate R-gene and resistance gene analog (RGA) sequences that were PCR-amplified using degenerate oligonucleotide primers designed from such conserved domains (Kanazin *et al.*, 1996; Leister *et al.*, 1996; Yu *et al.*, 1996). The extensive use of degenerate primers in combination with gene mining from databanks of several plant genome sequencing initiatives are the most common methods to uncover novel genomic and cDNA sequences related to different R-gene classes. A number of resistance gene analogs (RGAs) have been isolated and characterized from several plant genotypes. These NBS encoding regions have been shown to be genetically linked to known R-genes, or indeed to be fragments of known R genes themselves (Kanazin *et al.*, 1996; Yu *et al.*, 1996; Aarts *et al.*, 1998; Collins *et al.*, 1998; Shen *et al.*, 1998; Hayes and Saghai-Marroof 2000; Donald *et al.*, 2002; Penuela *et al.*, 2002).

2.3 *In silico* genome wide identification and characterization of transcription factors

2.3.1 Transcription factors

Regulation of gene expression is critical for a variety of processes such as growth, development, differentiation, metabolic regulation and adaptation to surrounding environment in plants (Liansen *et al.*, 1999). Transcription, the first step in the expression of any gene, plays a central role in the regulation of the expression of genes. Transcription is controlled by numerous transcription factors that mediate the effects of intercellular and extracellular signals. Therefore, the analysis of transcription factors is essential for an understanding of mechanism of gene expression and is important in all areas of plant science (Sainz *et al.*, 1997).

Transcription factors (TFs) are master regulators that control gene clusters. A single TF can control the expression of many target genes through a specific binding of the TF to the *cis*-acting element in the promoters of respective target genes. This type of transcriptional regulatory system is called regulon. Several major regulons that are active in response to abiotic stress have been identified in *Arabidopsis* and rice (Huang *et al.*, 1996)

Dehydration-responsive element binding protein 1 (DREB1)/C-repeat binding factor (CBF) and DREB2 regulons function in ABA-independent gene expression, whereas the ABA responsive element (ABRE) binding protein (AREB)/ABRE binding factor (ABF) regulon functions in ABA-dependent gene expression. In addition to these major pathways, other regulons, including the NAC and MYB/MYC regulons, are involved in abiotic stress responsive gene expression. Recent studies demonstrated that DREB1/CBF, DREB2, AREB/ABF, and NAC regulons have important roles in response to abiotic stresses in rice (Sainz *et al.*, 1997).

Plants can develop numerous physiological and biochemical strategies to cope with adverse conditions. The major events of plant response to dehydration stresses are perception and transduction of the stress signals through signaling components, resulting in activation of a large number of stress-related genes and synthesis of diverse functional proteins that finally lead to various physiological and metabolic responses. Well characterized proteins involved in the protection of plant cells from dehydration stress damage include molecule chaperons, osmotic adjustment proteins, ion channels, transporters, and antioxidation or detoxification proteins. The

expression of these functional proteins is largely regulated by specific transcription factors (Huang *et al.*, 1996).

Although plants share fundamental mechanisms of life with other eukaryotic kingdoms, relating to their immobility, they possess distinct systems of adaptation to the environment, defense against pathogens, growth of specific organs such as flowers, *etc.* TFs are involved in the control of all such plant-specific reactions, and, very interestingly, many of them showed no obvious sequence similarity to those of other eukaryotes or bacteria. In 1500 probable specific TFs identified in the *Arabidopsis thaliana* genome, 45 per cent are classified into those plant-specific TFs. It should be noted that these transcription factors were identified and classified according to their DNA-binding domains (DBDs) and that, therefore, the names of the DBDs, *e.g.*, AP2/ERF (or EREBP), WRKY, NAC, are also used as the names of the TF families. More than 30 families of TFs have been predicted for *Arabidopsis*. Members of DREB or CBF, MYB, bZIP, and zinc-finger families have been well characterized with roles in the regulation of plant defense and stress responses. Most of these TFs regulate their target gene expression through binding to the cognate *cis*-elements in the promoters of the stress-related genes.

2.3.2 General features of plant TFs

Many TFs have been examined by X-ray crystallography and NMR spectrometry. The functional domains of plant TF genes are usually derived by comparing amino acid sequences deduced from cDNA clones with their animal counterparts. Study of putative functional domains, mutational and functional analysis has demonstrated that a typical plant TF gene consists of a DNA binding domain (DBD), an oligomerization domain (OD), a transcription regulation domain (RD) and a nuclear localization signal (NLS). Some TF genes lack either a transcription regulation domain or a specific DNA binding region (Washburn *et al.*, 1997; De-Vetten and Ferl, 1995; Hill *et al.*, 1996). In addition, there are also reports demonstrating novel functional domains in plant TF genes (Liansen *et al.*, 1999). DNA binding domains The DNA-binding domain of plant TF genes are basic and contain amino acid residues that contact DNA bases at *cis*-acting elements, and these determine the specificity of the protein. Other residues enhance TF genes binding by contacting DNA nonspecifically through interaction with either phosphate or deoxyribose moieties (Huang *et al.*, 1996). The base recognition residues are often highly conserved; *e.g.*, one arginine residues in bZIP domains (Aukerman *et al.*, 1991); either one or two cysteine and histidine residues in zinc finger motifs (Li and Thomas, 1998); and single arginine and lysine residues in MADS (*Mcm*, *Agamous*, *Defence* and *Serum response factor*) domains (Huang *et al.*, 1996) are nearly identical within plants, animals and fungi. Each plant TF genes has only one type of DNA binding domain, occurring in either single or multiple copies. For instance, most plant MYB related proteins have two myb domains, where as the potato *MybSt1* (*solanum tuberosum* 1) (Baranowskij *et al.*, 1994) and the *Arabidopsis* CCA1 (Wang *et al.*, 1997) each contain one copy.

2.3.2.1 Oligomerization domains

Many plant TF genes form hetero and homo-oligomers, affecting DNA-binding specificity the affinity of TF genes for promoter elements (Katagiri *et al.*, 1992; Guiltinan and Miller, 1994) and nuclear localization (Sainz *et al.*, 1997). Oligomers are either stabilized by hydrophobic interactions between coiled coils and B-sheets, or by reactions between hydrophilic residues, wherein in the alignment of residues affect oligomerization by altering ionic environments (Huang *et al.*, 1996). The oligomerization domain of bZIP type factor is characterized by several regulatory spaced leucine residues and by a zipper-like structure. In bHLH type factor, a helix-loop-helix composition appears (Sainz *et al.*, 1997), while MADS factors have oligomerization domains that form two α -helices and two β -pleated sheets.

2.3.2.2 Transcription regulation domains

TF genes belongs to same family generally have distinct actions because of differences in their regulation domains, regions of the proteins that tend to diverge from one another

(Yanagisawa and Sheen, 1998). Regulation domains of TF genes function as either repressor or activators, depending on whether they inhibit or stimulate the transcription of target genes. Repression of gene expression may occur via exclusion of activators from target promoters by competitive binding between TF genes for the same *cis*-acting element.

2.3.2.3 Nuclear localization signals

Plants TF genes contain nuclear localization signals (NLSs) characterized by a core peptide enriched in arginine (R) and lysine (K) for proteins that selectively enter nucleus (Bouliskas, 1994). Flanking residues influence the action of the basic core. Within plant TF genes, the NLSs vary in sequence, organization and number.

2.3.3 Classification of TF genes

Classification of TF genes depends on their structural features, families sometimes subdivided according to the number and spacing of conserved residues in the most similar domain (Guilfoyle *et al.*, 1998; Takatsuji, 1998; Riechmann and Meyerowitz, 1998).

2.3.3.1 Basic leucine zipper (bZIP)

bZIP class of DNA binding protein bind specifically to a core ABRE sequence (T/G/C)ACGTG (Guiltinan *et al.*, 1990; Izawa *et al.*, 1993; Oeda *et al.*, 1991). Sequence comparisons and functional analysis of the promoters of LEA and LEA related genes such as the wheat *Em*, rice *Osem* and *Rab16A-D* and barley *HVA22* led to identification of Absciscic Acid Responsive Elements (ABRE), required for the induction of transcription by ABA (Guiltinan *et al.*, 1990; Mundy *et al.*, 1990). Hattori *et al.* (1995) showed that the *Osem* promoter has 3 ACGTG-containing sequences, motif A, motif B and motif A', all of which contribute to a greater or lesser extent to the responsiveness of the promoter to ABA. In addition to these motifs, a sequence designated as region1 (CE3-coupling element 3) appears to function together with motif A as an ABA-response complex. Nakagawa *et al.* (1996) isolated a bZIP protein, *OsBZ8* from rice embryo cDNA library; *OsBZ8* had structural features typical of the GBF (G-box Binding Factor)-type bZIP proteins and binds to G-box and G-box like sequences that include ABREs, which are identified in the promoters of the ABA-inducible genes, *viz.*, *Em*, *OsEm*, and *RAB16*.

2.3.3.2 Homeodomain-leucine zipper (HD-ZIP) proteins

Homeodomain-leucine zipper (HD-ZIP) proteins are TF genes as yet found only in plants. Soderman *et al.* (1996) characterized *AtHB-7* in *Arabidopsis*. Induction of *AtHB7* was shown to be mediated strictly *via* ABA, since no induction was detected in the ABA deficient mutants, *aba-3* subjected to drought treatment. Induction levels in AB insensitive mutants, *abi2* and *abi3* were similar to the wild type response. These results indicate that *AtHB-7* is transcriptionally regulated in an ABA-dependent manner and may act in a signal transduction pathway which mediates a drought response. Basic Helix turn Helix (bHTH)-MYB/Basic helix loop helix (bHLH)-MYC MYB is an acronym derived from "myeloblastosis", an old name for a type of leukemia. MYB factors represent a family of proteins which includes a conserved domain, the MYB DNA-binding domain, found in both plants and animals. The first MYB gene identified was the 'oncogene' *v-Myb* from the avian Myeloblastosis virus (AMV). This acutely transforming retrovirus causes rapidly fatal monoblastic leukemia in chickens. Characteristics of a MYB repeat are three regularly spaced tryptophan residues which form a tryptophan cluster I, the three dimensional helix-turn-helix structures. The three repeats are referred to as R1, R2 and R3 (Stracke *et al.*, 2001). Plants MYB-related genes constitute a large family and play a wide variety of roles in the regulation of gene expression. MYB genes are involved in the ABA responsive expression of drought-inducible genes in the vegetative tissues of higher plants. The MYB domain binds to a specific DNA sequence (C/TAACG/TG, for most organisms) that is closely related to the E-box hexanucleotide. Once bound, MYB proteins function to facilitate transcription activation (Biedenkapp *et al.*, 1988).

2.3.3.3 WRKY

WRKY proteins which are unique to plants contain either one or two WRKY domains, a 60-amino acid region highly conserved among the family members. They play a key role in regulating the pathogen induced defense responses (Wu *et al.*, 2005), abiotic stress responses (Fowler and Thomashow, 2002; Seki *et al.*, 2002; Mare *et al.*, 2004) and are involved in various physiological processes including senescence, trichome development and biosynthesis of secondary metabolites (Eulgem *et al.*, 2000).

2.3.3.4 AP2/EREBP

AP2 (APETALA2) and EREBPs (Ethylene-responsive element binding proteins) are the prototypic members of a family of TF genes unique to plants, whose distinguishing characteristic is AP2 DNA-binding domain. AP2/EREBP genes form a large multigene family and they play a variety of roles throughout the plant life cycle being key regulators of several developmental processes, like floral organ identity, determination or control of leaf epiderma cell identity, part of the mechanisms used by plants to respond to various types of biotic and environmental stress (Riechmann and Meyerowitz, 1998). These proteins contain either one (EREBP subfamily) or two (AP2 subfamily) copies of an approximately 70-amino acid domain termed the AP2 repeat because of its initial description in the floral homeotic protein *APETALA2* (AP2). DNA-binding specificity has been determined for three types of AP2/EREBP proteins that contain an AP2 repeat. The first class contains proteins, which bind to ethylene response elements (ERE) or GCC boxes (tobacco EREBPs, *Arabidopsis* AtEBP and AtERF 1-5, and tomato *Pti4-6*) found in the promoters of ethylene- inducible pathogenesis related genes. The second class includes proteins that bind to the C-repeat or dehydration response element (DRE) in the promoter of genes that are turned on in response to low temperatures and/or water deficit (CBF1, CBF2, CBF3/DREB1A and DREB2A). The C-repeat/DREs contain the core sequence CCGAC. The third class of AP2/EREBP-like proteins that have been shown to bind DNA consists of *Arabidopsis* RAV1 and RAV2. RAV1 and RAV2 are highly related proteins that contain two DNA binding motifs: a single AP2 repeat and a B3-like domain (Sakuma *et al.*, 2002; Liu *et al.*, 1998 and Stockinger *et al.*, 1997).

2.3.3.5 ARF

Auxin-response factors (ARFs) bind with specificity to TGTCTC auxin-response elements (AuxREs), which are found in promoters of primary/early auxin-response genes. The ARF1 protein contains an amino-terminal DNA-binding domain (DBD), which has some sequence similarity to a carboxyl-terminal B3 domain found in the maize transcription factor *VIVIPAROUS1* (VP1) and its relatives. ARF1 contains a carboxyl-terminal domain (CTD) related to motifs III and IV found in the CTDs of Aux/IAA proteins, which are short-lived nuclear proteins encoded by a family of primary/early auxin-response genes. The CTDs in ARF1 and Aux/IAA proteins facilitate dimerization among the members of both ARF and Aux/IAA protein families (Ulmasov *et al.*, 1999).

2.3.3.6 bHLH

The basic/helix-loop-helix (bHLH) proteins are a super-family of TF genes that have been well characterized in non-plant eukaryotes (Atchley and Fitch, 1997; Littlewood and Evan, 1998; Ledent and Vervoort, 2001). The reports indicate that bHLH proteins are important regulatory components in transcriptional networks in mammalian systems, controlling a diversity of processes from cell proliferation to cell lineage establishment (Grandori *et al.*, 2000; Massari and Murre, 2000). This family is defined by the bHLH signature domain, which consists of 60-amino acids with two functionally distinct regions. The basic region, located at the N-terminal end

of the domain, is involved in DNA binding and consists of 15 amino acids with a high number of basic residues. The HLH region at the C-terminal end functions as a dimerization domain and is constituted mainly of hydrophobic residues that form two amphipathic-helices separated by a loop region of variable sequence and length. Outside of the conserved bHLH domain, these proteins exhibit considerable sequence divergence. Structural analysis has shown that the interaction between the HLH regions of two separate polypeptides leads to the formation of homodimers and/or heterodimers and that the basic region of each partner binds to half of the DNA recognition sequence. The core DNA sequence motif recognized by the bHLH proteins is a consensus hexanucleotide sequence known as the E-box (5'-CANNTG-3'). There are different types of E-boxes, depending on the identity of the two central bases. One of the most common is the palindromic G-box (5'-CACGTG-3'). Certain conserved amino acids within the basic region of the protein provide recognition of the core consensus site, whereas other residues in the domain dictate specificity for a given type of E-box. In addition, flanking nucleotides outside of the hexa nucleotide core have been shown to play a role in binding specificity, and there is evidence that a loop residue in the protein plays a role in DNA binding through elements that lie outside of the core recognition sequence (Toledo-ortiz *et al.*, 2003).

2.3.3.7 YABBY

Members of the YABBY gene family are characterized by two conserved domains, a C2C2 zinc finger-like domain towards the amino terminus and a helix-loop-helix, which called as YABBY domain, sequence similarity of the first two helices of the HMG box towards the carboxyl end of the protein. In addition to the conserved YABBY and zinc finger domains, *YAB1*, *YAB2*, *YAB3* and *YAB5* display some sequence similarity on the carboxyl side of the YABBY domain. The region between the YABBY and zinc finger domains is variable amongst family members. Database searches suggest that this family may be plant-specific, as genes with a similar arrangement of zinc finger and YABBY domains do not occur in either *Caenorhabditis elegans* or *Saccharomyces cerevisiae*. The YABBY genes are expressed in a polar manner in all lateral organs produced by apical and flower meristems. Their transcripts are detectable only in the abaxial domains of lateral organs when their primordia emerge and begin to differentiate. Loss of polar expression of these genes results in loss of polar differentiation of cell types in lateral organs, and gain-of-function alleles result in abaxialization of lateral organs. The expression of these genes is precisely correlated with abaxial cell fate in mutants in which abaxial cell fates are found ectopically, reduced or eliminated. The members of this gene family act redundantly to specify abaxial identity in lateral organs produced by the apical and flower meristems in *Arabidopsis* (Siegfried *et al.*, 1999).

2.3.3.8 NAC

The NAC protein family comprises a variety of plant proteins that are identified by the presence of a highly conserved N-terminal NAC domain, accompanied by diverse C-terminal domains. NAC is an acronym derived from the names of the three genes first described as containing the domain, namely NAM (no apical meristem), *ATAF1*, 2 and *CUC2* (cup-shaped cotyledon) (Souer *et al.*, 1996; Aida *et al.*, 1997). NAC proteins have been implicated in transcriptional control in a variety of plant processes. Functions include involvement in the development of the shoot apical meristem and floral organs, and in the formation of lateral roots (Souer *et al.*, 1996; Aida *et al.*, 1997; Xie *et al.*, 2000). Additionally, NAC proteins have been implicated in responses to stress and viral infections (Xie *et al.*, 1999; Ren *et al.*, 2000; Collinge and Boller, 2001). Several NAC proteins have been shown to function as transcription activators (Ren *et al.*, 2000; Xie *et al.*, 2000; Duval *et al.*, 2002). The DNA-binding ability of two of these proteins, NAC1 and has been localized to the NAC domain, whereas the C-terminal regions constitute transcriptional activation domains (Xie *et al.*, 2000; Duval *et al.*, 2002). Several NAC genes are hormone inducible (Hoth *et al.*, 2002; Xie *et al.*, 2002; Greve *et al.*, 2003). NAC domains have also been implicated in interactions with other proteins such as viral proteins (Xie *et al.*, 1999; Ren *et al.*, 2000) and RING finger proteins (Xie *et al.*, 2002; Greve *et al.*, 2003). For example, interactions between the auxin-inducible NAC1 protein, involved in lateral root formation, and the RING domain protein *SINAT5* attenuate the auxin signal through ubiquitination

and degradation of NAC1 (Xie *et al.*, 2002). Recently, a NAC protein from *Arabidopsis* (*AtNAC*) was identified as an interaction partner of another RING protein (Greve *et al.*, 2003). An examination of interactions between different RING domains and *AtNAC* suggested that RING and NAC interactions may also regulate pathways controlled by the plant stress hormone abscisic acid. HAP2 and HAP5 proteins that bind to the CCAAT motif were first characterized in the yeast *Saccharomyces cerevisiae* through analysis of mutants with reduced levels of expression of the *CYC1* gene (encoding iso-1-Cyt c). The *CYC1* promoter comprises two UAS, one of which (UAS2) contains an inverted CCAAT motif that is required for UAS2-directed transcription. Activation of transcription from UAS2 requires HAP2, HAP3 and HAP5, which form a heterotrimeric CCAAT-box-binding complex. The HAP complex appears to control expression of genes important for mitochondrial biogenesis (Edwards *et al.*, 1998). CCAAT box-related motifs have also been identified in the promoters of a variety of vertebrate genes. A range of TF genes has been shown to bind to different CCAAT boxes, with varying levels of specificity and each is thought to play a distinct role in gene expression or DNA replication. Direct homologs of the yeast HAP complex (called NF-Y, CP1, or CBF) have been identified in vertebrates (Romier *et al.*, 2003). Plant NF-Y function also appears to be important for responses to drought stress. Although a specific mechanism remains unclear, over expression of NF-YB1 and its ortholog in maize *ZmNF-YB2*, leads to enhanced drought resistance (Nelson *et al.*, 2007). Although the ability of NF-YB1 to promote drought resistance is clear, no loss-of-function data were reported to demonstrate an actual biological role in *Arabidopsis*. In contrast, a recent publication shows both over-expression and loss-of-function data for NFYA5 (Li *et al.*, 2008). Over-expression of NF-YA5 reduced drought susceptibility, anthocyanin production, and stomatal aperture, while *nf-ya5* mutants had the expected opposite phenotype in each instance.

2.3.3.9 High mobility group (HMG)

High mobility group (HMG) proteins are families of relatively low molecular weight non-histone components in chromatin. The animal HMG proteins have been classified in three families: HMG-1/2, HMG-14/17 and HMG-I/Y proteins. Members of the HMG-1/2 family contain a conserved protein motif known as the HMG box which binds to a variety of non-B forms of DNA, such as cruciform DNA. The HMG-14/17 proteins form complexes with nucleosomes on actively transcribed genes where their presence is associated with sensitivity to DNase I. The HMG-I/Y proteins were originally detected in rapidly dividing and undifferentiated cells of human and rodents and were shown to bind to the minor groove of A/T-rich DNA with a conserved DNA-binding motif called the AT-hook. HMG1/2 are proteins of about 200 amino acid residues with a highly acidic C-terminal section which is composed of an uninterrupted stretch of from 20 to 30 aspartic and glutamic acid residues, the rest of the protein sequence is basic (Gupta *et al.*, 1997).

2.3.3.10 MADS

The best studied plant MADS-box TF genes are those involved in floral organ identity determination. Analysis of homeotic floral mutants resulted in the formulation of a genetic model, named the ABC model, which explains how the combined functions of three classes of genes (A, B and C) determine the identity of the four flower organs. Recently, it was shown that the *Arabidopsis* B and C genes, which control petal, stamen, and carpel development are functionally dependent on three highly similar MADS-box genes, *SEP1*, *SEP2* and *SEP3* (Nam *et al.*, 2003). Interestingly, when mutant knockout alleles of the three *SEP* genes were combined in a triple *sep1, sep2, sep3* mutant was revealed loss of petal, stamen, and carpel resulting in a flower composed of only sepals. The *SHP* genes provide another example of MADS-box gene redundancy. *shp1* and *shp2* single mutants do not exhibit any phenotypic effect, whereas in the double mutant, development of the dehiscence zone is disturbed in the fruit, resulting in a failure to release seeds (Parenicova *et al.*, 2003). It has been proposed that there are at least 2 lineages (type I and type II) of MADS box genes in plants, animals, and fungi. Most of the well-studied plant genes are type II genes and have three more domains than type I genes from the N to the C terminus of the protein: intervening (I) domain (~30 codons), keratin-like coiled-coil (K) domain (~70 codons), and C terminal (C) domain (variable length). These genes are called the MIKC-type and are specific to plants. The MADS-box is a DNA binding domain of 58 amino acids that binds

DNA at consensus recognition sequences known as CArG boxes [CC(A/T)6GG] (Santelli and Richmond, 2000).

2.3.3.11 MBF1

Multi-protein bridging factor 1 (MBF1) is known to be a transcriptional co-activator that mediates transcriptional activation by bridging between an activator and a TATA-box binding protein (TBP). Tsuda *et al.* (2004) demonstrated that expression of three MBF1 from *Arabidopsis* partially rescues the yeast *mbf1* mutant phenotype, indicating that all of them function as co-activators for GCN4-dependent transcriptional activation. Each of the subtypes showed distinct tissue-specific expression patterns and responses to phyto-hormones. These observations suggest that even though they share a similar biochemical function, each MBF1 has distinct roles in various tissues and conditions.

2.3.3.12 PHD

The Plant Homeo Domain (PHD finger, a Cys4"CHis"CCys3 zinc finger), is found in many regulatory proteins from plants or animals which are frequently associated with chromatin-mediated transcriptional regulation. The PHD finger was originally identified by comparison of the maize homeodomain (HD) protein *ZmHOX1a* to its *Arabidopsis* relative HAT3.1 and named plant homeodomain (PHD) finger due to its association with the DNA binding HD in both genes. This motif often occurs in various regulatory genes, such as members of the trithorax (TRX-G) or polycomb (PC-G) groups and leukaemia-associated proteins (LAP finger) (Halbach *et al.*, 2000).

2.3.3.13 TRIHELIX

GT-2 is a plant transcriptional activator that contains two separate, but similar, trihelix DNA-binding domains. GT-1 is similar to GT-2, although it contains only one of such domains. cDNAs that encode GT-2 were isolated from rice (*OsGT2*) and *Arabidopsis* (*AtGT2*). Evidence is presented for the existence of an *Arabidopsis* gene family that is structurally related to *AtGT2*. Two members of this GT2-like family, *AtGTL1* and *AtGTL2*, have been isolated and characterized. Their sequences suggest that they evolved by a recent gene duplication event. Both *AtGT2* and *AtGTL* genes contain an intron in the amino terminal trihelix motif, indicating that this DNA-binding domain resulted from exon shuffling. RNA gel blot analysis using *AtGTL1* as a probe revealed four transcripts in the aerial part of the plant. All mRNA levels were significantly higher in siliques, suggesting that this gene family may function in fruit and/or seed development. To date, DNA-binding proteins characterized by the trihelix motif have been described only in plants, and may therefore be involved in plant-specific processes (Smalle *et al.*, 1998; Kitakura *et al.*, 2002).

2.3.3.14 WIRLY

Elicitor-induced activation of the potato pathogenesis-related gene PR-10a requires a 30-bp promoter sequence termed the ERE (elicitor response element) that is bound by the nuclear factor PBF-2 (PR-10a binding factor 2). Evidence demonstrates that inactive PBF-2 is stored in the nucleus of fresh tuber and becomes available for binding to the ERE upon elicitation. A protein with an apparent molecular mass of 24 kD (p24) is a DNA binding component of PBF-2. A cDNA encoding p24 has been cloned and encodes a novel protein with a potential transcriptional activation domain that could also act as a single-stranded DNA binding domain. Both PBF-2 and the cDNA-encoded protein bind with high affinity to the single-stranded form of the ERE in a sequence-specific manner. The inverted repeat sequence of the ERE, TGACAnnnnTGTCa, is critical for binding of this factor *in vitro* and for PR-10a expression *in vivo*, supporting the role of PBF-2 as a transcriptional regulator (Desveaux *et al.*, 2004).

Late embryogenesis abundant (LEA) proteins are a diverse and large group of polypeptides that play important roles in desiccation and freezing tolerance in plants (Liang *et al.*, 2016). The LEA family has been systematically characterized and 108 *BnLEA* genes were identified in the *B. napus* genome and classified into eight families based on their conserved domains. Protein sequence alignments revealed an abundance of alanine, lysine and glutamic acid residues in *BnLEA* proteins. The *BnLEA* gene structure has few introns (<3), and they are distributed unevenly across all 19 chromosomes in *B. napus*, occurring as gene clusters in chromosomes A9, C2, C4 and C5. More than two-thirds of the *BnLEA* genes are associated with segmental duplication. Synteny analysis revealed that most *LEA* genes are conserved, although gene losses or gains were also identified. These results suggest that segmental duplication and whole-genome duplication played a major role in the expansion of the *BnLEA* gene family. Expression profiles analysis indicated that expression of most *BnLEAs* was increased in leaves and late stage seeds.

Stage-specific transcription is a fundamental biological process in the life cycle of the Plasmodium parasite. Proteins containing the AP2 DNA-binding domain are responsible for stage-specific transcriptional regulation and belong to the only known family of transcription factors in Plasmodium parasites (Kaneko *et al.*, 2015). Comprehensive identification of their target genes will advance our understanding of the molecular basis of stage-specific transcriptional regulation and stage-specific parasite development. AP2-O is an AP2 family transcription factor that is expressed in the mosquito midgut-invading stage, called the ookinete, and is essential for normal morphogenesis of this stage. In this study, we identified the genome-wide target genes of AP2-O by chromatin immune precipitation-sequencing and elucidate how this AP2 family transcription factor contributes to the formation of this motile stage. The analysis revealed that AP2-O binds specifically to the upstream genomic regions of more than 500 genes, suggesting that approximately 10 per cent of the parasite genome is directly regulated by AP2-O. These genes are involved in distinct biological processes such as morphogenesis, locomotion, midgut penetration, protection against mosquito immunity and preparation for subsequent oocyst development. This direct and global regulation by AP2-O provides a model for gene regulation in plasmodium parasites and may explain how these parasites manage to control their complex life cycle using a small number of sequence-specific AP2 transcription factors.

Baloglu *et al.* (2014) have identified 64 bZIP transcription factor-encoding genes in the cucumber genome. Based on structural features of their encoded proteins, CsbZIP genes could be classified into 6 groups. Cucumber bZIP genes were expanded mainly by segmental duplication rather than tandem duplication. Although segmental duplication rate of the CsbZIP genes was lower than that of Arabidopsis, rice and sorghum, it was observed as a common expansion mechanism. Some orthologous relationships and chromosomal rearrangements were observed according to comparative mapping analysis with other species. Genome-wide expression analysis of bZIP genes indicated that 64 CsbZIP genes were differentially expressed in at least one of the ten sampled tissues. A total of 4 CsbZIP genes displayed higher expression values in leaf, flowers and root tissues. The *in silico* micro-RNA (miRNA) and target transcript analyses identified that a total of 21 CsbZIP genes were targeted by 38 plant miRNAs. CsbZIP20 and CsbZIP22 are the most targeted by miR165 and miR166 family members, respectively. They also analyzed the expression of ten CsbZIP genes in the root and leaf tissues of drought-stressed cucumber using quantitative RT-PCR. All of the selected CsbZIP genes were measured as increased in root tissue at 24th h upon PEG treatment. Contrarily, the down-regulation was observed in leaf tissues of all analyzed CsbZIP genes. CsbZIP12 and CsbZIP44 genes showed gradual induction of expression in root tissues during time points. This genome-wide identification and expression profiling provides new opportunities for cloning and functional analyses, which may be used in further studies for improving stress tolerance in plants.

Li *et al.* (2015) carried out a genome-wide identification and systematic analyses of 69 SlbZIP genes that distributes unevenly on the tomato chromosomes. This family can be divided into 9 groups according to the phylogenetic relationship among the SlbZIP proteins. Six kinds of intron patterns (a–f) within the basic and hinge regions are defined. The additional conserved motifs and their presence of the group specificity were also identified. Further, they predicted the

DNA-binding patterns and the dimerization property on the basis of the characteristic features in the basic and hinge regions and the leucine zipper, respectively, which supports our classification greatly and helps to classify 24 distinct subfamilies. Within the SlbZIP family, a total of 40 SlbZIP genes are located in the segmental duplicate regions in the tomato genome, suggesting that the segment chromosomal duplications contribute greatly to the expansion of the tomato SlbZIP family. Expression profiling analyses of 59 SlbZIP genes using quantitative RT-PCR and publicly available microarray data indicate that the tomato SlbZIP genes have distinct and diverse expression patterns in different tissues and developmental stages and many of the tomato bZIP genes might be involved in responses to various abiotic and biotic stresses as well as in response to light.

MicroRNAs (miRNA) are endogenous small RNAs that play essential roles in plant growth, development and response to biotic and abiotic stress. With the availability of draft genome sequence of pigeon pea, understanding miRNA repertoire of several crops has begun to be facilitated. Kompelli *et al.* (2015) have attempted to find miRNA sequences in pigeon pea using genome-wide computational approaches. Further whole genome sequence (WGS) based comparative studies using homology and secondary structure analysis was done. A total of 142 potential conserved miRNAs belonging to 48 families were identified and considered for this study. The size of these 48 miRNA families ranged from one to ten members while the length of miRNAs ranged from 19 nt to 24 nt. Furthermore, this criterion distinguishes miRNAs from other coding and non-coding RNAs. Among the miRNAs, Uracil was found to be dominant nucleotide base in the first position at the 5' end of the mature miRNAs. A total of 423 potential miRNA targets were identified for newly identified pigeon pea miRNAs using psRNA Target tool. These target genes include a number of transcription factors that control plant growth and development, linked to metabolic enzymes involved in stress response. We believe these identified miRNA target genes would help us to know more about the important roles of miRNAs, suggesting that genome-wide computational analysis is a good alternative strategy for identifying new miRNAs and their targets.

Based on the amino acid alignment and phylogenetic analyses, superfamily members were classified into four subfamilies: DREB (54), ERF (95), AP2 (27) and RAV (3), as well as one soloist. The physical and chemical characteristics of amino acids, interaction between AP2-ERF proteins, cis elements of promoter region of AP2-ERF genes and phylogenetic trees were predicted and analyzed. Additionally, expression levels of AP2-ERF genes were evaluated by *in silico* and qRT-PCR analyses. *In silico* micro-RNA target transcript analyses identified nearly all *PvAP2-ERF* genes as targets of by 44 different plant species' miRNAs were identified by Kavas *et al.* (2015). The most abundant target genes were *PvAP2/ERF-20-25-62-78-113-173*. miR156, miR172 and miR838 were the most important miRNAs found in targeting and BLAST analyses. Interactome analysis revealed that the transcription factor *PvAP2-ERF78*, an ortholog of Arabidopsis *At2G28550*, was potentially interacted with at least 15 proteins, indicating that it was very important in transcriptional regulation. Gene annotation, data mining for stress response, and phylogenetic analysis of members from bHLH and trihelix-GT transcription factors were done by Osorio *et al.* (2015) with at least 45 bHLH (from subgroup 25) and 63 trihelix-GT putative genes reside in the soybean genome. Among them, at least 14 bHLH and 11 trihelix-GT seem to be involved in responses to abiotic/biotic stresses. Phylogenetic analysis successfully clustered these with members from other plant species. Nevertheless, bHLH and trihelix-GT genes encompass almost three times more members in soybean than in Arabidopsis or rice, with many of these grouping into new clades with no apparent near orthologs in the other analyzed species.

MADS-box genes encode transcription factors that are involved in developmental control and signal transduction in eukaryotes. In plants, they are associated to numerous development processes most notably those related to reproductive development: flowering induction, specification of inflorescence and flower meristems, establishment of flower organ identity, as well as regulation of fruit, seed and embryo development. Genomic analyses of MADS-box genes in different plant species are providing new relevant information on the function and evolution of this transcriptional factor family. A total of 90 MADS-box genes were detected in the grapevine reference genome by Grimplet *et al.* (2016) by completing current gene annotations with a

genome-wide analysis based on sequence similarity. They performed a thorough in-depth curation of all gene models and combined the results with gene expression information including RNAseq data to clarify the expression of newly identified genes and improve their functional characterization. Curated data were uploaded to the ORCAE database for grapevine in the frame of the grapevine genome curation effort. This approach resulted in the identification of 30 additional MADS box genes. Among them, ten new MIKCC genes were identified, including a potential new group of short proteins similar to the SVP protein subfamily. The MIKC* subgroup contains six genes in grapevine that can be grouped in the S (4 genes) and P (2 genes) clades, showing less redundancy than that observed in *Arabidopsis thaliana*. Expression pattern of these genes in grapevine is compatible with a role in male gametophyte development. Most of the identified new genes belong to the type I MADS-box genes and were classified as members of the M α and M γ subclasses. Our analyses indicate that only few members of type I genes in grapevine have homology in other species and that species-specific clades appeared both in the M α and M γ subclasses. On the other hand, as deduced from the phylogenetic analysis with other plant species, genes that can be crucial for development of central cell, endosperm and embryos seem to be conserved in plants.

Exhaustive searches of pepper genome by Wu *et al.* (2016) revealed 33 potential CaDofs that were phylogenetically clustered into four sub groups. Twenty nine of the 33 Dof genes could be mapped on 11 chromosomes, except for chromosome7. The intron/ exon organizations and conserved motif compositions of these genes were also analyzed. Additionally, phylogenetic analysis and classification of the Dof transcription factor family in eight plant species revealed that *S. lycopersicum* and *C. annuum* as well as *O. sativa* and *S. bicolor* Dof proteins may have evolved conservatively. Moreover, comprehensive expression analysis of CaDofs using a RNA seq atlas and quantitative real-time polymerase chain reaction (qRT CR) revealed that these genes exhibit a variety of expression patterns. Most of the CaDofs were expressed in at least one of the tissues tested, whereas several genes were identified as being highly responsive to heat and salt stresses. Overall, this study describes the first genome-wide analysis of the pepper Dof family, whose genes exhibited different expression patterns in all primary fruit developmental stages and tissue types, as in response to abiotic stress. In particular, some Dof genes might be used as biomarkers for heat and salt stress.

A comprehensive genome wide analysis including chromosomal localization, gene structure analysis, multiple sequence alignment, phylogenetic analysis and promoter analysis of these *WRKY* genes was carried out to determine the functional homology in *Arabidopsis* by Sultan *et al.*, 2016. This analysis led to the classification of these *WRKY* family members into 3 major groups and subgroups and showed evolutionary relationship among these groups on the basis of their functional *WRKY* domain, chromosomal localization and intron/ exon structure. The proposed groups of these stress responsive *WRKY* genes and annotation based on their position on chromosomes can also be explored to determine their functional homology in other plant species in relation to different stresses. The result of this study provides indispensable genomic information for the stress responsive *WRKY* transcription factors in *Arabidopsis* and will pave the way to explain the precise role of various *AtWRKYs* in plant growth and development under stressed conditions.

2.3.4 Utilization of RGAs and TFs

Molecular isolation of resistant genes and TFs

The isolation of *R* genes was achieved with the development of technologies for cloning plant genes of unknown structure or molecular function. Several methods can be used for identifying and cloning genes that are differentially regulated including cDNA-RFLP (Durrant *et al.*, 2000), differential display PCR, and microarray and gene chip technologies (Maleck *et al.*, 2000; Seo *et al.*, 2007).

Several strategies to search for candidate genes for disease resistance could be developed. One of these consists of isolating genes that are expressed differentially in resistant and susceptible plants during the infection process. EST databases can be explored in search of orthologous resistance and defense genes (Lanaud *et al.*, 2003).

The identification, isolation and subsequent cloning of effective disease resistance genes for their use to enhance the protection level against various groups of pathogens in plants are an important aspect of crop improvement. Disease resistance genes (*R*-genes) have been characterized and cloned from many plant species (Mayank *et al.*, 2012). Various investigators have amplified resistance genes from genomes of diverse plant species using multiple combinations of primers with low degeneracy designed from consensus motifs (Aarts *et al.*, 1998, Collins *et al.*, 1998, Fenillet *et al.*, 1997, Kanazin *et al.*, 1996, Leister *et al.*, 1998, Shen *et al.*, 1998, Speulman *et al.*, 1998 and Yu *et al.*, 1996).

The RGAs are an efficient tool in identifying and isolating disease resistance genes and have got efficiency in building a durable resistance (Yong *et al.*, 2012). In order to know the exact function of RGAs they need to be characterized and linked with the genes actually conferring resistance phenotype. Regions of amino acid conservation in resistance gene encoded proteins have facilitated the isolation of RGA sequences from genomic DNA in many crop plants using polymerase chain reaction based techniques. With the advancement of molecular techniques, cloning specific genes has become a possibility apart from their specific deployment in selected crop plants. In this endeavor, cloning and characterization of plant disease resistance genes (*R*) has become a major long term strategy in several crop plants worldwide including flower crops (Jae, 2005). However, hardly there are any reports of cloning and characterization of RGAs in gladiolus.

There are several strategies employed to clone genes of interest. A method relying on PCR technology making use of conserved nature of *R* genes is relatively a preferred route. The amino acid residues conserved across specific group of genes can be used to design the degenerate primers which in combinations could yield partial genes sequences. Degenerate primers are designed based on known resistant genes (*R*-genes) and resistance gene analogs (RGAs) in combinations and are used to clone RGAs (Totad *et al.*, 2005). Most of the previously tried primer combinations resulted in amplicons of expected 500–600 bp sizes in sorghum along with few novel combinations. Restriction analysis of PCR amplicons of expected size revealed a group of fragments present in a single band indicating the heterogeneous nature of the amplicon. The nucleotide sequence of different cloned fragments was done and their predicted amino acid sequences compared to each other and to the amino acid sequences of known *R*-genes revealed significant sequence similarity.

RGAs have been successfully isolated using the PCR based approach from a wide range of plants including Potato (Leister *et al.*, 1996), Soybean (Kanazin *et al.*, 1996), *Arabidopsis* (Speulman *et al.*, 1998), Maize (Collins *et al.*, 1998), Rice (Leister *et al.*, 1998), Tomato (Ohmori *et al.*, 1998), Lettuce (Shen *et al.*, 1998), Bean (Rivkin *et al.*, 1999), Citrus (Deng *et al.*, 2000), Coffee (Noir *et al.*, 2001), Chick pea (Huettel *et al.*, 2002), Barrel medic (Zhu *et al.*, 2002), Cassava (Lopez *et al.*, 2002), Grapevine (Di Gaspero *et al.*, 2002), Peanut (Bertioli *et al.*, 2003), Cotton (Tan *et al.*, 2003), Pine (Liu and Ekramoddoullah, 2003), Strawberry (Martinez *et al.*, 2004), Oat (Irigoyen *et al.*, 2006), Buffalo grass and *Argostis* species (Budak *et al.*, 2006).

In order to clone and analyse Resistance Gene Analogs in 'Goldfinger' Banana, Dequan *et al.* (2010) isolated twenty fragments of RGAs of expected size (about 530 bp) and analysed the deduced amino acids of these RGAs. The analysis showed that, they shared the NB-ARC domain and belongs to the non-TIR-NBS class resistance gene candidates, containing 4 conservative amino acid domains, *i.e.* P-loop (GMGGVGKTT), Kinase-2 (LLVLDDIW), RNBS-B (CKVLFTTRS), and hydrophobic amino acids GLPL (GLPLALKVL). Other results revealed that sequence identity among the 20 RGAs ranged from 41.1 per cent to 99.3 per cent, while identity of the deduced amino acid sequences ranged from 33.2 per cent to 96.3 per cent. The phylogenetic analysis of the RGA nucleotide sequences and the deduced amino acids showed

that the 20 sequences could be divided into 5 distinct types. All of the amino acids deduced from the RGAs shared a homology of 28 per cent - 54 per cent with those deduced from the known wilt resistance genes such as Fom-2, I2C-1, I2C-2 and I2. This result to some degree indicated the conservation of disease resistance gene evolution and laid a base for the further cloning of wilt resistance genes in banana and related crops : such genes could also be used as molecular markers for screening candidate wilt resistance genes.

A total of 10 RGA fragments were identified, and their predicted amino acid sequences were compared to each other and to the amino acid sequences of known R-genes by Penuela *et al.* (2002) revealed significant sequence similarity. Phylogenetic analysis demonstrated that 10 RGAs dispersed along the phylogram on the two major branches of either TIR or non-TIR type of the NBS-LRR proteins. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis showed that transcripts of RGA1, RGA2, RGA5 and RGA23 were specifically induced after infection with *Plasmopara viticola*. These results suggest that RGA1, RGA2, RGA5 and RGA23 may play roles in defense mechanisms that confer resistance to downy mildew in grapevine.

Degenerate PCR primers targeting conserved motifs were tested on the wild cultivar *Musa acuminata* sub sp. *burmannicoides*, var. Calcutta 4, which is resistant to a number of fungal pathogens and nematodes. One hundred and seventy four resistance gene analogs (RGAs) were amplified and assembled into 52 contiguous sequences. Motifs present were typical of the non-TIR NBS-LRR RGA subfamily. A phylogenetic analysis of deduced amino-acid sequences for 33 RGAs with contiguous open reading frames (ORFs), together with RGAs from *Arabidopsis thaliana* and *Oryza sativa*, grouped most *Musa* RGAs within monocotyledon-specific clades. RFLPRGA markers were developed, with 12 displaying distinct polymorphisms in parentals and F₁ progeny of a diploid *M. acuminata* mapping population. Eighty eight BAC clones were identified in *M. acuminata* Calcutta 4, *M. acuminata* Grande Naine, and *M. balbisiana* Pisang Klutuk Wulung BAC libraries when hybridized to two RGA probes. Multiple copy RGAs were common within BAC clones, potentially representing variation reservoirs for evolution of new R-gene specificities (Miller *et al.*, 2011).

The first effort to sample and characterize R-gene candidate sequences in gerbera was done by Song *et al.* (2012) and revealed that the obtained sequences may provide a valuable entry point to obtain full-length or additional sequences of gerbera NB-LRR genes. The SCAR, CAPS and TRAP markers developed may be valuable for mapping of genes or quantitative trait loci responsible for disease resistance in gerbera.

The first report of NBS-LRR class RGAs in eggplants was reported by Zhuang, *et al.* (2012). Resistance gene analogs (RGAs) were isolated from cultivated and wild egg plants, *i.e.*, *Solanum melongena*, *S. aethiopicum*, *S. linnaeanum*, *S. integrifolium*, *S. sisymbriifolium* and *S. khasianum*. Sequence analysis indicated that the cloned eggplant RGAs belong to the non-TIR-NBS-LRR type, which are very similar to the R genes or the RGAs identified in other plant species, especially solanaceous plants, suggesting the existence of common ancestors.

Disease resistance gene candidates (RGCs) from six species of pear, including *Pyrus betulaefolia*, *P. bretschneideri*, *P. pyrifolia*, *P. ussuriensis*, *P. sinkiangensis*, *P. communis* and their interspecific hybrids were isolated using homolog based cloning method by Zhang *et al.* (2011). The deduced amino acid sequences of the 98 RGC displayed high diversity, ranging from 20 to 100 per cent, and could be divided into 17 distinct RGC families (RGC 1-RGC 17) based on the phylogenetic analysis with the threshold of 68 per cent identity.

Related sequences from soybean (*Glycine max* (L.) Merr.) were amplified using oligonucleotide primers designed for conserved sequences from coding regions of disease resistance genes *N* (tobacco), *RPS2* (*Arabidopsis*) and *L6* (flax) , and nine classes of resistance gene analogs (RGAs) were detected (Kanazin *et al.*, 1996). Genetic mapping of members of these classes located them to eight different linkage groups. Several RGA loci mapped near

known resistance genes. A bacterial artificial chromosome library of soybean DNA was screened using primers and probes specific for eight RGA classes and clones were identified containing sequences unique to seven classes.

R gene analogues (RGAs) from five species of banana (*Musa* spp.), i.e. wild species of Gongjiao (AA) and Xinyiyejiao (BB), cultivated species of Zhong shandajiao (ABB), Fenjiao (AAB) and Williams (AAA), respectively were isolated by Pei *et al.* (2007) using degenerate primers. A total of 91 sequences with uninterrupted open reading frame (ORFs) were typical of RGAs out of 208 clones sequenced, of which 33 sequences with identity of deduced amino acid sequence below 97 per cent were further analyzed. Based on the phylogenetic analysis by using MEGA software, the 33 sequences could be divided into 12 distinct *Musa* RGAs.

A pair of degenerate primers designed on the basis of the conserved nucleotide-binding-site motifs in many R genes were used by Huang *et al.* (2004) to amplified RGAs from single chromosomes of pomelo using products of Sau3A linker adaptor-mediated PCR (LAM-PCR) from a single chromosome as the templates for RGA amplification, and directly using a single chromosomal DNA molecule as the template. Sequencing and cluster analysis of RGA clones obtained from single chromosomes revealed the number, type and organization of R-gene clusters on the chromosomes.

Oligonucleotide primers, designed based on conserved regions of nucleotide binding site (NBS) motifs within previously cloned pathogen resistance genes, were used to amplify resistance gene analogues (RGAs) from dogwood by Shi *et al.* (2008). Twenty eight unique dogwood RGA sequences were identified and subdivided into 11 groups on the basis of nucleic acid sequence-identity of approximately 70 per cent or greater. Phylogenetic analysis of the predicted amino acid sequences grouped the RGAs into three distinct classes from which several subgroups were delineated based on nucleic acid sequences. These cloned RGAs may be useful as probes to map, characterize, and manipulate R genes of the dogwood genome.

Genomic DNA sequences sharing homology with NBS region of resistance gene analogs were isolated and characterized from *Pongamia glabra*, *Adenanthera pavonina*, *Clitoria ternatea* and *Solanum trilobatum* (Thirumalaiah et al., 2008). The presence of consensus motifs viz., kinase 1a, kinase 2, kinase 3a and hydrophobic domain provided evidence that the cloned sequences may belong to the NBS-LRR gene family. Conservation of tryptophan as the last residue of kinase-2 motif further confirmed their position in non-TIR NBS-LRR family of resistance genes.

Toll and interleukin-1 receptor (TIR) and nonTIR nucleotide binding site-leucine rich repeat (NBS-LRR) resistance gene analogues (RGAs) were obtained from chestnut rose (*Rosa roxburghii* Tratt) by two PCR-based amplification strategies (direct amplification and overlap extension amplification) with degenerate primers designed to the conserved P-loop, kinase-2, and Gly-Leu-Pro-Leu (GLPL) motifs within the NBS domain of plant resistance gene (R gene) products. Thirty-four of 65 cloned PCR fragments contained a continuous open reading frame (ORF) and their predicted protein products showed homology to the NBS-LRR class R proteins in the GenBank database. These 34 predicted protein sequences exhibited a wide range (19.5–99.4 per cent) of sequence identity among them (Xu *et al.*, 2005).

Seventy five RGAs were identified by Cao, *et al.* (2011) using two different degenerative primer pairs in the Honggen gansutao (*Prunus kansuensis*), a wild species of peach resistant to drought and nematodes. Through aligning their amino-acid sequences, P-loop and GLPL motifs were found in 48 RGAs with open reading frames (ORF). These RGAs and 17 RGAs from *Arabidopsis thaliana*, *Capsicum annuum* and *Solanum lycopersicum* were grouped into two classes by phylogenetic analysis: toll and interleukin-1 receptor (TIR) and non-TIR-NBS. Most Honggen gansutao RGAs were TIR-NBS. A semiquantitative RT-PCR analysis revealed transcript-level variations of 22 RGAs in the young leaves, flowers, fruits and roots of the Honggen gansutao, demonstrating their probable role in resistance against diseases attacking the organs.

Backiyarani *et al.* (2013) isolated RGAs from two banana cultivars viz., Karthobiumtham and Rose using degenerate primers designed from the conserved motifs of different plant resistance genes. A total of 40 sequences were hit with various R genes, of which 20 sequences were having uninterrupted open reading frame (ORFs). Based on the conserved domains like P loop, internal kinase 2, kinase 3a and hydrophobic domain motifs of the deduced amino acid sequences were grouped as NBS-LRR class of resistant genes. The phylogentic analysis of RGAs showed that all the Musa RGAs are grouped under non-TIR branch and grouped into six distinct Musa RGA cluster.

2.3.5 Engineering of disease resistance by using RGAs and TFs

For nearly a century breeders have been studying the inheritance of *R* genes and using classical breeding techniques for the interogression of *R* genes from wild populations into elite cultivars. Traditional breeding methods are very time consuming, development of resistant cultivars may take up to 15-20 years. RGAs can be utilized as a marker to be applied in marker assisted selection for early release of disease resistant varieties.

Many case studies have been reported on transfer of *R* genes within and across species to confer disease resistance. For e.g. *Bs2* gene in pepper which confers resistance to *Xanthomonas campestris* was transferred to tomato (Tai *et al.*, 1999) and *N* gene of tobacco resistant to *Tobacco Mosaic Virus* was transferred to tomato (Levy *et al.*, 2004).

Many *R* genes have a narrow range of resistance, often to only one or a few strains of a single pathogen species. Many *R* genes lack durability because they can be defeated by a single loss-of-function mutation in the corresponding *Avr* gene (thereby rendering the pathogen 'invisible'). Because individual *Avr* genes often make only incremental contributions to virulence, pathogens can afford to alter or discard an *Avr* gene with little or no fitness penalty. Unfortunately, *R* genes are often quickly defeated by co-evolving pathogens (Pink, 2002). Many *R* genes recognize only a limited number of pathogen strains and therefore do not provide broad-spectrum resistance. Pyramiding and multiline deployment have not been widely used, owing to the time required for breeding assortments of *R* genes into elite cultivars. However, recent molecular-level insights into the function of R proteins and downstream signal transduction pathways might provide strategies to remedy these deficiencies (McDowell and Woffenden, 2003).

Broad-spectrum resistance can be achieved by coordinate expression of an *R* gene and a corresponding *Avr* transgene, controlled by a pathogen-inducible promoter. This tactic enables induction of defense by multiple pathogens without pyramiding numerous *R* transgenes. Further, this system might circumvent the loss of durability commonly observed with *R* genes because the *Avr* gene is not under selective pressure for mutation (De Wit, 1992).

The most exciting approach towards engineering improved resistance to multiple diseases may be the development of new *R* genes having multiple specificities. The *Fen* (resistance to the insecticide Fenthion) and *Pto* genes are located in the same *R* gene cluster in the tomato genome and they are 86 per cent identical in nucleotide sequence. A functional gene was made by domain swapping of the two genes, thus raising the possibility of creating a hybrid gene containing multiple specificities. Another novel strategy, termed two-component approach, has been developed lately and holds lot of promise for introducing broad-spectrum resistance. This strategy involves generation of transgenic plants that express a pathogen *Avr* gene under the control of a heterologous infection-inducible promoter. If the plant carries the matching *R* gene, it will respond with an HR at the site of infection thus limiting the pathogen. The key to this approach is the identification of suitable promoters that respond or are induced only following infection by broad range pathogens.

2.4 Expressional analysis of selected RGAs and TFs using qRT-PCR

2.4.1 Expression profiling of RGAs and TFs

The study of gene function requires the ability to accurately quantify temporal and spatial patterns of gene expression. Traditional approaches, such as northern blots and RNase protection assays are in many cases unsuitable, as their low sensitivity required high concentrations of starting template (Sambrook and Russell, 2001; Bustin, 2000). The advent of real-time PCR has enabled rapid and reproducible high throughput RT-PCR quantification, with an unparalleled dynamic range and extremely high sensitivity. The first practical kinetic PCR technology, the 5'-nuclease assay was established in 1993 and combines the exponential PCR amplification of specific transcript with monitoring of newly synthesized DNA in each performed PCR cycle (Wong and Medrano, 2005). It is most sensitive method for the detection and quantification of gene expression levels, in particular for low abundant transcripts in tissues with low RNA concentrations, from limited tissue sample and for the elucidation of small changes in mRNA expression levels (Mackay *et al.*, 2002; Schmittgen *et al.*, 2000, and Fleige *et al.*, 2006). RT-PCR is a highly sensitive and specific method useful for the detection of rare transcripts or for the analysis of samples available in limiting amounts (Carding *et al.*, 1992). By the RT-PCR method, many studies have been conducted for the expression analysis of disease resistant genes in ground nut (Coelho *et al.*, 2006), sunflower (Radwan *et al.*, 2005) *etc.*

Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) is an efficient, specific and reproducible method for quantifying transcript expression levels, and is widely used to analyze mRNA in different organisms. Gene expression is most commonly quantified for measuring the amount of the transcripts of interest (Higuchi *et al.*, 1992). This allows continuous monitoring of the amplification kinetics of a PCR in real time measuring the specific fluorescence signal in each cycle. The *CT* is the fractional number of cycles in which the kinetic curve reaches a threshold amount of fluorescence. The amplification efficiency could be visualized in a half-logarithmic plot in which log transformed fluorescence values are plotted against the time (cycle number). Roughly, the efficiency represents the yield of the reaction.

Real-time quantitative PCR allow to sensitive, specific and reproducible quantitation of nucleic acid (Bustin, 2000). The real-time PCR is the technique of collecting data throughout the PCR process as it occurs, thus combining amplification and detection into a single step. PCR can be broken into four major phases. The 1st phase is hidden under background fluorescence where an exponential amplification is expected; 2nd phase with exponential amplification that can be detected and above the background; 3rd phase linear amplification efficiency and a steep increase of fluorescence; and finally 4th phase or plateau phase, defined as the attenuation in the rate of exponential product accumulation, which is seen concomitantly in later cycles (Kainz, 2000). The amount of amplified target is directly proportional to the input amount of target only during the exponential phase of PCR amplification. Hence, the key factor in the quantitative ability of kinetic RT-PCR is that it measures the product of the target gene within that phase (Schmittgen *et al.*, 2000; Tichopad *et al.*, 2003). In plateau stage, reaction components become limited and the fluorescence intensity is no longer useful for data calculation (Bustin, 2000). Two-step real-time PCR separates the reverse transcription reaction from the real-time PCR assays, allowing several different real-time PCR assays on dilutions of a single cDNA. Two-step real-time PCR is quite reproducible with Pearson correlation coefficients ranging from 0.974 to 0.988. A two step protocol may be preferred when using a DNA binding dye (such as SYBR green) because it is easier to eliminate primer-dimers through the manipulation of melting temperatures (*T_m*) (Vandesompele *et al.*, 2002).

2.4.2 Types of real-time quantification

The quantification strategy is the principal marker in gene quantification. Generally, two strategies can be performed in real-time RT-PCR. The levels of expressed genes may be measured by absolute or relative real-time PCR. Absolute quantification relates the PCR signal to input copy number using a calibration curve, while relative quantification measures the relative change in mRNA expression levels.

2.4.2.1 Absolute quantification

Absolute quantification uses serially diluted standards (recombinant plasmid DNA (recDNA), genomic DNA, RT-PCR product and commercially synthesized big oligonucleotide) of known concentrations to generate a standard curve. The standard curve produces a linear relationship between Ct and initial amounts of total RNA or cDNA, allowing the determination of the concentration of unknowns based on their Ct values. This method assumes all standards and samples have approximately equal amplification efficiencies (Souaze *et al.*, 1996).

2.4.2.2 Relative quantification

Relative quantification determines the changes in steady-state mRNA levels of a gene across multiple samples and expresses it relative to the levels of an internal control RNA. Relative quantification does not require standards with known concentrations and the reference can be any transcript, as long as its sequence is known. Ideally the reference gene should not be regulated or influenced by the experimental procedure. The accurate quantification of a true reference gene allows the normalization of differences in the amount of amplifiable RNA or cDNA in individual samples generated due to the following reasons *i.e.* different amounts of starting material, the quality of the starting material, differences in RNA preparation and cDNA synthesis. Since, the reference gene is exposed to the same preparation steps as the gene of interest. It is crucial to choose the appropriate reference gene for an exact comparison of mRNA transcription in different samples or tissues (Radonic *et al.*, 2005; Silver *et al.*, 2006). To calculate the expression of a target gene in relation to an adequate reference gene various mathematical models are established. Two types of relative quantification models are available:

Without efficiency correction

$$\text{Ratio} = 2^{-(\Delta \text{Ct sample} - \Delta \text{Ct control})}$$

With kinetic PCR correction efficiency

$$\text{Ratio} = \frac{(\text{E target})^{\Delta \text{Ct (sample} - \Delta \text{Ct control)}}}{(\text{E ref})^{\text{Ct control}}} \div \frac{(\text{E ref})^{\Delta \text{Ct (sample} - \Delta \text{Ct control)}}}{(\text{E ref})^{\text{Ct control}}}$$

Many PCR reactions do not have ideal amplification efficiencies, and calculations without an appropriate correction factor may overestimate starting concentration (Liu and Saint, 2002). When used appropriately, relative quantification can generate useful and biologically relevant information (Pfaffl, 2004). Although being extremely powerful technique, qPCR suffers from certain pitfalls, noteworthy the use of unreliable reference genes for the normalization step. Normalization has necessary for the correction of non-specific variations, such as inaccurate quantification of RNA and problems in the quality of RNA that can be trigger variable reverse transcription and PCR reactions. A number of strategies have been proposed to normalize qPCR data but normalization remains one of the most important challenges concerning this technique (Huggett *et al.*, 2005).

Scientists have been validated genes suitable for use as normalization controls by qPCR technique in *Arabidopsis thaliana* (Czechowski *et al.*, 2005), rice (Jain *et al.*, 2006), potato leaves (Nicot *et al.*, 2005), the parasitic plant *Orobancha ramose* (Gonzalez *et al.*, 2008), *Brachypodium distachyon* (Hanhong *et al.*, 2008), and grape (Reid *et al.*, 2006). In the Solanaceae, candidate genes for normalization have been determined based on EST abundance (Coker *et al.*, 2003), and qPCR followed by statistical analysis (Exposito *et al.*, 2008). The importance of reference genes from plant system can be analyzed by qPCR and has been recently emphasized even though the identification of these genes have been quite laborious (Brunner and Strauss, 2004; Reid *et al.*, 2006). Till date experiments have been carried for validating putative sorghum reference genes having data normalization for relative quantitation of expression of genes were

approached by identifying the most suitable reference gene among tested actin (AC1), β -tubulin and 18S rRNA in sorghum were selected according to their similarity to reference genes identified in *Arabidopsis* (Li *et al.*, 2008).

Zhongtang *et al.*, 2005 developed and validated the use of three real-time PCR assays to quantify the abundance of the three groups of tetracycline resistance genes: *tet* (A) and *tet* (C); *tet* (G); and *tet* genes encoding ribosomal protection proteins, including *tet* (M), *tet* (O), *tetB* (P), *tet* (Q), *tet* (S), *tet* (T), and *tet* (W). Gene-specific primer pairs were developed by Giulia *et al.* (2013) for each of the 31 *Mal d 1* genes and incorporated them into a qRT-PCR assay. The results from the application of the assay showed that 11 genes were not expressed in apple fruit. In addition, differential expression was observed among the *Mal d 1* genes that were expressed in the apple fruit. Moreover, the expression levels were tissue and cultivar dependent. qRT-PCR analysis was done by Wan *et al.* (2012) to show that, both salicylic and abscisic acids induce the expression of *Capsicum annuum* RGA genes, suggesting that they may primarily be involved in defense responses by activating signaling pathways.

A total of 195 RGAs were predicted and physically localized by Ghazi *et al.* (2009). Of these, 91.79 per cent expressed in rice, and 51.28 per cent expressed in wheat, which was the highest among other cereals. Among monocots, sugarcane showed the highest (78.92 per cent) expression, while among dicots, RGAs were maximally expressed in *Arabidopsis* (11.79 per cent). Interestingly, two of the chromosome 11-specific RGAs were found to be expressing in all the organisms studied. Eighty RGAs of chromosome 11 had significant homology with chromosome 12, which was the maximum among all the rice chromosomes. Thirty-one per cent of the RGAs used in polymerase chain reaction (PCR) amplification showed polymorphism in a set of rice genotypes. Actual gene expression analysis revealed post-inoculation induction of one RGA in the rice line IRBB-4 carrying the bacterial blight resistance gene *Xa-4*.

The expression profile of RGAs was determined by Gutierrez *et al.* (2012) using quantitative real-time polymerase chain-reaction (qPCR) in WR315 (resistant) and ILC3279 (susceptible) genotypes in response to *Foc* race 5 inoculation and demonstrated that, RGA05 and RGA07 were induced after *Foc* race 5 treatment at 2 days after inoculation (DAI) in the resistant genotype. In contrast RGA10 was induced in both resistant and susceptible plants, although the basal level of this gene was higher in the resistant genotype. On the contrary, no significant changes were observed for any of these genes at 7 DAI and suggested a role of some of the candidate genes in the early response against *Fusarium* wilt, mainly as part of the inducible defensive system. Thus, these genes could be a good start point for further studies such as candidate gene mapping or understand the bases for resistance in chickpea.

To analyze the expression levels of RGAs in both compatible and incompatible interactions across the infection time course in *Musa*. Emediato *et al.* (2013) designed specific primer pairs for reverse transcriptase (RT) PCR. This RT-PCR result revealed examples of constitutive RGA gene expression in both compatible and incompatible interactions across the infection time course, as well as a complete absence of gene expression. Examples of RGAs showing similar expression pattern in compatible and incompatible interactions were also apparent, with susceptible and resistant cultivars, for example, showing low levels of expression in early infection stages and up-regulation in late stages. In contrast, another RGA displayed constitutive early expression in the resistant cultivar, strong down-regulation in the intermediate infection stage, and recovery to higher levels in the final stage of the examined infection time course. Candidate gene application via plant transformation or marker-assisted selection will benefit *Musa* breeding programs for the generation of resistant genotypes.

To test the expression profile at transcriptional level Zhang *et al.* (2011) selected one representative RGC from 17 distinct RGC families from six species of pear and all the 17 selected RGCs could be detected at mRNA level. In response to *Venturia nashicola* inoculation or salicylic acid (SA) treatment, the expression level of RGC Pb-Zs1 (RGC 1) from pear scab-resistant cultivar 'Zaosu' pear changed while the expression level of RGC Pb-Dshs2 (RGC 8) from pear scab-susceptible pear cultivar 'Dangshansu' seemed less affected. These RGCs isolated could

help to understand the R-gene in pear species and will serve as a potential resource for future improvement of disease resistance in pear.

2.4.3 Tissue collection of diseased plants for qRT analysis

2.4.3.1 Rust

2.4.3.1.1 Nomenclature and history

The basidiomycetes *Uromyces appendiculatus* (Pers. Pers.) Unger var. *appendiculatus* was first described in Germany in 1795 by Persoon as *Uredo appendiculatus phaseoli*. Grafton *et al.* (1985) were of the view that rusts fungi, *U. phaseoli* RebenWint. *U. appendiculatus* Pers. Unger. var. *appendiculatus* and *U. phaseolivar. typica* are synonymous. In India, it was first reported from Pusa in 1918 (Butler, 1918).

2.4.3.1.2 Host parts affected and symptoms

The disease can be noticed on all aerial parts of the plant but it is most common on leaves. Initial symptoms are white to cream-colored circular specks under the epidermis (the developing sori). Rust-colored pustules develop as urediniospores which break through the epidermis on both the adaxial and abaxial leaf surfaces but they are more common on the abaxial leaf surfaces. Larger pustules often are surrounded by a chlorotic halo (Liebenberg and Pretorius, 2010).

Butler (1918) reported that the pathogen attacks mostly on leaves and rarely on stems and pods. In some cases, necrotic lesions may contain sporulating pustules (Fromme and Wingard, 1921; Harter and Zaumeyer, 1941). Vergas (1980) was of the view that the pathogen infects leaves, pods, petioles and rarely stems and branches. A ring of secondary and even tertiary pustules may develop on susceptible genotypes. Circular or irregular brown or gray to black necrotic lesions, ranging from less than 0.3 to 3 mm or more in diameter are found in the field but they are difficult to detect (Liebenberg and Pretorius, 2010).

2.4.3.1.3 Economic importance

Rust can cause major yield reduction and serious economic loss in bean crop. Total destruction of the crop, due to rust was reported in California (Fromme and Wingard, 1921) and also in parts of Colorado (Zaumeyer and Thomas, 1957). At CIAT Columbia, yield loss caused by rust was in the range of 18 to 45 per cent. Several studies have indicated that the yield reduction was as high as 78 per cent (Grafton *et al.*, 1985)

In Africa, serious bean yield losses are reported regularly as the crop cultivation is almost round the year providing a continuous supply of inoculum (Howland and McCartney, 1966). At times, even with the moderate levels of rust infection (0.7-31.6 pustules per sq cm) can cause substantial yield loss of 13-54 per cent (Venette and Jones, 1982). Whereas, Grafton *et al.* (1985) found that the epiphytotics of 1982 in USA had caused the yield loss upto 78 per cent.

Steadman *et al.* (1986a) reported a highly significant correlation between rust severity and yield, total number of pods, total number of seeds and seeds per pod, and indicated that early rust intensity could be used to predict yield loss. In Kenya, Kimani *et al.* (1990) cited yield losses varying between 18 to 100 per cent and Gridley (1990) made similar observation in East African countries. Lindgren *et al.* (1995) reported a method for predicting yield loss accurately based on the extent of rust severity recorded two weeks before pod maturity. In India, Mohan *et*

al. (1993) estimated the yield loss of 45 per cent due to this disease on beans under natural infection under Bangalore condition.

2.4.3.2 Mung Bean Yellow Mosaic Virus (MYMV)

2.4.3.2.1 Historical Aspects

The incidence of *MYMV* was first reported on mungbean from New Delhi in 1955 (Nariani, 1960). *MYMV* disease on mungbean has been reported from several other countries viz., Indonesia, Pakistan, Bangladesh, Sri Lanka, Thailand, Philippines, New Guinea, Columbia and Ethiopia. In India, the disease on mungbean has been reported from all most all mungbean-growing states. But, it has attained serious proportions in Uttar Pradesh, Uttaranchal, Madhya Pradesh, Chatisgarh, Bihar, Jharkand, Orissa, Delhi, Punjab, Haryana, Rajasthan, and Andhra Pradesh (Singh *et al.*, 2004). The first report of *MYMV* infection on french bean under field conditions was reported from Karnataka in 2004 (Annonymus, 2004).

2.4.3.2.2 Economic Importance

Yield loss due to *MYMV* in french bean has not been systematically documented in India. However, various workers have reported yield loss up to 100 per cent due to *MYMV* in mungbean and urdbean crops. Chenulu *et al.* (1979) and Marimuthu *et al.* (1981) reported yield loss between 10 and 100 per cent. Ahmad, (1990) found 62.9 per cent reduction in the number of pods in mungbean plants, which showed early disease symptoms *i.e.*, 20 days after sowing. Ghafoor *et al.* (2000) noticed the significant association of *MYMV* with all the characters of mungbean except seeds per pod, seed weight and harvest index. They also found the decrease of 12.2 per cent in branches, 15.4 per cent in pods, 14.0 per cent in biological yield and 13.9 per cent grain yield.

2.4.3.2.3 Symptoms

Honda and Ikegami, (1986) observed downward curling of leaves without yellow mosaic in young systemically infected leaflets but reported rare appearance of irregular chlorotic spots. Whereas, Dhingra and Ghosh (1993) reported irregular chlorotic spots on older leaves of french bean cv, Contender and they also inferred that the plants will take two weeks to express disease after infection. Mandal *et al.* (1998) reported appearance of golden yellow mosaic with mild leaf bending symptoms on cv, Contender. Biswas and Varma (2001) also observed symptoms of leaf crinkling, inward curling, small yellow patches in VL-67, Top Crop, and Contender cultivars of french bean, while studying of different agroinoculation methods for the transmission of cloned *MYMV* virus.

The initial symptoms of the disease are appearance of yellow specks on young leaves, with the next emerging trifoliate leaf exhibiting irregular yellow and green patches and slight puckering and reduced leaf size in mungbean (Nariani, 1960 and Nene *et al.*, 1972). The yellow patches coalesce to form larger patches that develop in to a yellow mottle; eventually the entire leaf may turn yellow. Maturity is delayed in the diseased plants; flower and pod production is severely reduced. Seeds that develop in severely infected plants were small and immature (Varma *et al.*, 1992). Singh *et al.* (2004) reported that necrotic center develops in the yellow spots of the leaves in some cultivars. In plants with the necrotic mottle, the size of the newer leaves was not reduced and there was no reduction in number and size of pods. The yellow mottle and necrotic mottle were host reaction to the virus cultivars. However, sometimes the yellow mottle symptoms were very clear on the pods, while rest of the plant may not show any symptoms. Symptom less infection in urdbean and mungbean was also observed (Biswas and Varma, 2001).

2.4.3.2.4 Causal organism and pathogenic variability

The disease is caused by *Mungbean Yellow Mosaic Virus (MYMV)*, a whitefly transmitted virus (WTG's). They are restricted to dicotyledonous plants and are assigned to the genus *Begomovirus* (formerly sub group III) within the family *Gemini viridae* (Mayo and Pringle, 1998; Jindal *et al.*, 1998). Srivastava *et al.* (1985) reported that the virus particles were isometric, paired, 18 x 30 nm in size and had single stranded DNA. These particles were limited to phloem-associated elements in infected plants. Honda *et al.* (1983) reported that the virus was a single stranded circular DNA with about 20 per cent of particle weight, M. Wt 0.8×10^6 , sedimentation coefficient of ($S_{20,w}$), at pH 7.0. Moringa *et al.* (1990) studied restriction endonuclease mapping and reported that there were two major DNA species namely DNA-1 and DNA-2. DNA-1 has 2715 nucleotides (22.5 per cent G, 27.1 per cent A, 20.7 per cent C, 29.7 per cent T; M. Wt 0.84×10^6) and DNA-2 has 2637 nucleotides (20.6 per cent G, 28.7 per cent A, 19.0 per cent C, 31.7 per cent T; M. Wt 0.81×10^6).

Honda *et al.* (1983) reported the relationships among the isolates of mungbean yellow mosaic virus from India, West Pakistan, Sri Lanka, and Thailand. The Thai isolate of the virus was transmissible by mechanical inoculation and its host range appeared to be restricted to seven species in the legumes. Whereas, on the contrary, other isolates from India, West Pakistan and Sri Lanka were not transmissible through sap (Nene, 1972; Honda and Ikegami, 1986). Moreover, an Indian isolate had a wider host range than Thai isolates, including certain species in the leguminosae, Compositae and Graminae (Nene, 1972).

Amin and Singh (1989), observed differential reaction of mungbean varieties, when they were subjected to multi location testing by infector row technique against *MYMV*. The varieties found resistant or tolerant at one location were found susceptible at the other and vice versa. Reddy *et al.* (2006) had also observed variable disease reaction types with different cultivars of mungbean and urdbean at different locations in Andhra Pradesh and based on these observations they reported the possibility of existence of different strains in *MYMV*. Mandal and Varma (1996) had differentiated eight natural variants of *MYMV* collected from different hosts and location by following host reactions of full length and restriction fragment probes to DNA- 1 and DNA – 2 by dot blot hybridization. The work on differential strains of *MYMV* in french bean has not been reported.

2.4.3.2.5 Transmission of virus

Nene, 1972; Ahmad and Harwood, 1973; Thogmeearkan *et al.* (1981) and Singh *et al.* (2004) reported that the virus was transmitted by whitefly *Bemisia tabaci* Genn and by grafting, but not transmissible through seeds, sap and soil borne. Studied the acquisition and inoculation of virus by adult white flies and reported that minimum time of 15 minutes for acquisition of virus and with a latent period of less than 4 hours was most effective for transmission. Raithi (1972) reported that a single viruliferous adult can transmit the virus and most efficient female and male adults in a population can retain infectivity for 10 days and 3 days respectively whereas, neither female nor male adult can retain infectivity throughout the lifespan. Dhingra and Ghosh (1993) and Raithi (1972) opined that female adults were over three times more efficient as vectors than males.

Nene (1972) and Chenulu *et al.* (1978) found acquiring of virus by the nymphs of *Bemisia tabaci* from diseased leaves and successful transmission at their adult stage. Chenulu *et al.* (1979) recorded minimum acquisition period of 15 to 30 minute and inoculation feeding period of minimum one hour for better results of transmission and they also added that a single whitefly was enough to transmit the virus. Whereas, variation in acquisition and inoculation time among varieties may be due to concentration of the virus in the host tissue or occurrence of virus inhibitors in the host or host palatability for the virus (Mandal *et al.*, 1998). However, Dhingra and Ghosh (1993) reported on 55 and 65 per cent of transmission of the virus in the presence of 5 and 10 adult flies per plant respectively whereas, it was reduced to 20 and 30 per cent in the presence of nymphs. They also grouped french bean as rank four according to DMRT ranking based on the mean transmission per cent of *MYMV* among different legumes viz., mungbean,

urdbean, soybean and pigeonpea. However, Raithi (1974), Ahmad and Harwood (1973) and Chenulu *et al.* (1978) confirmed the not transmissibility of virus by means of eggs.

Apart from this Biswas and Varma (2001), Usharani *et al.* (2004), reported on successful inoculation of virus by following agroinoculation method at sprouted seed and seedling stage of french bean and other legumes. The results indicated high infection in french bean, cowpea and mungbean than in black gram and soybean. They had also recorded less percent of transmission by means of agroinoculation method than the whitefly transmission.

III. MATERIAL AND METHODS

The present study involved development of functional mutants using chemical mutagen, EMS, cloning and characterization of Resistant Gene Analogues (RGAs), *in silico* genome wide identification of Transcriptional Factors (TFs) and expressional analysis of selected RGAs and TFs in french bean. The material and various methods adapted are described in this chapter.

Experiment 1: Induced mutation to create spectral variability in french bean

The present study, "Induced mutation to create spectral variability for functional mutants in french bean (*Phaseolus vulgaris* L.)" was carried out in a well adapted and most extensively grown 'Arka Anoop' variety of french bean (Plate 1). The crop was grown at College of Horticulture, Vegetable Research Block, UHS campus, Bengaluru.

3.1 Experimental material

3.1.1 Standardization of EMS concentration for LD₅₀

A set of 100 well-matured healthy seeds were subjected to treatment of different concentrations of EMS. Initially seeds were pre-soaked in distilled water for five hours at room temperature (28±2°C). After pre-soaking, water was drained out and surface moisture was removed by using filter paper. Thereafter, seeds were transferred to the freshly prepared aqueous solution (pH 8.5, adjusted by using borax), volume of which is about three times higher than that of volume of seeds with corresponding concentrations of EMS viz., 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 per cent. All seed-solution mixtures were kept at room temperature (28±2 °C) for six hours with an hour intermittent shaking, after the treatment seeds were washed thoroughly with distilled water. Germination percentile was recorded from the seeds germinated on moist blotting paper and in field when radical and plumule emerged out (Avinash *et al.*, 2010).



**Plate 1. Arka Anoop genotype used
for mutation**



Plate 2a. M_1 Population



Plate 2b. M_2 Population



Plate 2c. M_3 Population

Plate 2. General view of french bean mutant population evaluated under field condition

3.1.2 Generation of large-scale functional mutants' population

Using standardized LD₅₀ concentration, over 3000 seeds were treated to get around 1500 plants in M₁ generation. The untreated seeds pre-soaked in water were used as control. Both treated and control seeds were sown in the field to raise the M₁ generation.

3.1.3 Experimental layout

The M₁ generation along with control was raised during rainy season, 2014 at Vegetable Research Block, College of Horticulture, Bengaluru. Distance maintained between and within rows were 30 cm and 10 cm respectively, whereas the row length was three meter.

Observation of survival plants was taken and expressed in percentage. M₁ plants were harvested individually at maturity to obtain M₂ seed. Further, M₂ seeds were sown during October, 2014 in plants to progeny rows along with the control to generate M₃ seeds. The M₂ generation was screened for different morphological traits during the entire growing season. M₂ plants were selected and harvested individually (Plate 2). The plants showed variability for different characters (100) were further carried to raise M₃ generation. In M₃ seeds of all selected 100 plants were sown in plant to row manner, in each line have sown 20 seeds.

Final trial was conducted at Vegetable Research Block, College of Horticulture, Bengaluru during 2016, where the M₃ generation from selected plants seeds was raised in augmented block. Each plot consisted of single row of 1.5 m length and, row-to-row and plant-to-plant spacing of 30 cm and 10 cm, respectively along with the control. Recommended UHS package of practices was followed for raising the crops.

During 2015, 500 M₂ families comprising of 10000 mutant plants and during 2016, 2000 plants of M₃ were studied for different morphological traits.

3.1.4 Recording of data

Data in M₂ and M₃ generation were recorded on single plant basis and observations were recorded on the following traits:

3.1.4.1 Plant height (cm)

The height of the plant was measured from ground level to the tip of the growing point and expressed in centimeter at 60 days after sowing and the average was worked out.

3.1.4.2 Number of branches per plant

The total numbers of branches per plant were recorded from all plants in each line at 60 days after sowing and the average was worked out.

3.1.4.3 Days to 50 per cent flowering

The number of days taken from date of sowing to the date at which 50 per cent of the total number of plants flowered in each line was recorded.

3.1.4.4 Pod length (cm)

Five pods were chosen randomly from plant of each line and length of each pod was measured with scale in centimetres from base of the pod to its tip and average pod length was expressed in centimetres.

3.1.4.5 Pod width (cm)

The same set of pods used for measure pod length was used for measure pod width with vernier calipers and average pod width was worked out and expressed in millimeter.

3.1.4.6 Weight of ten pods (g)

Ten pods were randomly selected from individual plant were weighed by using balance and average was recorded.

3.1.4.7 Average number of seeds per pod

Five randomly selected pods from each plant of each line were cut longitudinally, seeds were separated and counted separately and average number of seeds per pod was worked out.

3.1.4.8 Tenderness of pods (tr)

The tenderness of five pods harvested from individual plants measured by firmness tester (tenderometer) and expressed in 'tr' values.

3.1.4.9 Total yield of pod per plant (g)

Total pod yield of individual plants recorded over successive harvest were weighed by using balance and average was recorded and expressed in grams.

3.1.4.10 Total yield per hectare (q)

Total pod yield was expressed in per hectare basis from the harvestable pod yield using the following formula.

3.1.4.11 Qualitative traits

3.1.4.11.1 Pod stringness

The observation recorded as stringed and stingless pods.

3.1.4.11.2 Plant growth habit

The observation recorded as bushy or pole type at the time of harvesting stage.

3.1.4.11.3 Leaf colour

The intensity of leaf color was recorded as green, dark green and light green.

3.1.4.11.4 Leaf character

The size of the leaf was recorded as broad, normal, thick and thin.

3.1.4.11.5 Flower color

The flower color was recorded as white and pink at flowering stage.

3.1.5 Statistical analysis

3.1.5.1 Components of variance

The genotypic and phenotypic components of variance were computed according to formulae given by Burton and Devane (1953). for the observed characters.

$$\text{Genotypic variance (Vg)} = \frac{\text{Tr.M.S.S.} - \text{E.M.S.S.}}{r}$$

Error variance (Ve) = E.M.S.S.

Phenotypic variance (Vp) = Vg+Ve

Number of replications/ blocks = r

3.1.5.2 Coefficient of variability

Genotypic and phenotypic coefficient of variability was computed according to Burton and Devane (1953).

$$\text{GCV (\%)} = \frac{\sigma_g}{\bar{X}} \times 100$$

$$\text{PCV (\%)} = \frac{\sigma_p}{\bar{X}} \times 100$$

Where,

$\bar{X}$ = Grand mean

r = Number of replications

σ_g = Genotypic standard deviation

σ_p = Phenotypic standard deviation

The PCV and GCV values are ranked as low, medium and high (Sivasubramanian and Menon, 1973) and are mentioned below:

Low - 0-10%

Moderate - 10-20%

High - >20%

3.1.5.3 Heritability (h^2)

Broad sense heritability was estimated based on the ratio of genotypic variance to the phenotypic variance and was expressed in percentage (Hanson *et al.*, 1956).

$$h^2 (\%) = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where, Vg = Genotypic variance

V_p = Phenotypic variance

Heritability values are categorised as low, moderate and high are given below,

Low - 0-30%

Moderate - 30-60%

High - 60% and above

3.1.5.4 Genetic advance

Genetic advance (GA) was computed according to the formula given by Johnson *et al.* (1955).

$$\text{Genetic advance (GA)} = h^2 \times \sigma_p \times k$$

Where,

k = selection differential (2.06) at 5 per cent selection intensity

h^2 = Heritability in broad sense

σ_p = Phenotypic standard deviation

Genetic advance as per cent of mean (GAM) expressed in percentage was computed by the formula.

$$\text{GAM} = \frac{\text{GA}}{\bar{X}} \times 100$$

Where,

GA = Genetic advance

$\bar{X}$ = General mean

Genetic advance as percent of mean was classified as low, moderate and high (Johnson *et al.*, 1955) and values are given below:

Low - 0-10%

Moderate - 10-20%

High - 20% and above

3.1.5.5 Correlation coefficients

The correlation coefficients were calculated to determine the degree of association of the characters with yield and also among the different yield components.

Phenotypic coefficients of correlation between all pairs of characters were determined by using variance and covariance components as suggested by Al – Panse and Sukhantme (1957). The analysis was done using the SPAR-1 computer programme.

$$r_{p(x,y)} = \frac{\text{Cov}_p(x, y)}{\sqrt{\sigma_{px}^2 \cdot \sigma_{py}^2}}$$

Where,

r_p is phenotypic correlation

$\text{Cov}_p(x, y)$ is phenotypic covariance between the characters x and y

σ_{px}^2 is the phenotypic variance of the characters x and y

The calculated value of r was compared with 'r' table value with $(n - 2)$ degree of freedom at 5 and 1 per cent level of significance.

3.1.5.6 Student 't' test

To test the difference in quantitative characters between generation and control, this test was used. Mean difference between mutant plants in M₁, M₂ and M₃ generations were statistically analysed by following the student 't' test with mean frequency (Panse and Sukhatme, 1957). This method was used to test whether two means were significantly different. Mean differences were tested using table 't' value with calculated 't' value, which was calculated by following formula.

$$t = \frac{\overline{Xd}}{SE(\overline{Xd})} \text{ (at } n-1 \text{ d.f.)}$$

Where,

t = calculated 't' value; $\overline{Xd}$ = Mean difference and

$SE(\overline{Xd})$ = Standard error of mean difference and n = Number of related samples.

3.1.5.7 Contingency χ^2 test

The test as suggested by Panse and Sukhantme (1957) was used to find the association between the characters by using the following formula

$$\text{Actual } \chi^2 = \frac{1}{n_1 n_2} \sum \frac{(an_2 - a'n_1)^2}{(a + a')}$$

Where, χ^2 = chi-square value of the contingency table

a and a' = Frequencies in the r x c fold classification.

n₁ and n₂ = Total in the r x c classification.

a + a' = Totals of the classes;

Σ = Summation extends to all classes.

As the expected frequencies in the contingency tables were less than 5, alternative table χ^2 was calculated for comparison of actual χ^2 value to ascertain the level of significance (Lawal and Upton, 1984). The Pearson's goodness of fit statistics χ^2 for r x c contingency table was calculated as usual and compared with calculated table χ^2 value.

$$\text{i.e., Cal., T. } [\chi^2 = 1 - \frac{1}{n} (1 - d)^{-1}] \text{ T. } \chi^2 @ d_{0.005} \text{ at 5\% level of significance}$$

$$\text{Cal., T. } \chi^2 = [1 - 3/2n] \chi^2 @ d_{0.001} \text{ at 1\% level}$$

Where, Cal., T. χ^2 = calculated table chi-square

n = number of observations; d = Degrees of freedom (n-1) (r-1)

$\chi^2_{d_{0.005}}$ = Table χ^2 value at 'd' df. at 5 per cent level;

$\chi^2_{d_{0.001}}$ = Table χ^2 value at 'd' df. at 1 per cent level.

Experiment 2: Genome-wide identification, cloning and characterization of RGAs and TF genes from french bean

3.2 Cloning and structural characterization of NBS-LRR type RGAs from french bean

3.2.1 Preparation of genomic DNA

Total DNA was isolated from the rust resistant genotype Arka Anoop of french bean following CTAB protocol of Murry and Thompson (1980), with suitable modifications as described here.

About 4 g of leaf sample was ground with liquid nitrogen in a pre-chilled mortar and pestle. The ground tissue was transferred to a 2.0 ml eppendorf tubes and then 900 µl of 2X CTAB extraction buffer was added to each tube.

Contents were mixed well and incubated in water bath at 65°C for 45 minutes. After that the tubes were allowed to cool down to room temperature and then the contents were spun for 10 min at 10,000 rpm at 4°C.

About 600 µl of supernatant was transferred to fresh tube and the remaining was discarded. To the supernatant, equal volumes of chloroform: isoamyl alcohol (24:1) was added and mixed gently by inverting the tubes for 4-5 times.

This was centrifuged at 8000 rpm for 10 min at 4°C. Supernatant from the top aqueous phase was taken into a new centrifuge tube and lower chloroform phase was discarded. Another chloroform: isoamyl alcohol extraction step was performed to remove left over protein and other debris.

To the supernatant collected, two volumes of chilled isopropanol was added and kept at -20°C overnight for precipitation of DNA. It was then centrifuged for 10 minutes at 10,000 rpm at 4°C.

The supernatant was discarded carefully and DNA pellet was washed twice with 70 per cent alcohol and air-dried. Then the pellet was resuspended in 50 µl of T₁₀ E₁ (Appendix I).

3.2.2 Purification of genomic DNA

To remove RNA contamination, RNase (10 µg/ml) was added to DNA solution and incubated for 30 min at 37°C in water bath and stored at -20°C until further use.

3.2.3 Quantification of genomic DNA

Total DNA isolated was quantified by following the ethidium bromide spotting method as given by Sambrook and Russel (2001).

3.2.4 RGA degenerate primers

The degenerate primer pairs used for amplification of RGAs in genotype Arka Anoop are as follows

NBS name	Peptide domain	Primer sequences (5'→3')	Reference
FB_F1(F)	P-loop	GGDGTGGNAARACWAC	Deng <i>et al.</i> , 2000
FB_F2(R)	GLPL	AANGCHAGNGGYAANCC	Deng <i>et al.</i> , 2000
FB_F3(F)	P-loop	GGWATGGGWGGWRTHGGWAA RACHAC	Lee <i>et al.</i> , 2003
FB_F4(R)	GLPL	ARNWYYTTVARDGCVARWGGVA RWCC	Lee <i>et al.</i> , 2003
FB_F5(F)	P-loop	GGIGGIGTIGGIAAIACIAC	Echeverria <i>et al.</i> , 2008
FB_F6(R)	GLPL	AAGIGCTAAGIGGIAAGICC	Echeverria <i>et al.</i> , 2008
FB_F7(F)	Kinase	GTNYTNGAYGAYGTNTGG	Dequan <i>et al.</i> , 2010
FB_F8(R)	Kinase	TAGTTGTRAYDATDAYYYTRC	Dequan <i>et al.</i> , 2010
FB_F9(F)	P-loop	GGNGGNRTIGGIAARACIAC	Dequan <i>et al.</i> , 2010
F10(R)	GLPL	GAGGGCNARNGGNAAICC	Dequan <i>et al.</i> , 2010
FB_S1 (F)	NBS GGV/IGKT	GGT GGG GTT GGG AAG ACA ACG	Leister <i>et al.</i> , 1996
FB_S2 (F)	NBS GGV/IGKT	GGI GGI GTI GGI AAI ACI AC	Leister <i>et al.</i> , 1996
FB_AS1 (R)	P-loop (GGVGKTT) G L P L A/T L	CCA CGC TAG TGG CAA TCC	Leister <i>et al.</i> , 1996
FB_AS2 (R)	P-loop (GGVGKTT) G L P L A/T L	IAA IGC IAG IGG IAA ICC	Leister <i>et al.</i> , 1996
FB_AS3 (R)	P-loop (GGVGKTT) G L P L A/T L	IAG IGC IAG IGG IAG ICC	Leister <i>et al.</i> , 1996
FB_AS4 (R)	P-loop (GGVGKTT) G L P L A/T L	ARI GCT ARI GGI ARI CC	Leister <i>et al.</i> , 1996
FB_M (R)	Motif 1 (FLDIACF)	GAA GCA IGC GAT GTC IAG GAA	Lopez <i>et al.</i> , 2002
FB_T1 (R)	Toll/interleukin-1 receptor (TIR 1)(D/E) VFLSF (R/S) G	GAI GTN TTY TTI TCI TTY AGI GG	Lopez <i>et al.</i> , 2002
FB_T5 (R)	Toll/interleukin-1 receptor (TIR5) PVFYDVDP	IGG GTC IAG GTC GTA GAA IAC/IGG	Lopez <i>et al.</i> , 2002

Various combinations of primers were used to amplify the RGAs of size 500bp- 700bp. Since, most of the earlier reports on model systems and selected crop plants indicated that the RGAs isolated from different crops corresponding the RGAs of larger or smaller than 500bp sizes have not resulted in sequence homology to known resistance genes. Hence, in the present analysis of RGAs of about 500-700bp were considered for characterization.

Primer combination used for amplification of resistance gene analogs in french bean and cloned

Combination	Primers	Expected amplicon size (bp)
1E	S2+ AS2	~ 550 bp
2E	S2+AS3	~ 550 bp
3E	S2+AS4	~ 550 bp

4E	S1+AS1	~ 500 bp
5E	S1+M	~ 500 bp
6E	S2+AS1	~ 550 bp
7E	S1+AS4	~ 700 bp
8E	S2+M	~ 550 bp
9E	F1+F2	~ 650 bp
10E	F3+F4	~ 550 bp
11E	F5+F6	~ 550 bp
12E	F9+F10	~ 550 bp

3.2.5 Standardization of PCR condition

The degenerate primers are complex in nature. To amplify the RGAs, it is very crucial to optimize PCR components and conditions.

3.2.6 Standardization of primer concentration

Primer concentration ranging from 10 pM, 15 pM and 20 pM, were used to optimize the amplification. Based on the results, 10 pM was found to be good with all combinations of antisense primers. This concentration was used for large-scale amplification.

3.2.7 Standardization of annealing temperature

Since different primer combinations amplified at different annealing temperatures for PCR amplification of RGAs, it was essential to standardize the annealing temperature for each combination. PCR amplification of RGAs was optimized for different components using gradient PCR (Eppendorf master cycles gradient).

3.2.8 Reaction mixture for PCR amplification

Degenerate primers were used to amplify the different RGAs from the genotype Arka Anoop of french bean. The master mix was prepared afresh except primer addition to avoid possible handling errors (Appendix II).

The master mix (18 µl) was distributed to the 0.2 ml PCR tubes and 1 µl of sense primer and 1 µl of antisense primer each were added to make up the total reaction volume of 20 µl.

3.2.9 PCR amplification

A PCR condition used for amplification of RGAs is as follows. The annealing temperature was primer specific.

Steps	Process	Temp. (°C)	Duration (min)	No. of cycles
1.	Initial denaturation	95°C	4	1
2.	Final denaturation	95°C	1	
3.	Primer annealing	Primer combination specific	1	29

4.	Primer extension	72°C	2	1
5.	Final extension	72°C	30	
6.	Hold	4°C	-	

The product obtained after the PCR reactions were kept at 4°C till they were used for further analysis.

3.2.10 Separation of amplified products

After the set programme was over the contents of the tubes were electrophoresed to separate different size products from each tube along with 2 µl of loading dye (Appendix III) were separated on 1 per cent agarose gel along with 100 bp DNA ladder as marker. Gel was prepared using 1x TAE buffer from 50x TAE buffer and agarose (Appendix III). The electrophoresis was done at 60 V for 2 hrs. The gels were observed under a mid range UV transilluminator and gel image was documented using gel documentation system (UV tec, Cambridge, England).

3.2.11 Elution of PCR fragment

Large-scale amplification was done to elute the PCR fragments. The major amplification fragment corresponding to 500 to 700 bp region was excised from the 1.0 per cent low melting agarose gel using a sharp sterile scalpel blade by keeping the gel at the low intensity UV transilluminator. The agarose gel piece containing the band was collected in the sterile pre weighed 2 ml microcentrifuge tube. The Qiagen Gel extraction kit was used to elute the DNA from the agarose piece. To the pre- weighed tube, 3 volumes of QG buffer of gel weighed was added which helps in melting the agarose when kept at 50° C for 10 minutes. To this 1 volume of gel weight isopropanol was added. The melted agarose was then loaded into spin filter column and spun at 12000 rpm for 1 min. Lower liquid solution was discarded. To the filter column, 500 µl of QG buffer was again added to remove traces of agarose and spun at 12000 rpm for 1 min. Then 750 µl of PE buffer was added to the column and spun at 12000 rpm for 1 min. As per the kit specifications DNA is now bound to the silica membrane of the column. Then, DNA was eluted in 20 µl of elution buffer. The same procedure was used to elute the different PCR fragments amplified by different degenerate primers.

3.2.12 Quantification of eluted PCR products

The purified PCR fragments were quantified by UV quantification as described earlier (3.2.3).

3.2.13 Cloning of PCR products

Eluted and purified PCR fragments of size 500 to 700 bp were ligated to pTZ57R/ T vector (pTZ57R/ T is a cloning vector of size 2868 bp) as described in InsT/A clone™ PCR product cloning kit (#K1214) (Plate 4). The vector supplied after cleaving with *Eco321* (an Isoschizomer of *EcoRV*) and treated with terminal deoxynucleotidyl transferase to create 3' ddT overhangs at both ends. When PCR fragments with 3'-A overhang is ligated into the vector, a circular molecule with two nicks is produced. The circular plasmid with insert was directly transformed in *E. coli* DH5α. The same procedure was followed for all PCR amplified; gel eluted and purified RGA fragments.

3.2.14 Ligation reaction

Looking into the conversion table for the amount of PCR fragment required per ligation reaction (Appendix III), an optional molar ratio of ends of 1:3 of vector: insert was calculated. The components of the ligation mix (Appendix IV) were mixed into a 0.5 ml micro- centrifuge tube and were incubated at 4°C overnight. A control ligation reaction was performed using control PCR fragment provided in the kit by adding components in Appendix IV.

3.2.15 Preparation of competent cells

The competent cells of *E. coli* DH5 α were prepared following the protocol described in Sambrook and Russell (2001) with minor modification as described below.

An isolated colony from *E. coli* DH5 α plate was inoculated into 5 ml Luria broth and incubated at 37°C overnight at 200 rpm. The next day, the culture was diluted to 1:100 using Luria broth *i.e.*, 0.5 ml of culture was added to 50 ml of Luria broth. It was incubated for 2 to 3 hours till it attained an OD of 0.3 to 0.4 at 600 nm. The culture was chilled in ice for 30 min and 25 ml of culture was dispensed into two 50 ml centrifuge tubes. The cells were pelleted at 6000 rpm for 5 min, the supernatant was discarded and pellet was suspended in 12.5 ml of ice-cold 0.1 M calcium chloride. The centrifuge tubes were again kept in ice for 45 min and later centrifuged at 4000 rpm for 10 min. The pellet was dispensed in 1 ml of 0.1 M calcium chloride and to this 88 μ l of dimethyl sulfoxide (DMSO) was added. About 200 μ l of cell suspension was distributed to each pre-chilled 1.5 ml micro centrifuge tubes and immediately used.

3.2.16 Transformation of *E. coli* DH5 α

About 100 μ l of freshly prepared competent cells were taken in a chilled centrifuge tube and 7 μ l of ligation mixture was added into the tube and was mixed gently. The mixture was chilled in ice for 45 min and heat shock was given by shifting the chilled mixture into water bath at 42°C for 2 min immediately, it was transferred onto ice to chill for 5 minutes. To this, 800 μ l of Luria broth was added and incubated at 37°C at 200 rpm for 45 minutes, to allow bacteria to recover and express the antibiotic marker encoded by plasmid. The culture was centrifuged at 13,000 rpm for 1 min and about 700 μ l of supernatant was discarded and the pellet was resuspended in the remaining supernatant and spread on pre-warmed Luria agar plates with ampicillin, X- gal and IPTG (Appendix V) and incubated overnight at 37°C.

3.2.17 Selection of recombinant clones

After overnight incubation, the recombinant clones were identified by the blue-white assay. Since X-gal is substrate for the enzyme β -galactosidase (*lacZ* Gene), *lacZ* breaks the X-gal into its derivatives, which is blue in colour. IPTG is an inducer for *lacZ*, white colonies will appear on plates, which will be recombinants. About 15 white colonies from each transformation were randomly picked up and streaked on fresh Luria agar plates containing ampicillin at 100 μ l/ 100 ml, X-gal and IPTG and incubated at 37°C overnight.

Approximately 150 white colonies were revived, labelled and screened by colony PCR technique using M13 forward and reverse primers.

3.2.18 Confirmation of clones

White colonies picked up from blue white assay were further analysed for the presence of desired DNA fragment in the transformed bacteria. It was done by two methods as described below.

3.2.19 Plasmid isolation from recombinants

Following the protocol described by Sambrook and Russel (2001) plasmid isolation of recombinants was carried out as follows.

Streaked white colonies were inoculated to 2.0 ml Luria broth with ampicillin (100 µl/100 ml) and incubated overnight at 37° C at 200 rpm. 1.5 ml of overnight culture was centrifuged at 13000 rpm for 30 seconds at 4°C. Bacterial pellet was then resuspended in 100 µl of ice-cold alkaline lysis solution I (Appendix VI) by vigorous vortexing. To this, 200 µl of freshly prepared alkaline lysis solution II (Appendix VI) was added and mixed by inverting the tubes for five times. 150 µl of ice cold alkaline lysis solution III (Appendix VI) was added to this and mixed by inverting the tubes several times and incubated on ice for 4 to 5 minutes. The bacterial lysate was centrifuged at 13000 rpm for 5 min at 4°C and the suspension was treated with equal volume of phenol: chloroform (Appendix II). The top aqueous layer was transferred to fresh tube and plasmid DNA was recovered by adding two volumes of absolute alcohol. Solution was mixed by inverting and allowed to stand at room temperature for 2 minutes. The DNA was precipitated by centrifugation for 13000 rpm at 4° C. The DNA pellet was washed with 70 per cent ethanol and air-dried. Finally the pellet was dissolved in 50 µl of T₁₀E₁ (pH 8.0) containing 20 µl per ml DNase free RNase.

Plasmid DNA was visualized on 0.8 per cent agarose gel as described earlier. Simultaneously, plasmid from blue colony was also isolated and loaded along with plasmid isolated from white colonies as a control.

3.2.20 PCR confirmation of recombinants

The recombinant clones confirmed with plasmid profile were taken and plasmid DNA was used as template along with control vector for further confirmation with M13 forward and reverse primers in a PCR reaction. Approximately 150 white colonies were revived, labelled and screened by colony PCR technique using M13 forward and reverse primers.

3.2.21 Sequencing and analysis of RGA clones

Out of many clones, a random set of 8 clones from all 12 primer combinations were sent for sequencing. In all 90 different RGA clones were sequenced. These sequences were analysed for similarity with other resistance genes encoding conserved motifs using BLAST search. Multiple alignments of nucleotide and amino acid sequence were carried out using ClustalW. Domain identification using Pfam software, Pair wise identity, homology among the known resistance genes with cloned RGAs and E-value were considered during the analysis.

Further, based on annotation studies and phylogenetic analysis 10 RGA sequences were selected and subjected for primer design (Appendix XI). The designed primers were used to study the expression status of these genes for rust and *MYMV*.

3.3 *In silico* genome-wide identification of Transcription factor genes from french bean

3.3.1 Genome sequence dataset retrieval

Initially, the draft genome protein sequence of french bean/common bean used in this study was downloaded from publically available National Centre for Biotechnology Information (NCBI) (ftp://ftp.ncbi.nlm.nih.gov/genomes/PLANTS/Phaseolus_vulgaris) resource.

3.3.1.2 Identification and classification of TFs in draft genome of French bean

The protein sequence of TFs from a plant model system *Arabidopsis thaliana* was downloaded from publically available plant transcription factor database (<http://planttfdb.cbi.pku.edu.cn/>). Considering the reference sequences of *Arabidopsis thaliana*, a similarity search was performed against french bean using BLAST built at Bioedit tool, based on the E-value cutoff of $1e^{-5}$ with identity value cut off >80% and query coverage 80%, have been selected with an top 10 hits from each query sequence in draft genome of french bean.

3.3.1.3 Identification of functional domains of sequences

Top 10 hits of each sequence were then subjected for DNA binding domain identification using smart pfam. The transcription factors defining feature like DNA-binding domains presence or absence was typically employed as the major criteria for identification of TFs and moreover, for classification of the TFs into individual TF families (Harrison, 1991). By following are the criteria several public databases of plant TFs have been established, such as PlantTFDB (v. 3.0) (Zhang *et al.*, 2011) and PlnTFBS (v. 3.0) (Pérez-Rodríguez 2010). By considering the results, we have selected the sequences, which had TFs domains. Those were subjected for primer designing with primer3plus software. More primers are selected from biotic stress related TFs, those primer sequences were again blast search performed in NCBI for further confirmation. Corresponding french bean sequences of all the selected genes will be catalogued and will be further used for characterization of structural and functional features to characterize them. Based on the annotated data, we have selected 20 TF genes of different families and primers were designed with preliminary requirement of qRT-PCR primers standards using Primer 3plus software. The analysis used to predict transcription factors and classification is depicted. Further, based on annotation studies and phylogenic analysis 10 RGA sequences were selected and subjected for primer design (Appendix XII). The designed primers were used to study the expression status of these genes for rust and MYMV.

3.4 Expressional quantification of selected french bean RGAs and TFs

3.4.1 Disease induction and tissues collection from pot experiment

French bean genotypes resistant (Arka Anoop) and susceptible (Arka Sharath) to rust were raised in pots containing a mixture of soil, sand and well decomposed Farm Yard Manure (FYM) in the ratio of 2:1:1. The filled pots were kept in polyhouse. The pot mixture was sterilized before use. In replicated trials one seed was sown in each pot. Rust spores were collected from infected plants in farmer's field and dissolved in water at 10^3 concentration and spread on pots one month after sowing. Control pots were maintained without inoculation both in resistant and susceptible genotypes. Both from susceptible and resistant french bean genotypes the tissues were collected from leaf tissues. Under virulent pathogen challenging situations, tissues from different stages (15 and 30 days after post inoculation and till the completion of the disease infestation) targeting different stages of disease manifestation were collected both from

challenged and control plant. The tissues were frozen with liquid nitrogen and stored at -80°C for isolation of total RNA.

Similarly to collect tissues from *MYMV* infected plants, the insect (vector) transmission protocol developed by Aidawaati *et al.* (2002) was used. *MYMV* transmission experiment with *Bamisia tabaci* were conducted using rectangular nylon cages with mesh top. Around 50-100 *B. tabaci* per plant were introduced into the cage through a hole made. After 24 and 48 h acquisition access period *B. tabaci* adults were removed from *MYMV* agro infected french bean plants and transferred to separate cage containing healthy without virus inoculated french bean (Arka Anoop). After 24 h inoculated access period *B. tabaci* were removed and tissues were collected targeting different stages of disease manifestation both from challenged and control plants ie., Arka Anoop plants (7th, 15th and 30th DAI). The tissues were frozen and stored at -80°C for isolation of total RNA.

3.4.2 Preparation of cDNA

3.4.2.1 Isolation of total RNA

Total RNA was isolated from inoculated and uninoculated leaf tissues of Arka Anoop genotypes using TRIzol reagent.

Frozen tissue sample approximately 0.5-1.0 g of tissue was grounded into fine powder in a pre-chilled mortar and pestle and the ground tissue was transferred into a pre-chilled 50 ml conical tube and 5-10 ml of TRIzol reagent (1ml of TRIzol/100 mg of tissue) was added.

- Contents were mixed well with vortex and 1 ml of the mixture was transferred into labeled, *RNase*-free 1.5 ml tubes to incubate for 5 min at room temperature.
- Chloroform 200 μl per 1 ml of TRIzol was added and vortexed for 20 sec and incubated the content for 10 min at room temperature and centrifuged at 13,200 rpm for 10 min at 4°C .
- The aqueous phase was carefully transferred into a new *RNase*-free 1.5 ml tube without disturbing the other phases and the tube was placed on ice as soon as transferred.
- Equal volume of isopropanol was added and mixed gently by inverting 2-3 times and incubated it on ice for 30 min and centrifuged at 13,200 rpm for 20 min at 4°C .
- A very small pellet was visible at the bottom of the tube - the total RNA.
- The supernatant was decanted and allowed to stand upside down on kimwipes (Kim tech science, Canada) for 5 min.
- The pellet was washed with 75 per cent ethanol (500 μl).
- The liquid was decanted and the inside of the tube was wiped to dry with a clean Kimwipe, without touching the pellet.
- The pellet was resuspended in 500 μl of *RNase*-free water and was incubated on ice for at least an hour and pipetted occasionally for dissolving the pellet.
- The content was centrifuged at 13,200 rpm for 20 min at 4°C and the supernatant was transferred into a new *RNase*-free 1.5 ml tube.
- It was precipitated with 10 per cent 3M sodium acetate and equal volume of isopropanol.
- Contents were incubated on ice for 1 hr or overnight at -80°C .

3.4.2.2 Purification of total RNA

The total RNA was treated to remove DNA using Turbo DNA-freeTM kit (cat#AM1907 Ambion, USA) as per the manufacturer's instruction. In order to eliminate the genomic DNA, 20 μg of total RNA was digested with *RNase*-free *DNase*I and finally the total RNA was precipitated

into desired volume of water. The quantity and quality of total RNA was checked using NanoDrop ND- 1000 spectrophotometer (NanoDrop Technologies, USA). The total RNA was subjected to Nanodrop ND-1000 using RNase-free water as blank: absorbance was recorded at 260/280 and 260/230. Further, the sample was fractionated over a formaldehyde agarose gel (Appendix X). Absence of genomic contamination was subsequently confirmed by PCR with total RNA as template (Caldana *et al.*, 2007).

3.4.2.3 Preparation of cDNA

Single stranded cDNA was prepared by using SuperScript® VILO™ cDNA Synthesis Kit (Cat.no.11754-050, Invitrogen) as per the manufacturer's protocol.

For a single reaction, the following components were combined in a tube on ice. For multiple reactions master mix was prepared without RNA.

5X VILO™ Reaction Mix	4 µl
10X SuperScriptR Enzyme Mix	2 µl
RNA (up to 2.5 µg)	x µl
DEPC-treated water	to make up 20 µl final mixture

The tube contents were mixed gently and incubated at 25° C for 10 minutes followed by incubation at 42° C for 60 minutes.

The reaction was terminated at 85° C at 5 minutes and was stored at -20° C until use.

3.4.3 Standardisation of qRT- PCR condition

3.4.3.1 Primer designing

From the 10 selected sequences of RGAs and 20 sequences of TFs, the primer pairs were designed using Primer3Plus software and primers were synthesized by Eurofins Genomics India Pvt. Ltd. Bengaluru. A predicted melting temperature (T_m) of 60+2°C, primer lengths of 20-24 nucleotides, guanine-cytosine (GC) contents of 45-55 per cent and PCR amplicon length of 90-200 base pairs (bp) were adopted for designing the primer pairs. The specificity of primer pairs were reconfirmed by searching homology in NCBI, BLAST search. The primer sequences are given in Appendix IX.

3.4.3.2 Template concentration standardization

In real-time quantification of any gene expression, template concentration is one of the key factors to consider. The different concentration 10 ng, 1 ng, 0.1 ng, 0.01 ng and 0.001 ng of internal control *actin* gene were used to optimize the amplification. Template concentration at 10ng gave amplification with low Ct value and high fluorescence value. Hence, this concentration was used as standard concentration for the rest of the reactions.

3.4.3.3 Primer concentration standardization

In real-time quantitation of any gene expression, primer concentration is one of the key factors. Primer concentrations ranging from 150 nM, 200 nM, 250 nM, 300 nM, 350 nM, and 400 nM were used to optimize the amplification. Primer at 200nM concentration gave single melting curve, low Ct value, high fluorescence value and no primer dimer when loaded on gel. Hence, this concentration of primer was used for rest of the experiment.

3.4.3.4 Annealing temperature

It is essential to determine optimal annealing temperature for each primer pair before their use. Hence, the gradient PCR was carried out with -5°C of their predicted annealing temperatures. A temperature that gave bright single amplicon was selected for that particular pair of primer. Annealing temperature of each primer was standardized.

3.4.3.5 qRT-PCR reaction

The master mix of different components of real-time PCR was prepared fresh to avoid handling errors. The reaction mixture of 10 μl containing 1.0 ng cDNA, 200 nM of each gene specific primer and 5 μl of 2x SYBR green reagents (Cat.#4368706, Ambion, USA) were used in the experiment. Individual components of reaction mixture were standardized for 10 μl reaction volume.

3.4.3.6 Internal controls

For data normalization, the transcripts of stably expressed (reference genes) genes are commonly used. In Arabidopsis, the house keeping genes like *18S rRNA*, *EF-1 α* , β - *actin* and *ubiquitin (UBQ)* have served as good reference genes (Caldana *et al.*, 2007 and Czechowski *et al.*, 2004). In our experiment we selected *Arabidopsis thaliana* housekeeping gene *actin* as an internal control.

3.4.3.7 Setting baseline and threshold level

The points of measurement in terms of baseline and threshold level should be accurately determined to reflect the quantity of a particular target within a reaction. During PCR, changing the reaction conditions and environment can influence fluorescence. In general, the level of fluorescence in any well corresponds to the amount of target present in it. Fluorescence levels may fluctuate due to changes in the reaction medium creating a background signal. The background signal is most evident during the initial cycles of PCR prior to significant accumulation of the target amplicon. During these early PCR cycles, the background signal in all wells is used to determine the “baseline fluorescence” across the entire reaction plate. For data analysis, more accurate measurements of fluorescence are needed which are attained when the target amplification is sufficiently above the background signal. The Applied Biosystems step one RT-PCR detection software sets the default baseline from 3-15 cycles. To determine if the baseline needs to be adjusted, it is important to determine which reaction in the assay emerges earliest above the baseline. The highly abundant gene 18S rRNA started to amplify very early in 20th PCR cycle. Therefore, baseline was not changed and set default between 3-15 cycles. The threshold value is a numerical value assigned for each run, which reflects a statistically significant point above the calculated baseline. Once the baseline has been set correctly, the software automatically sets the threshold at 10 standard deviations above the mean baseline fluorescence. It was ensured that threshold line would be placed in the exponential phase to increase the precision and quality of the experimental data and same baseline and threshold default setting was used for all PCR reactions.

3.4.4 Relative gene quantitation analysis

The two most commonly used methods to analyze data from real-time PCR experiments are absolute quantification and relative quantification. Absolute quantification determines the input copy number, usually by relating the PCR signal to a standard curve and the relative quantification relates the PCR signal of the target transcript in a treatment group to that of other samples such as an untreated control. In this experiment, the relative quantification approach was

used to measure the expression level of RGAs in control and pathogen challenged leaf and root tissues.

The mathematical model delta-delta Ct method (Livak and Schmittgen, 2001) was used to determine relative expression ratio (fold change). In real-time PCR, fluorescence was recorded at each cycle to monitor the generation of amplified product. For proper calculation of initial target levels, differences in efficiency of amplification (E) must be taken into consideration. Even small differences in amplification efficiencies (E) will get added up making large apparent differences in mRNA levels. The absolute quantification requires a set up of standard curves from which PCR efficiency will be deduced; the disadvantages of standard curves are (i) the extra efforts and cost needed to set up additional samples (ii) Non matching E due to presence of inhibitors and serial dilutions. The relative quantification with PCR efficiency correction was adopted to calculate the fold change expression.

PCR efficiency of all the RGAs was obtained from the exponential phase of each individual amplification plot using the equation $(1+E) = 10^{\text{slope}}$ (Ramakers *et al.*, 2003). The LinReg PCR (<http://www.bioinfo@amc.uva.nl>; subject: LinRegPCR) software based on the above equation proposed a linear regression on the log fluorescence per cycle number data as an assumption-free method was used to calculate starting concentrations of mRNA and PCR efficiencies for each sample. The log-linear part of the PCR data was determined for each sample by selecting a lower and an upper limit of a “window of linearity”. Linear regression analyses was used to calculate the intercept and the slope, log (No) and log (eff.) respectively, from the straight line that fits best to the included data points. The individual PCR efficiency follows from the slope of the linear regression line ($\text{Eff} = 10^{\text{slope}}$) and used as a quality check to exclude possible contained samples. To ensure unambiguous selection of data point within the “window of linearity”, the lines consisting of at least 4 and not more than 6 data points with the highest R² value (0.99) and slope close to the maximum slope were selected.

3.4.4.1 Processing the raw fluorescence data

Pre-requisite for LinRegPCR to achieve maximum PCR efficiency is background corrected fluorescence data points of each well. Raw fluorescence data was obtained from the Applied Biosystems stepone RT-PCR and this background was due to residual fluorescence of the dye, differences in tube transparency, dust, noise of the electronics etc. In majority of cases, a variable background makes a near-linear contribution to the curves generated by the amplifier and it should be subtracted from the raw fluorescence without distorting the data considerably. For background correction, the baseline fluorescence data was collected from 3-15 cycles. The fluorescence increments (raw fluorescence -Y₀) were normalized to reaction fluorescence background (Y₀) for each sample reaction as below (Yu *et al.*, 2006).

3.4.4.2 Normalized fluorescence = raw fluorescence -Y₀/ Y₀

The proposed method minimized the influence of the initial vertical background shift of reaction. The background corrected or normalized fluorescence data was used to calculate PCR efficiency by LinRegPCR software. The calculated PCR efficiency was used to derive fold expression of TFs gene using the following method:

$$\text{Ratio} = \frac{(E \text{ target})^{-\Delta Ct}}{(E \text{ control})^{-\Delta Ct}}$$

E target = PCR efficiency of target gene in sample.

E control = PCR efficiency of target gene in control.

Δ Ct = (Ct of target gene - Ct of reference gene)

3.4.4.3 Real-time PCR condition

An Applied Biosystems stepOne RT-PCR instrument was used for all real-time PCR amplifications. The optimal PCR conditions used for amplification are given as follows.

Real-time PCR amplification conditions

Stage	Step	Temperature (°C)	Duration (min. & sec.)	No. of Cycles
I.	Initial denaturation	95°C	10 min.	1
II.	Denaturation	95°C	15 sec.	
III.	Annealing (vary with primer)	Primer specific	20 sec.	35
IV.	Extension	68°C	20 sec.	1
V.	Melting curve	95°C	10 min.	

3.4.5 Statistical analysis

Use of statistical analysis on real-time PCR data is a new subject in gene quantitation and it is gaining importance. The statistical data analysis in gene quantitation is performed on the basis of classical standard parametric test such as correlation coefficient, t-tests, standard deviation and standard error. The technical precision or reproducibility of real-time PCR measurement was assessed by performing replicate measurements in separate PCR runs (technical and biological replicates). Correlation coefficient was calculated between two technical and two biological replicates (technical replicate: same pool of cDNA; biological replicate: two different pools of cDNA obtained independently from different batch of total RNA) to determine the reproducibility of the experiment. The standard deviation of ΔCt was calculated from the standard deviation of the target and reference values using the formula $S = (S1^2 + S2^2)^{1/2}$ ($S1$ = standard deviation of target gene and $S2$ = standard deviation of reference gene). Further, t-test was used to assess the level of significance of TFs expression value between the stressed sample and control sample at $\alpha = 0.01$. Microsoft Excel programme was used to calculate above statistical parameters.

IV. EXPERIMENTAL RESULTS

The results obtained in the study on “Development of functional mutants, cloning, characterisation and expressional analysis of selected Resistance Gene Analogs (RGAs) and Transcription Factor (TF) genes in french bean (*Phaseolus vulgaris* L.)” are presented here under

4.1 Induced mutation to create spectral variability in french bean

The experiment was conducted with the aim of developing spectral variability in french bean genotype ‘Arka Anoop’ using EMS, estimating the genetic variability parameters and correlation between different quantitative characters related to yield and its attributing traits in the M₂ and M₃ populations of french bean.

4.1.1 Standardization of EMS concentration for LD₅₀

The seeds treated with mutagen were subjected for germination per cent count as influenced by different EMS concentration treatments. Minimum germination percentage (16.85%) was recorded at 0.6 per cent of EMS; maximum germination percentage (86.52%) was recorded in 0.01 per cent of EMS treatment. It was found that 0.2 per cent of EMS recorded a germination per cent of 57.30 followed by 46.07 per cent at 0.3 percent of EMS. So the LD₅₀ was calculated as 0.25 per cent in french bean. The germination percentage was found to be inversely related to the dose of chemical mutagen ethyl methyl sulphonate, i.e., the germination percentage decreased with the increase of the dose (Table 1a).

4.1.2 Genetic analysis of quantitative parameters for assessment of mean, range and standard error in M₂ generation

The ramification of mean, range, minimum and maximum revealed significant differences among mutants for all the characters studied. Mutations on genetic variability in french bean was conspicuous. Upshots showed variable responses to mutagenic treatment for all traits, which indicated that the EMS treatment induced mutation to a great extent. Mean, range, minimum and maximum of all the 11 characters are presented in Table 1b.

Table 1a. Estimation of LD₅₀ concentration for ethyl methyl sulphonate

Treatment (per cent)	No. of seeds sown	No. of seeds germinated	Per centile germination
0.01	100	77	86.52
0.02	100	74	83.15
0.05	100	69	77.53
0.1	100	60	67.42
0.2	100	51	57.30
0.3	100	41	46.07
0.4	100	29	32.58
0.5	100	19	21.35
0.6	100	15	16.85
Control (Water)	100	89	100.00

The mean plant height documented was 55.6 cm with a range of 50 cm to 60 cm among 10000 M₂ plants. Similarly, number of branches per plant, ranged from 4 to 9 branches per plant with mean value of 5.23 branches per plant among individuals of the population. Whereas, observation for days to 50 per cent flowering, the mean value was 40.58 days, with a minimum of 18.63 days and a maximum of 48 days with a range of 29.37 days was recorded. Further, the pod length parameters recorded a wide range of variation among the individuals of mutant populations in M₂ generation. The mean pod length was found to be 15.16 cm with a range between 14.3 cm and 16.3 cm. Similarly, for pod width, mean value was at 8.4 mm with a minimum of 6.19 mm and a maximum of 8.71 mm. Mean value for the days to pod maturity was 44 days with a range of 42 to 47 days.

The mean weight of pods noted was 57.22 g, which ranges between 32.67 g and 67.47 g whereas, the average number of seeds per pod ranged 5.47 to 6.9 with a mean value of 6.29 seeds per pod across the individual of M₂ population. Similarly, mean tenderness recorded was 36.66 tr with a minimum of 26.83 tr and to a maximum of 58.25 tr. In case of pod yield per plant, the mean value recorded was at 92.4 g with a range of 65.67 g to 99.5 g. However, mean pod yield per hectare documented was 205.34 qt less yield of 145.93 qt and more of 221.11 qt.

4.1.3 Assessment of genetic variability parameters of yield and yield component traits in M₂ generation

The result of eleven quantitative parameters tested for genetic variability estimation viz., phenotypic variance and genotypic variance, phenotypic co-efficient of variation (%), genotypic co-efficient of variation (%), heritability (%), genetic advance and genetic advance as percent of mean in M₂ population results are presented in Table 1c and Figure 1a.

Table 1b. Genetic analysis of quantitative parameters for assessment of mean, range and standard error in field evaluated M₂ generation of french bean during 2014-15 at Bengaluru (n= 10000)

Sl. no.	Parameters	Mean	Standard error (±)	Range	Minimum	Maximum
1	Plant height (cm)	55.60	0.25	10.00	50.00	60.00
2	Number of branches/ plant	5.23	0.21	5.00	4.00	9.00
3	Days to 50 per cent flowering	40.58	0.26	29.37	18.63	48.00
4	Pod length (cm)	15.16	0.20	2.00	14.30	16.30
5	Pod width (mm)	8.40	0.00	2.52	6.19	8.71
6	Days to pod maturity	44.00	0.22	5.00	42.00	47.00
7	Weight of 10 pods (g)	57.22	0.31	34.8.	32.67	67.47
8	Average number of seeds/ pod	6.29	0.20	1.43	5.47	6.90
9	Tenderness (tr)	36.66	1.95	31.42	26.83	58.25
10	Pod yield/ plant (g)	92.40	3.85	33.83	65.67	99.50
11	Pod yield/ ha (q)	205.34	2.69	75.18	145.93	221.11

4.1.3.1 Plant height (cm)

The variance due to phenotype (8.4) was higher than the variance due to genotype (8.15) for the plant height. The lower coefficient of variability at phenotypic and genotypic levels were 5.21 and 5.14 per cent, respectively coupled with high heritability (97%) were recorded for this trait. The values of genetic advance and GAM were 4.1 and 7.37 per cent.

4.1.3.2 Number of branches per plant

Significant difference among the mutants for number of branches per plant was noticed for all genetic variability parameters. The phenotypic and genotypic variances were 0.64 and 0.44 respectively. The moderate level of coefficient of variability at phenotypic and genotypic level was (15.33% and 12.63%, respectively) recorded for no. of branches per plant. The heritability of this character was high (68%) and predicted genetic advance and GAM was 0.95 and 18.13 per cent respectively.

4.1.3.3 Days to 50 per cent flowering

Phenotypic variance of 44.75 and genotypic variance of 44.49 were pragmatic for days to 50% flowering with 16.44 % of genotypic coefficient of variation (moderate) and moderate phenotypic coefficient of variation (16.48%). Genetic advance of 9.57 and moderate genotypic over mean of 23.59% with a high heritability of 99% were observed.

4.1.3.4 Pod length (cm)

The variance due to phenotype (0.56) was higher than the variance due to genotype (0.36) for pod length. Analysis predicted low phenotypic coefficient of variation and genotypic coefficient of variation (4.94 and 3.94%, respectively) coupled with 64% of moderate heritability. Genetic advance of 0.86 and low genotypic over mean of 5.65% were noticed.

4.1.3.5 Pod width (cm)

The phenotypic and genotypic variances for pod width were 0.24 and 0.23, respectively. The coefficients of variability at phenotypic and genotypic levels were low (5.8 and 5.75 per cent, respectively). The values of heritability, genetic gain and GAM for the population obtained were 98, 0.69 and 8.26 per cent respectively.

Table 1c. Assessment of genetic variability parameters of yield and yield component traits in field evaluated M₂ generation of french bean during 2014-15 at Bengaluru (n=10000)

Sl. no.	Parameters	PV	GV	PCV (%)	GCV (%)	h² (%)	GA	GAM (%)	CV (%)
1	Plant height (cm)	8.40	8.15	5.21	5.14	97	4.1	7.37	2.40
2	Number of branches/ plant	0.64	0.44	15.33	12.63	68	0.95	18.13	15.57
3	Days to 50 per cent flowering	44.75	44.49	16.48	16.44	99	9.57	23.59	6.28
4	Pod length (cm)	0.56	0.36	4.94	3.94	64	0.86	5.65	3.81
5	Pod width (mm)	0.24	0.23	5.80	5.75	98	0.69	8.26	7.10
6	Days to pod maturity	7.22	7.00	6.11	6.01	97	3.8	8.63	3.11
7	Weight of 10 pods (g)	119.07	118.77	19.07	19.05	98	15.64	27.34	5.70
8	Average number of seeds/ pod	0.28	0.08	8.45	4.43	28	0.40	6.37	10.44
9	Tenderness (tr)	71.46	69.51	23.06	22.74	97	11.97	32.64	7.83
10	Pod yield/ plant (g)	89.15	85.3	10.22	10.00	96	13.26	14.35	3.20
11	Pod yield/ ha (q)	457.4	454.71	10.42	10.38	99	30.61	14.91	2.15

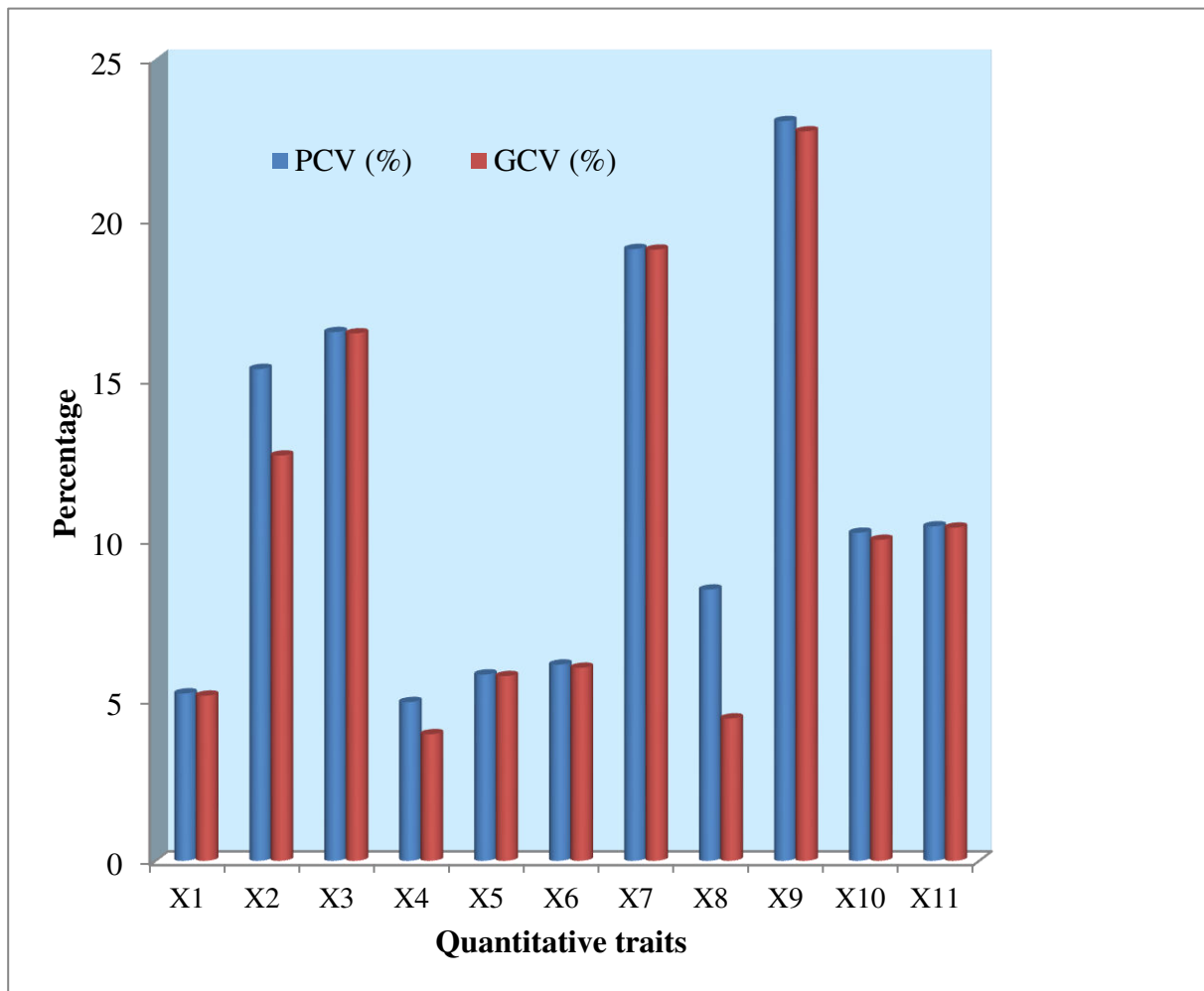


Figure 1a. Genetic variability parameters (PCV % and GCV %) for eleven quantitative components in M_2 population of Arka Anoop

X1: Plant height (cm)

X2: Number of branches/ plant

X3: Days to 50 per cent flowering

X4: Pod length (cm)

X5: Pod width (mm)

X6: Days to pod maturity

X7: Weight of ten pods (g)

X8: Average number of seeds/ pod

X9: Tenderness (tr.)

X10: Pod yield/ plant (g)

X11: Pod yield/ ha. (qt)

4.1.3.6 Days to pod maturity

The variance due to phenotype (7.22) was higher than the variance due to genotype (7). Estimates of phenotypic coefficient of variation (6.11) and genotypic coefficient of variation (6.01), respectively was low. However, high heritability (97%) along with low genetic advance of 3.8 and genotypic over mean of 8.63 per cent were noticed for days to pod maturity.

4.1.3.7 Weight of ten pods

Phenotypic variance of 119.07 and genotypic variance of 118.77 were recorded for Weight of ten pods with 19.05 per cent of moderate genotypic coefficient of variation and phenotypic coefficient of variation 19.07 per cent. Low Genetic advance of 15.64 and genotypic over mean 27.34 per cent with a high heritability of 98 percentage were observed.

4.1.3.8 Average number of seed per pod

The variance due to phenotype (0.28) was higher than the variance due to genotypic (0.08) for average number of seeds per pod. Estimated phenotypic coefficient of variation and genotypic coefficient of variation was low (8.45 and 4.43, respectively) coupled with low heritability (28%). Genetic advance of 0.4 and genotypic over mean of 6.37 percent were noticed.

4.1.3.9 Tenderness (tr)

Phenotypic variance of 71.46 and genotypic variance of 69.51 were observed for tenderness with high level of (22.74%) genotypic coefficient of variation and phenotypic coefficient of variation (23.06%). Genetic advance of 11.97 and genotypic over mean 32.64% was low with a high heritability of 97% were observed in the population.

4.1.3.10 Pod yield per plant (g)

Phenotypic variance of 89.15 and genotypic variance of 85.3 were observed for pod yield per plant with 10 per cent of moderate genotypic coefficient of variation and phenotypic coefficient of variation 10.22 per cent. Moderate genetic advance of 13.26 and genotypic over mean 14.35 per cent with a high heritability of 96 per cent were observed.

4.1.3.11 Pod yield per ha (q)

The variance due to phenotype (457.4) was higher than the variance due to genotypic (454.71) for pod yield per ha. Estimates of phenotypic coefficient of variation and genotypic coefficient of variation (10.42 and 10.38, respectively) coupled with high heritability (99%) was recorded among the mutants. High Genetic advance of 30.61 per cent and genotypic over mean of 14.91 per cent were noticed.

4.1.4 Phenotypic correlation coefficients for yield and yield component traits in M₂ generation of french bean

The results pertaining to character association studies among 11 quantitative characters in M₂ population are presented in Table 1d.

4.1.4.1 Plant height (cm)

Plant height showed significant positive correlations at phenotypic level with number of branches per plant (0.855), days to 50 per cent flowering (0.090), pod width (0.080), days to pod maturity (0.055), pod yield per plant (0.263) and pod yield per ha (0.535). It was positive but not significantly correlated with pod length (0.011), Weight of ten pods (0.031), average number of seeds per pod (0.041) and tenderness (0.029).

4.1.4.2 Number of branches per plant

Significant correlation with positive relation for number of branches per plant was found for days to 50 per cent flowering (0.045), pod yield per plant (0.253) and pod yield per ha (0.524). Whereas, pod length (0.007), pod width (0.035), days to pod maturity (0.038), Weight of ten pods (0.034), average number of seeds per pod (0.038), tenderness (0.021) had positive but no significant relation.

Table 1d. Phenotypic correlation coefficients for yield and yield component traits in field evaluated M₂ generation of french bean during 2014-15 at Bengaluru

Parameters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
X1	1.000										
X2	0.855*	1.000									
X3	0.090*	0.045*	1.000								
X4	0.011	0.007	0.022	1.000							
X5	0.080*	0.035	0.008	0.124*	1.000						
X6	0.055*	0.038	0.013	0.017	0.048*	1.000					
X7	0.031	0.034	0.028	0.302**	0.293**	0.031	1.000				
X8	0.041	0.038	0.021	0.026	0.019	0.032	0.191*	1.000			
X9	0.029	0.021	0.009	0.017	0.022	0.010	0.136*	0.038	1.000		
X10	0.263**	0.253**	0.080	0.228**	0.248**	0.024	0.277*	0.011	0.208*	1.000	
X11	0.535**	0.524**	0.011	0.420**	0.433**	0.024	0.277*	0.011	0.208*	1.000*	1.000

*** and ** Significant at 5 and 1 per cent level, respectively. Other values are non significant, Table 'r' (0.05) = 0.0438**

X1: Plant height (cm)

X2: Number of branches/ plant

X3: Days to 50 per cent flowering

X4: Pod length (cm)

X5: Pod width (mm)

X6: Days to pod maturity

X7: Weight of ten pods (g)

X8: Average number of seeds/ pod

X9: Tenderness (tr)

X10: Pod yield/ plant (g)

X11: Pod yield/ ha. (q)

4.1.4.3 Days to 50 per cent flowering

Days to 50 per cent flowering had positive correlations at phenotypic level with pod length (0.022), pod width (0.008), days to pod maturity (0.013), Weight of ten pods (0.028), average number of seeds per pod (0.021), tenderness (0.009), pod yield per plant (0.0794) and pod yield per ha (0.011) but, the results depicted the non significant correlation among traits at 5 per cent.

4.1.4.4 Pod length (cm)

Highly significant positive correlation was found between pod length, Weight of ten pods (0.3019), pod yield per plant (0.229) and pod yield per ha (0.420). Whereas, positive and non significant relation among pod length with pod width (0.124), average number of seeds per pod (0.026), tenderness (0.017), days to pod maturity (0.017) and days to 50 per cent flowering (0.022) was noticed.

4.1.4.5 Pod width (cm)

Significant positive correlation was found between pod width and pod length (0.124), Weight of ten pods (0.302), pod yield per plant (0.248), pod yield per ha (0.433), days to pod maturity (0.048). Positive and non significant relation among average number of seeds per pod (0.026) and tenderness (0.017) was noticed.

4.1.4.6 Days to pod maturity

Days to pod maturity showed non significant but positive correlations at phenotypic level with Weight of ten pods (0.031), average number of seeds per pod (0.032), tenderness (0.01), pod yield per plant (0.024) and pod yield per ha (0.024).

4.1.4.7 Weight of ten pods

Weight of ten pods showed significant positive correlations at phenotypic level with average number of seeds per pod (0.191), tenderness (0.136), pod yield per plant (0.277) and pod yield per ha (0.277).

4.1.4.8 Average number of seeds per pod

Average number of seeds per pod showed positive and non significant correlations at phenotypic level with tenderness (0.038), pod yield per plant (0.011) and pod yield per ha (0.011).

4.1.4.9 Tenderness (tr)

Tenderness showed significant positive correlations at phenotypic level pod yield per plant (0.208) and pod yield per ha (0.208).

4.1.4.10 Pod yield per plant (g)

Pod yield per plant showed significant positive correlations at phenotypic level pod yield per ha (1.000).

4.1.5 Comparative mean performance of yield and yield related component traits in M₂ generation of french bean

Mean values of eleven quantitative characters for the M₂ population were taken from the evaluated data. Comparison of mean values of control and individuals of M₂ population was tested with t-test and the results are presented in Table 1e and Figure 1b.

Mean values of M₂ population and control were compared for eleven quantitative characters. t- test result indicated the significant difference between the control and M₂ population at 5 per cent. The mean values found in control in comparison with M₂ population was, plant height (control=55.80 cm and M₂ population=51.36 cm), number of branches per plant (control=7.00 and M₂ population=5.23), days to 50 per cent flowering (control=32.20 and M₂ population=40.58), pod length (control=16.41 cm and M₂ population=15.16 cm), pod width (control=8.67 mm and M₂ population=8.4 mm), days to pod maturity (control=48.20 and M₂ population=44), Weight of ten pods (control=65.00 g and M₂ population=57.22 g), average number of seeds per pod (control=5.00 and M₂ population=6.29), tenderness (control=33.20 tr and M₂ population=36.66 tr), pod yield per plant (control=99.80 g and M₂ population=92.4 g) and pod yield per ha (control=221.78 qt and M₂ population=205.34 qt). The results revealed that, differences between means of the control and M₂ population were significant for all the quantitative characters at 5 per cent.

Table 1e. Comparative mean performance of yield and yield related component traits in field evaluated M₂ generation of french bean during 2014-15 at Bengaluru (n= 10000)

Sl. no.	Parameters	Response				't' Test
		Control (n=5)		M ₂ population		
		Mean	SD	Mean	SD	
1	Plant height (cm)	55.80	4.66	51.36	1.78	48.31*
2	Number of branches/ plant	7.00	1.22	5.23	0.66	28.48*
3	Days to 50 per cent flowering	32.20	0.84	40.58	6.49	15.10*
4	Pod length (cm)	16.41	0.89	15.16	0.33	28.79*
5	Pod width (mm)	8.67	0.47	8.40	0.35	74.29*
6	Days to pod maturity	48.20	0.85	44.00	1.87	19.04*
7	Weight of ten pods (g)	65.00	3.94	57.22	10.62	85.59*
8	Average number of seeds/ pod	5.00	0.71	6.29	0.43	25.58*
9	Tenderness (tr)	33.20	3.11	36.66	8.24	33.74*
10	Pod yield/ plant (g)	99.80	2.68	92.4	8.76	35.34*
11	Pod yield/ ha (q)	221.78	5.96	205.34	19.47	52.49*

*Significant at 5% level

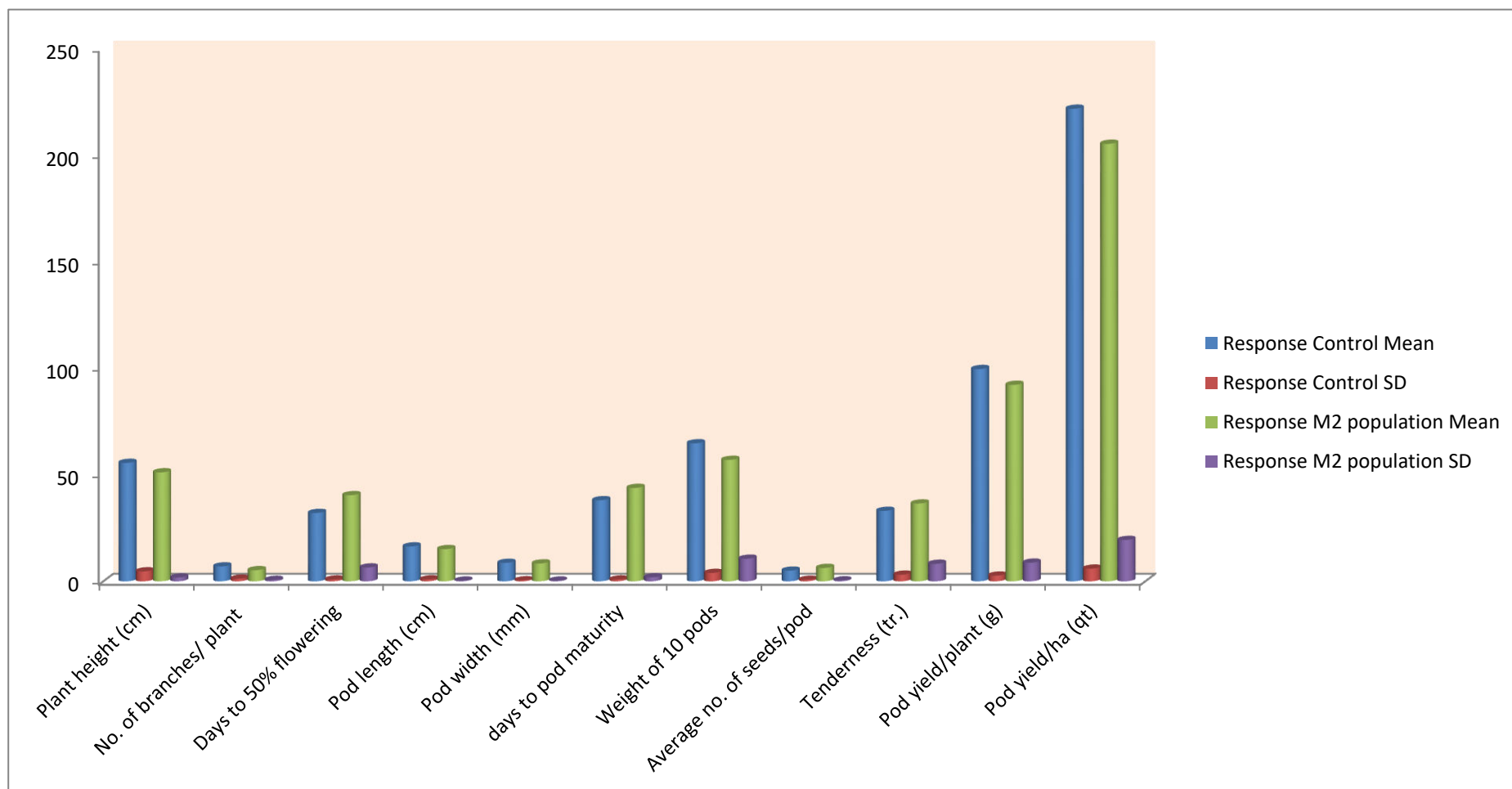


Figure 1b. Comparative mean performance of yield and yield related component traits in M₂ generation of french bean field evaluated during 2014-15 at Bengaluru (n= 10000)

Control: Arka Anoop

4.1.6 Genetic analysis of quantitative parameters for assessment of mean, range and standard error in M₃ generation

The mean plant height documented was 51.36 cm with a range of 46 cm to 63 cm among 2000 M₃ plants. Similarly, number of branches per plant, ranged from 6 to 3 branches per plant with mean value of 5.23 branches per plant among individuals of the population. Whereas, observation for days to 50 per cent flowering, the mean value was 40.36 days, with a minimum of 18.63 days and a maximum of 45 days with a range of 26.37 days was recorded. Further, the pod length parameters recorded a wide range of variation among the individuals of mutant populations in M₃ generation. The mean pod length was found to be 15.15 cm with a range between 12.66 cm and 17.00 cm. Similarly, for pod width, mean value was at 8.46 mm with a minimum of 5.78 mm and a maximum of 9.71 mm. Mean value for the days to pod maturity was 44 days with a range of 43 to 47 days (Table 1f).

The mean weight of pods noted was 57.16 g, which ranges between 32.07 g and 57.47 g. Whereas, the average number of seeds per pod ranged from 5.47 to 5.77 with a mean value of 6.28 seeds per pod across the individual of M₃ population. Similarly, mean tenderness recorded was 38.75 tr with a minimum of 26.34 tr and to a maximum of 51.25 tr. In case of pod yield per plant, the mean value recorded was at 93.23 g with a range of 48.35 g to 99.00 g. However, mean pod yield per hectare documented was 207.18 qt less yield of 145.93 qt and more of 221.11 qt.

Table 1f. Genetic analysis of quantitative parameters for assessment of mean, range and standard error in field evaluated M₃ generation of french bean during 2015-16 at Bengaluru (n= 2000)

Sl. no.	Parameters	Mean	Standard error (±)	Range	Minimum	Maximum
1	Plant height (cm)	51.36	0.21	17.00	46.00	63.00
2	Number of branches/ plant	5.23	0.18	6.00	3.00	9.00
3	Days to 50 per cent flowering	40.36	0.95	26.37	18.63	45.00
4	Pod length (cm)	15.15	0.01	4.34	12.66	17.00
5	Pod width (mm)	8.46	0.01	3.93	5.78	9.71
6	Days to pod maturity	44.00	0.56	4.00	43.00	47.00
7	Weight of 10 pods (g)	57.16	2.56	25.40	32.07	57.47
8	Average number of seeds/ pod	6.28	0.10	0.30	5.47	5.77
9	Tenderness (tr.)	38.75	1.89	24.91	26.34	51.25
10	Pod yield/ plant (g)	93.23	3.56	50.65	48.35	99.00
11	Pod yield/ ha (q)	207.18	4.68	75.18	145.93	221.11

4.1.7 Assessment of genetic variability parameters of yield and yield component traits in M₃ generation of french bean

The eleven quantitative parameters tested for genetic variability estimation viz., phenotypic variance and genotypic variance, phenotypic co-efficient of variation (%), genotypic co-efficient of variation (%), heritability (%), genetic advance and genetic advance as per cent of mean in M₃ population (2000 plants) and results are presented in Table 1g and Figure 1c.

4.1.7.1 Plant height (cm)

Higher phenotypic variance (3.15) than the genotypic variance (2.94) was observed for plant height. Estimates of phenotypic coefficient of variation and genotypic coefficient of variation found to be low with 3.45 and 3.34 per cent respectively coupled with high heritability (93%), genetic advance of 2.46 and low genotypic over mean of 4.79 per cent.

4.1.7.2 Number of branches per plant

No. of branches per plant had phenotypic variance 0.43 which was greater than the variance due to genotype (0.25). Moderate phenotypic coefficient of variation with 12.59 per cent and genotypic coefficient of variation of 9.54 per cent, low genetic advance of 0.72 and genotypic over mean of 13.69 per cent with moderate heritability of 57 per cent were observed for no. of branches per plant.

4.1.7.3 Days to 50 per cent flowering

Phenotypic variance of 46.11 and genotypic variance of 45.16 were noticed for days to 50 per cent flowering with moderate (16.65%) genotypic coefficient of variation and phenotypic coefficient of variation (16.82%). Genetic advance of 9.64 and high genotypic over mean of 23.90 per cent with a high heritability of 98 per cent were observed.

Table 1g. Assessment of genetic variability parameters of yield and yield component traits in field evaluated M₃ generation of french bean during 2015-16 at Bengaluru (n= 2000)

Sl. No.	Parameters	PV	GV	PCV (%)	GCV (%)	h ² (%)	GA	GAM (%)	CV (%)
1	Plant height (cm)	3.15	2.94	3.45	3.34	93	2.46	4.79	2.59
2	Number of branches/ plant	0.43	0.25	12.59	9.54	57	0.72	13.69	15.52
3	Days to 50 per cent flowering	46.11	45.16	16.82	16.65	98	9.64	23.90	6.46
4	Pod length (cm)	0.10	0.09	2.10	2.03	93	0.44	2.91	3.73
5	Pod width (mm)	0.04	0.04	2.50	2.35	89	0.29	3.38	5.43
6	Days to pod maturity	3.50	2.94	4.25	3.90	84	2.46	5.59	3.11
7	Weight of ten pods (g)	113.99	111.43	18.68	18.47	98	15.15	26.51	5.72
8	Average number of seeds/ pod	0.18	0.08	6.79	4.55	45	0.41	6.54	10.40
9	Tenderness (tr)	179.40	177.51	34.56	34.38	99	19.12	49.34	9.44
10	Pod yield/ plant (g)	40.47	36.91	6.82	6.52	91	8.72	9.35	2.71
11	Pod yield/ ha (q)	199.84	195.16	6.82	6.74	98	20.05	9.68	1.82

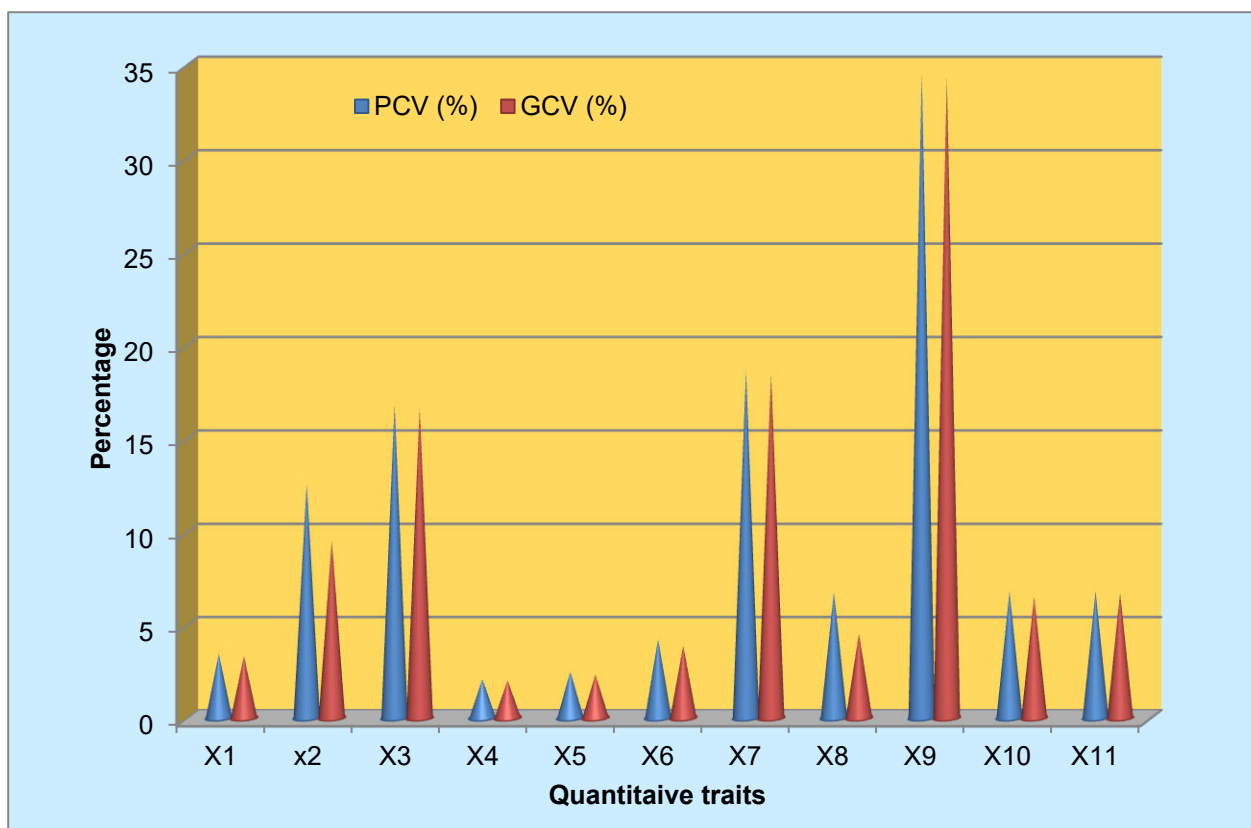


Figure 1c. Genetic variability parameters (PCV % and GCV %) for eleven quantitative components in M_3 population of Arka Anoop

X1: Plant height (cm)

X7: Weight of ten pods (g)

X2: Number of branches/ plant

X8: Average number of seeds/ pod

X3: Days to 50 per cent flowering

X9: Tenderness (tr.)

X4: Pod length (cm)

X10: Pod yield/ plant (g)

X5: Pod width (mm)

X11: Pod yield/ ha. (qt)

X6: Days to pod maturity

4.1.7.4 Pod length (cm)

The variance due to phenotype (0.10) was higher than the variance due to genotypic (0.09). Estimated value depicted low phenotypic coefficient of variation and genotypic coefficient of variation 2.10 and 2.03 per cent respectively. With respect to heritability it was high (93%) with 0.44 per cent of genetic advance and low genotypic over mean (2.91%).

4.1.7.5 Pod width (cm)

The phenotypic and genotypic variances for pod width were 0.04. Phenotypic coefficient of variation and genotypic coefficient of variation estimated as low (2.50% and 2.35%), respectively coupled with higher heritability (89%). Genetic advance of 0.29 and genotypic over mean of 3.38 per cent were noticed as low.

4.1.7.6 Days to pod maturity

The variance due to phenotype (3.50) was higher than the variance due to genotype (2.94). Low estimate of phenotypic coefficient of variation and genotypic coefficient of variation (4.25 and 3.90 per cent respectively) were observed. Heritability (84%) was high with low genetic advance of 2.46 and genotypic over mean of 5.59 per cent for days to pod maturity were noticed.

4.1.7.7 Weight of ten pods

Phenotypic variance of 113.99 and genotypic variance of 111.43 were estimated for Weight of ten pods with 18.47 per cent of genotypic coefficient of variation and phenotypic coefficient of variation of 18.68 per cent (moderate). High genetic advance of 15.15 and genotypic over mean 26.51 per cent with a high heritability of 98 per cent were observed.

4.1.7.8 Average number of seed per pod

The variance due to phenotype (0.18) and genotype (0.08) with phenotypic coefficient of variation and genotypic coefficient of variation 6.79 and 4.55 per cent respectively were observed for average number of seeds per pod with moderate (45%) heritability, 0.41 genetic advance and low (6.54%) genotypic over mean.

4.1.7.9 Tenderness (tr)

Phenotypic variance of 179.40 and genotypic variance of 177.51 were recorded for tenderness with 34.38 per cent of genotypic coefficient of variation and phenotypic coefficient of variation of 34.56 per cent was found to be high. Genetic advance of 19.12 and high genotypic over mean 49.34 per cent with a high heritability of 99 per cent were observed for tenderness.

4.1.7.10 Pod yield per plant (g)

Phenotypic variance of 40.47 and genotypic variance of 36.91 were pragmatic for pod yield per plant with 6.52 per cent of genotypic coefficient of variation and phenotypic coefficient of variation of 6.82 per cent. Genetic advance of 8.72 and low genotypic over mean 9.35 per cent with a high heritability of 91 per cent were observed.

4.1.7.11 Pod yield per ha (q)

The variance due to phenotype (199.84) was higher than the variance due to genotypic (195.16). Estimate of phenotypic coefficient of variation and genotypic coefficient of variation observed were 6.82 and 6.74 per cent, respectively coupled with 98 per cent heritability, 9.68 per cent genetic genotypic over mean of 1.82 per cent.

4.1.8 Phenotypic correlation coefficients for yield and yield component traits in M₃ generation

The results pertaining to correlation coefficient (r) estimates between different growth parameters in M₃ generations are presented in Table 1h.

4.1.8.1 Plant height (cm)

Plant height showed significant positive correlations at 5% level at phenotypic level with number of branches per plant (0.866), days to 50% flowering (0.075), pod width (0.117), days to pod maturity (0.050), Weight of ten pods (0.094), average number of seeds per pod (0.035), tenderness (0.055) and positive with non significant relation was observed for pod length (0.012), pod yield per plant (0.01) and pod yield per ha (0.01).

Table 1h. Phenotypic correlation coefficients for yield and yield component traits in field evaluated M₃ generation of french bean during 2015-16 at Bengaluru

Measures	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
X1	1.000										
X2	0.866*	1.000									
X3	0.075*	0.035	1.000								
X4	0.012	0.017	0.001	1.000							
X5	0.117*	0.111*	0.032	0.112*	1.000						
X6	0.050*	0.208*	0.013	0.009	0.012	1.000					
X7	0.094*	0.076*	0.011	0.032	0.041	0.032	1.000				
X8	0.035*	0.044*	0.037	0.037	0.025	0.003	0.365	1.000			
X9	0.055*	0.051*	0.018	0.026	0.025	0.020	0.301	0.077*	1.000		
X10	0.010	0.035	0.003	0.016	0.002	0.047*	0.547*	0.104*	0.408*	1.000	
X11	0.010	0.035	0.003	0.016	0.002	0.047*	0.547*	0.404*	0.408*	1.000*	1.000

*Significant at 5% level, Other values are non significant,

Table 'r' (0.05) = 0.0438

X1: Plant height (cm)

X5: Pod width (mm)

X9: Tenderness (tr)

X2: Number of branches/ plant

X6: Days to pod maturity

X10: Pod yield/ plant (g)

X3: Days to 50 per cent flowering

X7: Weight of ten pods (g)

X11: Pod yield/ ha. (q)

X4: Pod length (cm)

X8: Average number of seeds/ pod

4.1.8.2 Number of branches per plant

Number of branches per plant showed significant (5%) positive correlations at phenotypic level with pod width (0.111), days to pod maturity (0.208), Weight of ten pods (0.076), average number of seeds per pod (0.044) and tenderness (0.051) and non significant with direct relation was existed with pod length (0.017), pod yield per plant (0.035), pod yield per ha (0.035) and days to 50% flowering (0.035).

4.1.8.3 Days to 50 per cent flowering

Days to 50 per cent flowering exhibited positive relation at phenotypic level with pod length (0.001), pod width (0.032), days to pod maturity (0.013), average number of seeds per pod (0.037), Weight of ten pods (0.011), tenderness (0.018) and pod yield per ha (0.003), yield per plant (0.003) but it was not significant relationship.

4.1.8.4 Pod length (cm)

Pod length showed significant positive correlations at 5% level with pod width (0.112) and had positive but non significant correlation with Weight of ten pods (0.032), average number of seeds per pod (0.037) and tenderness (0.026) days to pod maturity (0.009), pod yield per plant (0.016) and pod yield per ha (0.016) and days to 50% flowering (0.011).

4.1.8.5 Pod width (cm)

Pod width showed direct correlations at 5% level with Weight of ten pods (0.041), average number of seeds per pod (0.025) and tenderness (0.025), days to pod maturity (0.012), pod yield per plant (0.002) and pod yield per ha (0.002).

4.1.8.6 Days to pod maturity

Significant and positive correlation was observed for pod yield per plant (0.047) and pod yield per ha (0.047). But with days to pod maturity, with Weight of ten pods (0.032), tenderness (0.020) and average number of seeds per pod (0.003) had positive correlation.

4.1.8.7 Weight of ten pods

Weight of ten pods showed significant positive correlations at phenotypic level with pod yield per plant (0.547) and pod yield per ha (0.547). Direct relation was existed with average number of seeds per pod (0.365) and tenderness (0.301).

4.1.8.8 Average number of seed per pod

Average number of seeds per pod showed positive correlations at phenotypic level with pod yield per plant (0.104) and pod yield per ha (0.404) and tenderness (0.077) significantly at 5%.

Table 1i. Comparative mean performance of yield and yield related component traits in field evaluated M₃ generation of french bean during 2015-16 at Bengaluru (n= 2000)

Sl. no.	Parameters	Response				't' Test
		Control (n=5)		M ₃ population		
		Mean	SD	Mean	SD	
1	Plant height (cm)	54.00	6.63	51.36	1.77	66.70*
2	Number of branches/ plant	7.20	1.30	5.23	0.66	33.49*
3	Days to 50 per cent flowering	39.60	2.19	40.36	6.79	5.01*
4	Pod length (cm)	16.08	1.07	15.15	0.32	129.97*
5	Pod width (mm)	8.66	0.06	8.46	0.21	42.59*
6	Days to pod maturity	59.40	1.34	44.00	1.87	62.18*
7	Weight of ten pods (g)	66.31	2.45	57.16	10.68	38.31*
8	Average number of seeds/ pod	6.40	0.41	6.28	0.43	12.48*
9	Tenderness (tr)	33.88	3.52	38.76	13.40	16.29*
10	Pod yield/ plant (g)	99.00	3.00	93.23	6.36	40.57*
11	Pod yield/ ha (q)	215.56	6.67	207.18	14.14	26.50*

*Significant at 5% level

4.1.8.9 Tenderness (tr)

Similarly for tenderness showed significant positive correlations at phenotypic level pod yield per plant (0.408) and pod yield per ha (0.408).

4.1.8.10 Pod yield per plant (g)

Pod yield per plant showed significant positive correlations at phenotypic level pod yield per ha (1.000).

4.1.9 Comparative mean performance of yield and yield related component traits in M₃ generation

Mean values for eleven quantitative characters for the M₃ population was taken from the evaluated data. Comparison of mean values of control and M₃ population based on t-test are presented in Table 1i.

Mean values of M₃ population and control were compared for eleven quantitative characters. The mean values of control in comparison with M₃ population was, plant height (control=54.00 cm and M₃ population=51.36 cm), number of branches per plant (control=7.20 and M₃ population=5.23), days to 50 per cent flowering (control=39.60 and M₃ population=40.36), pod length (control=16.08 cm and M₃ population=15.15 cm), pod width (control=8.66 mm and M₃ population=8.46 mm), days to pod maturity (control=59.40 and M₃ population=44.00), Weight of ten pods (control=66.31 g and M₃ population=57.16 g), average number of seeds per pod (control=6.40 and M₃ population=6.28), tenderness (control=33.88 tr and M₃ population=38.76 tr), pod yield per plant (control=99.00 g and M₃ population=93.23 g) and pod yield per ha (control=215.56 qt and M₃ population=207.18 qt) were observed. The results revealed that, differences between means of the control and M₃ population were significant for all the quantitative characters at 5 per cent (Figure 1d).

4.1.10 Comparative analysis and frequency distribution between mean qualitative component traits in M₂ and M₃ generation

M₂ and M₃ populations were characterized with respect to qualitative traits like stringiness, plant type, leaf colour, leaf character and flower pigmentation. The plants were grouped based on the variability present in each one of the qualitative characters under different sub descriptors in both M₂ and M₃ population (Plate 3 to 5).

In order to estimate frequency distribution pattern for qualitative characters among the M₂ (10000) and M₃ (2000) generations, frequency distribution with χ^2 test was employed. The results showed the significant variation between the generations for all mentioned qualitative characters. The data is presented in Table 1j.

All the 10000 plants (100%) of M₂ showed string pod character and about 1995 (99.8%) of 2000 plants in M₃ showed stringed pods. However, none of the plant exhibited stringlessness in M₂ but found 5 plants (0.20%) of M₃ without string in the pods.

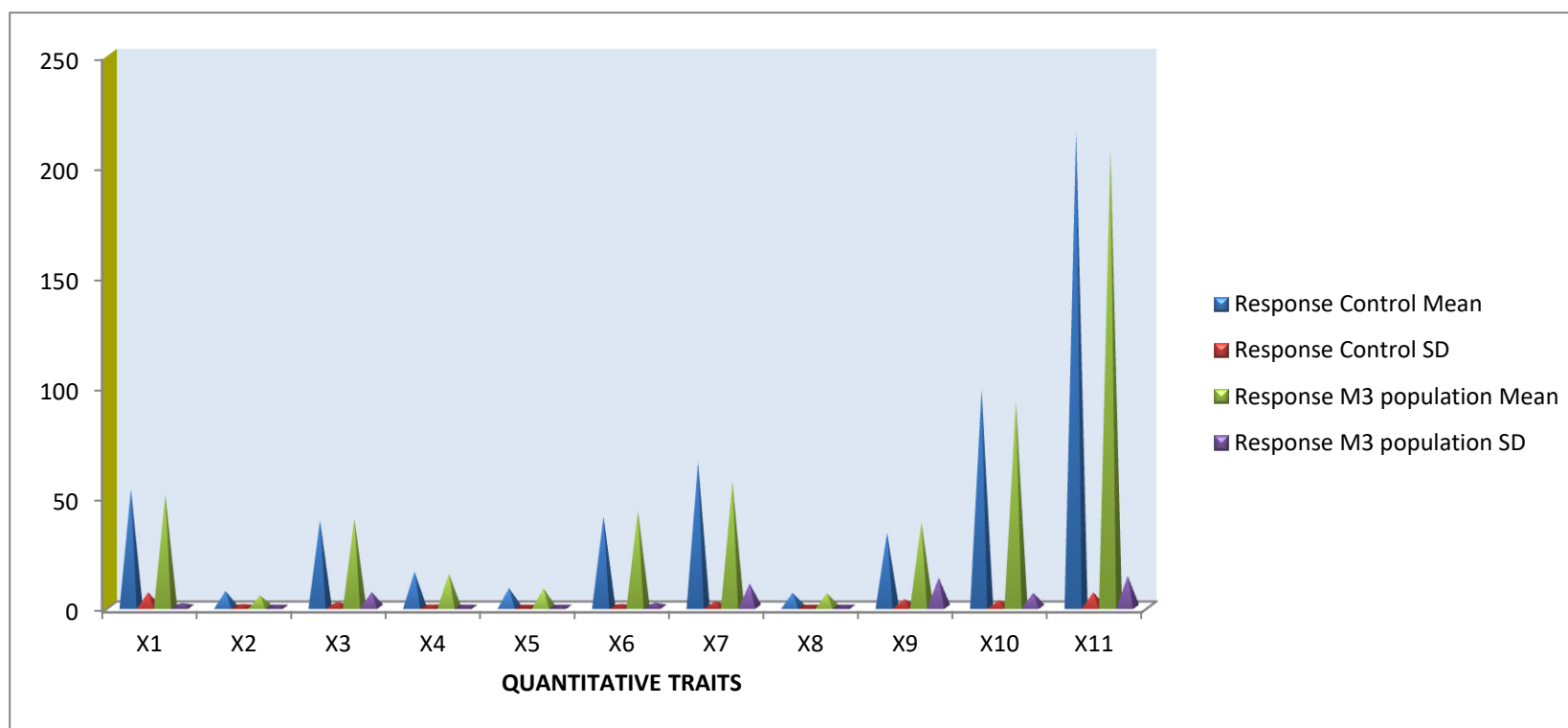


Figure 1d. Comparative mean performance of yield and yield related component traits in M₃ generation of french bean field evaluated during 2015-16 at Bengaluru (n= 2000)
Control: Arka Anoop

X1: Plant height (cm)

X5: Pod width (mm)

X9: Tenderness (tr.)

X2: Number of branches/ plant

X6: Days to pod maturity

X10: Pod yield/ plant (g)

X3: Days to 50 per cent flowering

X7: Weight of ten pods (g)

X11: Pod yield/ ha. (qt)

X4: Pod length (cm)

X8: Average no. of seeds/ pod

Table 1j. Comparative analysis and frequency distribution between mean qualitative component traits in field evaluated M₂ and M₃ generation french bean at Bengaluru

Parameters	Category	Response				χ^2 test
		M ₂ (n=10000)		M ₃ (n=2000)		
		N	Per cent	N	per cent	
Stringness	Stringed	10000.00	100.00	1995.00	99.80	25.01*
	String less	0.00	0.00	5.00	0.20	
Plant type	Bush	10000.00	100.00	1997.00	99.90	15.00*
	Pole	0.00	0.00	3.00	0.10	
Leaf colour	Green	8305.00	83.00	1611.00	80.60	9.44*
	Dark green	658.00	6.60	136.00	6.80	
	Light green	1037.00	10.40	253.00	12.60	
Leaf character	Normal	8869.00	88.70	1816.00	90.80	15.60*
	Thick	671.00	6.70	92.00	4.60	
	Thin	200.00	2.00	43.00	2.20	
	Broad	260.00	2.60	49.00	2.40	
Flower	White	9039.00	90.4	1816.00	90.90	0.33 ^{NS}
	Pink	961.00	9.60	184.00	9.10	

*Significant at 5% level,
NS : Non-significant



Plate 3a. Thin leaved mutant plants



Plate 3b. Rough leaved mutants



Plate 3c. Mutant plants showing variegated leaf character



Plate 3d. Mutant plants showing broad leaf character



Plate 3e. Pole type mutants



Plate 3f. Mutant plant with curved leaves

Plate 3g. Mutant free from *MYMV* infestation

Plate 3. Variation in the mutant plants for leaf characters



Plate 4. Mutation observed for flower colour in M₂ plant



Plate 5a. Mutant plants showing variation in pod width



Plate 5b. Varied pod shape in mutant plants

Plate 5. Variation in the mutant plants for pod characters

With respect to plant type, all 1000 (100%) plants of M₂ population showed bushy nature. However, none of the plant exhibit pole category, where in M₃ population about 1997 (99%) plants showed the bushy nature and 3 (0.1%) were pole type.

There was a significant variation between the M₂ and M₃ population for leaf characters. All type of leaf characters viz., normal, thick, thin and broad green was observed in both M₂ and M₃ populations. 8869 plants (88.7%) of M₂ and 1816 plants (90.8%) of M₃ population showed normal leaf character as in case of control plants. 671 plants (6.7%) of M₂ and 92 plants (4.5%) of M₃ showed thick leaf character. 200 plants (2%) in M₂ population and in M₃, 43 plants (2.2%) showed thin leaf character. However, 2.6 per cent (260 plants) in M₂ population and 2.4 per cent (49 plants) in M₃ population exhibits broad leaf character.

With respect to flower characters, about 9039 plants (90.4%) in M₂ population and 1816 plants (90.9%) in M₃ population showed white flower character; 961 plants (9.6%) in M₂ population and 184 plants (9.1%) in M₃ population showed pink flower character.

4.2 Cloning and structural characterization of NBS-LRR type resistant gene analogs (RGAs) from french bean

4.2.1 PCR amplification of RGAs

A set of degenerate primers were used to amplify corresponding RGAs from genomic DNA of genotype Arka Anoop of french bean (Plate 6). In order to isolate most of RGAs, a number of novel combinations of degenerate primers were tried. Optimization of PCR conditions for amplification of RGAs is a pre-requisite as primers were degenerate in nature. Primer concentration of 10 pM gave good amplification. The annealing temperature was optimized using gradient function of PCR (Eppendorf master cycler gradient). At optimum temperature, only a few minor bands were observed along with the desired one (Plate 7). At an annealing temperature of 56°C, F3 + F4, F7 + F8 and S1 + AS3 primer combinations produced desired amplicons with very few or no other bands. Similarly, 50°C was optimum for S2 + AS1, S2 + AS2, S2 + AS3, S2 + AS4, S1 + AS1, S1 + AS2, S1 + M, S2 + M, F1 + F2, F3 + F4, F5 + F6 and F9 + F10. In all combinations of primers tried typical RGA amplicon of desired size was found. S1 + T1, S2 + T1, F1 + T1, F2 + T1, S1 + AS4, S1 + AS3 combination of primers did not amplify any region from french bean DNA.

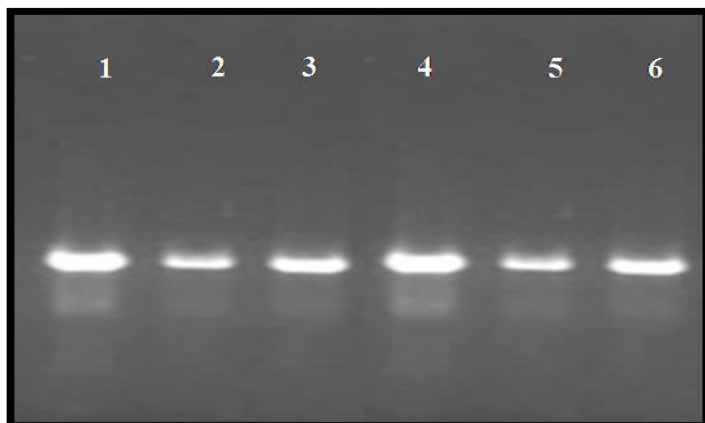


Plate 6. Genomic DNA isolated from french bean genotype Arka Anoop

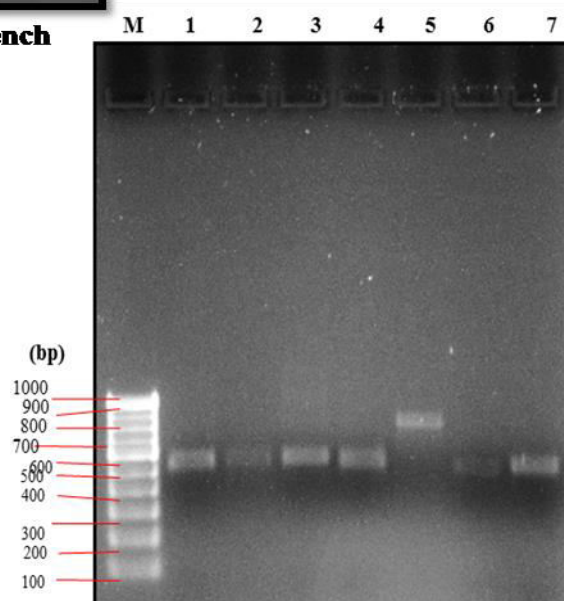


Plate 7. PCR amplification of RGAs with degenerative primers

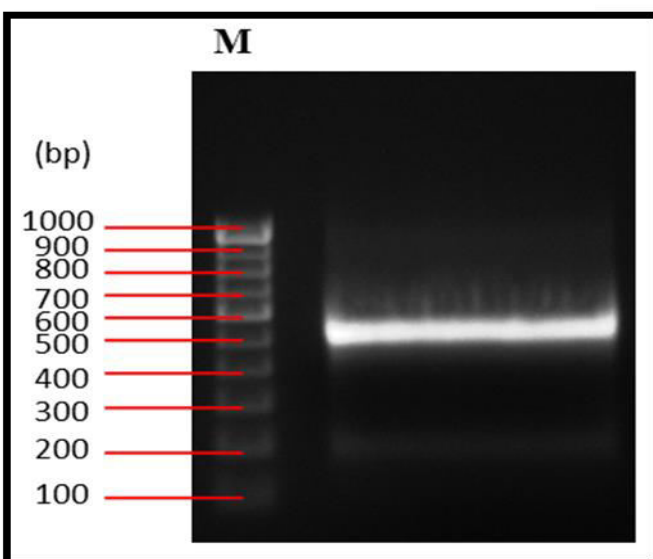


Plate 8. Large scale amplification of PCR product for elution

4.2.2 Elution of PCR products

Typical 500 to 700bp RGA amplicons were considered for elution from each combination of primer pairs. A large-scale amplification of the RGA from the genome was carried out using similar thermal cycler condition. The PCR product of 100µl volume was loaded on the 1.0 per cent low melting EEO agarose gel. The expected fragment, corresponding to 500 to 700 bp was excised from the gel using a sharp sterile blade by keeping the gel at low intensity UV transilluminator. The eluted DNA was purified and the purified product was checked on the gel before ligation to quantify the DNA. The concentrations of eluted DNA were approximately 40 ng per µl, 55 ng per µl, 62 ng per µl, 48 ng per µl, 55 ng per µl, 59 ng per µl, 60 ng per µl, 55 ng per µl, 56 ng per µl and 49 ng per µl (Plate 8).

4.2.3 Cloning and sequencing of french bean RGAs

Eluted and purified PCR were ligated into ddt site of pTZ57R perT vector as described in Ins T perA cloneTM PCR product cloning kit (# K1214). About 7µl of ligation mixture of the ligated product was used for transformation in cells of *E. coli* strain *DH5α* after making them competent (Plate 9).

4.3.1 Confirmation of clones

Recombinant cells were selected based on blue-white colony assay. The plate containing the blue and white colonies is shown in plate 10. Out of several transformations resulted from each primer combination, reasonably good number of white colonies were recorded. These transformed white colonies were randomly picked and streaked on Luria plates containing ampicillin, X-gal and IPTG. Upon repeated blue-white assay with cold incubation, some of the white colonies turned blue. Stable white colonies were further confirmed by plasmid and PCR confirmation. Plasmid profile of isolated recombinant clones indicated the upward movement (Plate 11). The amplification of 15 plasmids from each primer combination using M13 forward and reverse primers confirmed the recombinant nature by producing the expected size amplicons (Plate 12). In all the clones tested a single expected band was observed.

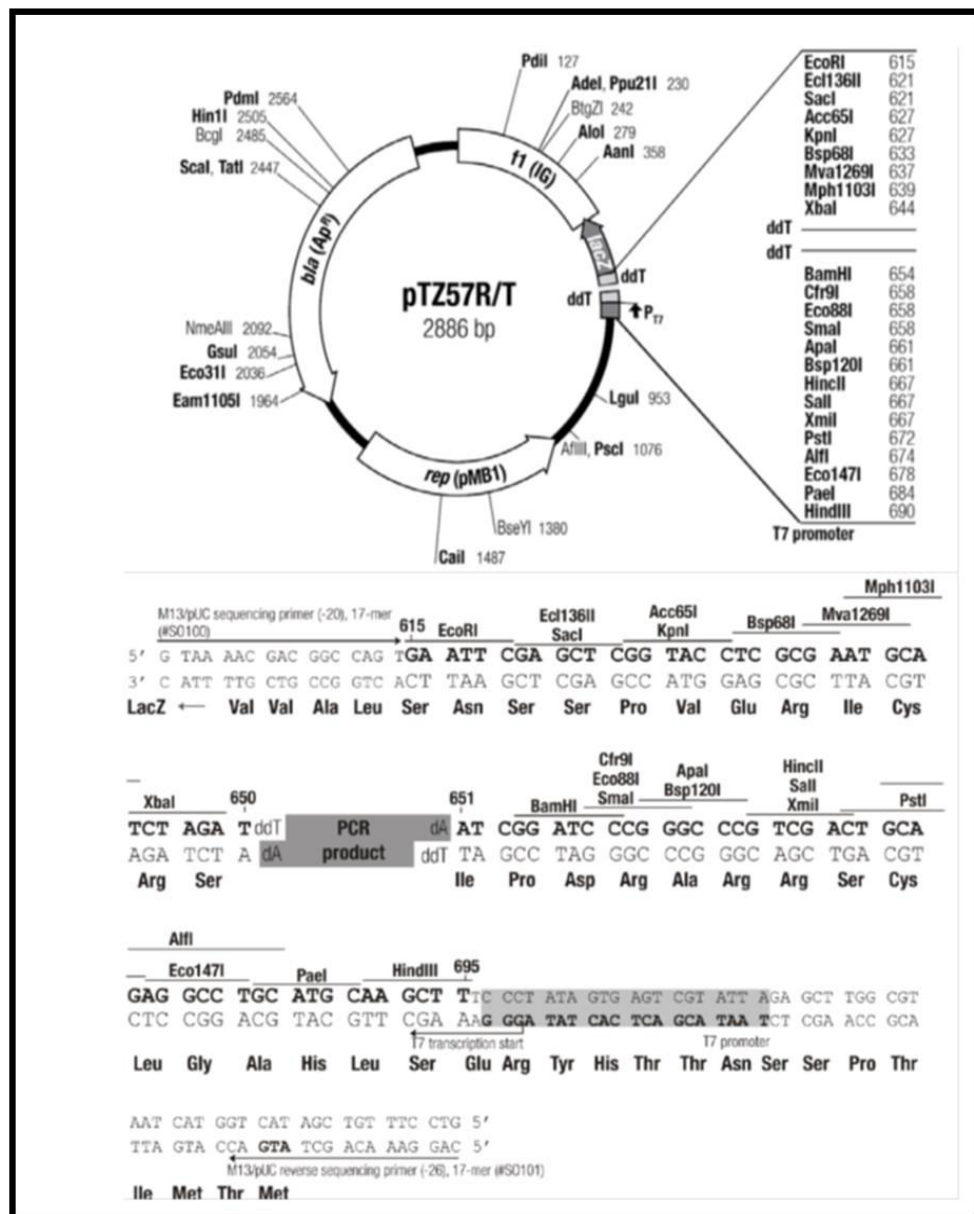
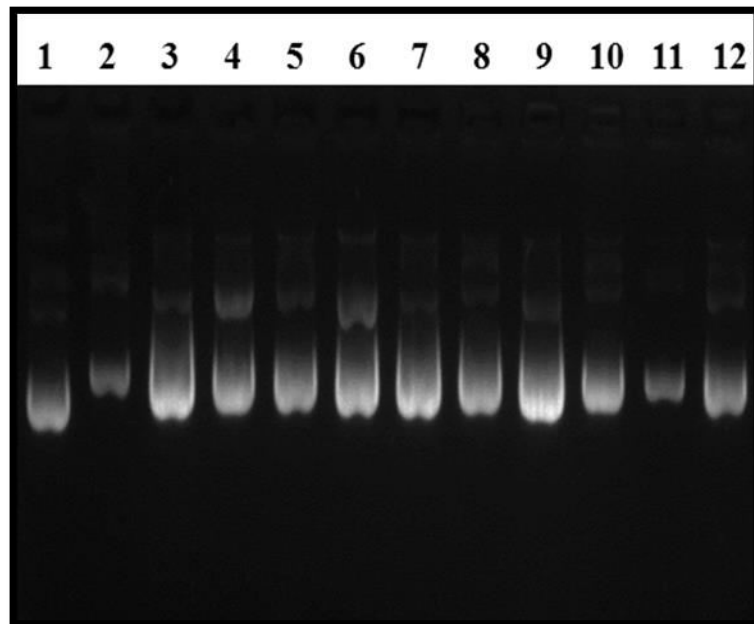


Plate 9. Map of pTZ57R/T cloning vector: Unique restriction sites are indicated



Plate 10. Blue and white colony assay



1-12 plasmids of different recombinants

Plate 11. Plasmid miniprep for recombinants for combination of presence of insert in clones

4.3.2 Sequencing of clones

A total of 90 clones representing different degenerate primer combinations were sequenced at SciGenomics India Pvt. Ltd. Bengaluru. The sequence information of these clones is presented in Appendix VIII.

4.3.4 Sequence analysis of french bean RGA clones

4.2.4.1 Nucleotide homology search

Homology search was carried out for each RGA sequences using default setting of BLASTn with nucleotide database of genbank (NCBI). Out of the 90 sequences, 38 were used for homology search. Rests of the sequences were either vector contaminations or unknown sequences, and were not taken for further analysis. The search results indicated extensive similarities that were recently cloned from other plant species. Significantly high similarity of french bean RGAs was noticed with the resistance genes or RGAs COHFBRGA1 showed 100% homology with resistant gene OB9 of *Phaseolus vulgaris* and with resistance Protein Non-TIR 07 Gene of *P. vulgaris* COHFBRGA2, COHFBRGA25, COHFBRGA26, COHFBRGA9 had 100 per cent homology. There was 96 per cent similarity among the NBS LRR disease resistance protein gene of *Glycine max* and COHFBRGA9 with significantly low E value. Similarly with COHFBRGA33, COHFBRGA34, COHFBRGA36 and COHFBRGA37, PNTR gene and PRG gene of *Arachis hypogaea* showed 100 per cent similarity. NBS-LRR type disease resistance protein (RGA) gene of *Pisum sativum* showed 100% similarity with COHFBRGA38 with low E value. COHFBRGA24, COHFBRGA25, COHFBRGA26, COHFBRGA27, COHFBRGA28, COHFBRGA31 showed maximum similarity with resistance protein gene of *Phaseolus vulgaris* var. *aborigineus*. Isocitrate lyase gene and Arabinose Kinase of *Arabidopsis thaliana*, COHFBRGA18 (100%) and COHFBRGA38 (50%) showed similarity respectively. COHFBRGA27 and NBS-LRR class resistance protein gene of *Clitoria ternatea* had high similarity 9100%). Whereas, Retrovirus-Related Polyprotein KAS-1 of *Arabidopsis thaliana* and COHFBRGA11 had only 39% homology. Whereas, COHFBRGA35 did not share any significant similarity with known resistance genes. Other GenBank accessions showing high similarity to the elucidated french bean RGAs along with the per cent similarity and E value are listed in the Table 2a.

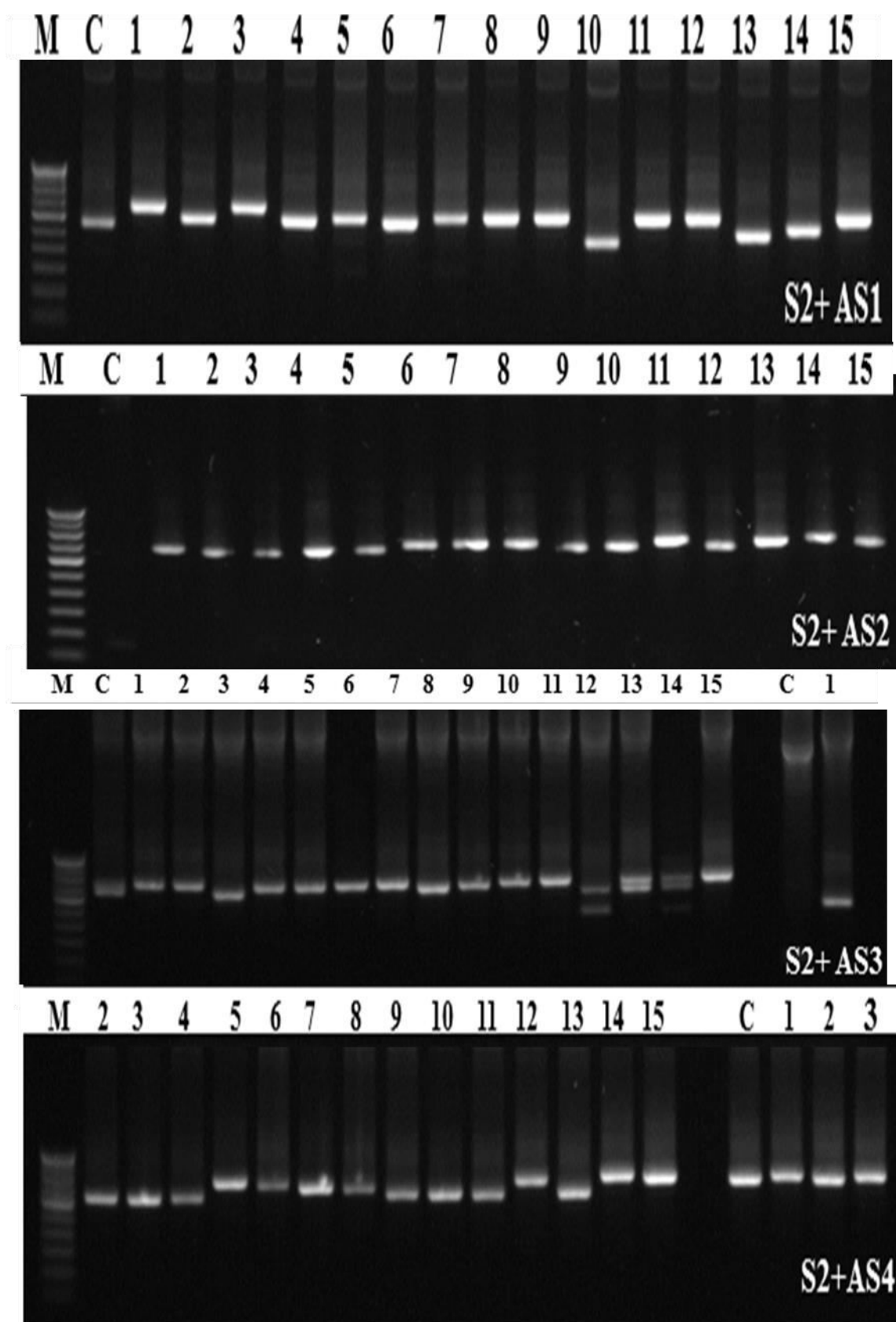


Plate 12a. Colony PCR amplification for presence of insert in primer combinations S2 +AS1, S2 +AS2, S2+AS3 and S2+AS4

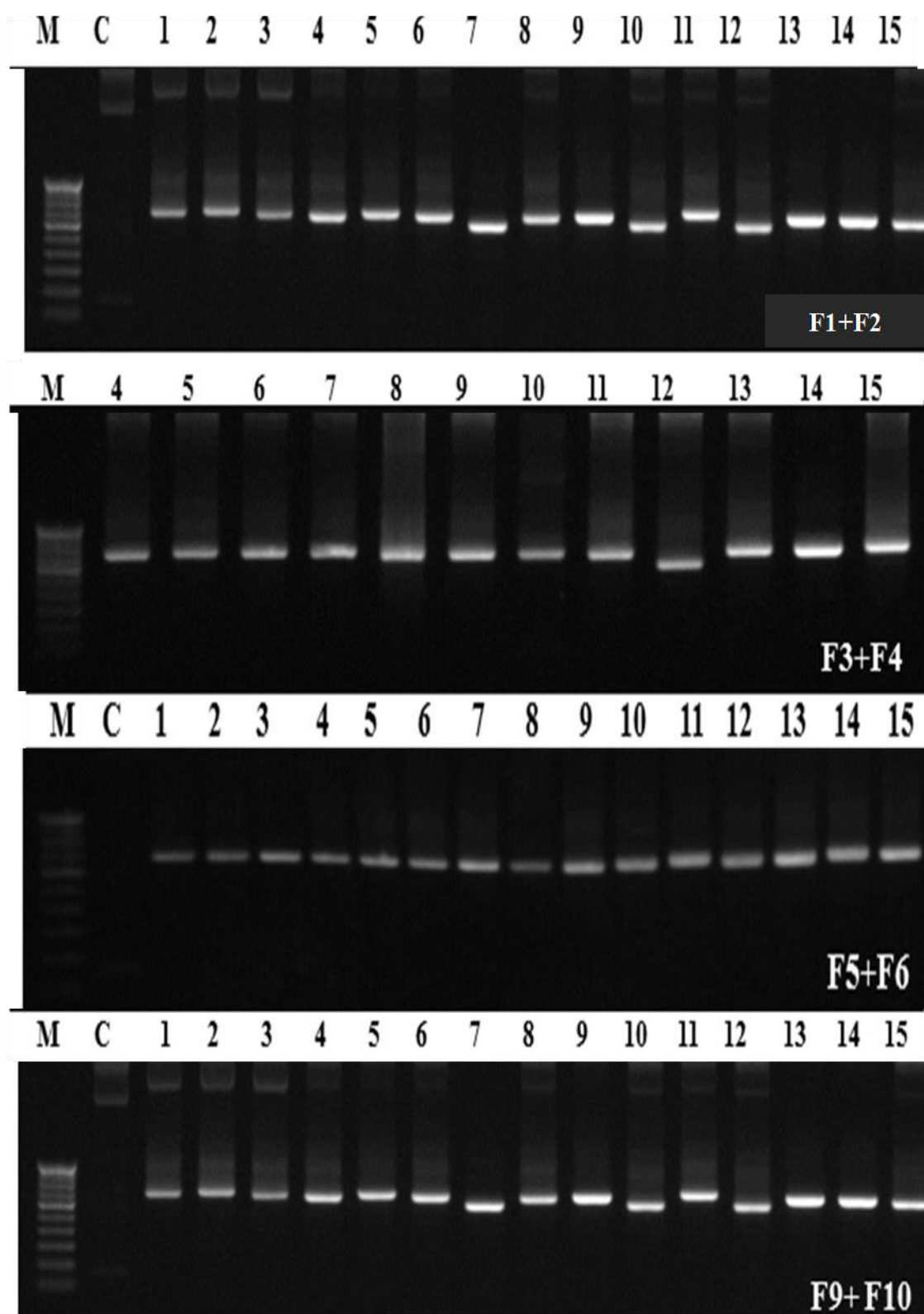


Plate 12b. Colony PCR amplification for presence of insert in primer combinations F1+F2, F3+F4 , F5+F6 and F9+F10

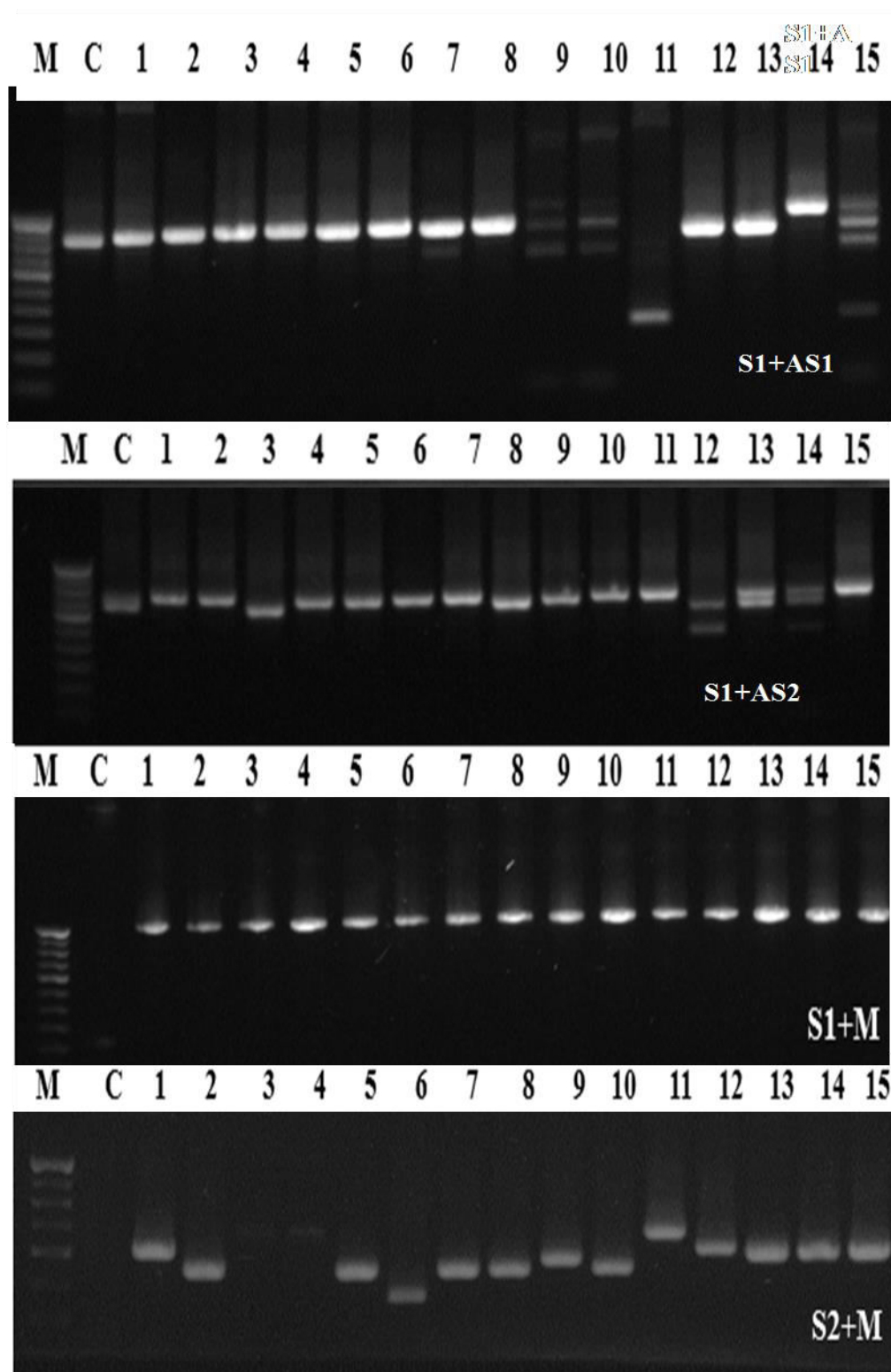


Plate 12c. Colony PCR amplification for presence of insert in primer combinations S1+AS1, S1+AS2, S1+M and S2+M

4.2.4.2 Protein homology search and analysis

The nucleotide sequences of RGAs were used for protein similarity search across the SwissProt database. For this purpose, BLASTx algorithm was used which converts given nucleotide sequence into protein by itself and searches for protein homology. The results of the search for similarity between french bean resistant gene sequences and GenBank accessions carried out using BLASTx algorithm are presented in Table 2b.

The protein database search for elucidated RGAs of french bean resulted in higher similarity with the putative resistance protein of *Glycine max*, putative resistance protein non-TIR 07 of *Phaseolus vulgaris*, NBS-LRR type disease resistance protein of *Pisum sativum*. Catalase protein of Maize, Puroindoline B Protein of *Triticum estivum* had 100 and 96 % similarity with COHFBGA19 and COHFBGA17 respectively. Many of the french bean RGA sequences shared 37 to 100 per cent similarity with disease resistance protein of *Arabidopsis thaliana*, *Triticum macha*, *Cicer arietinum*, *Vigna radiata* Var. *Radiata* *Brassica napus*. COHFBGA35 revealed no significant similarity with any disease resistance protein. Other GenBank accessions showing high similarity to the elucidated french bean RGAs along with the per cent similarity and E value are listed in the Table 2b.

4.2.4.3 Identification of resistance gene family domain in the cloned sequences

The protein sequences of the clones were then subjected for domain identification. For this purpose, Pfam software in NCBI database was used. The sequences showed significance result for the presence of resistance gene family domains (Plate 13). Result showed that out of 38 sequences, 30 sequences confirmed the presences of resistance gene analogue family domains and where remaining eight sequences failed to match with any of the RGAs domain families. Among 30 sequences which had RGA domain, COHFBGA2, COHFBGA4, COHFBGA12, COHFBGA13, COHFBGA14, COHFBGA17, COHFBGA20, COHFBGA21, COHFBGA25, COHFBGA34, COHFBGA35, COHFBGA3 and COHFBGA38 confirmed the presence of P-Loop NTPase superfamily domain with low E value. Sequences like COHFBGA38, COHFBGA29, COHFBGA30 and COHFBGA32 were showing the identity of protein kinase domain, another important group of RGA TIR domain was identified in COHFBGA26, COHFBGA27, COHFBGA33 and COHFBGA37 sequences. Only two sequences COHFBGA3 and COHFBGA15 showed presence of helicase domain and remaining sequences did not significantly matched with RGA families but had domains of catalase (COHFBGA16, COHFBGA19, COHFBGA22), beta- lactamase (COHFBGA28), TIM phosphate binding family (COHFBGA18) and esterase (COHFBGA1) which also involved in disease resistance mechanism in plants (Table 2c).

Table 2a. GenBank nucleotide (Blastn) accessions showing significant homology to cloned french bean RGAs and their functional annotation

Sl. No.	Accessions	Functional description	Organism	Maximum score	Total score	Query cover (%)	E value	Identity (%)
COHFBRGA1	KYP38547.1	GPI Inositol-Deacylase A	<i>Cajanus cajan</i>	26.6	26.6	33%	3.00E-04	53%
COHFBRGA2	CAC81058.1	Mitochondrial F1 ATP Synthase Beta Subunit	<i>Arabidopsis thaliana</i>	40.8	40.8	21%	3.00E-04	69%
COHFBRGA3	CAA37921.1	Unnamed Protein Product	<i>Arabidopsis thaliana</i>	28.5	28.5	16%	3.2	35%
COHFBRGA4	KRH70834.1	Hypothetical Protein GLYMA_02G112700	<i>Glycine max</i>	30.4	30.4	33%	6.7	37%
COHFBRGA5	AAC15224.2	Putative resistance protein KNBS4	<i>Glycine max</i>	353	353	100%	8e-124	100%
COHFBRGA6	CAD59768.1	Putative Reverse Transcriptase	<i>Cicer arietinum</i>	73.9	73.9	28%	6.00E-17	100%
COHFBRGA7	AFM54546.1	Putative resistance protein non-TIR 07	<i>Phaseolus vulgaris</i>	342	342	100%	3e-119	100%
COHFBRGA8	CAD59768.1	Putative Reverse Transcriptase	<i>Cicer arietinum</i>	73.9	73.9	28%	6.00E-17	100%
COHFBRGA9	CAD59768.1	Putative Reverse Transcriptase	<i>Cicer arietinum</i>	74.3	74.3	25%	8.00E-17	100%
COHFBRGA10	CAA74753.1	Putative Arabinose Kinase	<i>Arabidopsis thaliana</i>	31.2	31.2	19%	0.3	50%
COHFBRGA11	S23312	Retrovirus-Related Polyprotein KAS-1 - Retrotransposon Ta1 (Fragment)	<i>Arabidopsis thaliana</i>	28.1	28.1	15%	7.00E-04	39%
COHFBRGA12	CAC81058.1	Mitochondrial F1 ATP Synthase Beta Subunit	<i>Arabidopsis thaliana</i>	39.3	39.3	7%	0.003	89%
COHFBRGA13	CAC81058.1	Mitochondrial F1 ATP Synthase Beta Subunit	<i>Arabidopsis thaliana</i>	39.3	39.3	7%	0.003	89%
COHFBRGA14	CAC81058.1	Mitochondrial F1 ATP Synthase Beta Subunit	<i>Arabidopsis thaliana</i>	39.3	39.3	8%	0.003	89%
COHFBRGA15	CAA37921.1	Unnamed Protein Product	<i>Arabidopsis thaliana</i>	28.5	28.5	15%	2.2	35%
COHFBRGA16	A55092	Catalase (EC 1.11.1.6) CAT-2 - (Fragment)	<i>Maize</i>	60.5	60.5	11%	1.00E-08	100%
COHFBRGA17	CAQ43070.2	Putative Puroindoline B Protein	<i>Triticum macha</i>	64.3	64.3	13%	3.00E-16	94%
COHFBRGA18	BAH03206.1	Peroxisomal isocitrate lyase	<i>Glycine max</i>	278	706	33%	2e-80	77%
COHFBRGA19	A55092	Catalase (EC 1.11.1.6) CAT-2	<i>Maize</i>	60.5	60.5	10%	1.00E-08	100%
COHFBRGA20	XP_013708970.1	Helicase MPN_020-Like	<i>Brassica napus</i>	49.7	49.7	9%	5.00E-05	96%
COHFBRGA21	CAC81058.1	Mitochondrial F1 ATP Synthase Beta Subunit	<i>Arabidopsis thaliana</i>	42	42	15%	0.013	69%

COHFBRGA22	A55092	Catalase (EC 1.11.1.6) CAT-2	<i>Maize</i>	60.5	60.5	10%	2.00E-08	100%
COHFBRGA23	XP_013690297.1	PREDICTED: DNA Cross-Link Repair 1A Protein-Like	<i>Brassica napus</i>	39.7	39.7	7%	0.15	100%
COHFBRGA24	XP_014504967.1	PREDICTED: L-Arabinokinase	<i>Vigna radiata var. radiata</i>	31.6	31.6	15%	2.5	55%
COHFBRGA25	CAC81058.1	Mitochondrial F1 ATP Synthase Beta Subunit	<i>Arabidopsis thaliana</i>	39.7	39.7	6%	0.064	69%
COHFBRGA26	CAD59768.1	Putative Reverse Transcriptase	<i>Cicer arietinum</i>	73.9	73.9	7%	4.00E-15	100%
COHFBRGA27	CCH47205.1	Hypothetical Protein	<i>Lupinus Angustifolius</i>	88.2	88.2	9%	1.00E-19	90%
COHFBRGA28	CAQ43070.2	Putative Puroindoline B Protein[	<i>Triticum macha</i>	102	102	11%	2.00E-22	96%
COHFBRGA29	XP_013708970.1	Helicase protien	<i>Brassica napus</i>	87	87	9%	4.00E-17	95%
COHFBRGA30	CAL37000.1	Putative Reverse Transcriptase	<i>Platanus X Acerifolia</i>	63.5	63.5	6%	2.00E-10	100%
COHFBRGA31	CAQ43070.2	Putative Puroindoline B Protein	<i>Triticum macha</i>	102	102	10%	2.00E-22	96%
COHFBRGA32	ABA55626.1	Beta Lactamase	<i>Synthetic Construct</i>	47.8	47.8	3%	0.001	79%
COHFBRGA33	XP_013690297.1	Toll/interleukin-1 receptor-like protein	<i>Glycine max</i>	65.5	65.5	11%	1.00E-09	82%
COHFBRGA34	CAQ43070.2	Putative Puroindoline B Protein	<i>Triticum macha</i>	102	102	10%	2.00E-22	96%
COHFBRGA35	-	-	-	-	-	-	-	-
COHFBRGA36	CCH47205.1	Hypothetical Protein	<i>Lupinus Angustifolius</i>	88.2	88.2	12%	3.00E-19	90%
COHFBRGA37	CAQ43070.2	Putative Puroindoline B Protein	<i>Triticum macha</i>	102	102	10%	2.00E-22	96%
COHFBRGA38	AGM38898.1	Putative NBS-LRR type disease resistance protein	<i>Pisum sativum</i>	347	347	99%	4e-121	100%

Table 2b. GenBank proteins (Blastx) accessions showing significant homology to cloned french bean RAGs and their functional description

Sl. No.	Accessions	Functional description	Organism	Maximum score	Total score	Query cover (%)	E value	Identity (%)
COHFBRGA1	AF363804.1	Putative Disease Resistance Protein OB9 (RGA) Gene	<i>Phaseolus vulgaris</i>	37.4	37.4	11%	0.74	100%
COHFBRGA2	JQ313585.1	Clone 07N55C1038 Putative Resistance Protein Non-TIR 07 Gene.	<i>Phaseolus vulgaris</i>	39.2	39.2	5%	0.56	100%
COHFBRGA3	EF180027.1	Clone 8 Resistance Protein Gene.	<i>Phaseolus vulgaris</i> var. <i>aborigineus</i>	41	41	3%	0.29	100%
COHFBRGA4	AF363802.1	Putative Disease Resistance Protein OB5 (RGA) Gene.	<i>Phaseolus vulgaris</i>	39.2	39.2	6%	0.59	93%
COHFBRGA5	AF060192.2	Putative Resistance Protein KNBS4 Gene.	<i>Glycine max</i>	42.8	42.8	10%	0.028	96%
COHFBRGA6	JQ313585.1	Clone 07N55C1038 Putative Resistance Protein Non-TIR 07 Gene.	<i>Phaseolus vulgaris</i>	39.2	39.2	5%	0.52	100%
COHFBRGA7	JQ313585.1	Clone 07N55C1038 Putative Resistance Protein Non-TIR 07 Gene.	<i>Phaseolus vulgaris</i>	39.2	39.2	4%	0.59	100%
COHFBRGA8	DQ910861.1	NBS-LRR Class Resistance Protein Gene.	<i>Clitoria ternatea</i>	39.2	39.2	5%	0.54	100%
COHFBRGA9	JQ313585.1	Clone 07N55C1038 Putative Resistance Protein Non-TIR 07 Gene.	<i>Phaseolus vulgaris</i>	39.2	39.2	5%	0.59	100%
COHFBRGA10	CAA74753.1	Putative Arabinose Kinase	<i>Arabidopsis thaliana</i>	31.2	31.2	19%	0.3	50%
COHFBRGA11	S23312	Retrovirus-Related Polyprotein KAS-1 - Retrotransposon Ta1 (Fragment)	<i>Arabidopsis thaliana</i>	28.1	28.1	15%	7.00E-04	39%
COHFBRGA12	CAC81058.1	Mitochondrial F1 ATP Synthase Beta Subunit	<i>Arabidopsis thaliana</i>	39.3	39.3	7%	0.003	89%
COHFBRGA13	CAC81058.1	Mitochondrial F1 ATP Synthase Beta Subunit	<i>Arabidopsis thaliana</i>	39.3	39.3	7%	0.003	89%
COHFBRGA14	CAC81058.1	Mitochondrial F1 ATP Synthase Beta Subunit	<i>Arabidopsis thaliana</i>	39.3	39.3	8%	0.003	89%
COHFBRGA15	CAA37921.1	Unnamed Protein Product	<i>Arabidopsis thaliana</i>	28.5	28.5	15%	2.2	35%
COHFBRGA16	A55092	Catalase (EC 1.11.1.6) CAT-2 gene	<i>Maize</i>	60.5	60.5	11%	1.00E-08	100%

COHFBRGA17	CAQ43070.2	Putative Puroindoline B Protein gene	<i>Triticum macha</i>	64.3	64.3	13%	3.00E-16	94%
COHFBRGA18	M83534.1	lisocitrate lyase gene	<i>Arabidopsis thaliana</i>	41.0	41.0	5%	0.082	100%
COHFBRGA19	A55092	Catalase (EC 1.11.1.6) CAT-2	<i>Maize</i>	60.5	60.5	10%	1.00E-08	100%
COHFBRGA20	XP_013708970.1	PREDICTED: Uncharacterized ATP-Dependent Helicase	<i>Brassica napus</i>	49.7	49.7	9%	5.00E-05	96%
COHFBRGA21	CAC81058.1	Mitochondrial F1 ATP Synthase Beta Subunit	<i>Arabidopsis thaliana</i>	42	42	15%	0.013	69%
COHFBRGA22	A55092	Catalase (EC 1.11.1.6) CAT-2	<i>Maize</i>	60.5	60.5	10%	2.00E-08	100%
COHFBRGA23	XP_013690297.1	PREDICTED: DNA Cross-Link Repair 1A Protein gene	<i>Brassica napus</i>	39.7	39.7	7%	0.15	100%
COHFBRGA24	EF186913.1	Clone 2 resistance protein gene.	<i>Phaseolus vulgaris var. aborigineus</i>	42.8	42.8	5%	0.051	100%
COHFBRGA25	EF180027.1	Clone 8 resistance protein gene.	<i>Phaseolus vulgaris var. aborigineus</i>	41	41	5%	0.18	100%
COHFBRGA26	AF363802.1	Putative disease resistance protein OB5 (RGA) gene.	<i>Phaseolus vulgaris</i>	41	41	5%	0.18	100%
COHFBRGA27	EF180030.1	Clone 11 resistance protein gene.	<i>Phaseolus vulgaris var. aborigineus</i>	39.2	39.2	4%	0.61	100%
COHFBRGA28	EF180026.1	Clone 7 resistance protein gene.	<i>Phaseolus vulgaris var. aborigineus</i>	39.2	39.2	4%	0.61	100%
COHFBRGA29	AF305391.1	Clone 37 putative resistance gene analog pseudogene, partial sequence	<i>Glycine max</i>	41	81.9	5%	0.18	100%
COHFBRGA30	AF305388.1	Clone 35 putative resistance gene analog gene.	<i>Glycine max</i>	41	80.1	5%	0.18	100%
COHFBRGA31	EF180027.1	Clone 8 resistance protein gene.	<i>Phaseolus vulgaris var. aborigineus</i>	41	41	4%	0.19	100%
COHFBRGA32	EF186913.1	Clone 2 resistance protein gene.	<i>Phaseolus vulgaris var. aborigineus</i>	42.8	42.8	6%	0.077	88%
COHFBRGA33	AY747434.1	PNTRP1B04 resistance protein PNTR gene.	<i>Arachis hypogaea</i>	39.2	39.2	4%	1.2	100%
COHFBRGA34	AY747555.1	PRGA3T7H07 resistance protein PRG gene.	<i>Arachis hypogaea</i>	44.6	44.6	5%	0.024	100%
COHFBRGA35	No							
COHFBRGA36	AY747450.1	PNTRP2B04 resistance protein	<i>Arachis hypogaea</i>	41	41	5%	0.3	100%

COHFBRGA37	AY747546.1	PNTR gene. PRGA3T7E10 resistance protein PRG gene.	<i>Arachis hypogaea</i>	41	41	5%	0.3	100%
COHFBRGA38	KC633410.1	Ym.IV.2 putative NBS-LRR type disease resistance protein (RGA) gene.	<i>Pisum sativum</i>	37.4	37.4	19%	0.68	100%

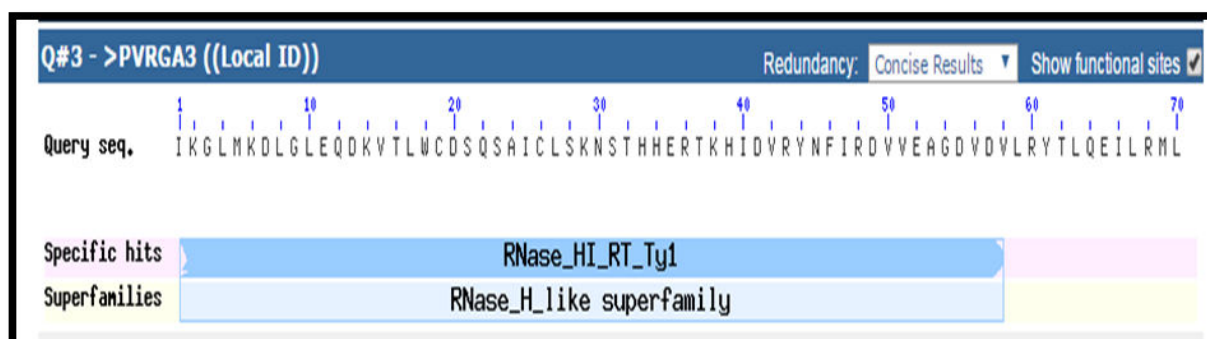
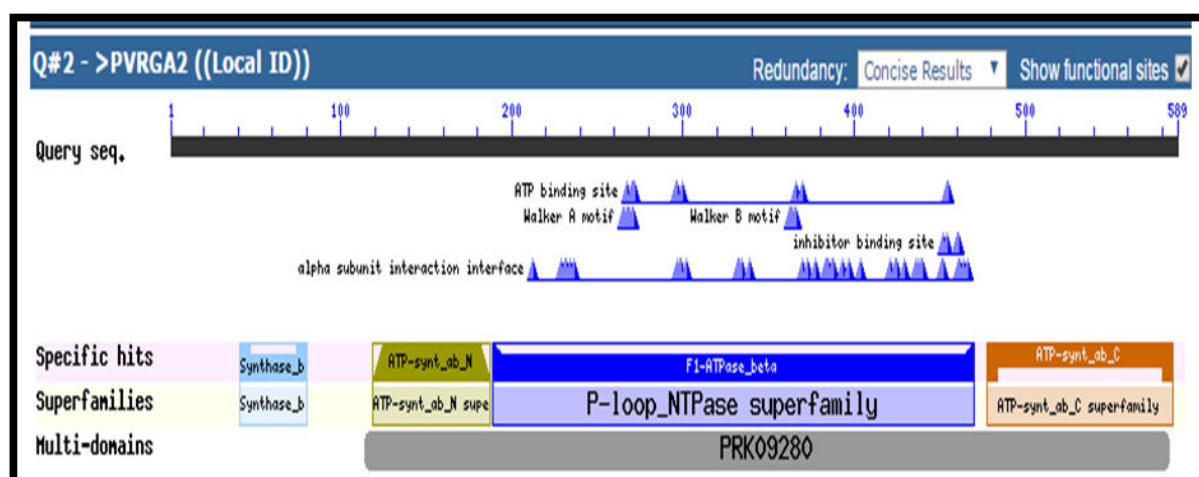
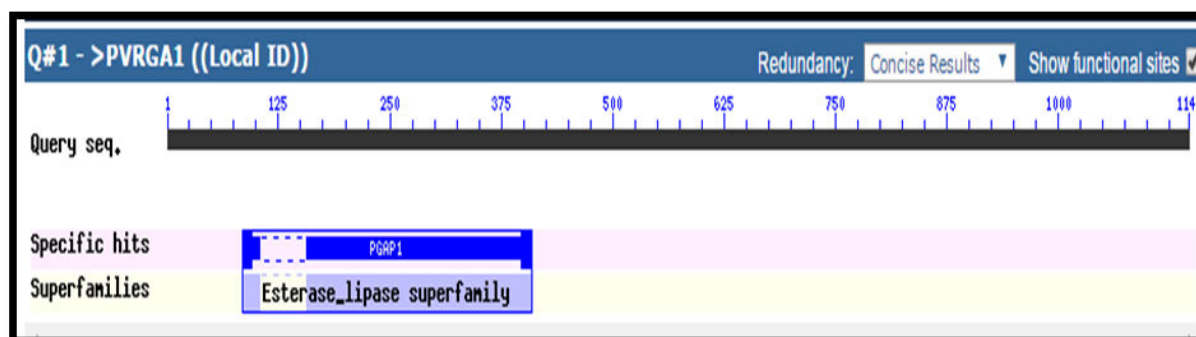


Plate 13a. Identification of RGAs conserved domains in the cloned sequences

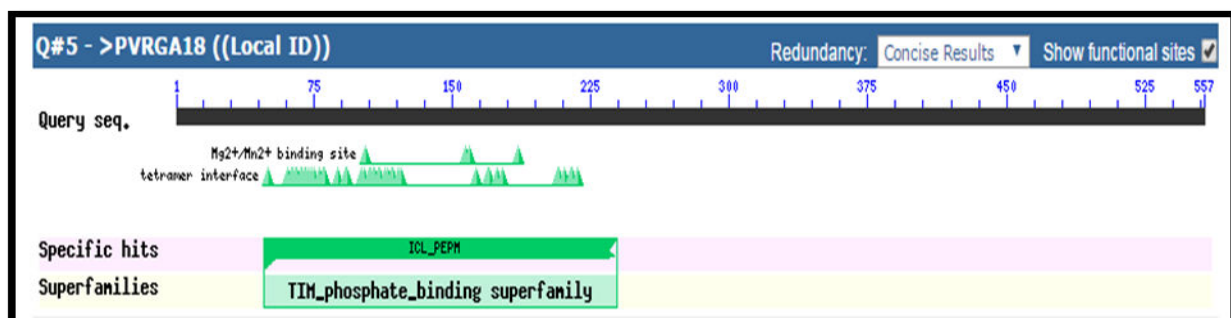
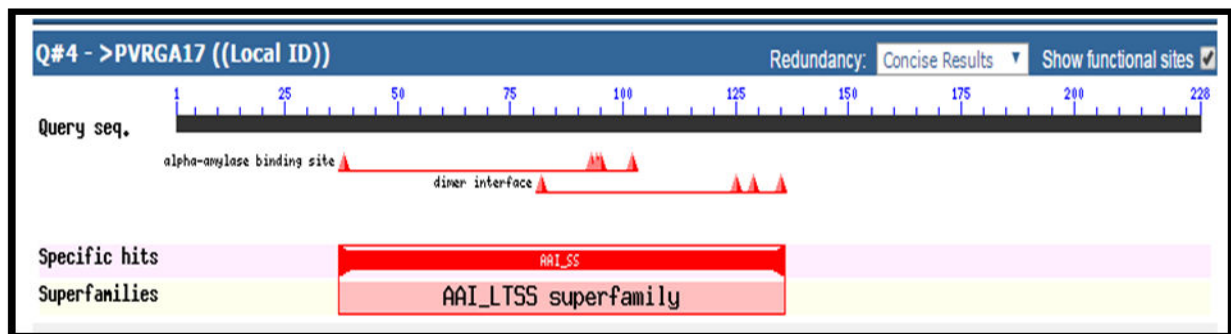
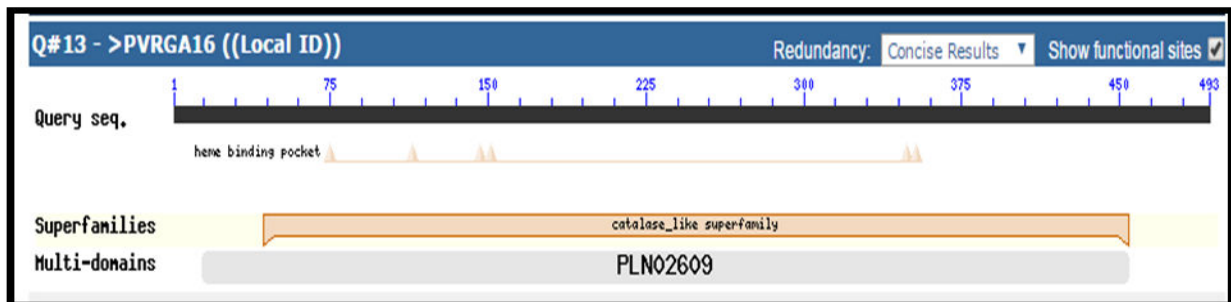
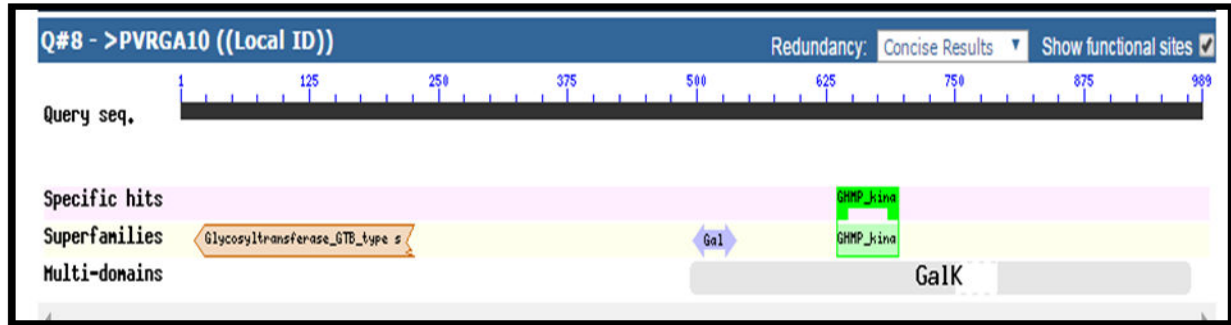


Plate 13b. Identification of RGAs conserved domains in the cloned sequences

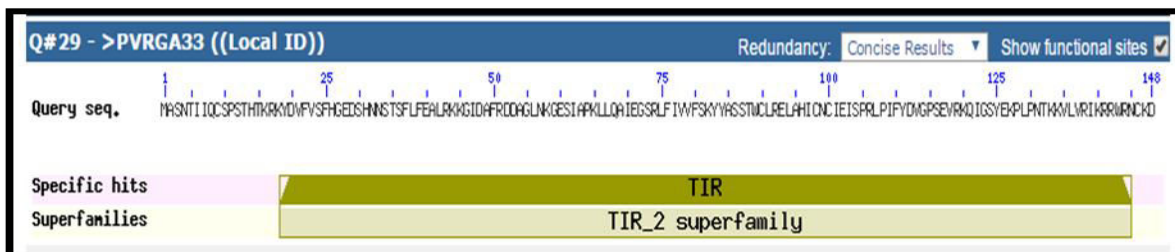
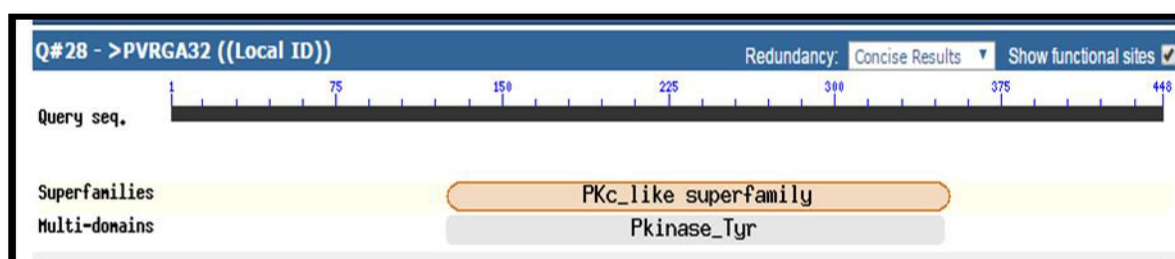
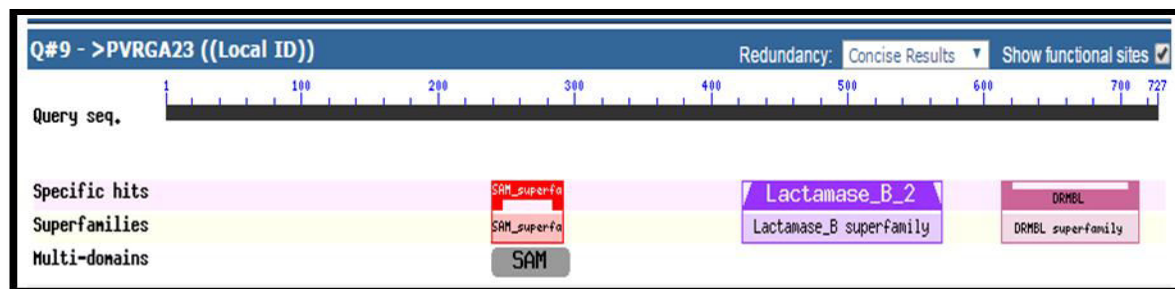


Plate 13c. Identification of RGAs conserved domains in the cloned sequences

4.2.5 Alignment of french bean RGAs with known R genes

Elucidated RGA sequences of french bean were translated into polypeptide by Expasy. These proteins were then aligned with the known disease resistance genes like 5 resistance genes of different crops like N gene of tobacco (RPP5)-AAF08790.1, R gene of *Papevar somnifera* CAQ43070.2, R gene of banana (NP1880651), XP013690297 of Arabidopsis and potato RGA2 (BAA25068.1). Out of 38, 33 clone sequences showed more homology with the known resistance genes. The amino acid residues of known resistance genes of *P. somnifera* CAQ43070.2 from 216 to 392 (GI: U15605), banana from 273 to 458 of the NP1880651 (GI: U27081), Arabidopsis from 182 to 355 of XP013690297., 216 to 389 of AAF08790.1 (GI: U14158, GI: U97106) and potato from 326 to 508 of RGA2 (GI: AB002266) were considered for alignment. ClustalW multiple alignment of deduced amino acid sequences of french bean NBS-LRR sequence and analogous domains in known R gene is presented in Figure 2a. The sequences showed more homology with P-Loop NTP, *kinase* and *TIR* motifs of known R genes.

4.2.6 Phylogenetic analysis of the RGA sequences

The 33 unique cloned sequence *P. vulgaris* RGAs and 5 resistance genes of different crops like N gene of tobacco (RPP5)- AAF08790.1, R gene of *Papevar somnifera* CAQ43070.2, R gene of banana (NP1880651), XP013690297 of Arabidopsis and potato RGA2 (BAA25068.1) taken as reference were subjected for phylogenetic cluster analysis using Megha 6.06 software. Percent amino acid sequence identities of pairs of unique *P. vulgaris* RGAs ranged from 10 % to 100%. The phylogenetic analysis of RGA deduced amino acids revealed that the 33 sequences could be divided into six distinct types. Phylogenetic tree based on Neighbour-Joining method of the deduced amino acid sequence of french bean NBS-LRR and analogous domains of known resistance genes is presented in Figure 2b. A group of ten french bean RGA sequences (COHFBGA17, 34, 29, 31, 33 and 37) formed a major cluster (A) with N gene of tobacco (RPP5) and R gene of *Papevar somnifera* CAQ43070.2. Second major cluster (B) comprised five french bean RGA sequences (COHFBGA25, 16, 19, 38 and 30) with R gene of banana and potato RGA2, third cluster (C) comprised of five french bean RGA sequences (COHFBGA27, 23 and 28) with XP013690297 of Arabidopsis, fourth (D), fifth (E) and sixth (F) cluster comprised seven, five and three french bean RGA sequences respectively. These three clusters did not form group with any of the reference genes. The two most similar pairs of sequences were in family C, in which RGA sequences COHFBGA32 and COHFBGA36 were 100% identical. These french bean RGA sequences of shared at least 2.90 to 98 per cent similarity with all the known disease resistance genes used for comparison.

Table 2c. Significant homology of selected RGAs of french bean and their domain identification using *Pfam*.

Query	PSSM-ID	From	To	E-Value	Bit score	Accession	Domain family name
COHFBRGA1	277541	84	410	3.31E-78	256.904	cl21494	Esterase_lipase superfamily
COHFBRGA2	277521	189	470	0	600.755	cl21455	P-loop_NTPase superfamily
COHFBRGA3	271754	1	58	4.21E-25	91.3757	cl14782	Helicase_ superfamily
COHFBRGA4	253483	370	621	1.31E-84	272.326	pfam05970	P-loop_NTPase superfamily
COHFBRGA5	-	-	-	-	-	-	-
COHFBRGA6	-	-	-	-	-	-	-
COHFBRGA7	-	-	-	-	-	-	-
COHFBRGA8	-	-	-	-	-	-	-
COHFBRGA9	-	-	-	-	-	-	-
COHFBRGA10	272091	635	695	8.27E-11	59.4892	cl21671	Protein kinase superfamily
COHFBRGA11	-	-	-	-	-	-	-
COHFBRGA12	277521	189	470	0	600.755	cl21455	P-loop_NTPase superfamily
COHFBRGA13	277521	189	470	0	600.755	cl21455	P-loop_NTPase superfamily
COHFBRGA14	277521	189	470	0	600.755	cl21455	P-loop_NTPase superfamily
COHFBRGA15	271754	1	58	4.21E-25	91.3757	cl14782	Helicase superfamily
COHFBRGA16	263790	43	454	0	586.571	cl09506	Catalase
COHFBRGA17	277446	37	136	3.76E-23	90.5398	cl07890	P-loop_NTPase superfamily
COHFBRGA18	277523	48	239	2.70E-44	158.035	cl21457	TIM_phosphate_binding superfamily
COHFBRGA19	263790	43	454	0	586.571	cl09506	Catalase_like superfamily
COHFBRGA20	277521	64	186	2.58E-33	118.494	cl21455	P-loop_NTPase superfamily
COHFBRGA21	277521	189	470	0	600.755	cl21455	P-loop_NTPase superfamily

COHFBRGA22	263790	43	454	0	586.571	cl09506	Catalase_ superfamily
COHFBRGA23	188886	239	291	1.61E-17	78.05	cd09487	SAM_ superfamily
COHFBRGA24	277460	15	232	0.00075	41.6035	cl10013	Glycosyltransferase-GTB-type superfamily trehalose-6-phosphate.
COHFBRGA25	277521	189	470	0	600.755	cl21455	P-loop_ NTPase superfamily
COHFBRGA26	277584	18	145	3.53E-27	100.09	cl22869	TIR superfamily
COHFBRGA27	277584	18	145	3.53E-27	100.09	cl22869	TIR superfamily
COHFBRGA28	254258	613	713	1.25E-23	96.9972	pfam07522	β-lactamase superfamily
COHFBRGA29	276297	125	352	8.88E-64	209.051	cl21453	Protein kinase superfamily
COHFBRGA30	276297	125	352	8.88E-64	209.051	cl21453	Protein kinase superfamily
COHFBRGA31	-	-	-	-	-	-	-
COHFBRGA32	276297	125	352	8.88E-64	209.051	cl21453	Protein kinase superfamily
COHFBRGA33	277584	18	145	3.53E-27	100.09	cl22869	TIR superfamily
COHFBRGA34	215633	2	170	2.26E-32	123.061	PLN03210	'P-Loop NTPase' superfamily
COHFBRGA35	215633	2	170	2.26E-32	123.061	PLN03210	'P-Loop NTPase' superfamily
COHFBRGA36	215633	2	170	2.26E-32	123.061	PLN03210	'P-Loop NTPase' superfamily
COHFBRGA37	277446	37	136	3.76E-23	90.5398	cl07890	TIR superfamily
COHFBRGA38	215633	2	170	2.26E-32	123.061	PLN03210	'P-Loop NTPase' superfamily

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COHFBRGA4
COHFBRGA1
COHFBRGA18
COHFBRGA26-----MSELT--HINCVALTARFPVGKP-----VVPAAALMN-RPTRGE
COHFBRGA30-----MSELT--HINCVALTARFPVGKP-----VVPAAALMN-RPTRGE
COHFBRGA16-----MSELT--HINCVALTARFPVGKP-----VVPAAALMN-RPTRGE
COHFBRGA19-----MSELT--HINCVALTARFPVGKP-----VVPAAALMN-RPTRGE
COHFBRGA20-----MSELT--HINCVALTARFPVGKP-----VVPAAALMN-RPTRGE
CAQ43070.2-----MSELT--HINCVALTARFPVGKP-----VVPAAALMN-RPTRGE
COHFBRGA37-----MSELT--HINCVALTARFPVGKP-----VVPAAALMN-R-----
COHFBRGA34-----MSELT--HINCVALTARFPVGKP-----VVPAAALMN-R-----
COHFBRGA29
XA1      KGFEEVTHDITTDVADSAGRTAGMQAVIAV-SLNCVALALRYHDGSPE----TLGPRVASVYTGGNWTVETLPSLIRD-GIKSGH
L6      KGFEEVTHDITTDVADSAGRTAGMQAVIAV-SLNCVALALRYHDGSPE----TLGPRVASVYTGGNWTVETLPSLIRD-GIKSGH
COHFBRGA2
COHFBRGA12-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
COHFBRGA13-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
COHFBRGA14-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
COHFBRGA21-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
COHFBRGA25-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
COHFBRGA3-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
COHFBRGA15-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
COHFBRGA33-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
AAF08790.1-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
COHFBRGA10-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
COHFBRGA22-----MKTFLFL-ALLALVASTTFAQYSE-----VGGWYN-EVGGGG
COHFBRGA23
COHFBRGA28      RVCQVIGAIVDVRFEDEGLPPIMTSLEVQDHPTRLVLEVSHHLGQN-----VVRTIAMDGTGLVRGRKVLNTG
XP_013690297      RVCQVIGAIVDVRFEDEGLPPIMTSLEVQDHPTRLVLEVSHHLGQN-----VVRTIAMDGTGLVRGRKVLNTG
RPG5      RVCQVIGAIVDVRFEDEGLPPIMTSLEVQDHPTRLVLEVSHHLGQN-----VVRTIAMDGTGLVRGRKVLNTG
COHFBRGA27      RVCQVIGAIVDVRFEDEGLPPIMTSLEVQDHPTRLVLEVSHHLGQN-----VVRTIAMDGTGLVRGRKVLNTG
COHFBRGA31      RVCQVIGAIVDVRFEDEGLPPIMTSLEVQDHPTRLVLEVSHHLGQN-----VVRTIAMDGTGLVRGRKVLNTG
COHFBRGA32      RVCQVIGAIVDVRFEDEGLPPIMTSLEVQDHPTRLVLEVSHHLGQN-----VVRTIAMDGTGLVRGRKVLNTG
RVCQVIGAIVDVRFEDEGLPPIMTSLEVQDHPTRLVLEVSHHLGQN-----VVRTIAMDGTGLVRGRKVLNTG
RVCQVIGAIVDVRFEDEGLPPIMTSLEVQDHPTRLVLEVSHHLGQN-----VVRTIAMDGTGLVRGRKVLNTG
-----VTLWCD--SQSAICLSK----NSTHHERTKHIDVRYN-----FIRDVVEAGDVDVLR
-----VTLWCD--SQSAICLSK----NSTHHERTKHIDVRYN-----FIRDVVEAGDVDVLR
QAIEGSRLEFIVVFSKYYASSTWCLRELAHICNIEIS--PRLPIFYD-----VGPSEVRK-QIGSYE
SAIREARISIVIFSKNYASSTWCLNELVEIHKCFNDLGQMVIPVFYD-----VDPSEVRK-QTGEFG
QSPRLKIRKVLDDCGAVQADALTVDRLASLEKYVETAVVPRAEILETEVEWLHSIKADFVSDVVPVACRAAADAGIRSVCTNFS
QSPRLFIRKVLDDCGAVQADALTVDRLASLAKYSETAVKPRAKILAQSEWLNSIKADLVSDVVPVACRAAADAGIRSVCTNFS
SGSHALGSILPSSSSTPCIPSSVDCSSAGDSCSLAAEVGNDGVLV-----GGEEKKGECFKANR
SGSHALGSILPSSSSTPCIPSSVDCSSAGDSCSLAAEVGNDGVLV-----GGEEKKGECFKANR
SGSHALGSILPSSSSTPCIPSSVDCSSAGDSCSLAAEVGNDGVLV-----GGEEKKGECFKANR
-----VTLWCD--SQSAICLSK----NSTHHERTKHIDVRYN-----FIRDVVEAGDVDVLR
-----VTLWCD--SQSAICLSK----NSTHHERTKHIDVRYN-----FIRDVVEAGDVDVLR
QAIEGSRLEFIVVFSKYYASSTWCLRELAHICNIEIS--PRLPIFYD-----VGPSEVRK-QIGSYE
SAIREARISIVIFSKNYASSTWCLNELVEIHKCFNDLGQMVIPVFYD-----VDPSEVRK-QTGEFG
QSPRLKIRKVLDDCGAVQADALTVDRLASLEKYVETAVVPRAEILETEVEWLHSIKADFVSDVVPVACRAAADAGIRSVCTNFS
QSPRLFIRKVLDDCGAVQADALTVDRLASLAKYSETAVKPRAKILAQSEWLNSIKADLVSDVVPVACRAAADAGIRSVCTNFS

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Figure 2a. Multiple sequence alignment of deduced amino acid sequences of French bean NBS-LRR sequences and analogous domains in R genes using ClustalW analysis

4.3 *In silico* genome-wide identification of Transcription factor genes from french bean

For identification and characterization of transcription factors at whole genome level each study uses its own pipeline. Since, as such no universal pipeline is available for this kind of analysis. However, existing pipeline is based on the similar tool; BLAST searches against a plant database. In order to identify the *P. vulgaris* transcription factor coding genes the model plant Arabidopsis TFs was employed as query to perform a blast search against the *P. vulgaris* draft genome ([ftp: per perftp.ncbi.nlm.nih.gov pergenomes perPLANTS perPhaseolus_vulgaris](ftp://per.perftp.ncbi.nlm.nih.gov/pergenomes/perPLANTS/perPhaseolus_vulgaris)). There are totally 58 TFs families identified in *P. vulgaris* using *Arabidopsis* as a reference plant system and in each family they have characterized number of TFs that are listed in Appendix VIII.

The genome-wide identification of TFs gene families in *P. vulgaris* were done based on homology with TF families from Arabidopsis identified from the transcription factor database 3.0 (plntfdb.bio.uni-potsdam.de per). Subsequently, a BLASTP search was performed using these proteins in the Phytozome v9.1 database to identify putative TF proteins ([www. phytozome.net](http://www.phytozome.net) per). All hits with expected values less than 1.0 were retrieved and redundant sequences were removed using the decrease redundancy tool ([web.expasy.org perdecrease_redundancy](http://web.expasy.org/perdecrease_redundancy)), only unique IDs were considered for further work. Among 58 families of TFs, *Phaseolus vulgaris* showed maximum gene homology for NAC TF family members followed by family BHLH was having homology for 62 genes. SIFA, HSF, NZ were having no homology for any of the members of the family. Further, the result obtained from homology study is present in the Table 3a.

Each unique sequence was checked for the presence of the conserved domain by Pfam ([http: per perpfam.sanger.ac.uk](http://per.pfam.sanger.ac.uk)) search. Among all 58 transcription families, in Phaseolus transcription factor domain was observed only for members of 24 families (Figure 3a). MYB family was having highest members with respective TF domain i.e., 102 members of this family in Phaseolus confirmed the presence of TF conserved domain (Table 3b and Appendix IX). Whereas, the members of TFs families like NF YC, NIN like, NZZ, RAV, SAP, SRS, STAT, TALE, Tri Helix, MIKC and other in french bean did not show any conserved domains during the analysis.

The GO slim analysis conducted through Blast2Go showed the putative participation of TFs family proteins in diverse biological process (Table 3c.). Among all TFs, only few members of few families could be annotated for their functionality (Figure 3b). In the french bean, only 8 families of TFs viz., AP2, B3, BHLH, Bzip, C2H2, ERF and MYB exhibited the functional annotation, whereas for remaining families there was no information on functional annotation. Among these 8 families which showed functional annotation of respective families, BHLH family had more number of members having function similarity (56), followed by C2H2 (51) and bZIP family members.

Table 3a. Number of transcription factor genes predicted using *Arabidopsis thaliana* and homologous genes in *Phaseolus vulgaris*

Sl. No	Family	<i>Arabidopsis thaliana</i>	<i>Arabidopsis thaliana</i>	<i>Phaseolus vulgaris</i>	<i>Phaseolus vulgaris</i>
		Raw IDs	Unique IDs	Raw IDs	Unique IDs
1	AP2	30	18	21	11
2	ARF	37	14	43	15
3	ARRB	21	66	29	8
4	B3	77	7	30	12
5	BBR BBC	7	8	21	3
6	BES 1	15	153	25	5
7	bHLH	222	73	159	60
8	bZIP	128	100	182	42
9	C2H2	118	50	192	57
10	C3H	67	6	93	28
11	CAMAT	10	17	13	5
12	CO LIKE	22	8	29	9
13	CPP	10	8	11	4
14	DBB	14	48	23	9
15	DH ZIP	60	36	36	16
16	DOF	47	7	38	16
17	E2F	17	6	21	5
18	EIL	7	122	9	5
19	ERF	139	17	70	33
20	FAR 1	28	42	23	13
21	G2 LIKE	64	30	93	22
22	GATA	41	23	23	10
23	GE BP	23	34	8	4
24	GRAS	37	9	60	32
26	HB OTHERS	13	7	5	3
27	HB PHD	3	2	2	2
28	HRT	2	2	1	1
29	HSF	24	24	0	1
30	LBD	50	43	42	19
31	LSD	13	3	4	21
32	LYF	1	1	1	1
33	MIKC	76	41	152	3
34	MYTPE	70	65	1	20

35	MYB RELATED	97	62	69	1
36	MYB	166	142	237	24
37	NAC	139	110	207	102
38	NF X1	2	2	1	64
39	NF YA	22	10	8	4
40	NF YB	27	13	63	9
41	NF YC	22	14	65	7
42	NIN LIKE	18	14	8	1
43	NZZ	1	1	0	5
44	RAV	7	6	10	2
45	S1 FA	4	3	0	1
46	SAP	1	1	2	10
47	SBP	30	17	26	6
48	SRS	15	12	20	1
49	STAT	4	2	3	25
50	TALE	33	21	111	12
51	TCP	33	24	33	23
52	TRI HELIX	35	30	42	2
53	VOZ	3	2	8	1
54	WHIRLY	4	3	2	10
55	WOX	18	16	14	42
56	WRLY	90	72	112	7
57	YABBY	8	6	17	7
58	ZF HD	19	15	16	0

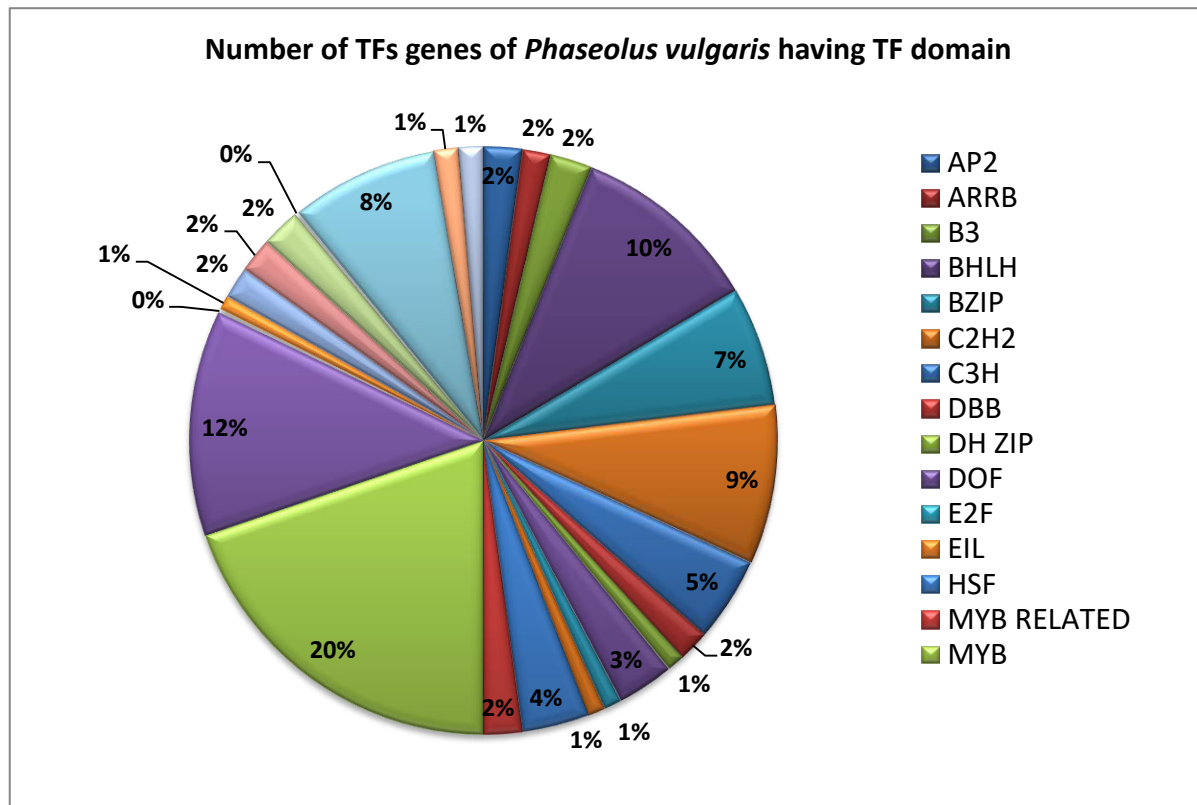


Figure 3a: Details of transcription factor families identified (%) in *Phaseolus vulgaris* having TFs domains.

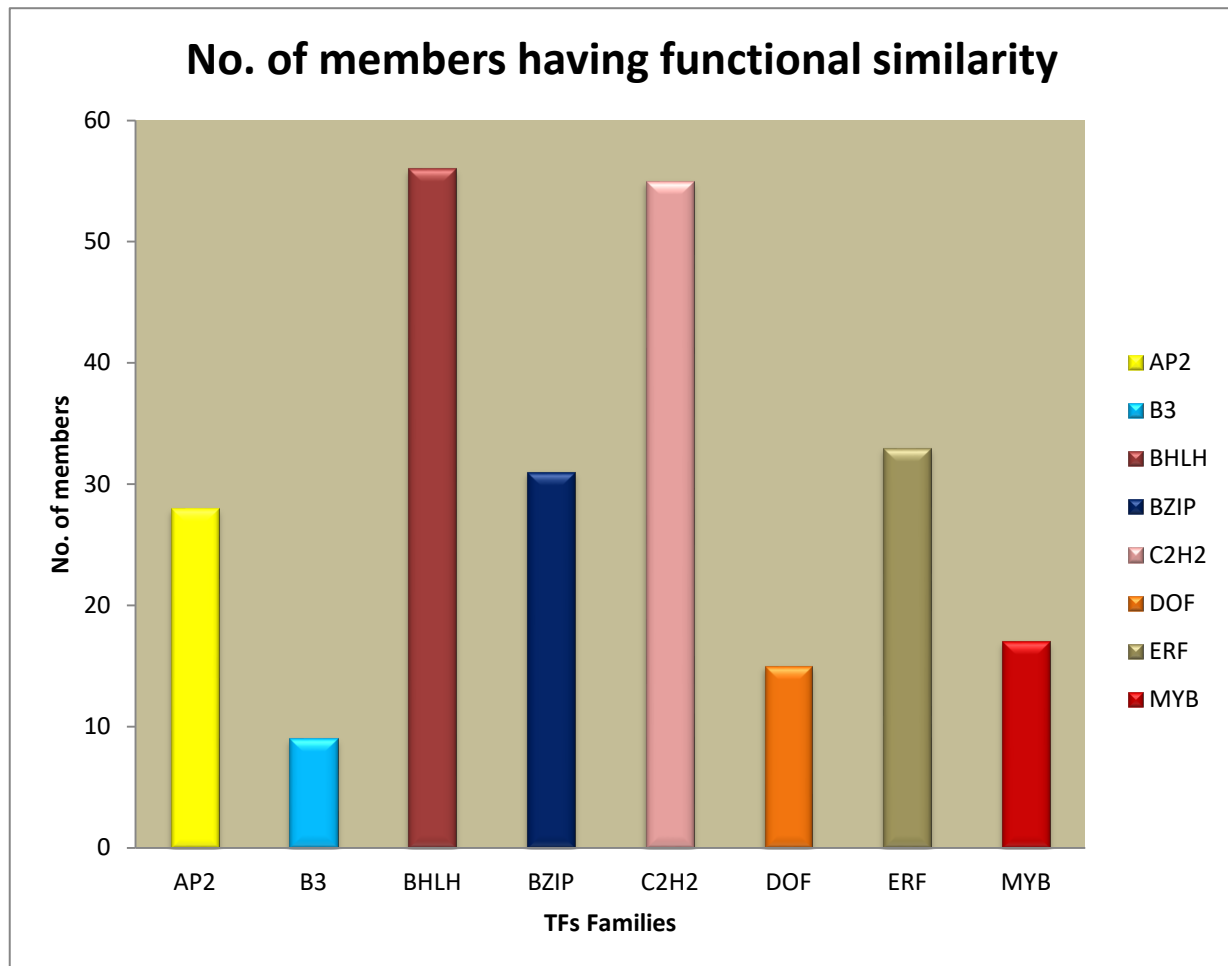


Figure 3b. GenBank protein accessions showing significant homology of selected transcription factor families in french bean genome and their annotation

Table 3b. Details of transcription factor families identified in *Phaseolus vulgaris* having TFs domains

Sl. No	Family	Number of IDs showing domain
1	AP2	11
2	ARF	0
3	ARRB	8
4	B3	12
5	BBR BBC	0
6	BES 1	0
7	BHLH	54
8	BZIP	34
9	C2H2	45
10	C3H	24
11	CAMAT	0
12	CO LIKE	0
13	CPP	0
14	DBB	9
15	DH ZIP	5
16	DOF	16
17	E2F	5
18	EIL	5
19	ERF	0
20	FAR 1	0
21	G2 LIKE	0
22	GATA	0
23	GE BP	0
24	GRAS	0
25	GRAS	0
26	HB OTHERS	0
27	HB PHD	0
28	HRT	0
29	HSF	19
30	LBD	0
31	LSD	0
32	LYF	0
33	MIKC	0
34	MYTPE	0
35	MYB RELATED	11
36	MYB	102
37	NAC	64
38	NF X1	1
39	NF YA	4

40	NF YB	9
41	NF YC	0
42	NIN LIKE	0
43	NZZ	0
44	RAV	0
45	S1 FA	0
46	SAP	0
47	SBP	10
48	SRS	0
49	STAT	0
50	TALE	0
51	TCP	11
52	TRI HELIX	0
53	VOZ	0
54	WHIRLY	1
55	WOX	0
56	WRKY	42
57	YABBY	7
58	ZF HD	7

Table 3c. GenBank protein (Blastx) accessions showing significant homology of selected transcription factor families in french bean genome and its functional annotation

Sequence name	Annotation	Length (bp)	Bit-score	E value
Phvul.010G130200.1	gi 571524489 ref XP_006598829.1 PREDICTED: AP2-like ethylene-responsive transcription factor AIL1 isoform X1 [<i>Glycine max</i>]	580	919.072	0
Phvul.003G241900.1	gi 571537169 ref XP_006600938.1 PREDICTED: floral homeotic protein APETALA 2-like isoform X1 [<i>Glycine max</i>]	517	829.706	0
Phvul.003G153100.1	gi 571441034 ref XP_006575322.1 PREDICTED: AP2-like ethylene-responsive transcription factor At1g16060 [<i>Glycine max</i>]	368	575.474	0
Phvul.003G144500.1	gi 356546106 ref XP_003541472.1 PREDICTED: AP2-like ethylene-responsive transcription factor AIL5 [<i>Glycine max</i>]	526	774.622	0
Phvul.009G206500.1	gi 734368165 gb KHN18668.1 AP2-like ethylene-responsive transcription factor [<i>Glycine soja</i>]	346	471.855	5.65E-166
Phvul.005G138300.1	gi 734397476 gb KHN30231.1 Ethylene-responsive transcription factor RAP2-7 [<i>Glycine soja</i>]	475	790.801	0
Phvul.005G183700.1	gi 356522262 ref XP_003529766.1 PREDICTED: AP2-like ethylene-responsive transcription factor At1g16060 isoform X1 [<i>Glycine max</i>]	321	325.479	1.69E-109
Phvul.011G058000.1	gi 356541922 ref XP_003539421.1 PREDICTED: AP2-like ethylene-responsive transcription factor PLT2 isoform X2 [<i>Glycine max</i>]	544	863.218	0
Phvul.011G071100.1	gi 571492484 ref XP_006592243.1 PREDICTED: ethylene-responsive transcription factor RAP2-7-like isoform X2 [<i>Glycine max</i>]	485	726.472	0
Phvul.008G253600.1	gi 356522262 ref XP_003529766.1 PREDICTED: AP2-like ethylene-responsive transcription factor At1g16060 isoform X1 [<i>Glycine max</i>]	302	422.165	5.20E-148
Phvul.001G174400.1	gi 356503696 ref XP_003520641.1 PREDICTED: floral homeotic protein APETALA 2-like [<i>Glycine max</i>]	459	711.835	0
Phvul.010G011600.1	gi 356504356 ref XP_003520962.1 PREDICTED: auxin response factor 9 isoform X1 [<i>Glycine max</i>]	693	1197.57	0
Phvul.003G128800.1	gi 734365364 gb KHN17743.1 Auxin response factor 7 [<i>Glycine soja</i>]	1163	811.601	0
Phvul.003G128800.1	gi 357479369 ref XP_003609970.1 auxin response factor 2 [<i>Medicago truncatula</i>]	1163	793.882	0
Phvul.005G134500.1	gi 356543474 ref XP_003540185.1 PREDICTED: auxin response factor 18-like [<i>Glycine max</i>]	698	1242.25	0
Phvul.011G073600.1	gi 734327168 gb KHN05725.1 Auxin response factor 4 [<i>Glycine soja</i>]	792	1439.86	0
Phvul.008G118400.1	gi 356520917 ref XP_003529106.1 PREDICTED: auxin response factor 9-like [<i>Glycine max</i>]	659	1158.67	0
Phvul.008G242400.1	gi 571441516 ref XP_006575466.1 PREDICTED: auxin response factor 8-like isoform X2 [<i>Glycine max</i>]	844	1548.87	0
Phvul.004G177600.1	gi 734419812 gb KHN40318.1 Auxin response factor 5 [<i>Glycine soja</i>]	1106	1632.46	0

Phvul.006G181200.1	gi 356549269 ref XP_003543018.1 PREDICTED: auxin response factor 3-like [<i>Glycine max</i>]	734	1170.22	0
Phvul.006G221500.1	gi 356543365 ref XP_003540131.1 PREDICTED: auxin response factor 1-like [<i>Glycine max</i>]	664	1303.5	0
Phvul.002G177600.1	gi 734350481 gb KHN12428.1 Auxin response factor 6 [<i>Glycine soja</i>]	913	1487.63	0
Phvul.003G228600.1	gi 356565422 ref XP_003550939.1 PREDICTED: two-component response regulator- like APRR2 [<i>Glycine max</i>]	559	917.531	0
Phvul.009G253200.1	gi 356556050 ref XP_003546340.1 PREDICTED: two-component response regulator ARR2-like isoform X1 [<i>Glycine max</i>]	672	1104.35	0
Phvul.009G194200.1	gi 734359273 gb KHN15214.1 Two-component response regulator ARR12 [<i>Glycine soja</i>]	676	1131.32	0
Phvul.001G046300.1	gi 734432589 gb KHN46344.1 Two-component response regulator ARR12 [<i>Glycine soja</i>]	591	838.565	0
Phvul.002G004800.2	gi 356540587 ref XP_003538769.1 PREDICTED: two-component response regulator- like APRR2 isoform X1 [<i>Glycine max</i>]	560	875.544	0
Phvul.010G120900.2	gi 571464885 ref XP_003529866.2 PREDICTED: B3 domain-containing protein Os03g0120900-like [<i>Glycine max</i>]	476	469.544	8.12E-161
Phvul.010G070200.1	gi 734426225 gb KHN43730.1 B3 domain-containing protein [<i>Glycine soja</i>]	906	1664.82	0
Phvul.003G011300.1	gi 356575102 ref XP_003555681.1 PREDICTED: B3 domain-containing transcription factor VRN1-like isoform X1 [<i>Glycine max</i>]	435	772.696	0
Phvul.009G064100.1	gi 356510383 ref XP_003523918.1 PREDICTED: B3 domain-containing protein At5g42700-like [<i>Glycine max</i>]	228	363.229	7.53E-127
Phvul.011G050600.1	gi 734387565 gb KHN25328.1 B3 domain-containing transcription factor VRN1 [<i>Glycine soja</i>]	430	716.457	0
Phvul.008G244300.1	gi 734420805 gb KHN41001.1 Putative B3 domain-containing protein [<i>Glycine soja</i>]	401	554.673	0
Phvul.007G230700.1	gi 922401589 ref XP_013467931.1 B3 DNA-binding domain protein [<i>Medicago truncatula</i>]	353	251.906	1.13E-79
Phvul.001G193100.1	gi 922346448 ref XP_013450138.1 B3 DNA-binding domain protein [<i>Medicago truncatula</i>]	297	244.588	5.22E-78
Phvul.008G065600.1	gi 356568386 ref XP_003552392.1 PREDICTED: beta-amylase 7-like [<i>Glycine max</i>]	700	1137.87	0
Phvul.002G123700.1	gi 356531537 ref XP_003534334.1 PREDICTED: beta-amylase 8-like [<i>Glycine max</i>]	657	1221.45	0
Phvul.010G024800.1	gi 734327734 gb KHN05778.1 Transcription factor bHLH62 [<i>Glycine soja</i>]	585	792.727	0
Phvul.010G120000.1	gi 356520219 ref XP_003528761.1 PREDICTED: transcription factor bHLH57 [<i>Glycine max</i>]	382	604.364	0
Phvul.010G114800.1	gi 734329630 gb KHN06316.1 Transcription factor bHLH13 [<i>Glycine soja</i>]	630	908.286	0
Phvul.010G147600.1	gi 571464437 ref XP_006583061.1 PREDICTED: transcription factor bHLH68-like isoform X1 [<i>Glycine max</i>]	317	520.39	0

Phvul.003G181900.1	gi 734367743 gb KHN18389.1 Transcription factor BIM1 [<i>Glycine soja</i>]	532	821.231	0
Phvul.003G072000.1	gi 356526880 ref XP_003532044.1 PREDICTED: transcription factor bHLH79 [<i>Glycine max</i>]	264	446.817	1.00E-158
Phvul.003G285700.1	gi 1142619 gb AAB00686.1 phaseolin G-box binding protein PG1 [<i>Phaseolus vulgaris</i>]	642	992.645	0
Phvul.003G140800.1	gi 734365256 gb KHN17635.1 Transcription factor bHLH35 [<i>Glycine soja</i>]	240	358.992	9.11E-125
Phvul.003G094700.1	gi 356529573 ref XP_003533364.1 PREDICTED: transcription factor BEE 1-like [<i>Glycine max</i>]	273	485.723	6.62E-174
Phvul.003G033900.1	gi 571434765 ref XP_006573288.1 PREDICTED: transcription factor FAMA-like [<i>Glycine max</i>]	422	650.203	0
Phvul.003G286400.1	gi 734319687 gb KHN03619.1 Transcription factor bHLH93 [<i>Glycine soja</i>]	332	440.654	2.57E-154
Phvul.009G162000.1	gi 351723481 ref NP_001238560.1 inducer of CBF expression 1 [<i>Glycine max</i>]	495	496.893	1.34E-171
Phvul.009G033800.1	gi 734391821 gb KHN27371.1 Transcription factor bHLH96 [<i>Glycine soja</i>]	305	439.884	1.76E-154
Phvul.009G235700.1	gi 358248852 ref NP_001239951.1 transcription factor bHLH35-like [<i>Glycine max</i>]	244	407.142	8.90E-144
Phvul.009G023500.1	gi 734405983 gb KHN33752.1 Transcription factor bHLH63 [<i>Glycine soja</i>]	404	546.969	0
Phvul.009G145800.1	gi 356515677 ref XP_003526525.1 PREDICTED: transcription factor bHLH93-like [<i>Glycine max</i>]	328	530.02	0
Phvul.009G137400.1	gi 356509301 ref XP_003523389.1 PREDICTED: transcription factor SPEECHLESS-like [<i>Glycine max</i>]	338	538.495	0
Phvul.005G091000.1	gi 357452673 ref XP_003596613.1 BHLH domain class transcription factor [<i>Medicago truncatula</i>]	231	306.99	1.44E-104
Phvul.005G089000.1	gi 356546916 ref XP_003541866.1 PREDICTED: transcription factor bHLH123-like isoform X1 [<i>Glycine max</i>]	458	761.911	0
Phvul.005G130500.1	gi 223702432 gb ACN21647.1 putative basic helix-loop-helix protein BHLH21 [<i>Lotus japonicus</i>]	323	432.95	3.95E-154
Phvul.011G026500.1	gi 734326388 gb KHN05467.1 Transcription factor bHLH121 [<i>Glycine soja</i>]	276	383.645	3.91E-136
Phvul.011G153000.1	gi 734394550 gb KHN28608.1 Transcription factor bHLH123 [<i>Glycine soja</i>]	466	744.191	0
Phvul.011G178100.1	gi 356525604 ref XP_003531414.1 PREDICTED: transcription factor bHLH121-like [<i>Glycine max</i>]	282	389.037	5.89E-138
Phvul.008G144300.1	gi 571488817 ref XP_006591039.1 PREDICTED: transcription factor UNE10-like [<i>Glycine max</i>]	478	602.438	0
Phvul.008G133600.1	gi 356527316 ref XP_003532257.1 PREDICTED: transcription factor bHLH62 [<i>Glycine max</i>]	570	845.114	0
Phvul.008G177500.1	gi 734391821 gb KHN27371.1 Transcription factor bHLH96 [<i>Glycine soja</i>]	316	397.127	3.66E-140
Phvul.008G158800.1	gi 734414688 gb KHN37435.1 Transcription factor SPEECHLESS [<i>Glycine soja</i>]	352	439.499	4.72E-156

Phvul.004G166300.1	gi 734422530 gb KHN41646.1 Transcription factor bHLH68 [<i>Glycine soja</i>]	333	471.085	1.77E-168
Phvul.007G179100.1	gi 922345865 ref XP_013449847.1 ABA-inducible bHLH-type transcription factor [<i>Medicago truncatula</i>]	506	729.554	0
Phvul.007G257100.1	gi 734397570 gb KHN30254.1 Transcription factor bHLH130 [<i>Glycine soja</i>]	383	539.265	0
Phvul.007G256800.2	gi 356498448 ref XP_003518064.1 PREDICTED: transcription factor bHLH48-like [<i>Glycine max</i>]	344	507.294	5.63E-180
Phvul.007G156200.1	gi 356536868 ref XP_003536955.1 PREDICTED: transcription factor PIF1-like [<i>Glycine max</i>]	517	483.797	7.77E-166
Phvul.007G181900.1	gi 734362461 gb KHN16145.1 Transcription factor bHLH96 [<i>Glycine soja</i>]	337	508.064	0
Phvul.007G101900.1	gi 728826119 gb KHG06567.1 Transcription factor bHLH30 -like protein [<i>Gossypium arboreum</i>]	338	398.282	3.57E-137
Phvul.007G176900.1	gi 357440517 ref XP_003590536.1 basic helix loop helix protein BHLH4 [<i>Medicago truncatula</i>]	280	403.675	3.24E-141
Phvul.001G150300.1	gi 519898392 gb AGP03686.1 basic helix-loop-helix (bHLH) DNA-binding superfamily protein, partial [<i>Glycine soja</i>]	575	794.653	0
Phvul.001G085500.1	gi 351723971 ref NP_001238577.1 inducer of CBF expression 2 [<i>Glycine max</i>]	427	476.093	3.45E-165
Phvul.001G138200.1	gi 363808336 ref NP_001242250.1 transcription factor bHLH48-like [<i>Glycine max</i>]	367	625.55	0
Phvul.001G168700.1	gi 918463563 gb ALA09126.1 bHLH transcription factor, partial [<i>Glycine max</i>]	497	624.394	0
Phvul.001G121200.1	gi 356571913 ref XP_003554115.1 PREDICTED: transcription factor bHLH106-like [<i>Glycine max</i>]	247	336.65	1.06E-115
Phvul.001G138600.1	gi 356504823 ref XP_003521194.1 PREDICTED: transcription factor bHLH130-like [<i>Glycine max</i>]	396	523.857	0
Phvul.001G156400.1	gi 223702398 gb ACN21630.1 putative basic helix-loop-helix protein BHLH3 [<i>Lotus japonicus</i>]	292	416.001	1.43E-145
Phvul.001G031300.1	gi 922397437 ref XP_013465951.1 transcription factor [<i>Medicago truncatula</i>]	429	591.652	0
Phvul.L009100.1	gi 734388815 gb KHN25939.1 Transcription factor bHLH89 [<i>Glycine soja</i>]	513	766.148	0
Phvul.006G153300.1	gi 922388414 ref XP_013462737.1 basic helix loop helix (BHLH) DNA-binding family protein [<i>Medicago truncatula</i>]	191	317.775	5.18E-110
Phvul.006G196600.1	gi 356557617 ref XP_003547112.1 PREDICTED: transcription factor ILR3-like [<i>Glycine max</i>]	215	310.071	2.26E-106
Phvul.006G195000.2	gi 571516465 ref XP_006597381.1 PREDICTED: transcription factor bHLH112-like isoform X2 [<i>Glycine max</i>]	451	684.485	0
Phvul.006G093900.1	gi 734320727 gb KHN04014.1 Transcription factor bHLH96 [<i>Glycine soja</i>]	321	543.888	0
Phvul.006G114000.1	gi 802694529 ref XP_012083197.1 PREDICTED: transcription factor bHLH13-like [<i>Jatropha curcas</i>]	615	707.597	0
Phvul.006G028500.1	gi 955385608 ref XP_014626636.1 PREDICTED: transcription factor PIF4 [<i>Glycine</i>	550	879.011	0

	<i>max]</i>			
Phvul.002G311700.1	gi 734422530 gb KHN41646.1 Transcription factor bHLH68 [<i>Glycine soja</i>]	347	570.081	0
Phvul.002G110500.1	gi 922359892 ref XP_013453871.1 BHLH transcription factor [<i>Medicago truncatula</i>]	550	729.939	0
Phvul.002G079400.1	gi 356500821 ref XP_003519229.1 PREDICTED: transcription factor bHLH79-like isoform X1 [<i>Glycine max</i>]	269	343.969	4.32E-118
Phvul.002G141500.1	gi 571478541 ref XP_003534274.2 PREDICTED: transcription factor MYC2-like [<i>Glycine max</i>]	728	881.708	0
Phvul.002G282300.1	gi 571453047 ref XP_006579339.1 PREDICTED: inducer of CBF expression 3 isoform X1 [<i>Glycine max</i>]	432	491.5	3.45E-170
Phvul.002G271000.1	gi 357519927 ref XP_003630252.1 basic helix loop helix (BHLH) DNA-binding family protein [<i>Medicago truncatula</i>]	281	236.884	1.37E-75
Phvul.010G108800.1	gi 51870705 dbj BAD42432.1 bZip transcription factor [<i>Psophocarpus tetragonolobus</i>]	426	659.448	0
Phvul.003G252000.1	gi 359806400 ref NP_001240983.1 bZIP transcription factor bZIP115 [<i>Glycine max</i>]	339	486.108	7.48E-172
Phvul.003G028800.1	gi 734384039 gb KHN24211.1 Transcription factor HBP-1b(c1) [<i>Glycine soja</i>]	462	594.734	0
Phvul.003G273900.1	gi 13775107 gb AAK39130.1 AF369790_1bZIP transcription factor 2 [<i>Phaseolus vulgaris</i>]	417	791.186	0
Phvul.003G291800.1	gi 571467246 ref XP_006583882.1 PREDICTED: bZIP transcription factor TRAB1-like [<i>Glycine max</i>]	408	546.199	0
Phvul.009G065500.2	gi 543177367 gb AGV54705.1 bZIP transcription factor 6 [<i>Phaseolus vulgaris</i>]	415	592.423	0
Phvul.005G068500.1	gi 356549689 ref XP_003543224.1 PREDICTED: basic leucine zipper 61 [<i>Glycine max</i>]	332	435.261	2.99E-152
Phvul.005G097800.1	gi 356549868 ref XP_003543312.1 PREDICTED: basic leucine zipper 61-like [<i>Glycine max</i>]	329	461.84	7.44E-163
Phvul.005G153900.1	gi 734405496 gb KHN33298.1 Putative transcription factor PosF21 [<i>Glycine soja</i>]	434	478.019	9.17E-166
Phvul.005G124100.1	gi 734404142 gb KHN32878.1 TGACG-sequence-specific DNA-binding protein TGA-2.1 [<i>Glycine soja</i>]	486	793.497	0
Phvul.005G011600.1	gi 356515347 ref XP_003526362.1 PREDICTED: ABSCISIC ACID-INSENSITIVE 5-like protein 2 [<i>Glycine max</i>]	289	330.872	7.78E-112
Phvul.011G038200.1	gi 734387673 gb KHN25436.1 Putative transcription factor PosF21 [<i>Glycine soja</i>]	561	748.429	0
Phvul.011G093700.1	gi 955353406 ref XP_014620106.1 PREDICTED: transcription factor TGA6 isoform X3 [<i>Glycine max</i>]	460	764.607	0
Phvul.011G035700.1	gi 351725099 ref NP_001235033.1 bZIP transcription factor bZIP122 [<i>Glycine max</i>]	265	413.305	1.91E-145
Phvul.011G203400.1	gi 351721009 ref NP_001237963.1 bZIP transcription factor bZIP99 [<i>Glycine max</i>]	467	811.216	0
Phvul.011G116800.1	gi 351722123 ref NP_001236977.1 bZIP transcription factor bZIP50 [<i>Glycine max</i>]	329	385.956	1.42E-132

Phvul.011G053500.1	gi 734405496 gb KHN33298.1 Putative transcription factor PosF21 [<i>Glycine soja</i>]	337	306.99	4.31E-100
Phvul.011G186800.3	gi 356556732 ref XP_003546677.1 PREDICTED: ABSCISIC ACID-INSENSITIVE 5-like protein 2 [<i>Glycine max</i>]	323	440.269	1.42E-154
Phvul.008G247800.1	gi 357491489 ref XP_003616032.1 bZIP transcription factor [<i>Medicago truncatula</i>]	363	419.083	1.00E-144
Phvul.004G097200.1	gi 351720971 ref NP_001237194.1 bZIP transcription factor bZIP131 [<i>Glycine max</i>]	332	343.969	5.11E-116
Phvul.004G053300.1	gi 734408204 gb KHN34586.1 ABSCISIC ACID-INSENSITIVE 5-like protein 2 [<i>Glycine soja</i>]	303	318.931	6.97E-107
Phvul.007G025500.1	gi 734347579 gb KHN11437.1 Transcription factor HBP-1b(c1) [<i>Glycine soja</i>]	462	701.434	0
Phvul.007G229600.1	gi 734353834 gb KHN13511.1 Protein ABSCISIC ACID-INSENSITIVE 5 [<i>Glycine soja</i>]	421	463.766	3.05E-160
Phvul.001G119300.1	gi 571445313 ref XP_003521109.2 PREDICTED: bZIP transcription factor 17 [<i>Glycine max</i>]	783	1002.28	0
Phvul.001G189400.1	gi 734353834 gb KHN13511.1 Protein ABSCISIC ACID-INSENSITIVE 5 [<i>Glycine soja</i>]	393	358.221	2.85E-119
Phvul.001G101000.1	gi 357509087 ref XP_003624832.1 bZIP transcription factor [<i>Medicago truncatula</i>]	313	417.927	9.51E-146
Phvul.001G123300.1	gi 571553040 ref XP_006603764.1 PREDICTED: bZIP transcription factor bZIP96 isoform X2 [<i>Glycine max</i>]	460	733.021	0
Phvul.001G140100.1	gi 734332448 gb KHN07408.1 Transcription factor HBP-1b(c1) [<i>Glycine soja</i>]	483	782.326	0
Phvul.006G109700.2	gi 734409831 gb KHN35355.1 Common plant regulatory factor 1 [<i>Glycine soja</i>]	424	651.744	0
Phvul.006G078500.1	gi 922380435 ref XP_013460447.1 bZIP transcription factor [<i>Medicago truncatula</i>]	416	449.899	9.20E-156
Phvul.002G136000.1	gi 13775109 gb AAK39131.1 AF369791_1bZIP transcription factor 3 [<i>Phaseolus vulgaris</i>]	397	693.345	0
Phvul.010G071300.1	gi 922326181 ref XP_013442483.1 C2H2-type zinc finger protein, putative [<i>Medicago truncatula</i>]	329	258.84	2.62E-82
Phvul.010G138200.1	gi 357513641 ref XP_003627109.1 C2H2-like zinc finger protein, putative [<i>Medicago truncatula</i>]	500	455.292	1.96E-154
Phvul.003G185100.1	gi 922371657 ref XP_013457987.1 DnaJ heat shock family protein, putative [<i>Medicago truncatula</i>]	627	644.81	0
Phvul.003G027900.1	gi 590701032 ref XP_007046299.1 C2H2-like zinc finger protein [<i>Theobroma cacao</i>]	308	378.637	1.20E-130
Phvul.005G099800.1	gi 734413224 gb KHN36669.1 Zinc finger protein NUTCRACKER [<i>Glycine soja</i>]	463	748.043	0
Phvul.005G124800.1	gi 356547134 ref XP_003541972.1 PREDICTED: zinc finger protein WIP3-like [<i>Glycine max</i>]	308	484.952	2.81E-172
Phvul.005G020400.1	gi 356525655 ref XP_003531439.1 PREDICTED: zinc finger protein WIP2-like [<i>Glycine max</i>]	323	497.664	1.34E-176
Phvul.005G130300.1	gi 918463661 gb ALA09175.1 C2H2-Zn transcription factor, partial [<i>Glycine max</i>]	296	310.457	1.12E-103

Phvul.005G025300.1	gi 356499400 ref XP_003518529.1 PREDICTED: zinc finger protein 695-like [<i>Glycine max</i>]	362	553.903	0
Phvul.005G148600.1	gi 959092844 ref NP_001304490.1 zinc finger protein WIP2-like [<i>Glycine max</i>]	336	416.772	3.87E-145
Phvul.011G142000.1	gi 357474845 ref XP_003607708.1 C2H2-like zinc finger protein [<i>Medicago truncatula</i>]	439	676.396	0
Phvul.008G143900.1	gi 922326181 ref XP_013442483.1 C2H2-type zinc finger protein, putative [<i>Medicago truncatula</i>]	384	372.474	4.64E-126
Phvul.008G122800.1	gi 590701032 ref XP_007046299.1 C2H2-like zinc finger protein [<i>Theobroma cacao</i>]	262	352.829	2.96E-121
Phvul.004G084300.1	gi 571480644 ref XP_006588359.1 PREDICTED: C2-H2 zinc finger protein isoform X1 [<i>Glycine max</i>]	512	713.375	0
Phvul.007G139600.1	gi 922404386 ref XP_013469274.1 C2H2-type zinc finger protein [<i>Medicago truncatula</i>]	333	358.607	1.94E-121
Phvul.007G090500.1	gi 571480644 ref XP_006588359.1 PREDICTED: C2-H2 zinc finger protein isoform X1 [<i>Glycine max</i>]	512	913.679	0
Phvul.007G253000.1	gi 356500501 ref XP_003519070.1 PREDICTED: zinc finger AN1 and C2H2 domain-containing stress-associated protein 16-like [<i>Glycine max</i>]	285	467.233	3.05E-166
Phvul.001G134900.1	gi 734332418 gb KHN07378.1 Zinc finger AN1 and C2H2 domain-containing stress-associated protein 11 [<i>Glycine soja</i>]	286	500.36	3.29E-179
Phvul.001G026700.1	gi 357437641 ref XP_003589096.1 C2H2-type zinc finger protein [<i>Medicago truncatula</i>]	220	246.514	7.82E-81
Phvul.006G064800.1	gi 922379920 ref XP_013460192.1 C2H2-like zinc finger protein [<i>Medicago truncatula</i>]	386	487.649	6.54E-171
Phvul.002G082200.1	gi 590699390 ref XP_007045914.1 C2H2-like zinc finger protein, putative [<i>Theobroma cacao</i>]	507	339.732	5.94E-109
Phvul.010G084000.1	gi 959092322 ref NP_001304643.1 zinc finger CCCH domain-containing protein 1 [<i>Glycine max</i>]	329	555.444	0
Phvul.003G102600.2	gi 356563888 ref XP_003550189.1 PREDICTED: zinc finger CCCH domain-containing protein 58-like [<i>Glycine max</i>]	483	665.611	0
Phvul.003G207900.1	gi 734356326 gb KHN14074.1 Zinc finger CCCH domain-containing protein 43 [<i>Glycine soja</i>]	485	749.199	0
Phvul.005G022000.1	gi 734419114 gb KHN39938.1 Zinc finger CCCH domain-containing protein 30 [<i>Glycine soja</i>]	633	1094.72	0
Phvul.005G101500.1	gi 959092915 ref NP_001304454.1 zinc finger CCCH domain-containing protein 2-like [<i>Glycine max</i>]	391	493.041	5.56E-173
Phvul.005G019900.2	gi 734395860 gb KHN29218.1 Zinc finger CCCH domain-containing protein 69 [<i>Glycine soja</i>]	363	658.292	0
Phvul.011G018600.1	gi 955350118 ref XP_014619454.1 PREDICTED: zinc finger CCCH domain-containing protein 46 isoform X2 [<i>Glycine max</i>]	363	444.891	1.33E-154
Phvul.011G125300.1	gi 356542977 ref XP_003539940.1 PREDICTED: zinc finger CCCH domain-	362	410.223	2.49E-141

	containing protein 2-like [<i>Glycine max</i>]			
Phvul.011G180700.1	gi 734416239 gb KHN38167.1 Zinc finger CCCH domain-containing protein 37 [<i>Glycine soja</i>]	484	701.819	0
Phvul.011G078600.1	gi 734417054 gb KHN38771.1 Zinc finger CCCH domain-containing protein 24 [<i>Glycine soja</i>]	862	1413.28	0
Phvul.011G211700.1	gi 734415207 gb KHN37619.1 Zinc finger CCCH domain-containing protein 30 [<i>Glycine soja</i>]	707	1102.04	0
Phvul.008G260900.1	gi 959092278 ref NP_001304583.1 zinc finger CCCH domain-containing protein 66-like [<i>Glycine max</i>]	689	1143.26	0
Phvul.008G223400.1	gi 922357162 ref XP_003616654.2 ARM repeat CCCH-type zinc finger protein [<i>Medicago truncatula</i>]	683	901.738	0
Phvul.008G054800.1	gi 950960994 ref XP_014497785.1 PREDICTED: zinc finger CCCH domain-containing protein ZFN-like isoform X1 [<i>Vigna radiata</i> var. <i>radiata</i>]	426	840.491	0
Phvul.004G122600.1	gi 356559882 ref XP_003548225.1 PREDICTED: zinc finger CCCH domain-containing protein 48-like isoform X1 [<i>Glycine max</i>]	424	776.933	0
Phvul.004G173600.1	gi 734419850 gb KHN40356.1 Zinc finger CCCH domain-containing protein 20 [<i>Glycine soja</i>]	354	558.91	0
Phvul.007G254100.1	gi 734432364 gb KHN46294.1 Zinc finger CCCH domain-containing protein 30 [<i>Glycine soja</i>]	700	1092.8	0
Phvul.007G033200.1	gi 922407901 ref XP_013470126.1 zinc finger CCCH domain protein [<i>Medicago truncatula</i>]	365	501.13	4.69E-177
Phvul.007G259300.1	gi 356504857 ref XP_003521211.1 PREDICTED: zinc finger CCCH domain-containing protein 31-like isoform X2 [<i>Glycine max</i>]	293	457.218	7.26E-162
Phvul.007G118500.1	gi 734324206 gb KHN04981.1 Zinc finger CCCH domain-containing protein ZFN-like [<i>Glycine soja</i>]	444	711.449	0
Phvul.007G165200.1	gi 734433028 gb KHN46585.1 Zinc finger CCCH domain-containing protein 32 [<i>Glycine soja</i>]	466	746.117	0
Phvul.001G141900.1	gi 734332464 gb KHN07424.1 Zinc finger CCCH domain-containing protein 14 [<i>Glycine soja</i>]	295	494.197	1.67E-176
Phvul.002G306100.1	gi 922338621 ref XP_013447300.1 zinc finger CCCH domain protein [<i>Medicago truncatula</i>]	359	486.493	5.26E-171
Phvul.002G065400.1	gi 922402249 ref XP_013468260.1 zinc finger (CCCH-type) family protein [<i>Medicago truncatula</i>]	359	548.125	0
Phvul.002G056300.1	gi 558695566 gb AHA84136.1 homeobox-leucine zipper protein HAT4 [<i>Phaseolus vulgaris</i>]	290	314.694	9.44E-106
Phvul.002G008600.1	gi 734411940 gb KHN36326.1 Homeobox-leucine zipper protein HAT22 [<i>Glycine soja</i>]	292	428.328	1.09E-150
Phvul.010G052200.1	gi 356504593 ref XP_003521080.1 PREDICTED: homeobox-leucine zipper protein HAT3-like [<i>Glycine max</i>]	305	306.605	4.51E-102
Phvul.003G067700.1	gi 734404196 gb KHN32882.1 Homeobox-leucine zipper protein ATHB-13 [<i>Glycine</i>	278	502.286	2.84E-180

	<i>soja</i>]			
Phvul.003G232000.1	gi 734411940 gb KHN36326.1 Homeobox-leucine zipper protein HAT22 [<i>Glycine soja</i>]	299	351.288	3.78E-120
Phvul.005G040000.1	gi 356525608 ref XP_003531416.1 PREDICTED: homeobox-leucine zipper protein HOX11-like [<i>Glycine max</i>]	377	424.091	4.06E-146
Phvul.008G045100.1	gi 734347994 gb KHN11572.1 Homeobox-leucine zipper protein HAT3 [<i>Glycine soja</i>]	292	247.284	4.41E-80
Phvul.004G017500.1	gi 734382154 gb KHN23555.1 Homeobox-leucine zipper protein HAT5 [<i>Glycine soja</i>]	284	483.411	1.12E-172
Phvul.007G260900.1	gi 922401109 ref XP_013467691.1 homeobox associated leucine zipper protein [<i>Medicago truncatula</i>]	327	348.206	2.50E-118
Phvul.001G254000.1	gi 734329901 gb KHN06556.1 Homeobox-leucine zipper protein ATHB-16 [<i>Glycine soja</i>]	296	358.221	1.66E-122
Phvul.010G013500.1	gi 922362306 ref XP_003604971.2 Dof domain zinc finger protein [<i>Medicago truncatula</i>]	333	307.375	3.31E-101
Phvul.003G189300.1	gi 734367912 gb KHN18558.1 Dof zinc finger protein DOF3.3 [<i>Glycine soja</i>]	472	719.153	0
Phvul.003G200600.1	gi 356513607 ref XP_003525503.1 PREDICTED: dof zinc finger protein DOF5.6-like [<i>Glycine max</i>]	328	499.59	1.36E-177
Phvul.003G182100.1	gi 351723985 ref NP_001236530.1 Dof4 [<i>Glycine max</i>]	287	362.073	2.62E-124
Phvul.003G248500.1	gi 751814087 gb AJG01748.1 Dof38, partial [<i>Cajanus cajan</i>]	233	266.159	1.38E-88
Phvul.009G204000.1	gi 351723985 ref NP_001236530.1 Dof4 [<i>Glycine max</i>]	318	223.016	2.60E-69
Phvul.009G186600.1	gi 734314876 gb KHN01870.1 Dof zinc finger protein DOF3.3 [<i>Glycine soja</i>]	480	617.846	0
Phvul.009G178300.1	gi 734399546 gb KHN30912.1 Dof zinc finger protein DOF5.6 [<i>Glycine soja</i>]	336	510.76	0
Phvul.011G071900.1	gi 734378351 gb KHN22036.1 Dof zinc finger protein DOF2.1 [<i>Glycine soja</i>]	298	373.629	1.15E-128
Phvul.011G064800.1	gi 356542453 ref XP_003539681.1 PREDICTED: dof zinc finger protein DOF3.6 [<i>Glycine max</i>]	309	395.971	2.87E-137
Phvul.008G037000.1	gi 734348039 gb KHN11617.1 Dof zinc finger protein DOF5.2 [<i>Glycine soja</i>]	503	701.049	0
Phvul.008G099400.1	gi 922343783 ref XP_013448812.1 Dof domain zinc finger protein [<i>Medicago truncatula</i>]	361	206.453	1.23E-61
Phvul.001G080800.1	gi 734324227 gb KHN05002.1 Dof zinc finger protein DOF4.6 [<i>Glycine soja</i>]	355	374.015	4.94E-128
Phvul.006G114900.1	gi 351722579 ref NP_001236737.1 Dof18 [<i>Glycine max</i>]	254	375.941	3.80E-131
Phvul.002G170600.1	gi 356577235 ref XP_003556733.1 PREDICTED: cyclic dof factor 1-like [<i>Glycine max</i>]	464	572.007	0
Phvul.009G016300.1	gi 571458603 ref XP_006581173.1 PREDICTED: transcription factor E2FC-like isoform X1 [<i>Glycine max</i>]	533	568.54	0
Phvul.011G097700.1	gi 357471621 ref XP_003606095.1 E2F transcription factor [<i>Medicago truncatula</i>]	452	692.96	0

Phvul.007G269500.1	gi 734317999 gb KHN02830.1 Transcription factor E2FA [<i>Glycine soja</i>]	467	743.036	0
Phvul.005G150100.1	gi 734397359 gb KHN30114.1 ETHYLENE INSENSITIVE3-like 3 protein [<i>Glycine soja</i>]	592	1010.36	0
Phvul.008G207000.1	gi 734388923 gb KHN26027.1 Protein Ethylene Insensitive3 [<i>Glycine soja</i>]	611	1058.51	0
Phvul.006G011300.1	gi 955078108 ref NP_001304198.1 protein ETHYLENE INSENSITIVE3-like [<i>Vigna radiata</i>]	624	1169.07	0
Phvul.006G011200.1	gi 955078108 ref NP_001304198.1 protein ETHYLENE INSENSITIVE3-like [<i>Vigna radiata</i>]	619	1116.29	0
Phvul.002G253900.1	gi 734395722 gb KHN29080.1 ETHYLENE INSENSITIVE3-like 5 protein [<i>Glycine soja</i>]	469	638.647	0
Phvul.010G124700.1	gi 351725369 ref NP_001236578.1 ethylene responsive protein [<i>Glycine max</i>]	384	594.349	0
Phvul.010G124700.1	gi 545627565 gb AGW45314.1 AP2/ERF [<i>Lotus corniculatus</i>]	384	514.612	0
Phvul.010G154900.1	gi 357455223 ref XP_003597892.1 ethylene response factor [<i>Medicago truncatula</i>]	197	227.254	1.44E-74
Phvul.009G262200.1	gi 351723365 ref NP_001238300.1 EREBP/AP2 transcription factor [<i>Glycine max</i>]	355	422.165	3.00E-146
Phvul.009G029600.1	gi 918463511 gb ALA09100.1 AP2-EREBP transcription factor, partial [<i>Glycine max</i>]	353	372.089	4.84E-127
Phvul.009G161000.1	gi 734400162 gb KHN31248.1 Ethylene-responsive transcription factor ERF114 [<i>Glycine soja</i>]	258	224.172	4.64E-71
Phvul.009G196900.1	gi 356510515 ref XP_003523983.1 PREDICTED: ethylene-responsive transcription factor WIN1-like [<i>Glycine max</i>]	211	233.032	6.82E-76
Phvul.009G089300.1	gi 657971770 ref XP_008377674.1 PREDICTED: ethylene-responsive transcription factor ERF003-like [<i>Malus domestica</i>]	191	212.231	1.25E-68
Phvul.009G093600.1	gi 734331482 gb KHN07081.1 Ethylene-responsive transcription factor SHINE 3 [<i>Glycine soja</i>]	197	265.003	2.63E-89
Phvul.005G105200.1	gi 734413179 gb KHN36624.1 Ethylene-responsive transcription factor ERF053 [<i>Glycine soja</i>]	407	513.457	2.71E-180
Phvul.L006500.1	gi 356515442 ref XP_003526409.1 PREDICTED: ethylene-responsive transcription factor SHINE 2-like [<i>Glycine max</i>]	191	238.039	1.19E-78
Phvul.011G118600.1	gi 734402349 gb KHN31960.1 Ethylene-responsive transcription factor ERF053 [<i>Glycine soja</i>]	416	506.138	2.14E-177
Phvul.008G271100.1	gi 356552847 ref XP_003544774.1 PREDICTED: ethylene-responsive transcription factor 3 [<i>Glycine max</i>]	219	169.474	9.80E-51
Phvul.008G172200.1	gi 918463511 gb ALA09100.1 AP2-EREBP transcription factor, partial [<i>Glycine max</i>]	327	253.062	1.18E-80
Phvul.008G141000.1	gi 959092749 ref NP_001304470.1 ethylene-responsive transcription factor ERF039-like [<i>Glycine max</i>]	309	222.246	3.40E-69
Phvul.008G214700.1	gi 959092543 ref NP_001304374.1 ethylene-responsive transcription factor 3 [<i>Glycine max</i>]	204	200.29	3.08E-63

Phvul.007G127800.1	gi 356576468 ref XP_003556353.1 PREDICTED: ethylene-responsive transcription factor 1B-like [<i>Glycine max</i>]	209	297.745	1.35E-101
Phvul.001G046900.1	gi 734321387 gb KHN04157.1 Ethylene-responsive transcription factor ERF003 [<i>Glycine soja</i>]	199	239.195	7.99E-79
Phvul.001G251200.1	gi 959092454 ref NP_001304409.1 ethylene-responsive transcription factor ERF060-like [<i>Glycine max</i>]	295	304.294	2.83E-101
Phvul.001G187100.1	gi 734316769 gb KHN02251.1 Ethylene-responsive transcription factor ERF024 [<i>Glycine soja</i>]	188	144.05	1.72E-42
Phvul.006G047800.1	gi 922379280 ref XP_013459877.1 ethylene response factor [<i>Medicago truncatula</i>]	207	180.259	4.31E-55
Phvul.002G035900.1	gi 734406929 gb KHN34158.1 Ethylene-responsive transcription factor ERF021 [<i>Glycine soja</i>]	182	285.804	1.05E-97
Phvul.002G055800.1	gi 959092864 ref NP_001304366.1 ethylene-responsive transcription factor 5-like [<i>Glycine max</i>]	304	270.396	2.53E-88
Phvul.002G055700.1	gi 353078622 gb AEQ64867.1 ethylene response factor 11 [<i>Medicago sativa</i>]	270	301.212	4.43E-101
Phvul.002G254500.1	gi 734416307 gb KHN38186.1 Ethylene-responsive transcription factor RAP2-4 [<i>Glycine soja</i>]	318	306.22	2.76E-99
Phvul.003G028000.1	gi 922356454 ref XP_003614475.2 myb-like transcription factor family protein [<i>Medicago truncatula</i>]	373	404.831	5.51E-139
Phvul.005G047600.2	gi 734438017 gb KHN48860.1 Myb family transcription factor APL [<i>Glycine soja</i>]	371	703.745	0
Phvul.011G094300.1	gi 734375910 gb KHN21148.1 Myb family transcription factor APL [<i>Glycine soja</i>]	394	667.152	0
Phvul.001G163900.1	gi 734345474 gb KHN10772.1 Myb family transcription factor APL [<i>Glycine soja</i>]	488	771.541	0
Phvul.001G119500.1	gi 357509257 ref XP_003624917.1 MYB-like transcription factor family protein [<i>Medicago truncatula</i>]	311	338.961	1.05E-117
Phvul.002G132500.1	gi 734348271 gb KHN11849.1 Myb family transcription factor APL [<i>Glycine soja</i>]	269	492.271	1.39E-179
Phvul.002G084100.1	gi 734349785 gb KHN12199.1 Myb family transcription factor APL [<i>Glycine soja</i>]	344	554.673	0
Phvul.002G137300.1	gi 131054094 gb ABO32773.1 MYB transcription factor MYB34 [<i>Medicago truncatula</i>]	373	464.922	6.28E-162
Phvul.006G169600.1	gi 351722555 ref NP_001237504.1 MADS-box protein [<i>Glycine max</i>]	245	423.705	2.80E-150
Phvul.002G147600.1	gi 359806214 ref NP_001240951.1 MADS-box protein SVP-like [<i>Glycine max</i>]	227	432.18	3.45E-154
Phvul.002G172300.2	gi 734408516 gb KHN34832.1 MADS-box transcription factor 1 [<i>Glycine soja</i>]	242	471.855	2.14E-169
Phvul.010G012500.1	gi 734434507 gb KHN47436.1 Myb-related protein 3R-1 [<i>Glycine soja</i>]	562	602.823	0
Phvul.010G096400.1	gi 356506267 ref XP_003521908.1 PREDICTED: myb-related protein Zm38-like [<i>Glycine max</i>]	278	424.091	1.72E-149
Phvul.010G053200.1	gi 734407814 gb KHN34496.1 Transcription factor GAMYB [<i>Glycine soja</i>]	315	442.965	1.04E-155
Phvul.010G009900.1	gi 9972157 gb AAG10600.1 AF299140_1MYB-related transcription factor PHAN1	348	423.32	1.09E-146

	[<i>Pisum sativum</i>]			
Phvul.010G009800.1	gi 9972157 gb AAG10600.1 AF299140_1MYB-related transcription factor PHAN1 [<i>Pisum sativum</i>]	352	452.981	3.17E-158
Phvul.003G284000.1	gi 734391497 gb KHN27247.1 Transcription factor MYB39 [<i>Glycine soja</i>]	346	446.432	9.09E-156

4.4 Expressional quantitation of selected french bean RGAs and TFs

Expressional quantitation of selected TF genes and RGA genes in manifestation of rust and *MYMV* diseases

Based on the predicted features and functions, 20 candidate genes belong to transcription factor families and 10 cloned genes belongs to RGAs were selected. Though these TFs and RGAs have direct and indirect role in biotic stress tolerance in plants, not much gene experimental data is available even in model systems. Besides this, the qRT-PCR data of our laboratory previously carried out on some of these genes was also considered finally in pinning down on genes for further expressional quantification. Out of 30 gene specific primers 17 primer pairs of TF genes and all 10 RGA genes yielded expected amplicon and 3 primer pairs yielded no PCR product from 7 DAF, 15 DAF and 30 DAI cDNA after 40 cycles of real-time PCR reaction.

4.4.1 Total RNA isolation and its RNA integrity

The total RNA was isolated from leaf tissues from both rust and *MYMV* infected plants corresponding to 7 DAI, 15 DAI and 30 DAI using TRIZOL reagents (Plate 14 to 15). The integrity of total RNA was examined by electrophoresing the individual RNA samples on denaturing, formaldehyde 1% agarose gel stained with ethidium bromide which clearly showed the presence of two bright bands corresponding to ribosomal 28S *rRNA* and 18S *rRNA* with a ratio of intensities of ~ 2:1 (Plate 16). The quantity of RNA was checked on NanoDrop ND-1000 spectrophotometer™ which recorded a ratio of ~ 1.9 at 260 per 280 absorbance indicating the purity of RNA for further use in downstream reactions. RNA preparations are usually contaminated with low amounts of genomic DNA, which can result in non-specific amplification. To eliminate genomic DNA contamination, about 10 µg of total RNA from each treatment was treated with *DNase I* enzyme. Actual confirmation of complete degradation of genomic DNA in RNA preparations was done through PCR amplification where total RNA was used as template. As expected, there was no PCR amplification from the total RNA preparations indicating an absence of genomic DNA traces (Plate 17). cDNA from different stage of green leaf area fractionized on 1.2 per cent agarose gel (Plate 18 to 19). Predicted french bean gene specific primers were used in the PCR reaction.



Plate 14a. Healthy plants with out disease inoculation of rust



Plate 14b. Inoculation with rust



2nd DAI



7th DAI



15th DAI



30th DAI

Plate 14 c. Rust symptoms at disease development at different stages

Plate 14. General view of pot culture for rust infected tissues collection

Plate 14. General view of pot culture for rust infected tissues collection



Plate 15a. Healthy plants with out disease inoculation of *MYMV*



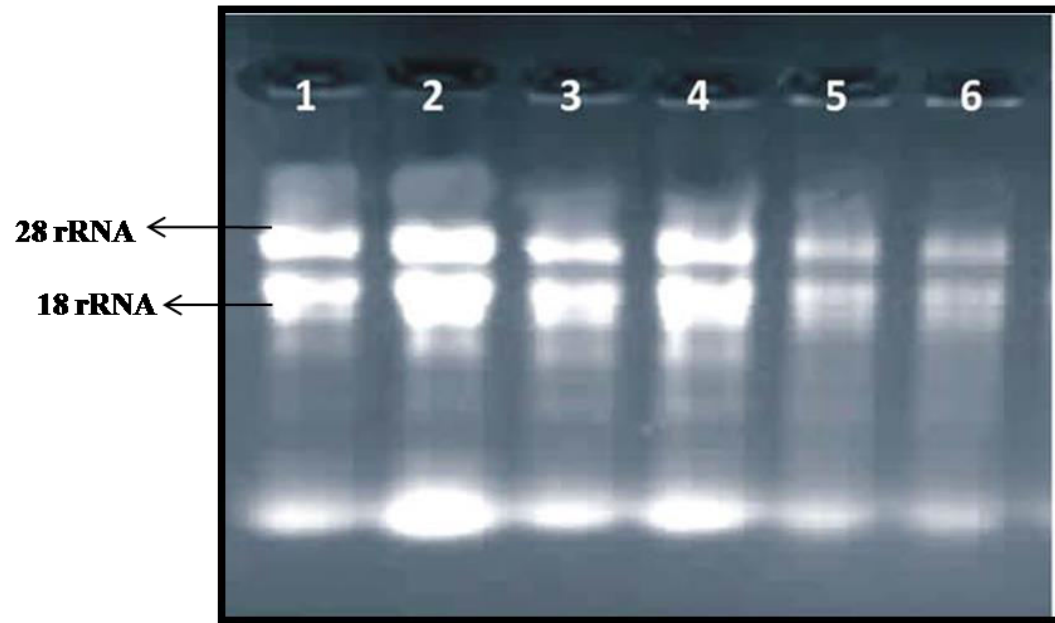
Plate 15b. Inoculation with *MYMV*



Plate 15c. Disease free and infected plant showing *MYMV* symptom 30DAI

Plate 15. General view of pot culture for *MYMV* infected tissues collection

Plate 15c. General view of pot culture for MYMV infected tissues collection



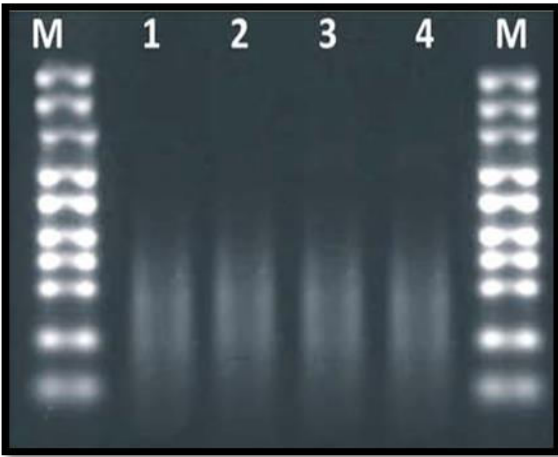
1-3- TOTAL RNA samples of rust infected leaf
 4-6- TOTAL RNA samples of *MYMV* infected leaf

Plate 16. Confirmation of integrity and quality of total RNA on formaldehyde denaturing agarose gel



M: 100 bp ladder
 1-12: Rust infected leaf samples
 13-22: MYMV infected leaf samples

Plate 17. PCR confirmation of predicted genes with total RNA for complete degradation of genomic traces upon *Dnase I* treatment



M: 1000 bp ladder

Plate 18a. cDNA from different stage of rust infected leaf sample

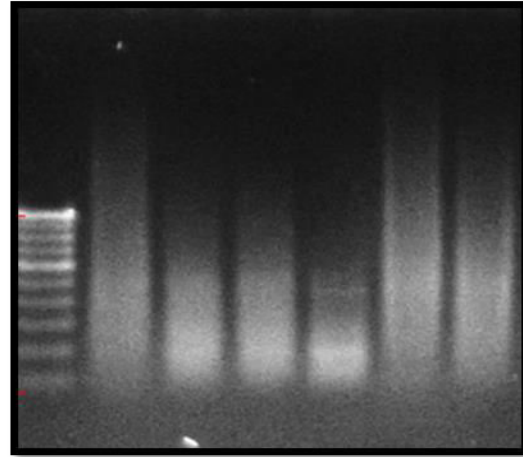


Plate 18b. cDNA from different stage of *MYMV* infected leaf sample

Plate 18. cDNA from different stages of disease infected leaf samples on 1.2 per cent agarose



Plate 19. PCR confirmation with DNA of all predicted genes on 2.5% agarose gel

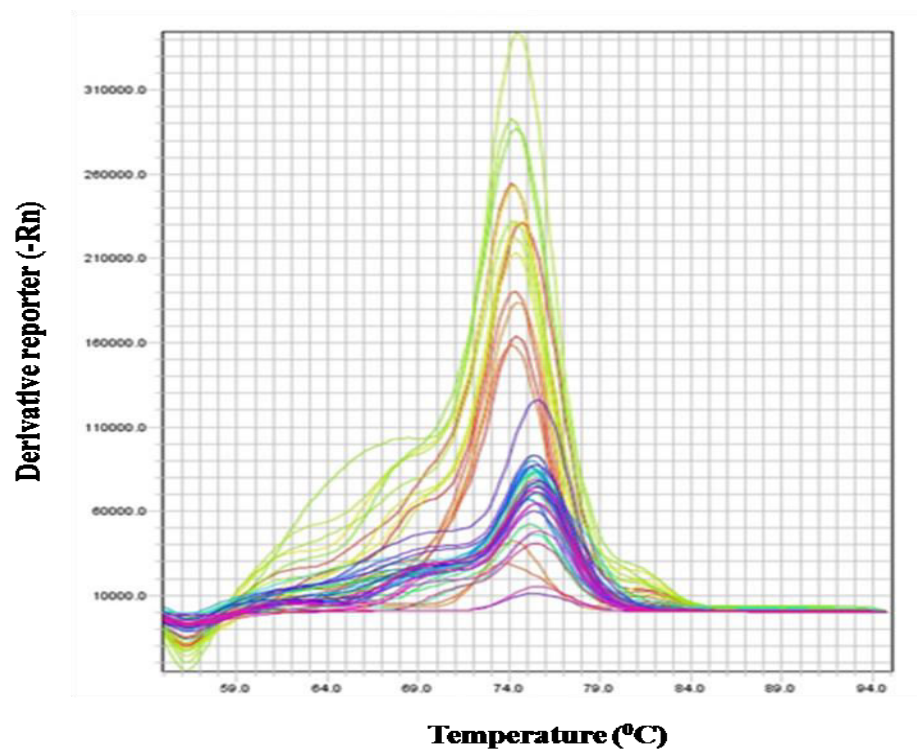


Plate 20. Melting curve of TF genes and R genes



Plate 21. Separation of qRT product of a gene in disease inoculated leaf area samples with endogenous controls (M: 100bp ladder)

4.4.2 Selection of reference genes

Endogenous reference genes are used for data normalisation in qRT-PCR, especially when relative quantitation approach is followed. In order to identify the most suitable reference genes in french bean, a set of three genes viz. *actin* (AC1), *β -tubulin* and *elongation factor- 1 α* were selected. The gene expression stability measure (M) was estimated through qRT-PCR in a set of 3 different cDNA samples corresponding to 7 DAI, 15 DAI and 30 DAI. On a model-based variance estimation approach, the M value, which should be < 1.5 for a stably expressed gene, was in the order of magnitude 0.298, 0.311 and 0.326 for, (AC1), *β -tubulin* and *elongation factor- 1 α* respectively. Based on M value *actin* gene was selected as endogenous reference gene for rest of qRT-PCR experiments.

4.4.3 Standardization of real-time conditions and data precision

The qRT-PCRs were performed in an optical 48-well plates with an Eppendorf Master cycler ep realplex detection system, using the Power SYBR® Green master mix to monitor DNA synthesis. Subsequently, the average PCR efficiencies were computed for each individual primer pairs across all analyzed samples. Out of 20 TF genes and 10 RGA genes tested, 14 genes PCR efficiency between 1.43 and 1.52, and 7 genes between 1.54 and 1.63, 6 genes between 1.65 and 1.70, and remaining 3 showed lower values 1.7 and 1.77. The specificity of the PCR reactions was determined by loading the PCR products on a 4 per cent agarose gel. All the genes yielded specific sized amplicons as predicted. However, in 3 genes no detectable PCR amplicons were noticed. Melting curve analyses was performed for all PCR products to confirm the occurrence of specific amplification peaks and an absence of primer-dimer formation (Plate 20 and 21). The technical precision or reproducibility of real-time PCR was assessed by performing replicate measurements in separate PCR runs using the same pool of cDNA (technical replicates) and two different pools of cDNA obtained independent two different batches of total RNA samples under same conditions (biological replicates). Precision, as reflected by the correlation coefficient (r) was > 0.970 for technical replicate and r > 0.968 for biological replicates 7 DAI, 15 DAI and 30 DAI leaf tissues. The scattered plot depicting correlation coefficient between duplicate measurements of cDNA levels from same and different pool of total RNA harvested from same treatment is presented in Figure 4a and 4b.

4.4.4 Relative status of expression profiling selected TF and RGA genes

4.4.4.1 Expression profiling of selected TF genes for rust disease manifestation in french bean

The expression level of selected 17 TF genes for rust manifestation of french bean was measured in both resistant and susceptible genotype leaf tissues separately at 15 DAI and 45 DAI. All the 17 selected genes were expressed in both leaf tissues at different days after inoculation with rust. 15 TF genes of french bean viz., AP2-2, AP2-3, AP2-4, AP2-5, B3-1, B3-2, B3-3, BHLH-1, BHLH-2, BHLH-3, BHLH-4, BHLH-5, BZIP-1, BZIP-2 and BZIP-3 were up-regulated in leaf of resistant genotypes at disease inoculation condition at 15th and 30th days and BZIP-4 was down-regulated in leaf of resistant genotypes at disease inoculation condition at 15th day and up regulated at 30th day. AP-1 was up-regulated in leaf of resistant genotype at 15th day of disease infection and was down-regulated at 30th day of infection. Where as in case of susceptible genotype for all TF genes except B3-2 were down-regulated in leaf of susceptible genotype at 15th DAI and B3-2 was up-regulated. At 30th DAI, 12 TF genes namely, AP2-1, AP2-2, AP2-3, AP2-4, AP2-5, B3-1, B3-3, BHLH-1, BHLH-2, BZIP-1, BZIP-2 and BZIP-3 were down-regulated in susceptible genotype and in case of B3-2, BHLH-3, BHLH-4, BHLH-5 and BZIP-4 were up-regulated in this condition (Table 4a).

TF genes up-regulating and down-regulating in leaf tissues of both resistant and susceptible genotypes at 15 DAI and 30DAI in pathogen challenged conditions is illustrated in Figure 4. At 15 DAI, in case of pathogen challenged leaf of resistant genotypes, the 16 TF genes (AP2-1, AP2-2, AP2-3, AP2-4, AP2-5, B3-1, B3-2, B3-3, BHLH-1, BHLH-2, BHLH-3, BHLH-4, BHLH-5, BZIP-1, BZIP-2 and BZIP-3) were

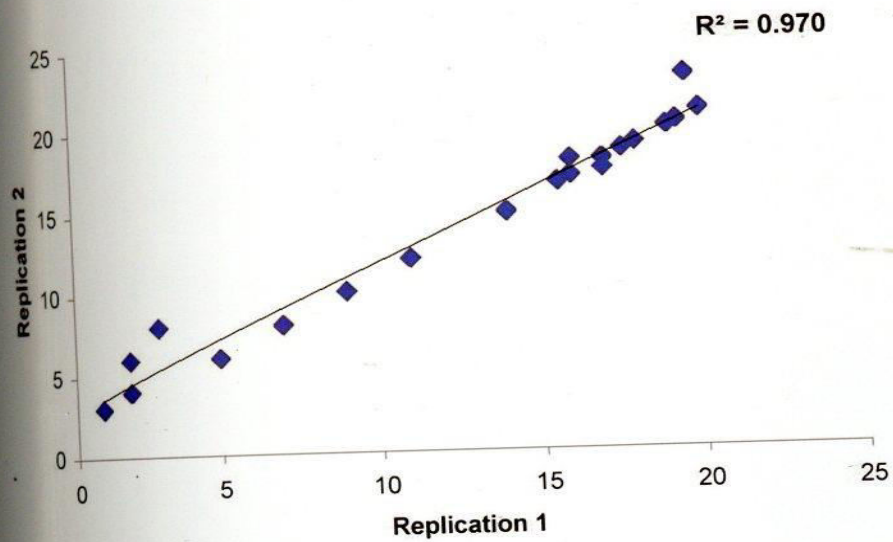


Figure 4a. Technical precision of real time PCR reflected as correlation coefficient between the duplicate measurements of cDNA levels of genes from the same reverse transcription reaction (biological replicates)

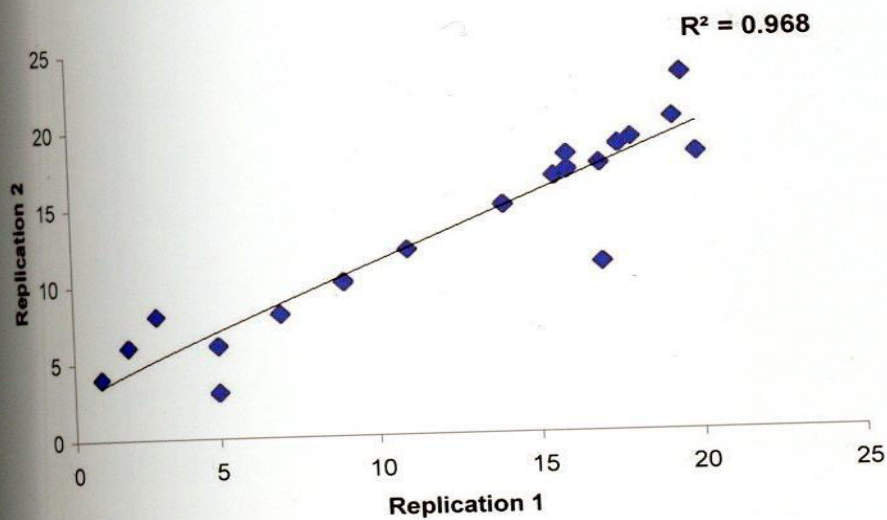


Figure 4b. Technical precision of real time PCR reflected as correlation coefficient between the duplicate measurements of cDNA levels of genes from the same reverse transcription reaction (technical replicates)

Table 4a. Relative change in the expression pattern of selected TF genes found in rust manifested leaf tissue of different genotypes of french bean

Sl. No.	TF genes	Normalize Δ Ct values (Resistance)				Normalize Δ Ct values (Susceptible)				Fold expression				Log10 ratio				Cal. t value			
		15 DAIR	15 DAIS	30 DAIR	30 DAIS	15 DAIR	15 DAIS	30 DAIR	30 DAIS	15 DAIR	15 DAIS	30 DAIR	30 DAIS	15 DAIR	15 DAIS	30 DAIR	30 DAIS	15 DAIR	15 DAIS	30 DAIR	30 DAIS
1	AP2-1	-1.86	1.48	1.02	0.19	-1.86	3.30	1.02	3.06	5.75	3.30	0.78	0.34	0.76	-0.55	-0.11	-0.47	0.09	0.30	0.30	0.44
2	AP2-2	-2.84	1.09	-0.49	-0.45	-2.84	2.19	-0.49	2.41	1.66	2.19	4.43	0.40	0.22	-0.33	0.65	-0.40	0.09	0.30	0.30	0.45
3	AP2-3	-1.67	2.82	0.18	-0.01	-1.67	3.31	0.17	2.85	1.48	3.31	5.60	0.98	0.17	-0.15	0.75	-0.01	0.09	0.30	0.30	0.45
4	AP2-4	-4.26	-0.04	-2.13	-2.07	-4.26	1.10	-2.13	0.80	2.41	1.10	55.26	0.56	0.38	-0.34	1.74	-0.25	0.09	0.30	0.30	0.46
5	AP2-5	1.38	2.83	4.15	2.18	1.38	4.81	4.15	5.04	3.07	4.81	1.43	0.22	0.49	-0.60	0.15	-0.67	0.09	0.31	0.31	0.46
6	B3-1	-1.42	1.88	0.77	-0.05	-1.42	3.69	0.76	2.82	4.02	3.69	29.75	0.52	0.60	-0.54	1.47	-0.28	0.09	0.31	0.31	0.47
7	B3-2	-0.55	3.51	1.52	0.55	-0.55	2.45	1.52	3.41	1.44	2.45	35.29	1.07	0.16	0.32	1.55	0.03	0.09	0.31	0.31	0.48
8	B3-3	-2.79	0.30	0.39	-0.16	-2.79	2.88	0.39	2.71	6.14	2.88	154.46	0.19	0.79	-0.78	2.19	-0.72	0.09	0.31	0.31	0.48
9	BHLH-1	-7.10	-4.01	-4.59	-4.95	-7.10	-2.51	-4.60	-2.09	1.31	-2.51	97.93	0.26	0.12	-0.45	3.99	-0.58	0.09	0.31	0.31	0.49
10	BHLH-2	-7.13	-3.41	-4.16	-4.95	-7.13	-2.93	-4.16	-2.09	1.33	-2.93	14.48	0.40	0.12	-0.14	4.16	-0.40	0.10	0.31	0.31	0.49
11	BHLH-3	2.60	4.24	4.00	-1.29	2.60	10.04	4.00	1.57	116.04	10.04	101.39	6.35	2.07	-1.75	2.01	0.80	0.10	0.31	0.31	0.50
12	BHLH-4	1.65	4.24	2.77	-0.12	1.65	6.46	2.77	2.74	3.40	6.46	47.41	2.83	0.53	-0.67	2.68	0.45	0.20	0.31	0.31	0.74
13	BHLH-5	0.68	0.00	3.72	-4.72	0.68	3.44	3.72	-1.85	1.36	3.44	49.82	3.61	0.13	-1.04	2.69	0.56	0.19	0.31	0.31	0.75
14	BZIP-1	-2.39	-0.25	1.28	0.78	-2.39	4.75	1.27	3.65	20.76	4.75	53.52	0.07	1.32	-1.50	3.73	-1.17	0.19	0.31	0.31	0.74
15	BZIP-2	-0.49	0.94	0.28	0.38	-0.49	3.44	0.28	3.24	1.21	3.44	21.31	0.20	0.08	-0.75	4.33	-0.69	0.23	0.31	0.31	0.80
16	BZIP-3	-2.63	0.72	0.28	-1.69	-2.63	3.09	0.28	1.17	2.66	3.09	42.62	0.73	0.42	-0.72	4.63	-0.14	0.22	0.32	0.32	0.80
17	BZIP-4	2.37	4.05	2.64	0.63	2.37	7.37	2.64	3.49	0.67	7.37	16.65	1.48	-0.18	-1.00	4.22	0.17	0.22	0.32	0.32	0.81

DAIR: Days after inoculation in resistance genotype

DAIS: Days after inoculation in susceptible genotype

Δ Ct: Normalized Ct value

Δ Ct: (Ct of target gene-Ct of reference gene)

Table t value (1 %, df: 3) = 5.8409

up-regulated with a fold change range of 1.21 to 116.04 and BZIP-4 was down regulated with a fold change of 0.67. Whereas, at 30 DAI in resistant genotype 16 TF genes (AP-2, AP-3, AP-4, AP-5, B3-1, B3-2, B3-3, BHLH-1, BHLH-2, BHLH-3, BHLH-4, BHLH-5, BZIP-1, BZIP-2, BZIP-3 and BZIP-4) were up-regulated with a fold change range of 0.78 to 154.46. In case of susceptible genotype, for all TF genes except B3-2 were down-regulated in leaf of susceptible genotype at 15th DAI with a fold change ranged 1.10 to 10.04 and B3-2 was up-regulated with fold change of 1.44. At 30th DAI, 12 TF genes namely, AP2-1, AP2-2, AP2-3, AP2-4, AP2-5, B3-1, B3-3, BHLH-1, BHLH-2, BZIP-1, BZIP-2 and BZIP-3 were down-regulated with fold change of 0.07 to 0.98. Up-regulated genes viz., B3-2, BHLH-3, BHLH-4, BHLH-5 and BZIP-4 had fold change of 1.07, 6.35. The height fold increase of 116.04 was observed by BHLH-3 AT 15 DAI in resistant genotype. In case of 30 DAI heights fold expression observed for B3-3 (154.46) and the least was 1.12 (BZIP-2) and 0.78 (AP2-1) at 15 and 30 DAI in resistant genotype. For susceptible genotype, at 15 and 30 DAI maximum fold change was observed for BHLH-3 (10.04 and 6.35 respectively) and minimum was observed by AP2-4 (1.10) and BZIP-1 (0.07) for 15 and 30 DAI respectively (Figure 4c).

4.4.4.2 Expression profiling of selected TF genes for *MYMV* disease manifestation in french bean

The expression level of selected 17 TF genes for *MYMV* of french bean was measured in susceptible genotype leaf tissues at 7, 15 and 30 DAI. All the 17 selected genes were expressed in leaf tissues at different days after inoculation with *MYMV*. 10 TF genes of french bean viz., AP2-1, AP2-2, AP-4, AP-5, B3-1, B3-2, BHLH-2, BHLH-3, BHLH-5 and BZIP-2 were up-regulated in leaf of susceptible genotypes at disease inoculation condition at 7th DAI with a fold change ranged between 1.123 to 67.039 and AP2-3, B3-3, BHLH-1, BHLH-4, BZIP-1 and BZIP-3 were down-regulated with the fold change ranged 0.01 to 0.897, but BZIP-4 did not expressed at this condition. At 15th DAI, 10 TF gene were up-regulated viz., AP2-1, AP2-2, AP2-4, B3-1, BHLH-1, BHLH-2, BHLH-3, BHLH-5, BZIP-3 and BZIP-4 were up-regulated with a range of 1.316 to 450.027 fold change, 7 TF genes namely, AP2-3, AP2-5, B3-2, B3-3, BHLH-4, BZIP-1 and BZIP-2 were down regulated with 0.020 to 0.974 range fold change expression in leaf of susceptible genotype. In case at 30th DAI 14 TF genes (AP2-1, AP2-2, AP2-4, B3-1, B3-2, BHLH-1, BHLH-2, BHLH-3, BHLH-4, BHLH-5, BZIP-1, BZIP-2, BZIP-3 and BZIP-4) with fold change range of 0.131 to 172.924 showed up-regulation and AP2-3, AP2-5 and B3-3 were down-regulated (fold change ranged between 0.582 to 156.038) (Figure 4d).

The height fold increase of 67.039 (BHLH-2), 450.027 (BZIP-3) and 172.924 (BZIP-1) at 7, 15 and 30 DAI in susceptible genotype respectively and the least was observed in BZIP-4 (0.00), BZIP-1 (0.020) and B3-3 (0.131) respectively (Table 4b).

4.4.4.3 Expression profiling of selected RGA genes for rust disease manifestation in french bean

The expression level of selected 10 RGA genes of french bean was measured in both resistant and susceptible genotype rust inoculated leaf tissues separately at 15 DAI and 45 DAI. All the 10 genes at 30 DAI and except COHFBGA2 at 15 DAI were up-regulated in resistant genotype. COHFBGA1, COHFBGA2, COHFBGA25, COHFBGA9 and COHFBGA38 were down-regulated in susceptible genotype at 15 and 30 DAI. Whereas, COHFBGA4, COHFBGA9 and COHFBGA27 (15 DAI) and COHFBGA9 at 30 DAI showed up-regulation. COHFBGA3 up-regulated at 30 DAI but expressed inversely at 15 DAI in susceptible genotype. Similarly, there was inverse trend in the expression status of COHFBGA4 and COHFBGA27. There was down-regulation of these genes at 30 DAI and were up-regulated at 15 DAI. But COHFBGA26 was down-regulated in susceptible genotype at 15 DAI and did not express at 30 DAI (Table 4c).

RGA genes up-regulating and down-regulating in leaf tissues of both resistant and susceptible genotypes at 15 DAI and 30DAI in pathogen challenged conditions is illustrated in Figure 4. At 15 DAI, in case of pathogen challenged leaf of resistant genotypes, the 9 COHFBGA genes (COHFBGA1 to COHFBGA38 except COHFBGA2) were up-regulated with a fold change range of 0.79 to 169.01 and COHFBGA2 was down regulated with a fold change of 0.79. Whereas, at 30 DAI in the resistant genotype, all RGA genes were up-regulated with a fold change range of 20.01 (COHFBGA9) to 115.69

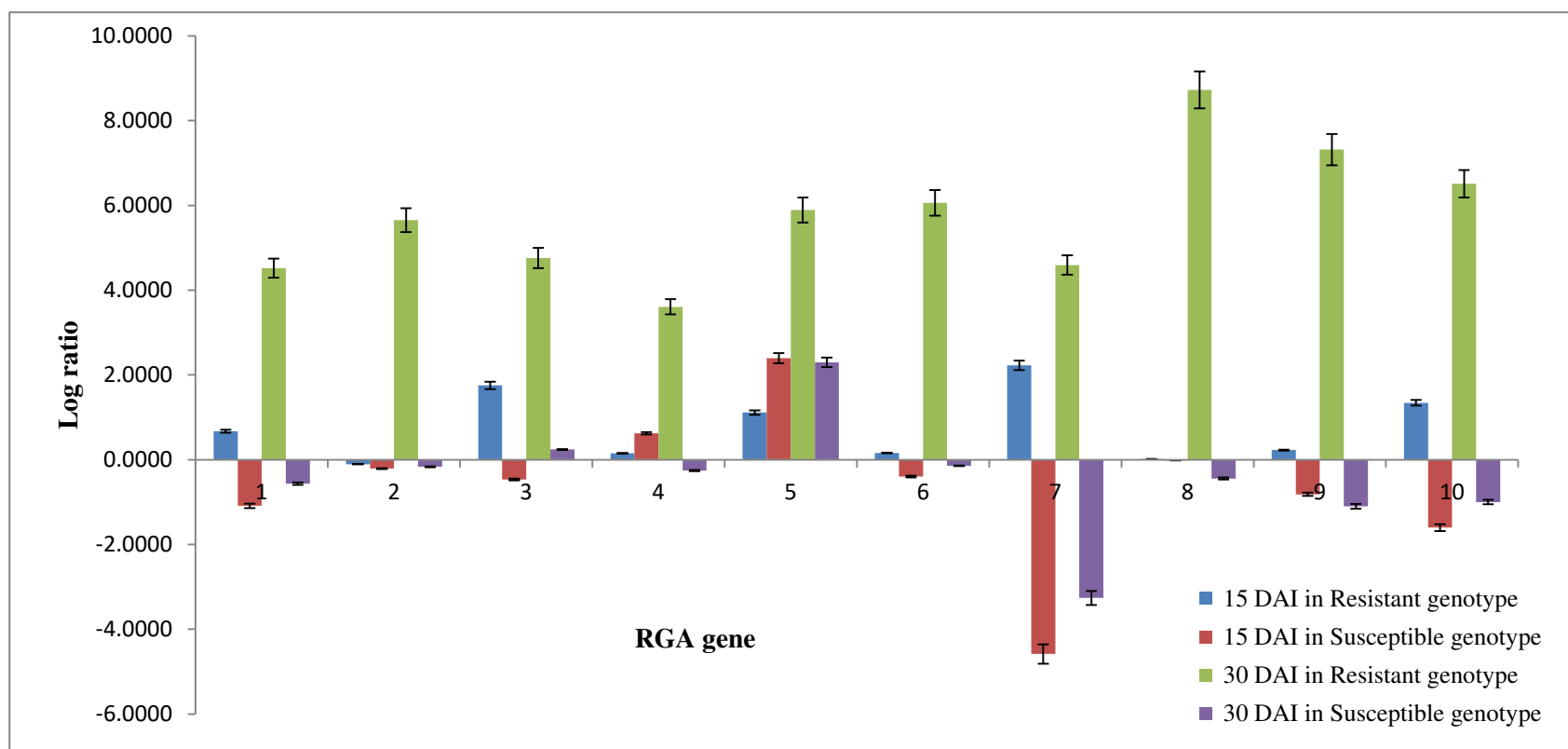


Figure 4c. Relative change in the expression pattern of selected RGA genes found in rust manifested leaf tissue at 15 and 30 days after inoculation of resistant and susceptible genotypes in french bean

1	COHFBRGA1	6	COHFBRGA25
2	COHFBRGA2	7	COHFBRGA26
3	COHFBRGA3	8	COHFBRGA27
4	COHFBRGA4	9	COHFBRGA32
5	COHFBRGA9	10	COHFBRGA38

(COHFBRGA25). In case of susceptible genotype, 5 RGA genes with the fold change ranged between 1.59 to 11.10 (15 DAI) and 7 RGA genes with 0.10 (COHFBRGA38) to 19.29 (COHFBRGA9) were down-regulated. Highest fold expression was found at 15 DAI in resistance genotype by COHFBRGA26 and lowest noticed in susceptible genotype at 30 DAI by COHFBRGA38 (Figure 4e).

4.4.4.4 Expression profiling of selected RGA genes for *MYMV* disease manifestation in french bean

Similarly, the same 10 RGA genes were tested under *MYMV* infested condition in french bean. All 10 selected RGA genes were expressed in leaf tissues at different days after inoculation with *MYMV*. COHFBRGA-2, 25, 27 and 38 genes of french bean were up-regulated in leaf of susceptible genotypes at disease inoculation condition at all the intervals with a fold change ranged between 5.048 to 110.268 (7 DAI), 3.915 to 121.312 (15 DAI) and 26.367 to 88.851 (30 DAI). COHFBRGA-26 was down-regulated among all the intervals of disease development in susceptible genotype with highest fold change of 0.553 at 15 DAI and range was 0.048 to 0.423. At 7 and 15 DAI, COHFBRGA-1 was down-regulated with 0.01 to 4.139 range of fold expression and it was up-regulated at 30 DAI (0.005). Down-regulation of COHFBRGA-3 was observed at 7 DAI with 0.559 fold change and it was inversely expressed at both 15 and 30 DAI 41.897 and 86.366 fold changes was observed for both intervals. Whereas, in case of COHFBRGA-4 down-regulation exhibited for 15 and 30 DAI with fold change of 0.795 and 0.031 respectively, but was up-regulated at 7 DAI with 40.534 fold expressions. Down-regulation at 7 and 30 DAI and up-regulation at 15 DAI was notice for COHFBRGA-32 with fold change ranged between 0.025 to 6.88. But COHFBRGA-9 did not get expressed in 7 and 15 DAI and expressed only at 30 DAI with 5.851 fold change (Table 4d and Figure 4f).

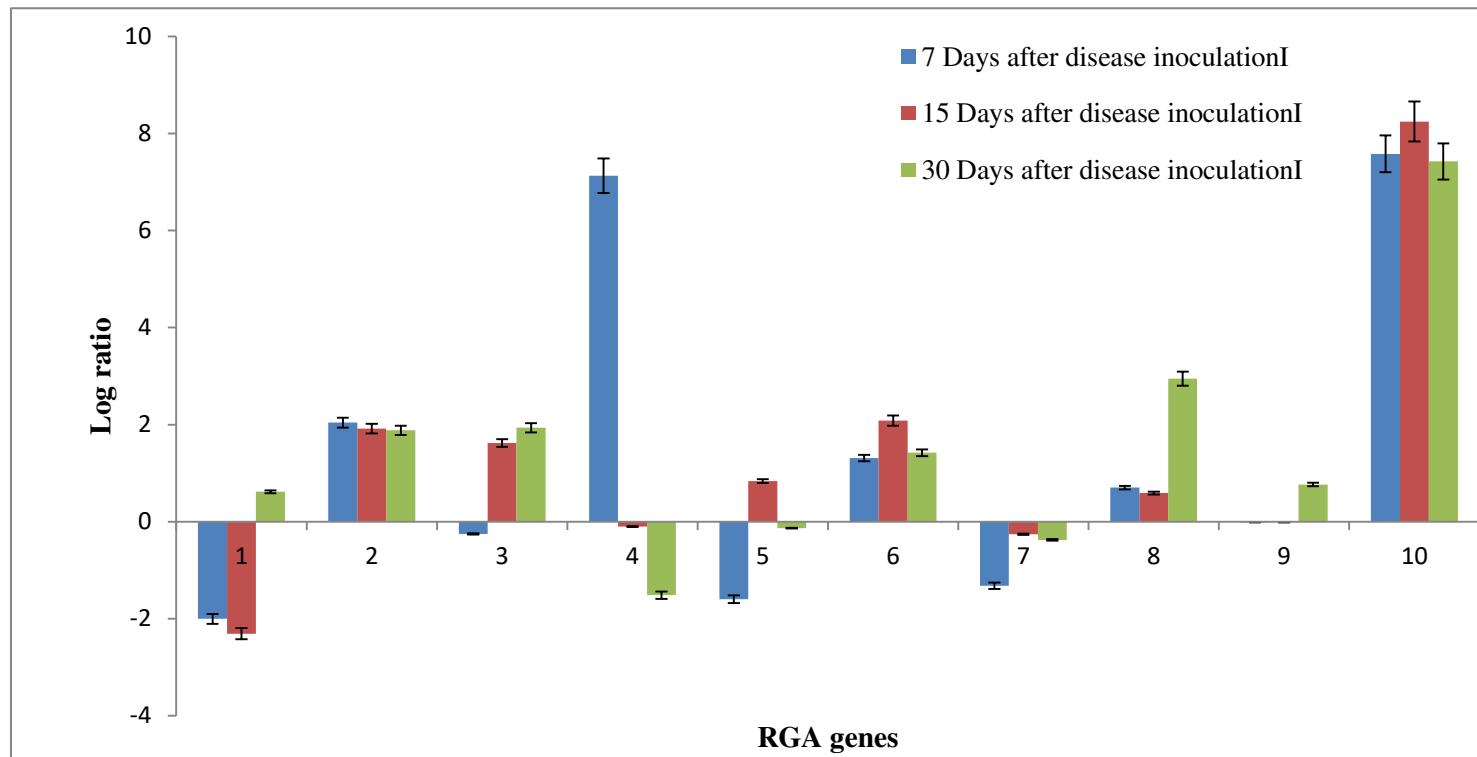


Figure 4d. Relative change in the expression pattern of selected RGA genes found in *MYMV* manifested leaf tissue at 7, 15 and 30 days after inoculation in french bean

1	COHFBRGA1	6	COHFBRGA25
2	COHFBRGA2	7	COHFBRGA26
3	COHFBRGA3	8	COHFBRGA27
4	COHFBRGA4	9	COHFBRGA32
5	COHFBRGA9	10	COHFBRGA38

Table 4b. Relative change in the expression pattern of selected TF genes found in *MYMV* manifested leaf tissue at 7, 15 and 30 DAI in french bean

Sl. No.	Genes	Normalize Δ Ct values (control)			Normalize Δ Ct values			Fold expression			Log10 ratio			Cal. t value		
		7DAI	15DAI	30DAI	7DAI	15DAI	30DAI	7DAI	15DAI	30DAI	7DAI	15DAI	30DAI	7DAI	15DAI	30DAI
1	AP2-1	6.191	0.355	1.764	1.473	1.764	0.194	2.186	1.335	1.755	0.340	0.125	0.244	0.376	0.325	0.173
2	AP2-2	2.601	2.181	1.006	1.188	1.006	-0.448	3.142	1.724	7.261	0.497	0.237	0.861	0.376	0.326	0.173
3	AP2-3	2.840	1.792	2.412	2.878	2.412	-0.010	0.665	0.309	0.950	-0.177	-0.511	-0.022	0.376	0.326	0.173
4	AP2-4	2.290	0.716	-0.084	0.862	-0.084	-2.066	50.173	66.463	74.300	1.701	1.823	1.871	0.377	0.327	0.173
5	AP2-5	6.511	5.971	4.149	4.532	4.149	2.177	1.523	0.092	0.582	0.183	-1.039	-0.235	0.377	0.327	0.173
6	B3-1	5.139	0.699	1.397	1.765	1.397	-0.045	10.264	1.894	4.066	1.011	0.277	0.609	0.378	0.328	0.174
7	B3-2	5.124	2.318	1.978	2.446	1.978	0.553	2.271	0.383	0.953	0.356	-0.416	-0.021	0.379	0.328	0.174
8	B3-3	3.630	0.595	0.484	0.341	0.484	-0.156	0.443	0.191	0.131	-0.354	-0.719	-0.884	0.379	0.329	0.174
9	BHLH-1	-0.835	-1.903	-3.092	-1.413	-3.092	-4.950	0.215	1.180	1.260	-0.668	0.072	0.100	0.380	0.329	0.174
10	BHLH-2	-3.632	-2.853	-4.616	-3.550	-4.616	-4.950	67.039	78.619	96.680	4.826	5.993	5.377	0.381	0.330	0.175
11	BHLH-3	12.483	15.292	12.911	12.855	12.911	-1.290	1.972	3.985	71.818	0.295	0.601	4.438	0.385	0.334	0.172
12	BHLH-4	13.834	14.906	13.454	11.779	13.454	-0.123	0.010	0.202	125.370	-1.985	-0.695	3.099	0.386	0.334	0.172
13	BHLH-5	5.185	11.144	10.171	9.076	10.171	-4.715	37.370	4.039	83.777	1.573	0.606	5.520	0.387	0.336	0.172
14	BZIP-1	14.300	12.185	13.622	11.454	13.622	0.784	0.897	0.020	172.924	-0.047	-1.705	2.238	0.389	0.336	0.167
15	BZIP-2	11.297	7.959	8.218	9.061	8.218	0.376	1.123	0.974	52.061	0.051	-0.011	1.717	0.390	0.337	0.167
16	BZIP-3	9.229	8.180	6.078	7.551	6.078	-1.687	0.522	450.027	156.038	-0.283	2.653	4.178	0.392	0.339	0.168
17	BZIP-4	6.612	14.892	12.191	15.532	12.191	0.627	0.000	1.316	49.900	-4.092	0.119	3.613	0.395	0.341	0.169

DAI: Days after inoculation

Δ Ct: Normalized Ct value

Δ Ct: (Ct of target gene-Ct of reference gene)

Table t value (1 %, df: 3) = 5.8409

Table 4c. Relative change in the expression pattern of selected R genes found in rust inoculated leaf tissue of different genotypes of french bean

Sl. No.	R gene	Normalize Δ Ct values (control)				Normalize Δ Ct values				Fold expression				Log ₁₀ ratio				Cal. t value			
		15 DAIR	15 DAIS	30 DAIR	30 DAIS	15 DAIR	15 DAIS	30 DAIR	30 DAIS	15 DAIR	15 DAIS	30 DAIR	30 DAIS	15 DAIR	15 DAIS	30 DAIR	30 DAIS	15 DAIR	15 DAIS	30 DAIR	30 DAIS
1	COHFBRGA1	1.45	3.03	2.64	2.04	1.45	6.65	2.64	4.90	4.72	6.65	33.31	0.27	0.67	-1.09	4.52	-0.56	0.21	0.32	0.32	0.81
2	COHFBRGA2	-1.07	0.91	-0.11	-1.40	-1.07	1.59	-0.12	1.46	0.79	1.59	44.85	0.68	-0.10	-0.21	5.65	-0.17	0.20	0.32	0.32	0.82
3	COHFBRGA3	-0.10	4.67	3.84	1.00	-0.10	6.22	3.84	3.86	56.90	6.22	57.88	1.75	1.76	-0.47	4.76	0.24	0.20	0.33	0.33	0.82
4	COHFBRGA4	6.66	11.85	8.68	9.84	6.66	9.77	8.67	12.70	1.42	9.77	40.60	0.56	0.15	0.62	3.61	-0.26	0.32	0.33	0.33	0.98
5	COHFBRGA9	0.80	11.85	2.08	1.35	0.80	3.90	2.08	4.21	12.98	3.90	78.49	19.29	1.11	2.39	5.89	2.30	0.30	0.34	0.34	0.98
6	COHFBRGA25	0.98	2.67	2.52	0.27	0.98	3.98	2.52	3.13	1.45	3.98	115.69	0.72	0.16	-0.40	6.06	-0.14	0.34	0.35	0.35	0.30
7	COHFBRGA26	4.60	-4.16	8.40	3.82	4.59	11.07	8.40	6.68	169.01	11.07	39.30	0.00	2.23	-4.58	4.59	-3.26	0.35	0.37	0.37	0.32
8	COHFBRGA27	-8.15	-3.01	-4.34	-4.41	-8.15	-2.97	-4.34	-1.55	1.04	-2.97	53.77	0.36	0.02	-0.01	8.73	-0.44	0.32	0.40	0.40	0.36
9	COHFBRGA32	-0.01	0.16	1.35	0.96	-0.01	2.87	1.35	3.82	1.69	2.87	20.01	0.08	0.23	-0.82	7.32	-1.10	0.57	0.40	0.40	0.45
10	COHFBRGA38	3.17	5.79	5.03	6.24	3.17	11.10	5.02	9.10	22.04	11.10	32.55	0.10	1.34	-1.60	6.51	-1.00	0.35	0.37	0.46	0.47

DAIR: Days after inoculation in resistance genotype

DAIS: Days after inoculation in susceptible genotype

Δ Ct: Normalized Ct value

Δ Ct: (Ct of target gene-Ct of reference gene)

Table t value (1 %, df: 3) = 5.8409

Table 4d. Relative change in the expression pattern of selected R genes found in *MYMV* manifested leaf tissue at 7, 15 and 30 DAI in french bean

Sl. No.	Genes	Normalize Δ Ct values (control)			Normalize Δ Ct values			Fold expression			Log ₁₀ ratio			Cal. t value		
		7DAI	15DAI	30DAI	7DAI	15DAI	30DAI	7DAI	15DAI	30DAI	7DAI	15DAI	30DAI	7DAI	15DAI	30DAI
1	COHFBRGA1	1.939	12.588	12.629	12.927	12.629	2.040	0.010	0.005	4.139	-2.002	-2.308	0.617	0.397	0.343	0.169
2	COHFBRGA2	6.276	4.960	4.089	5.500	4.089	-1.404	110.268	82.983	76.283	2.042	1.919	1.882	0.401	0.347	0.171
3	COHFBRGA3	12.285	10.464	4.850	10.462	4.850	1.002	0.559	41.897	86.366	-0.253	1.622	1.936	0.405	0.351	0.172
4	COHFBRGA4	9.623	10.238	7.434	-24.446	7.434	9.837	40.534	0.795	0.031	7.131	-0.099	-1.514	0.411	0.356	0.174
5	COHFBRGA9	-0.756	7.104	4.809	7.889	4.809	1.349	0.025	6.880	0.734	-1.600	0.838	-0.134	0.363	0.363	0.363
6	COHFBRGA25	2.575	7.591	0.902	2.661	0.902	0.272	20.634	121.312	26.367	1.315	2.084	1.421	0.374	0.374	0.374
7	COHFBRGA26	7.028	7.825	4.993	8.231	4.993	3.817	0.048	0.553	0.423	-1.322	-0.257	-0.374	0.391	0.391	0.391
8	COHFBRGA27	3.841	4.138	2.576	4.028	2.576	-4.409	5.048	3.915	88.851	0.703	0.593	2.949	0.423	0.423	0.423
9	COHFBRGA32	6.363	4.545	5.386	4.886	5.386	0.959	0.972	0.976	5.851	-0.013	-0.010	0.767	0.568	0.599	0.568
10	COHFBRGA38	4.844	5.351	3.508	5.722	3.508	6.240	38.116	68.619	79.239	7.581	8.248	7.425	0.675	0.565	0.357

DAI: Days after inoculation

Δ Ct: Normalized Ct value

Δ Ct: (Ct of target gene-Ct of reference gene)

Table t value (1 %, df: 3) = 5.8409

V. DISCUSSION

Low bean yield in most of the developing countries and all over the world is mainly due to three factors viz., biological (inferior genotypes, diseases, insect pests, weeds *etc.*), edaphic (poor soil fertility) and climatic (drought, high temperature *etc.*). Diseases, however, are one of the most important factors associated with low bean yields in bean producing regions of the world. Among the diseases, rust is wide spread and can reduce yields considerably.

Among the various *Phaseolus* beans, french bean is one of the most important legume vegetables in India. The crop is seriously affected by rust caused by *Uromyces phaseoli* resulting in yield loss up to 80 per cent (Grafton *et al.*, 1985). The disease is severe in bean growing areas of South India and also in other parts of the world.

Being a self pollinated crop, in french bean the genetic variability is rather limited. Moreover, interspecific and inter varietal incompatibilities pose problems in crossing and getting desirable recombination. Thus mutation, place an important role in creating genetic variability.

The products of stress-inducible genes, include the enzymes responsible for the synthesis of various antioxidants, proteins, chaperones and detoxification enzymes and another group of gene products involved in gene expression and signal transduction pathways includes transcription factors, protein kinases and enzymes of metabolic pathways. Further, a thorough analysis of available information indicates that there are interconnection between biotic and abiotic signal transduction pathways (Yamaguchi-Shinozaki and Shinozaki, 2006).

Development of plant organs is determined by differential gene expression which can be regulated at different levels. The major mechanism of differential gene expression is transcriptional regulation which is controlled by transcription factors that bind to DNA *cis*-elements (Lee and Young, 2000). Identification and functional characterization of TFs at transcripts and whole genome level using computational approaches are the preliminary step in understanding the mechanism of TFs expression and regulation (He *et al.*, 2010). The rapid expansion in the next generation sequencing (NGS) technologies, a large volume of data in genome and transcriptome level has been developed and it will facilitates identification of putative TF in other genomes through computational studies (Riechmann *et al.*, 2000 and Martínez-Bueno *et al.*, 2004). The availability of TF families resource at genomic level in model plant such as *Arabidopsis thaliana* can serve as reference source to explore the TFs associated with biological processes in unknown TF families in genomes. In legume plants, however, genome-wide computational identification of TFs reported in few genomes. But, no TFs characterization has been done in french bean till-to-date.

With this background, present study was undertaken to create the variability through EMS induced mutation, cloning and characterization of RGAs, *insilico* genome wide identification and characterization of TFs and expression profiling of selected RGAs and TFs for manifestation of rust and MYMV in french bean. The out come of the experiments were discussed here under.

5.1 Induced mutation to create spectral variability in french bean

Prospects for genetic improvement in french bean through selection and recombination breeding methods are rather limited in owing to the prevalence of narrow genetic variability as such in the germplasm and the presence of inter-varietal and inter-specific incompatibilities, respectively (Singh, 2006). Keeping in view the development and release of about 20 mutant varieties of french bean in the world till date, mutation breeding seems to be the viable supplementary approach, as also this approach can at times yield very different and superior genotypes. Hence, the present study on “development of functional mutants using chemical mutagen EMS in french bean (*Phaseolus vulgaris* L.)” was undertaken to develop spectral variability for functional analysis through the expression assay of candidate genes and to prospect the improvement of economically important traits. Further, it was also envisaged to study the nature and magnitude of induced variation. Radiations as well as chemical mutagens have been extensively used for inducing mutations in leguminous crops. In the present study

ethyl-methane sulfonate (EMS) as a chemical mutagen was used for inducing variability in the background of popular cv., 'Arka Anoop'.

Seeds treated with EMS at 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 per cent doses were subjected for germination percentile count. Minimum germination was found with lowest concentration of EMS (0.01%) and maximum was with (0.6%). Data showed 57.3 percentile germination at 0.2 per cent and 46.07 percentile germination at 0.3 per cent EMS respectively and the average of these were calculated. Analysis of the data indicated that 0.25 per cent of EMS as LD₅₀ concentration for french bean. The germination percentile was found to be inversely related to the dose of chemical mutagen used: the germination percentile decreased with the increase of the dose of EMS. These results are in accordance with the earlier reported of Budennyi and Naumov (2004) and Dimova (2000) in french bean where they used EMS together with other mutagen. Further, 0.25 per cent EMS concentration was used to induce mutation in french bean var. 'Arka Anoop' to create large-scale variability.

The amount of variability existing in mutant population decides the progress in crop improvement programme. The utility of this material is decided by the extent of variability and heritability of mutant traits. Hence, M₂ and M₃ populations were studied to assess their genetic potential. In the present study represented lot of variation among the population for all the characters. It has been demonstrated earlier by Gregory (2006 and 2007), Frey (2005) and Brock (2000) that mutagenesis is as efficient as hybridization to create variability. We know that, it is important to understand the nature and magnitude of variation before initiating any breeding programme. Therefore, estimates of parameters of induced variability have been worked out and the results reveal that significant variation has been induced in 'Arka Anoop' variety of french bean for mutagenic treatment.

Low to moderate values of phenotypic coefficient of variation (PCV) have been observed for plant height, pod length and width, days to pod maturity, average number of seeds per pod in both M₂ and M₃ generations, while these were moderate for number of branches per plant, days to 50 per cent flowering, weight of 10 pods and pod yield. Only pod tenderness has exhibited high values of PCV in both M₂ and M₃ generations.

The coefficient of variation detects the extent of variability present for different characters and it does not indicate the heritable portion to obtain the magnitude of heritable portion of variability, it is essential to know the heritability estimates for different characters.

Heritability (h^2) is another important parameter of variability in the study of polygenic variation, the magnitude of which determines the response to selection in any crop breeding programme. In the present study, both M₂ and M₃ generations have exhibited higher estimates of heritability. However, the estimates of heritability in M₂ and M₃ generations have been high for plant height, days to 50% flowering, pod length, pod width, days to pod maturity, weight of 10 pods, pod yield and tenderness of pod, whereas high heritability found for number of branches per plant in M₃ but moderate heritability found in M₂ generation. Low to moderate heritability found in M₂ and M₃ generations for average number of seeds per pod.

In order to draw more relevant information, it is important to estimate genetic advance and make selection after taking into account both heritability and genetic advance. The estimates of genetic advance expressed as per cent mean were mostly trait dependent. The values have varied from low to high in both the generations. Genetic advance for plant height, pod length, pod width, days to pod maturity and average number of seeds per pod has been low in both M₂ and M₃. The estimate in M₃ generation is low for pod yield per plant and for hectare and it was moderate in M₂ generation. Whereas, number of branches per plants had moderate heritability both in M₂ and M₃ generations. High heritability range both in M₂ and M₃ has been estimated for days to 50 per cent flowering and weight of 10 pods and tenderness.

High heritability and low genetic advancement has been recorded for most of the traits which indicates the role of additive gene action and also that the characters were not much influenced by environmental effects and major part of variability was due to genotypic causes. This in turn also indicates that compare to hybridization, in mutation generation the trait will be stabilized in fewer generations. Therefore, selection of these traits for the improvement may be effective and less time consuming. But heritability coupled with genetic advance as percentage of mean will be more useful

than heritability alone in predicting the resultant effect for selecting the best individual as explained by Jaidev and Gupta (1997) in french bean. Increase in the values of genetic parameters in mutagenized population has been observed earlier by Ignacimuthu and Babu (1990), Nandi *et al.* (1996, 1998), Singh *et al.* (2001a) in mung bean and Singh *et al.* (2001b) in Urdbean. These findings are concerned with those of Patil *et al.* (1993) for yield per plant, plant height, pod length, Mishra and Dash (1991) for yield per plant, pod width, Korla *et al.* (1998) for pod length, Shah *et al.* (1999) for plant height and pod weight, Dikshit *et al.* (1999) for days to 50 per cent flowering, yield per plant, pods per plant, plant height, Pooran Chand (1999) for pods per plant. A Moderate heritable values captured with moderate genetic advance suggests the role of additive gene actions in the expressing traits.

A correlation study indicates the degree of interrelationship of plant characters for improvement of yield as well as important quality parameters in any breeding programme. Hence, understanding of the interrelationship between pod yield and yield influencing character is vital importance because this would facilitates effective selection for simultaneous improvement in one or more yield characters in mutant population. The intense and direction of association among the characters was measured by simple correlation.

In the present investigation correlations worked out for eleven quantitative characters in M_2 and M_3 generations revealed positive correlation has been estimated for almost all characters. These results are in accordance with earlier reports by Shah *et al.* (1986), Shah *et al.* (1999), Subhadeep and Korla (2004), Singh *et al.* (2000), Shinde and Dumbre (2001), Rai *et al.* (2004) and Atilla Dursum (2007). So, indirect selection for pod yield could be effectively done through direct selection of characters as indicated above. (Jayaram Gowda *et al.*, 2007 and Sumathi *et al.*, 2007).

The homogeneity of mean between the control and mutant population in both M_2 and M_3 generations for 11 quantitative characters was tested by using one sample t test. Studied characters in control (non mutated population) as compared with M_2 and M_3 generations for mean values by using one sample t test and in each trait we found significant difference. These results suggest that mean components of mutant populations are having variation for all the characters studied from that of control. Similar results were also observed by Ahloowalia *et al.* (2004), and Ashok and Khoushal (2003). These results suggest that in french bean and soya bean, mutant population of both generations developed from EMS is more diverse compared to untreated population. From these variability characteristics, it is evident that mutation thus can be employed to create adequate variability for all characters.

Qualitative characters are useful criterion for selection criteria, as they show high heritability and stable expression. Further, if qualitative characters show association with yield components, it can serve as a marker in selection process. The variability for twelve qualitative characters *viz.*, growth habit, flower and leaf pigmentation, leaf characters and pod string character were compared with that of M_2 and M_3 population using chi-square test. The study of all the qualitative characters clearly indicated that M_2 and M_3 generation exhibited the different frequency distribution. This result is because of the fact that mutation has possibility to occur in M_3 generation also. These results are in complimentary with the findings of Solanki and Sharma (2000) in lentil, and Yaqoob *et al.* (2003) in mung bean.

5.2 Cloning and structural characterization of NBS-LRR type Resistant Gene Analogs (RGAs) from french bean

The recent developments in the field of biotechnology have provided a number of tools to study the interaction between plants and potential pathogens at all stages of infestation. Many disease resistance (R) genes against distinct pathogen types have been isolated recently (Khan, 2009). Genetic transformation of susceptible genotypes with candidate genes will enable to assess the effectiveness of resistance genes. The molecular markers allow the dissection of monogenic and quantitative resistance trait and provide insight into the basic understanding of the function and evolution of resistance gene. The recent cloning of some resistance genes provides new opportunities for cloning related genes from crop species. Genome scanning for resistance gene analogs is expected to have immediate application for germplasm evaluation and disease resistance breeding. Since RGAs represent potentially useful genes, these can be effectively used in disease resistance breeding programme. Hence, attempts were made to clone and characterize RGAs from the genotype Arka Anoop genotype of french bean, which was found resistant to rust.

The recent cloning of genes for resistance against diverse pathogens from a variety of plants have revealed that many share conserved sequence motifs. These motifs provide the possibility of isolating numerous additional resistance genes using polymerase chain reaction with degenerate oligonucleotide primers. Various investigators have amplified resistance genes from genomes of diverse plant species using combination of primers with low degeneracy, designed from consensus motifs (Aarts *et al.*, 1998; Collins *et al.*, 1998; Leister *et al.*, 1996; Shen *et al.*, 1998 and Yu *et al.*, 1996).

Degenerate primers from other crops were used for amplification of french bean RGAs. In addition to 500-600 bp fragments, many bands of different sizes were also noticed in all primer combinations. But only the amplicons with the range of 500-700 bp were considered for further analysis. Since earlier efforts of characterizing longer (900-1000 bp) or shorter (300 bp) fragments did not yield any homology to known R-genes (Lopez *et al.*, 2002 and Aarts *et al.*, 1998). The amplicon of size other than 500-600 bp cloned and sequenced in Arabidopsis assuming as RGA resulted in no RGA identities (Aarts *et al.*, 1998). Similar observations were reported by Mago *et al.* (1998). Hence, 500-700 bp which also corresponds to average distance of regions of NBS- LRR are the prime fragments.

Eluted PCR fragments (500-700 bp) from each primer combination were cloned at ddt site of pTZ57R perT vector. The clones were confirmed both by plasmid profiling and PCR confirmation. Stable white colonies were further streaked on the plates and used for further analysis. As these RGAs are novel and conserved in nature, we expect more conserved motifs within these.

Computer aided similarity searches of DNA and amino acid sequences with the known sequences from database yielded information on structural and functional nature of the genes (Atschul *et al.*, 1997).

BLASTn and BLASTx search was done to find the similarity of the cloned sequences with other resistance gene sequences. The homology search of the french bean RGA sequences at the nucleotide level showed varied levels of similarity to the already known NBS-LRR domains of R genes and with other RGAs. Thus, the isolated RGAs from french bean have novel NBS-LRR motifs though they are similar to other RGAs. The search indicated the highest similarity (100 per cent) with NBS LRR disease resistance protein gene of *Phaseolus vulgaris* and 100 to 39 per cent similarity with NBS-LRR domains of *Glycine max*, *Pisum sativum*, *Arachis hypogaea*, , *Clitoria ternatea*, Maize, *Triticum macha* and *Arabidopsis thaliana* etc., It is notable that french bean RGAs were more similar to RGAs of both monocot and dicot species. The expected (E) value refers to the number of matches expected by chance alone. The low E value indicates a better match. The E value of near or equal to 1 indicates the sequence homology being not appropriate. When adopting these criteria, higher level of identity was between clones. Resistance protein Non-TIR 07 gene of *P. vulgaris* COHFBGA2, COHFBGA25, COHFBGA26 and COHFBGA32 had 100 per cent homology. There was 96 per cent similarity among the NBS LRR disease resistance protein gene of *Glycine max* and COHFBGA9 with significantly low E value. Similarly with COHFBGA33, COHFBGA34, COHFBGA36 and COHFBGA37, PNTR gene and PRG gene of *Arachis hypogaea* showed 100 per cent similarity. NBS-LRR type disease resistance protein (RGA) gene of *Pisum sativum* showed 100% similarity with COHFBGA38 with low E value. COHFBGA24, COHFBGA25, COHFBGA26, COHFBGA27, COHFBGA28, COHFBGA31 showed maximum similarity with resistance protein gene of *Phaseolus vulgaris* var. *aborigineus*. Whereas, COHFBGA35 did not share any significant similarity with known resistance gene. The sequences of clones differed significantly even though they were amplified by using the same primers. The sequence difference is due to the degeneracy of the primers used and structural and functional divergence of R genes found in french bean.

The BLASTx results of each french bean RGA also indicated the similarity with other crop RGAs / disease resistance genes. Many of the french bean RGA sequences shared 100 per cent similarity with disease resistance protein of *Phaseolus vulgaris*. Putative resistance protein non-TIR 07 of *Phaseolus vulgaris*, NBS-LRR type disease resistance protein of *Pisum sativum*. Catalase protein of Maize, Puroindoline B Protein of *triticum estivum* had 100 and 96 % similarity with COHFBGA19 and COHFBGA17 respectively. Many of the french bean RGA sequences shared 37 to 100 per cent similarity with disease resistance protein of *Arabidopsis thaliana*, *Triticum macha*, *Cicer arietinum*, *Vigna radiata* var. *Radiata Brassica napus*. COHFBGA35 revealed no significant similarity with any disease resistance protein.

There were clear differences observed in per cent similarity and E-value of the GenBank accessions with french bean RGA nucleotide and amino acid sequences. Nucleotide and protein search resulted in better similarity with the NBS-LRR disease resistance of many crop RGAs. The difference in the similarity percentage and E value between the nucleotide and amino acid level search is expected due to the codon degeneracy.

The domain search with Pfam database clearly indicated the presence of resistance gene domain of different families among the cloned sequences. Maximum number of cloned sequences (13 sequences) showed P-Loop NTPase superfamily domain, four sequences belongs to protein kinase, four sequences shared TIR family domain and two cloned genes had helicase family domain.

We employed ClustalW for the characterization and translation of sequences. These proteins were then aligned with the known disease resistance genes like 5 resistance genes of different crops like N gene of tobacco (RPP5)- AAF08790.1, R gene of *Papevar somnifera* CAQ43070.2 R gene of banana NP1880651, XP013690297 of Arabidopsis and potato RGA2 (BAA25068.1). The sequence showed more homology with P-Loop NTP and *kinase* of known R genes. This was expected as primers were based on the NBS-LRR motifs of *L6* of tobacco and flax, respectively. Within the NBS region, P-Loop NTP, *kinase* and *TIR* are the conserved regions. Isolated french bean RGA sequences also showed significant homology for P-Loop NTP and *kinase*. Out of 38 sequences, 33 were highly homologues to the known R genes. These homologues may constitute a super family of NBS containing genes, at least some of which may be involved in disease resistance in french bean. Sequencing of more and more clones could give a better insight into these aspects. Multiple sequence alignment of RGAs from many crop species has shown the presence of conserved motifs like kinases, P-Loop NTP and hydrophobic domain (Lopez *et al.*, 2002; Bhavani *et al.*, 2002 and Dequan *et al.*, 2010).

Studies of resistance gene clusters have revealed evidence of duplication, recombination, and rearrangement events leading to the expansion of clusters, growth of multigene families, and the potential for evolution of new resistance specificities (Chin *et al.*, 2001; Graham *et al.*, 2002 and Kruijt *et al.*, 2004). The 33 french bean RGAs grouped into six distinct families, each of which had identities with different RGAs and R genes from other plant species. Particular amino acid motifs conserved within french bean RGA families may indicate some functional specificity. However, closely related R genes do not necessarily confer resistance to the same pathogen. The potato Gpa2 and Rx1 genes are in the same cluster on potato chromosome 12 and are considered to be highly homologous but have very different specificities; Gpa2 is a nematode (*Caenorhabditis elegans* Miers) resistance gene, whereas Rx1 confers resistance to a virus (Vossen *et al.*, 2000). The french bean RGA families cluster A has significant similarity to N gene of tobacco (RPP5) and R gene of *Papevar somnifera* CAQ43070.2. All the *P. vulgaris* RGAs amplified with degenerate primers based on the P-loop and GLPL domains, had motifs consistent with the R gene classes. Distinct classes of RGAs were identified in french bean based on the presence of a conserved NBS-LRR motif within their predicted protein coding region. The number of class of RGAs has varied across species. Three classes of RGAs were reported to be present in common bean (Bhavani *et al.*, 2002) based on the dendrogram. Nine classes of RGAs were found in soybean (Kanazin *et al.*, 1996), five classes in banana (Dequan *et al.*, 2010), two classes in sorghum (Totad *et al.*, 2005). In the present study cluster D, E and F cluster showed more diversity in the sequences. These clones may even have different resistance gene function. All the RGAs may not code for functional proteins for disease resistance. A number of elucidated RGAs could be in the product of evolution and the detected domains need to be a part of a functional gene.

Some of the RGAs isolated in this study are novel to the french bean crop. Many other conserved domains are still to be identified. As more information becomes available, it is possible that some of the RGAs identified here could be used for mapping and for tagging resistance related trait. The study conducted here is first step towards a broader understanding of the structure and functional organization of R genes in french bean, and should assist in the eventual cloning and characterization of R genes against major pests and diseases of french bean.

5.3 *In silico* genome-wide identification of transcription factor genes from french bean

TFs are the master key regulators and further they are characterized into different families with specific DNA-binding domains (DBD), an oligomerization domain and a transcription regulatory domain (TRD) involved in the regulation of cell growth, cell cycle control, signal transduction, metabolic and physiological process, and response to adverse environmental stimuli by either activating or repressing transcription of the target genes (Calkhoven and Ab 1996; Latchman, 1997; Schwechheimer, 1998; Riechmann, 2000 and Wray *et al.*, 2003). Transcription factor family has been the subject of intensive studies. For instance, they have been identified and classified in *Arabidopsis* (Eulgem *et al.*, 2000). Identification and functional characterization of TFs at transcripts and whole genome level using computational approaches are the preliminary step in understanding the mechanism of TFs expression and regulation (He *et al.*, 2010). The rapid expansion in the next generation sequencing (NGS) technologies, a large volume of data in genome and transcriptome level has been developed and it will facilitates identification of putative TF in other genomes through computational studies (Riechmann *et al.*, 2000; Martínez-Bueno *et al.*, 2004).

The availability of TF families' resource at genomic level in model plant such as *Arabidopsis thaliana* can serve as reference source to explore the TFs associated with biological processes in unknown TF families in genomes. By keeping the *Arabidopsis* as reference, the genome wide identification of TFs in *P. vulgaris* by computational approaches lead to the identification of 58 numbers of TFs families. These members were then subjected to the Pfam and SMART domain searches to validate the presence of the TF related domains. By removal of different transcripts of the same gene, we identified putative genes with one domain in 24 TF families. Similar result was reported by Wray, *et al.* (2003) and Charoensawan *et al.* (2010) in soyabean and chilli for trihelix-GT and bHLH TFs respectively, here also transcription factors exhibited only one type of DNA-binding. Oligomerization domain, occasionally in multiple copies, but some contains two distinct types (Liu *et al.*, 1999).

The GO slim analysis conducted through Blast2Go showed the putative participation of TFs in diverse biological processes. Among all TFs, only few members of few families could annotate for their functionality. In the french bean, only 8 families of TFs viz., AP2, B3, BHLH, Bzip, C2H2, ERF and MYB exhibited the functional annotation, whereas for remaining families there was no information on functional annotation in the crop. Among these 8 families, which showed functional annotation of respective families, BHLH family had more number of members having function similarity (56), followed by C2H2 (51) and bZIP (31) family members. The AP2, B3, BHLH, Bzip, C2H2, ERF and MYB they were reported to be involved in biotic and abiotic stresses. Further some of the gene from these identified families were subjected for primer design and done expression profiling against rust and MYMV disease.

Genome-wide *in silico* identification, characterization and expression analyses of *Phaseolus vulgaris* transcription factors offer an insight into their potential role in stress-related mechanisms. In addition, we have demonstrated that the expression of few TF families was correlated with disease stress response in a cultivar-specific manner in common bean. The members of the TF gene families may also be used for genetic engineering applications in common bean, which is an economically important legume crop. In the study only few family have been annotated, but there are even many more family which are not having information on its functionality. Many other conserved domains are still to be identified. As more information becomes available, it is possible that generated information here could be used for mapping and for targeting resistance related traits. The study conducted here is first step towards a broader understanding of the structure and functional organization of TFs in french bean, and should assist in the eventual characterization of transcription factor genes against major pests and diseases of french bean.

5.4 Expressional dynamics of selected TF genes and resistant gene analogs in the manifestation of rust and *MYMV* in french bean

Responses to biotic stress require the production of important metabolic proteins such as those involved in synthesis of phenols, enzymes and of regulatory proteins operating in the signal transduction pathways, such as kinases or transcriptional factors (TFs). The transcriptional regulation of genes is directly controlled by a network of TFs and transcription factor binding sites (TFBS) (Chaves and Oliveira, 2004). TFs are proteins with a DNA domain that binds to the cis-acting elements present in the promoter of a target gene. They induce activators or repressors the activity of the RNA polymerase, thus regulating gene expression. TFs can be grouped into families according to their DNA-binding domain (Riechmann *et al.*, 2000). The presence or absence of transcription factors, activators and suppressors regulating transcription of target genes often involves a whole cascade of signaling events determined by tissue type, developmental stage or environmental condition (Wyrick and Young, 2002).

Transcription factors are master-control proteins in all living cells. They often exhibit sequence specific DNA binding and are capable of activating or repressing transcription of multiple target genes. In this way, they influence many biological processes, including cell cycle progression, metabolism, growth and development and responses to external environments (Hirt, 2000; Chen and Zhu, 2004). Alteration in the expression of TF genes normally results in dramatic changes to a plant (Latchman, 2003 and Broun, 2004). Similarly, RGAs represent potentially useful genes, these can be effectively used in disease resistance breeding programme. The present investigation was undertaken to get an insight on the expression levels of selected 20 genes of four transcription factor families and 10 RGA in leaf tissues of french bean for rust and *MYMV* disease.

Predicted features and functions of candidate genes belong to transcription factor families like *AP2*, *B3*, *bHLH*, and *bZIP* and 10 cloned RGA genes were selected in this experiment for their expression analysis. Though these TFs have direct and indirect role in biotic stress tolerance in plants, not much gene experimental data is available even in model systems. Out of 20 TF gene specific primers 17 primer pairs yielded expected amplicon and 3 primer pairs yielded no PCR product from 7 DAI, 15 DAI and 30 DAI cDNA after 40 cycles of real-time PCR indicating that the target genes were probably not expressed or expressed at very low level in these tissues and growth conditions. Whereas, all ten RGAs yielded expected amplicons. The total RNA from each treatment was treated with *DNase I* enzyme to eliminate traces of genomic DNA. Actual confirmation of complete degradation of genomic DNA in RNA preparation was done through PCR amplification using total RNA as template. There was no amplification from the total RNA preparation indicating absence of traces of genomic DNA as contamination. However, elimination of contaminating genomic DNA enzymatically is very important in gene expression analysis using qRT-PCR (Chini *et al.*, 2007). Presence of genomic DNA/genetic copies of genes seriously alter the precision of expression quantitation of genes in target tissues.

Generally, 18S rRNA, EF-1, α actin, β tubulin and ubiquitin (UBQ) genes are considered as good reference genes for any gene expression experiment (Caldana, 2007 and Czechowski *et al.*, 2004). The gene expression stability measure (M) was estimated to identify the most stable reference gene among actin (AC1), β -tubulin, 18S rRNA and elongation factor-1 through qRT-PCR in a set of 3 different cDNA samples corresponding to different interval of day after flowering *i.e.* 7 DAI, 15 DAI and 30 DAF tissues from french bean leaves inoculated with rust (where inoculated samples were collected from both resistant and susceptible genotypes at different intervals) and in *MYMV* (samples were collected from inoculated and control susceptible genotype as the resistance source is not available). The NormFinder software which uses model-based variance estimation approach was used; the M value should be <1.5. The M value, 0.298, 0.311 and 0.326 for *actin* (AC1), 18S rRNA and β -tubulin respectively, based on M value *actin* (AC1) gene was selected as endogenous reference gene for rest of qRT-PCR experiments. In several instances these gene has been tested and used as reference genes in qRT-PCR experiments, and the M values of these reports are within the range of present experimental results (Claus *et al.*, 2004; Ruth *et al.*, 2008 and Kakar *et al.*, 2008). It is the most stable combination indicating the absence of significant differences in the expression levels of reference genes in varied experimental conditions. In several instances of plant gene expression analysis by qRT-PCR these genes with similar combination have been adopted (Marino *et al.*, 2003).

PCR efficiency correction was used to calculate the fold change expression in the relative quantification of gene expression. The PCR efficiency of selected genes was calculated from the exponential phase of individual amplification plot using the equation $(1+E) = 10^{\text{slope}}$ (Ramakers *et al.*, 2003). Subsequently, the average PCR efficiencies were computed for each individual primer pairs across all analyzed samples. Out of 30 genes (including TF and RGAs) tested four genes PCR efficiency between 1.43 and 1.52, and seven genes between 1.54 and 1.63, five genes between 1.65 and 1.70, and remaining 3 showed lower values 1.7 and 1.77. The range of PCR efficiency determined was in consistent with the results reported by Kakar *et al.* (2008), Caldana *et al.* (2007) and Czechowski *et al.* (2004). Further, PCR efficiency was used to calculate final fold change of selected genes. The delta-delta Ct method (Livak and Schmittgen, 2001) was used to determine relative expression ratio of 27 genes (fold change). The delta-delta mathematical model of determining fold changes in the expression of genes is widely adopted in qRT-PCR (Czechowski *et al.*, 2004; Buchanan *et al.*, 2005; Caldana *et al.*, 2007 and Yang *et al.*, 2010). In this method an amplification efficiency of each gene specific primer pairs from the log slope of fluorescence versus cycle number in the exponential phase and the same is used to calculate fold expression using the delta-delta Ct method. Similarly, Caldana *et al.* (2007) and Yang *et al.* (2010) used delta-delta Ct method to calculate relative fold change in rice and common bean respectively.

The technical precision of qRT-PCR was assessed by performing replicated measurements in separate PCR runs. The same pool of cDNA to account the precision in technique employed and two different pools of cDNA obtained independently from two different batches of total RNA under same condition to test precision of biological responses of plant to different day after inoculation were used. Precision, as reflected by the correlation coefficient, was high in both cases; technical and biological replicates recorded correlation coefficient values greater than 0.970 and 0.968 in different day after inoculation tissues indicating high precision of technical and biological treatments and response of french bean tissues. A similar strategy to monitor the technical and biological precision of experiment was adopted by Czechowski *et al.* (2004) in *Arabidopsis thaliana* and by Kakar *et al.* (2008) in *Medicago* for TF genes to assign putative TF gene status. The specificity of the PCR reactions was determined by loading the PCR products on a 4% agarose gel. Most of the genes yielded specific sized amplicon as predicted. Among responded TF genes none of them were found to give unspecific PCR products in terms of either multiple or incorrect sized amplicon. Unless their specificity is achieved it is likely that some primer pair could co-multiply the members of same family or other sequences homologous within transcriptome, leading error in quantitation. Melting curve analyses was performed for all PCR products to confirm the occurrence of specific amplification peaks and the absence of primer-dimer formation. Melting curve analysis showed that all 27 genes were giving specific amplification and there was absence of primer-dimer formation. Similar technical and biological precision in drought stress experiments leading to qRT-PCR testing of TF gene was reported in *Arabidopsis* (Liu *et al.*, 2007; Huang *et al.*, 2008 and Seo *et al.*, 2009), soyabean (Stolf-Moreira *et al.*, 2010) and maize (Li and Wang, 2007 and Xiao-feng *et al.*, 2009).

The expression levels of 27 selected genes were analyzed in 7 DAI, 15 DAI and 30 DAI in comparison to controls. A differential up- and down-regulation of selected genes in response to different day after inoculation was recorded for both the diseases. This is in consistence with result obtained by Moreira *et al.* (2010), where the expression levels of DREB transcription factor (*GMDREB1*) and genes *viz.*, *GMGOLS*, *GMP1P1B*, *GMEREB*, and *GMDEFENSIN* in drought-tolerant (MG/BR46-Conquista) and drought-sensitive (BR16) genotypes of soybean was determined. Increase in the expression of *GMDREB1* in leaves of MG/BR46, under 1 and 3 hr drought treatments, respectively, two and three times greater than in the untreated soybean. In leaves of soybean cultivar BR16 under 1, 3 and 5 hr treatments, the increase in expression was two, two and five times greater than in the untreated soybean, respectively. However, fewer than 3 hr treatments, plant roots of MG/BR46 and BR16 showed reductions in the expression levels of the *GMDREB1*.

In present study, AP2-2, AP2-3, AP2-4, AP2-5, B3-1, B3-2, B3-3, BHLH-1, BHLH-2, BHLH-3, BHLH-4, BHLH-5, BZIP-1, BZIP-2 and BZIP-3 were up-regulated in leaf of resistant genotypes at rust inoculation condition at 15th and 30th days where as BZIP-4 was down-regulated in leaf of resistant genotypes at disease inoculation condition at 15th day and up regulated at 30th day. AP2-1, AP2-2, AP2-3, AP2-4, AP2-5, B3-1, B3-3, BHLH-1, BHLH-2, BZIP-1, BZIP-2 and BZIP-3 were down-regulated in susceptible genotype and in case of B3-2, BHLH-3, BHLH-4, BHLH-5 and BZIP-4 were up-regulated in this condition. In general, most of genes were up-regulated in leaf tissues of the resistant genes when compared to susceptible genotype inoculated with rust disease. Similarly, *HAHB- 4*, a member of HB

family, has been known to be responsive to stress in sunflower (Moreira *et al.*, 2010). The transgenic plants expressing the genes were more tolerant to water stress conditions, showing improved development, a healthier appearance and higher survival rates than wild-type plants. Since *HAHB-4* is a transcription factor, they proposed that it may participate in the regulation of the expression of genes involved in developmental responses of plants to desiccation. For *MYMV* manifested condition, AP2-1, AP2-2, AP-4, AP-5, B3-1, B3-2, BHLH-2, BHLH-3, BHLH-5 and BZIP-2 were up-regulated in leaf of susceptible genotypes at disease inoculation condition at 7th DAI. AP2-3, B3-3, BHLH-1, BHLH-4, BZIP-1 and BZIP-3 were down-regulated, but BZIP-4 did not express at this condition. 7 TF genes namely, AP2-3, AP2-5, B3-2, B3-3, BHLH-4, BZIP-1 and BZIP-2 were down regulated in susceptible genotype after disease inoculation. Hence, the pattern of expression of selected genes in the present study is in corroboration with earlier reports as for as response of tested genes in biotic stress in concern. These findings were consistent with the results of Czechowski *et al.* (2004), who examined various members of ARF, MADS and HB TF families in stressed root of *Arabidopsis thaliana*.

Expression of 10 RGA clones were analyzed in the leaf tissues of rust resistant and susceptible genotypes Arka Anoop and Arka Sharath respectively inoculated with rust (at 15 and 30 days after inoculation) and in case of *MYMV*, analysis was done for susceptible genotype inoculated with disease at 7, 15 and 30 DAI and control (as without inoculated at 7, 15 and 30 DAI) using real-time PCR. All the 10 RGAs were expressed in both leaf tissues at different days after inoculation with rust and *MYMV*. In case of rust disease, all the 10 genes at 30 DAI and except COHRGA2 at 15 DAI were up-regulated in resistant genotype. COHFBRGA1, COHFBRGA2, COHFBRGA25, COHFBRGA32 and COHFBRGA38 were down-regulated in susceptible genotype at 15 and 30 DAI. Whereas, COHFBRGA4, COHFBRGA9 and COHFBRGA27 (15 DAI) and COHFBRGA9 at 30 DAI showed up-regulation susceptible genotype. COHFBRGA3 up-regulated at 30 DAI but expressed inversely at 15 DAI in susceptible genotype. Similarly, the same 10 RGA genes were tested under *MYMV* infested condition in french bean. All 10 selected RGA genes were expressed in leaf tissues at different days after inoculation with *MYMV*. COHFBRGA-2, 6, 8 and 10 genes of french bean were up-regulated in leaf of susceptible genotypes at disease inoculation condition at all the intervals. COHFBRGA-3, 4, 5 and 7 was down-regulated among all the intervals of disease development compared to control. Whereas, COHFBRGA-9 expressed only at 30 DAI. Emediato *et al.* (2013) revealed that, gene expression for RGAs in both compatible and incompatible interactions across the infection time course with *Mycosphaerella musicola* in banana showed low levels of RGA expression in the early infection stages and up-regulation in late stages. In contrast, other RGAs displayed high expression levels in the resistant cultivar during the early infection stage, strong down-regulation in the intermediate infection stage, and recovery to higher levels in the final stages of the infection time course. Wang *et al.* (2006) reported that differences in transcript levels could be correlated with the reaction to susceptibility/resistance of the host plant to pathogen. Gao *et al.* (2006) found that some RGAs were expressed only after challenge with *Verticillium dahlia* in cotton.

Future line of work

1. M₃ generated population which are showing variability for different characters like stringless pod mutants, mutants showed late disease appearance for *MYMV*, broad and thick leaved mutants can be further evaluated and used in breeding programme as a source of variability for quality, diseases resistance breeding program.
2. Cloning and characterisation of full length saluted french bean RGAs/ R genes can be done using the information given in the present study.
3. Present study provides a bench for further transcription factor characterisation and functional validation for biotic stresses in french bean
4. Assessing expression of TF genes and RGAs in other tissues apart from leaf tissue at two time points to gain deeper understanding of these genes in manifestation of other disease.

VI. SUMMARY AND CONCLUSION

French bean, *Phaseolus vulgaris* L. ($2n = 22$) is a member of the family Fabaceae. It is an important legume vegetable with high nutritive value. Being a self pollinated crop, in french bean the genetic variability is rather limited. Moreover, interspecific and inter varietal incompatibilities pose problems in crossing and getting desirable recombination. Thus, it has been difficult to develop improved varieties by exploiting the limited genetic variability available through selection and recombination breeding. French bean has become an excellent crop model of choice for studying the genetic and physiological mechanisms of disease tolerance due to its adaptation to different environments, close degree of relatedness to other economically important crops, and relatively small genome.

Biotic stress majorly affects the plant growth and development pattern by suppressing cell division, organ growth, net photosynthesis, protein synthesis and alters hormonal balance in major plant tissue. Rust and MYMV are the important biotic stresses in crop and influences many agronomically important traits. Precise understanding of disease resistance mechanisms and development of molecular techniques for identifying these mechanisms may be beneficial in breeding programs for disease resistance. A number of plant genes and their products interact in the manifestation of either resistance or susceptibility to a pathogen. Cloning and characterization of such genes will be a long term solution towards development of resistant cultivars.

In the living organism, mechanisms at developmental, physiological and morphological stages allowing acclimation to adverse environmental changes are the result of gene expression modulation in the genome. Gene regulation at transcriptional level, many mechanisms are involved among them transcription factors (TFs) are the key players. The availability of TF families' resource at genomic level in model plant such as *Arabidopsis thaliana* can serve as reference source to explore the TFs associated with biological processes in unknown TF families in genomes. In legume plants, however, genome-wide computational identification of TFs reported in few genomes. Hence, the present study on "development of functional mutants, cloning and expressional analysis of resistance gene analogs and transcription factors in french bean (*Phaseolus vulgaris* L.)" was carried out at College of Horticulture, Bengaluru, during 2014-16.

Following is the summary of results obtained in the present study:

1. In french bean the LD₅₀ estimated as 0.25 per cent for ethyl methyl suphonate.
2. The mean, range and variance for all the characters among control and mutant plants (M₂ generation) were showed significant difference as it is segregating population.
3. Low to moderate values of phenotypic coefficient of variation (PCV) have been observed for plant height, pod length and width, days to pod maturity, average number of seeds per pod in both M₂ and M₃ generations, while these were moderate for number of branches per plant, days to 50 per cent flowering, weight of 10 pods and pod yield. Only Pod tenderness has exhibited high values of PCV in both M₂ and M₃ generations.
4. High heritability and low genetic advancement has been recorded for most of the traits in M₂ and M₃ generations, which indicates the role of additive gene action and also that the characters were not much influenced by environmental effects and major part of variability was due to genotypic causes.
5. In the present investigation correlations worked out for eleven quantitative characters in M₂ and M₃ generations revealed positive correlation has been estimated for almost all characters.
6. The study of all the qualitative characters clearly indicated that M₂ and M₃ generation exhibited the different frequency distribution. This result is because of the fact that mutation has possibility to occur in M₃ generation also.
7. In M₃, we have observed three pole type mutant plants but none of them yield flower and pods in later stages of growth and five mutants were free of MYMV infestation in the early stages however during pod maturity disease symptoms were recorded.
8. In the mutant generation we also observed broad and thick leaf mutants, these can be further used in developing drought resistance in french bean.

9. The resistant french bean genotype Arka Anoop was used for cloning of RGAs. Along with known combinations, a number of novel combinations of primers targeting conserved domains were tested for their ability to reveal RGAs in french bean. French bean RGAs of size 500-700 bp were considered for cloning and characterization. Over 15 transformed colonies from each primer combination was considered for further confirmation by colony PCR and sequencing.
10. A total of 90 colonies in all primer combinations were sequenced employing M13 forward and M13 reverse primer pairs.
11. *In silico* analysis was done to cure the sequences and to annotate them. A set of 38 sequences were found to be of R genes.
12. Sequence homology search for french bean RGAs at both nucleotide and amino acid sequences was carried out using BLASTn and BLASTx algorithm. There was significant similarity with NBS-LRR sequences of other crop species and with known R genes. There was a difference in the E value and per cent similarity of BLASTn and BLASTx results.
13. ClustalW alignment of translated proteins of french bean RGAs and known R genes was done. French bean RGAs revealed conserved region with the P-Loop-P, *kinase* TIR sequences in the NBS- LRR region. In order to infer relationship between the french bean RGAs and known R genes, phylogenetic tree analysis based on Neighbour-Joining method was done using Mega 6 software. Based on this, the isolated RGAs were grouped into six major clusters.
14. These RGAs can be further analysed and can be used as a tool for the dissection of disease resistance in french bean and this is a first study in french bean which is an initial step to a broader understanding of rust and *MYMV* resistance in french bean and should assist in the eventual cloning and characterization of R genes against diseases in french bean.
15. By keeping the Arabidopsis as reference, the genome wide identification of TFs in *P. vulgaris* by computational approaches leads to the identification of 58 numbers of TFs families.
16. Among 58 families of TFs, *Phaseolus vulgaris* showed maximum gene homology for NAC TF family members followed by family BHLH was having homology for 62 genes. SIFA, HSF, NZ were having no homology for any of the members of the family. TF family domain was observed only for members of 24 families and MYB family were having highest members with respective TF domain i.e., 102 members of this family in *Phaseolus* confirmed the presence of TF conserved domain.
17. In the french bean, only 8 families of TFs viz., AP2, B3, BHLH, Bzip, C2H2, ERF and MYB exhibited the functional annotation, whereas for remaining families there was no information on functional annotation in the crop. The study conducted here is first step towards a broader understanding of the structure and functional organization of TFs in french bean, and should assist in the eventual characterization of transcription factor genes against major pests and diseases of french bean.
18. Out of 30 genes (including TF and RGAs) tested four genes PCR efficiency between 1.43 and 1.52, and seven genes between 1.54 and 1.63, five genes between 1.65 and 1.70, and remaining 3 showed lower values 1.7 and 1.77.
19. AP2-2, AP2-3, AP2-4, AP2-5, B3-1, B3-2, B3-3, BHLH-1, BHLH-2, BHLH-3, BHLH-4, BHLH-5, BZIP-1, BZIP-2 and BZIP-3 were up-regulated in leaf of resistant genotypes at rust inoculation condition and BZIP-4 was down-regulated. AP2-1, AP2-2, AP2-3, AP2-4, AP2-5, B3-1, B3-3, BHLH-1, BHLH-2, BZIP-1, BZIP-2 and BZIP-3 were down-regulated in susceptible genotype and in case of B3-2, BHLH-3, BHLH-4, BHLH-5 and BZIP-4 were up-regulated.
20. In general, most of genes were up-regulated in leaf tissues of the resistant genes when compared to susceptible genotype inoculated with rust disease.
21. AP2-1, AP2-2, AP-4, AP-5, B3-1, B3-2, BHLH-2, BHLH-3, BHLH-5 and BZIP-2 were up-regulated in leaf of susceptible genotypes at *MYMV* disease manifested condition where as AP2-3, AP2-5, B3-2, B3-3, B3-3, BHLH-1, BHLH-4, BZIP-1, BZIP-2 and BZIP-3 were down regulated in susceptible genotype after disease inoculation.
22. All the 10 genes at 30 DAI and except RGA2 were up-regulated in resistant genotype. COHFBGA1, COHFBGA2, COHFBGA25, COHFBGA32 and COHFBGA38 were down-regulated in susceptible genotype. Whereas, COHFBGA4, COHFBGA9, COHFBGA27 and COHFBGA26 showed up-regulation in susceptible genotype. COHFBGA3 was up-regulated at 30 DAI but expressed inversely at 15 DAI in susceptible genotype in rust manifested condition.

23. For *MYMV* diseased condition, COHFBRGA-2, 25, 27 and 38 genes of french bean were up-regulated in leaf of susceptible genotypes in all the intervals. COHFBRGA-3, 4, 5 and 7 was down-regulated to control. Whereas, COHFBRGA-32 expressed only at 30 DAI.

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APPENDIX I

Reagents for total DNA isolation

Stock solutions

1 M Tris (pH 8.0)

Tris	:	121.1g
Water	:	800 ml
pH (adjusted with concentrated HCl)	:	8.0
Total volume	:	1000 ml

EDTA 0.5 M (pH 8.0)

EDTA	:	18.6 g
Water	:	80 ml
pH (adjusted with NaOH)	:	8.0
Total volume	:	100 ml

Sodium chloride 1M

Sodium chloride	:	58.44 g
Water	:	800 ml
Total volume	:	1000 ml

CTAB buffer (2%)

CTAB	:	2.00 g
Water	:	80 ml
Total volume	:	100 ml

Phenol: Chloroform (1:1)

Tris saturated phenol (pH > 7.8)

Chloroform and isoamyl alcohol (24:1 v/v), store at 4 °C.

DNase free RNase: 10mg/ml

Dissolved 10 mg of DNase free RNase in 1 ml of sterile distilled water and store at -20 °C.

Sodium acetate 3M

Dissolved 408.3 g of sodium acetate in 800 ml of distilled water, adjusted pH to 5.2 with glacial acetic acid or 7.0 with dilute acetic acid and made up the volume to 1 litre.

APPENDIX II

Master mix for PCR (20 µl volume) for each RGA amplification

PCR mix components	Volume
10x assay buffer with MgCl ₂	2.0 µl
dNTPs (2.5mM)	1.0 µl
Primer (sense)	1.0 µl
Primer (antisense)	1.0 µl
Template DNA (100 ng)	1.0 µl
Taq DNA polymerase (3U/ µl)	0.3 µl
Sterile double distilled water	13.7 µl
Total volume	20.0 µl

APPENDIX III

Gel electrophoresis

1. Loading dye composition

Loading dye (6x)	:	0.25% bromophenol blue
		40% (W/V) sucrose in water
		Store at 4 °C

2. Recipe for 1.0 per cent agarose gel (60 ml)

Agarose	:	600 mg
1xTAE	:	60 ml
EtBr (10 mg/ml)	:	3 µl

3. 50x TAE composition

Tris base	:	242 g
Glacial acetic acid	:	57.1 ml
0.5 M EDTA (pH 8.0)	:	100 ml

Total volume made upto 1000 ml with double distilled water

4. Recommended amount of PCR product for the ligation reaction

Length of PCR product (bp)	Optimal PCR product quantity for ligation reaction, (0.52 pmol ends)
100	17 ng
300	51 ng
500	86 ng
1000	172 ng
2000	343 ng
3000	515 ng
4000	686 ng
5000	858

5. Luria agar

Ingredients	Concentration (g/l)
Tryptone	10.0
Yeast extract	5.0
Sodium chloride	5.0
Agar	18.0
pH	7.2

APPENDIX IV

Ligation and control ligation reaction recipe

I. Ligation reaction recipe

Components	Volume
Vector pTZ57R/T (0.17 pmol ends)	3 μ l
5X Ligation buffer	6 μ l
PCR product (0.52 pmol ends)	variable*
Water, nuclease-free	to 29 μ l
T4 DNA ligase	1 μ l
Total volume	30 μ l

II. Control ligation reaction recipe

Components	Volume
Vector pTZ57R/T (0.17 pmol ends)	3 μ l
5X Ligation buffer	6 μ l
Control PCR fragment (0.52 pmol ends)	4 μ l
Water, nuclease-free	16 μ l
T4 DNA ligase	1 μ l
Total volume	30 μ l

APPENDIX V

Chemicals for transformation

1. IPTG (isopropyl- β -D- thiogalactopyranoside) 0.1 M

IPTG : 0.24 g

Water : 8.00 ml

Total volume : 10.00 ml

Filter sterilized and store at 4 °C. 40 μ l per plate was used

2. X-gal solution (2% w/v)

Dissolved X-gal at concentration of 20 mg/ml in N,N-dimethyl formamide. 40 μ l per plate was used.

3. Ampicillin (100 μ g/ml)- Amp₁₀₀

100 mg of ampicillin antibiotic dissolved in 1 ml sterile water, filter sterilized and stored at 4 °C. 100 μ l per 100 ml was used.

APPENDIX VI

Reagents for plasmid isolation

STE buffer

Tris Cl (pH 8.0)	:	10 mm
NaCl	:	0.1 M
EDTA (pH 8.0)	:	1.0 mM

Autoclaved and stored at 4 °C

Alkaline lysis solution I

Glucose	:	50 mM
Tris Cl (pH 8.0)	:	25 mM
EDTA (pH 8.0)	:	10 mM

Autoclaved and stored at 4 °C

Alkaline lysis solution II

NaOH	:	0.2 N
SDS	:	1% (w/v)

(Prepare fresh and use at room temperature)

Alkaline lysis solution III

5 M potassium acetate	:	50 ml
Glacial acetic acid	:	11.5 ml
Double distilled water	:	28.5 ml

Autoclaved and stored at 4 °C

APPENDIX VII

French bean RGA clone sequences

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Appendix VIII

Unique TF IDs of different families in french bean

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Phvul.011G024600	Phvul.001G159300	Phvul.011G063400	Phvul.003G054100	Phvul.005G141200
Phvul.005G089900	Phvul.007G195100	Phvul.004G143400	Phvul.003G203900	Phvul.004G116500
GRF	Phvul.007G195000	Phvul.003G195700	Phvul.009G174900	Phvul.009G075000
Phvul.001G031000	Phvul.010G084900	Phvul.009G183300	Phvul.002G159700	Phvul.003G232300
Phvul.001G187500	Phvul.003G236200	Phvul.006G123700	Phvul.002G292600	Phvul.002G008800
Phvul.007G222300	Phvul.002G012200	Phvul.002G078600	Phvul.008G236500	Phvul.010G053200
HB-other	Phvul.008G075900	Phvul.003G261500	Phvul.001G229900	Phvul.008G067300
Phvul.002G232200	Phvul.009G175200	Phvul.005G039500	Phvul.008G038200	Phvul.010G096400
Phvul.006G182000	Phvul.002G021200	Phvul.009G259400	Phvul.002G015100	Phvul.007G069400
HB-PHD	LFY	Phvul.001G214900	Phvul.003G240200	Phvul.007G215800
Phvul.009G236200	Phvul.009G160900	Phvul.007G162800	Phvul.009G068000	Phvul.005G109100
HRT	LSD	Phvul.001G174200	Phvul.007G192900	Phvul.006G192900
Phvul.004G171600	Phvul.003G230100	Phvul.005G166300	Phvul.005G115500	Phvul.011G110500
HSF	Phvul.002G006500	Phvul.003G222900	Phvul.006G045300	Phvul.005G087400
Phvul.003G283300	Phvul.002G252000	Phvul.008G240700	Phvul.011G084500	Phvul.002G060500
Phvul.002G138500	MICK	Phvul.007G154900	Phvul.007G147600	Phvul.003G214300
Phvul.009G065800	Phvul.008G170400	Phvul.002G269900	Phvul.005G131300	Phvul.002G221000
Phvul.001G022900	Phvul.002G172300	Phvul.008G262900	Phvul.001G179400	Phvul.002G040900
Phvul.009G078300	Phvul.001G263400	Phvul.008G107000	Phvul.008G102300	Phvul.007G023600
Phvul.001G037000	Phvul.008G027900	MYB	Phvul.010G009800	Phvul.008G226600
Phvul.001G154700	Phvul.004G042300	Phvul.007G273400	Phvul.010G009900	Phvul.003G013600
Phvul.007G174800	Phvul.003G182800	Phvul.001G161000	Phvul.006G065700	Phvul.003G036400
Phvul.004G163300	Phvul.010G087900	Phvul.007G108500	Phvul.001G215100	Phvul.001G061200
Phvul.007G278200	Phvul.003G039200	Phvul.001G107600	Phvul.004G029800	Phvul.005G147500
Phvul.007G061800	Phvul.003G281000	Phvul.009G187700	Phvul.008G038000	Phvul.008G146900
Phvul.002G155300	Phvul.011G005800	Phvul.008G041500	Phvul.002G170000	Phvul.004G057800
Phvul.004G119600	Phvul.008G183700	Phvul.003G190400	Phvul.004G012000	Phvul.001G211700
Phvul.006G034200	Phvul.002G147600	Phvul.004G028500	Phvul.008G155700	Phvul.006G061600
Phvul.002G019100	Phvul.009G037300	Phvul.001G221500	Phvul.011G019200	Phvul.003G148200
Phvul.007G223400	Phvul.008G190600	Phvul.007G206200	Phvul.002G139500	Phvul.001G025200
Phvul.002G242000	Phvul.006G169600	Phvul.001G219000	Phvul.003G284000	NAC
Phvul.006G171700	Phvul.005G000900	Phvul.007G208400	Phvul.006G120800	Phvul.009G125900
Phvul.007G067800	Phvul.002G243200	Phvul.004G090900	Phvul.002G184600	Phvul.001G072200
IBD	Phvul.009G203400	Phvul.007G122400	Phvul.004G046000	Phvul.002G275000
Phvul.007G092000	Phvul.001G068200	Phvul.002G088900	Phvul.011G059800	Phvul.009G152900
Phvul.005G137300	Phvul.008G027800	Phvul.003G067800	Phvul.011G212000	Phvul.009G152800
Phvul.011G072200	MT-type	Phvul.004G144900	Phvul.008G102000	Phvul.003G217600
Phvul.011G057500	Phvul.002G170400	Phvul.009G119900	Phvul.010G012500	Phvul.011G005700
Phvul.005G049700		Phvul.011G034900	Phvul.006G217200	Phvul.002G061000

Phvul.011G174300		Phvul.009G158200	Phvul.005G047400	Phvul.005G007900
Phvul.003G260500	Phvul.011G123400	SBP	TCP	Phvul.008G098800
Phvul.003G027100	Phvul.002G307000	Phvul.010G056000	Phvul.006G217800	Phvul.006G179900
Phvul.002G328700	Phvul.004G174000	Phvul.008G157200	Phvul.011G156900	Phvul.003G073400
Phvul.006G181500	Phvul.004G029900	Phvul.003G008800	Phvul.002G086600	Phvul.004G053700
Phvul.010G118700	Phvul.008G001000	Phvul.001G141000	Phvul.009G188100	Phvul.001G106700
Phvul.011G003900	Phvul.002G283600	Phvul.007G258400	Phvul.007G065900	Phvul.007G011600
Phvul.007G085600	Phvul.002G206300	Phvul.007G210600	Phvul.001G082900	Phvul.008G014000
Phvul.005G084600	Phvul.006G208600	Phvul.009G219200	Phvul.001G089100	WRLY
Phvul.002G224900	Phvul.009G156300	Phvul.003G120700	Phvul.003G190900	Phvul.008G039900
Phvul.005G084500	NFYA	Phvul.002G286000	Phvul.006G166600	Phvul.002G091100
Phvul.011G147800	Phvul.010G133300	Phvul.009G165100	Phvul.007G015000	Phvul.002G103400
Phvul.005G047300	Phvul.002G246600	SRS	Phvul.003G003300	Phvul.009G189700
Phvul.006G087000	Phvul.006G062200	Phvul.001G009800	Phvul.003G213300	Phvul.002G089700
Phvul.008G241200	Phvul.008G283100	Phvul.002G166700	Trihelix	Phvul.002G196800
Phvul.005G055400	NFYB	Phvul.009G013700	Phvul.001G258700	Phvul.006G123000
Phvul.006G188900	Phvul.009G155500	Phvul.003G258100	Phvul.007G079600	Phvul.006G147800
Phvul.011G148000	Phvul.002G273800	Phvul.008G200700	Phvul.004G095500	Phvul.002G081600
Phvul.003G045600	Phvul.001G174600	Phvul.006G030400	Phvul.004G095400	Phvul.005G181800
Phvul.002G170200	Phvul.007G163100	STAT	Phvul.007G079700	Phvul.010G161100
Phvul.007G089600	Phvul.007G134000	Phvul.011G173500	Phvul.007G079800	Phvul.002G016100
Phvul.005G044600	Phvul.006G139400	TALE	Phvul.007G000400	Phvul.003G240900
Phvul.L010000	Phvul.008G278900	Phvul.009G004800	Phvul.008G019400	Phvul.001G060200
Phvul.011G024700	Phvul.003G179300	Phvul.001G011000	Phvul.010G000200	Phvul.009G101900
Phvul.002G110900	Phvul.009G201500	Phvul.001G011100	Phvul.003G097500	Phvul.003G139500
Phvul.009G104400	NFYC	Phvul.002G024800	Phvul.008G049300	Phvul.009G233900
Phvul.001G059900	Phvul.006G093200	Phvul.003G250500	Phvul.007G222800	Phvul.008G251300
Phvul.008G194600	Phvul.007G181400	Phvul.001G195800	Phvul.001G187000	Phvul.006G074600
Phvul.006G023100	Phvul.005G050000	Phvul.007G267900	Phvul.007G224400	Phvul.008G090300
Phvul.005G059000	Phvul.009G166000	Phvul.005G119300	Phvul.001G185100	Phvul.010G062500
Phvul.001G100500	Phvul.005G014300	Phvul.011G099100	Phvul.007G224600	Phvul.006G053300
Phvul.004G076900	Phvul.008G204500	Phvul.009G060400	Phvul.004G161800	Phvul.007G177900
Phvul.009G186000	Phvul.011G196500	Phvul.008G114300	Phvul.005G171600	Phvul.001G173700
Phvul.003G189000	NFX1	Phvul.010G011900	Phvul.005G025700	Phvul.008G054100
Phvul.001G100200	Phvul.002G331600	Phvul.002G292000	Phvul.011G205900	Phvul.008G048100
Phvul.004G077400	NIN-like	Phvul.011G082200	Phvul.008G098300	Phvul.007G212900
Phvul.007G140300	Phvul.009G011200	Phvul.005G132700	Phvul.003G209600	Phvul.001G214400
Phvul.005G074500	Phvul.009G115800	Phvul.003G081800	Phvul.009G167400	Phvul.008G058300
Phvul.011G160400	Phvul.011G052100	Phvul.006G145800	VOZ	Phvul.010G104200
Phvul.004G143100	Phvul.007G071900	Phvul.001G032200	Phvul.003G073700	Phvul.001G218500
Phvul.002G316500	Phvul.004G114100	Phvul.003G062500	Phvul.006G180700	Phvul.011G101900
Phvul.004G156700	RAV	Phvul.002G064100	WHIRLY	Phvul.005G116000
Phvul.008G037900	Phvul.003G111800	Phvul.009G090500	Phvul.003G068800	Phvul.002G297100
Phvul.005G122500	Phvul.007G102800	Phvul.009G209500	WOX	Phvul.008G192800
Phvul.011G095500	SAP	Phvul.002G153300	Phvul.002G329500	Phvul.008G275300

Phvul.003G229600	Phvul.008G284100	Phvul.003G213500	Phvul.L002200	Phvul.009G195200
Phvul.005G100100		Phvul.003G174900	Phvul.001G023600	Phvul.001G042200
Phvul.003G192000	Phvul.010G079200			
Phvul.008G043000	Phvul.003G217700			
Phvul.007G075400	ZF-HD			
Phvul.005G005800	Phvul.003G091100			
YABBY	Phvul.004G028600			
Phvul.011G100300	Phvul.003G254200			
Phvul.005G117600	Phvul.008G041300			
Phvul.002G069700	Phvul.008G179200			
Phvul.003G039800	Phvul.009G037900			
Phvul.003G166100	Phvul.002G171000			

APPENDIX IX

Predicted TF families domain in *Phaseolus vulgaris*

Query	PSSM-ID	Accession	Envelop		Alignment		Family	Domain identification	Identity (%)	E value
			Start (bp)	Stop (bp)	From (bp)	To (bp)				
Phvul.001G174400	PF00847.15	CL0081	125	174	125	175	AP2	AP2 domain	53.6	1.80E-14
Phvul.003G144500	PF00847.15	CL0081	148	207	148	208	AP2	AP2 domain	46.1	3.90E-12
Phvul.003G153100	PF00847.15	CL0081	50	109	50	110	AP2	AP2 domain	45.5	6.10E-12
Phvul.003G241900	PF00847.15	CL0081	162	211	162	212	AP2	AP2 domain	53.3	2.10E-14
Phvul.005G138300	PF00847.15	CL0081	136	185	136	186	AP2	AP2 domain	54.3	1.10E-14
Phvul.005G183700	PF00847.15	CL0081	55	114	55	115	AP2	AP2 domain	51.1	1.00E-13
Phvul.008G253600	PF00847.15	CL0081	35	94	35	95	AP2	AP2 domain	51.2	9.60E-14
Phvul.009G206500	PF00847.15	CL0081	45	101	45	102	AP2	AP2 domain	38.8	7.60E-10
Phvul.010G130200	PF00847.15	CL0081	250	309	250	310	AP2	AP2 domain	49	4.90E-13
Phvul.011G058000	PF00847.15	CL0081	162	221	162	222	AP2	AP2 domain	47	2.00E-12
Phvul.011G071100	PF00847.15	CL0081	147	196	147	197	AP2	AP2 domain	53.5	1.90E-14
Phvul.001G193100	PF02362.16	CL0405	70	174	70	180	B3	B3 doamin	98.9	1.00E-28
Phvul.003G011300	PF02362.16	CL0405	7	96	5	98	B3	B3 doamin	55.4	3.60E-15

Phvul.006G079300	PF02362.16	CL0405	339	439	339	440	B3	B3 doamin	64.6	4.90E-18
Phvul.006G114300	PF02362.16	CL0405	134	234	134	236	B3	B3 doamin	71.5	3.30E-20
Phvul.007G230700	PF02362.16	CL0405	81	188	81	189	B3	B3 doamin	101.9	1.10E-29
Phvul.008G122100	PF02362.16	CL0405	584	681	583	685	B3	B3 doamin	68.4	3.20E-19
Phvul.008G244300	PF02362.16	CL0405	140	228	140	231	B3	B3 doamin	53.3	1.60E-14
Phvul.009G064100	PF02362.16	CL0405	128	218	128	219	B3	B3 doamin	55.4	3.60E-15
Phvul.009G260100	PF02362.16	CL0405	112	216	112	217	B3	B3 doamin	101	2.10E-29
Phvul.010G070200	PF02362.16	CL0405	321	420	321	422	B3	B3 doamin	56.4	1.80E-15
Phvul.010G120900	PF02362.16	CL0405	117	221	117	222	B3	B3 doamin	97.8	2.10E-28
Phvul.011G050600	PF02362.16	CL0405	6	95	6	98	B3	B3 doamin	52.7	2.50E-14
Phvul.001G031300	PF00010.21	CL0435	272	320	270	320	HLH	HLH domain	31.1	1.30E-07
Phvul.001G085500	PF00010.21	CL0435	241	283	237	284	HLH	HLH domain	36.1	3.60E-09
Phvul.001G121200	PF00010.21	CL0435	76	122	75	123	HLH	HLH domain	43.1	2.40E-11
Phvul.001G138200	PF00010.21	CL0435	211	258	208	258	HLH	HLH domain	28.8	6.80E-07
Phvul.001G138600	PF00010.21	CL0435	324	369	321	369	HLH	HLH domain	40.2	1.90E-10
Phvul.001G150300	PF14215.1	CL0435	9	191	9	191	bHLH-MYC_N	HLH domain	197.4	1.70E-58
Phvul.001G156400	PF00010.21	CL0435	141	185	139	186	HLH	HLH domain	30.3	2.40E-07
Phvul.001G168700	PF00010.21	CL0435	297	343	294	343	HLH	HLH domain	48.6	4.70E-13

Phvul.002G079400	PF00010.21	CL0435	152	199	149	199	HLH	HLH domain	33.3	2.80E-08
Phvul.002G110500	PF00010.21	CL0435	366	414	364	414	HLH	HLH domain	27.2	2.20E-06
Phvul.002G141500	PF14215.1	CL0435	118	299	118	299	bHLH-MYC_N	HLH domain	180.3	3.10E-53
Phvul.002G271000	PF00010.21	CL0435	151	191	147	192	HLH	HLH domain	26.3	4.10E-06
Phvul.002G282300	PF00010.21	CL0435	278	320	274	321	HLH	HLH domain	37.2	1.70E-09
Phvul.003G026800	PF00010.21	CL0435	169	215	167	216	HLH	HLH domain	44.1	1.20E-11
Phvul.003G033900	PF00010.21	CL0435	218	269	218	269	HLH	HLH domain	38	9.40E-10
Phvul.003G037600	PF00010.21	CL0435	46	95	45	95	HLH	HLH domain	50.1	1.60E-13
Phvul.003G072000	PF00010.21	CL0435	147	194	144	194	HLH	HLH domain	33.3	2.70E-08
Phvul.003G094700	PF00010.21	CL0435	166	213	163	213	HLH	HLH domain	23.8	2.50E-05
Phvul.003G140800	PF00010.21	CL0435	53	95	48	96	HLH	HLH domain	41.5	7.60E-11
Phvul.003G181900	PF00010.21	CL0435	232	281	231	281	HLH	HLH domain	54.9	5.00E-15
Phvul.003G285700	PF14215.1	CL0435	72	252	72	252	bHLH-MYC_N	HLH domain	178.7	9.20E-53
Phvul.003G286400	PF00010.21	CL0435	164	207	159	208	HLH	HLH domain	36.8	2.20E-09
Phvul.005G091000	PF00010.21	CL0435	74	121	71	122	HLH	HLH domain	35.3	6.60E-09
Phvul.005G130500	PF00010.21	CL0435	140	183	134	183	HLH	HLH domain	33.3	2.70E-08
Phvul.006G028500	PF00010.21	CL0435	353	399	350	399	HLH	HLH domain	55.3	3.70E-15
Phvul.006G093900	PF00010.21	CL0435	131	182	131	182	HLH	HLH domain	41	1.10E-10

Phvul.006G114000	PF14215.1	CL0435	52	237	52	237	bHLH-MYC_N	HLH domain	143.1	8.40E-42
Phvul.006G153300	PF00010.21	CL0435	2	49	1	50	HLH	HLH domain	33.9	1.80E-08
Phvul.006G196600	PF00010.21	CL0435	68	112	65	113	HLH	HLH domain	29.7	3.50E-07
Phvul.007G101900	PF00010.21	CL0435	156	202	154	203	HLH	HLH domain	44.9	6.50E-12
Phvul.007G156200	PF00010.21	CL0435	298	344	295	344	HLH	HLH domain	50.4	1.30E-13
Phvul.007G176900	PF00010.21	CL0435	130	174	128	175	HLH	HLH domain	30.4	2.20E-07
Phvul.007G179100	PF14215.1	CL0435	51	227	51	227	bHLH-MYC_N	HLH domain	124.9	3.40E-36
Phvul.007G181900	PF00010.21	CL0435	137	188	137	188	HLH	HLH domain	39.6	3.00E-10
Phvul.007G256800	PF00010.21	CL0435	187	234	184	234	HLH	HLH domain	27.9	1.30E-06
Phvul.007G257100	PF00010.21	CL0435	315	360	311	360	HLH	HLH domain	39.9	2.30E-10
Phvul.008G133600	PF00010.21	CL0435	380	427	377	427	HLH	HLH domain	28	1.20E-06
Phvul.008G144300	PF00010.21	CL0435	297	342	294	342	HLH	HLH domain	48.8	4.00E-13
Phvul.008G158800	PF00010.21	CL0435	107	157	106	157	HLH	HLH domain	47.4	1.10E-12
Phvul.008G177500	PF00010.21	CL0435	119	170	119	170	HLH	HLH domain	41.1	1.00E-10
Phvul.009G023500	PF00010.21	CL0435	212	259	209	259	HLH	HLH domain	30.8	1.60E-07
Phvul.009G033800	PF00010.21	CL0435	120	171	120	171	HLH	HLH domain	42	5.40E-11
Phvul.009G137400	PF00010.21	CL0435	102	153	102	153	HLH	HLH domain	42.6	3.30E-11
Phvul.009G145800	PF00010.21	CL0435	166	209	161	210	HLH	HLH domain	35.7	4.80E-09

Phvul.009G162000	PF00010.21	CL0435	311	353	307	354	HLH	HLH domain	36	3.90E-09
Phvul.009G207000	PF00010.21	CL0435	255	300	253	300	HLH	HLH domain	36.9	2.10E-09
Phvul.009G235700	PF00010.21	CL0435	53	95	48	96	HLH	HLH domain	42.7	3.10E-11
Phvul.010G024800	PF00010.21	CL0435	387	434	384	434	HLH	HLH domain	28	1.30E-06
Phvul.010G114800	PF14215.1	CL0435	51	241	51	241	bHLH-MYC_N	HLH domain	148.2	2.20E-43
Phvul.010G120000	PF00010.21	CL0435	181	232	181	232	HLH	HLH domain	41.9	5.70E-11
Phvul.011G026500	PF00010.21	CL0435	7	56	7	57	HLH	HLH domain	23.1	4.30E-05
Phvul.011G141300	PF00010.21	CL0435	80	127	78	128	HLH	HLH domain	36.8	2.30E-09
Phvul.011G178100	PF00010.21	CL0435	7	56	7	57	HLH	HLH domain	28.9	6.40E-07
Phvul.L009100	PF00010.21	CL0435	318	364	316	365	HLH	HLH domain	39.6	2.90E-10
Phvul.001G119300	PF00170.16	CL0018	274	334	272	335	bZIP_1	bZip doamin	42.2	5.40E-11
Phvul.001G123300	PF00170.16	CL0018	173	215	171	221	bZIP_1	bZip doamin	30.1	3.40E-07
Phvul.001G140100	PF00170.16	CL0018	192	222	189	236	bZIP_1	bZip doamin	33.7	2.60E-08
Phvul.001G189400	PF00170.16	CL0018	308	354	306	356	bZIP_1	bZip doamin	47.4	1.40E-12
Phvul.001G249300	PF00170.16	CL0018	76	107	74	123	bZIP_1	bZip doamin	35.2	8.60E-09
Phvul.002G136000	PF00170.16	CL0018	301	363	301	364	bZIP_1	bZip doamin	69.3	1.90E-19
Phvul.002G248500	PF00170.16	CL0018	244	290	236	291	bZIP_1	bZip doamin	24.5	1.90E-05
Phvul.003G028800	PF00170.16	CL0018	176	218	174	224	bZIP_1	bZip doamin	32.4	6.20E-08

Phvul.003G252000	PF00170.16	CL0018	257	319	257	328	bZIP_1	bZip doamin	74.8	3.70E-21
Phvul.003G273900	PF00170.16	CL0018	308	370	308	380	bZIP_1	bZip doamin	69	2.40E-19
Phvul.003G291800	PF00170.16	CL0018	328	381	326	386	bZIP_1	bZip doamin	42.8	3.60E-11
Phvul.004G053300	PF00170.16	CL0018	233	282	230	286	bZIP_1	bZip doamin	52.2	4.20E-14
Phvul.004G097200	PF00170.16	CL0018	174	233	172	233	bZIP_1	bZip doamin	30.9	1.90E-07
Phvul.005G011600	PF00170.16	CL0018	218	261	216	272	bZIP_1	bZip doamin	48.7	5.30E-13
Phvul.005G068500	PF07716.10	CL0018	178	225	176	230	bZIP_2	bZip doamin	35.3	7.20E-09
Phvul.005G097800	PF07716.10	CL0018	197	245	196	253	bZIP_2	bZip doamin	34.6	1.20E-08
Phvul.005G124100	PF00170.16	CL0018	182	216	180	228	bZIP_1	bZip doamin	32.1	7.80E-08
Phvul.005G153900	PF00170.16	CL0018	219	277	217	278	bZIP_1	bZip doamin	32.2	7.30E-08
Phvul.006G078500	PF00170.16	CL0018	217	275	215	276	bZIP_1	bZip doamin	32.8	4.90E-08
Phvul.006G109700	PF00170.16	CL0018	278	340	278	349	bZIP_1	bZip doamin	69.7	1.40E-19
Phvul.007G003600	PF00170.16	CL0018	157	199	155	205	bZIP_1	bZip doamin	30.1	3.30E-07
Phvul.007G025500	PF00170.16	CL0018	174	205	171	220	bZIP_1	bZip doamin	32.5	5.90E-08
Phvul.007G229600	PF00170.16	CL0018	340	382	338	388	bZIP_1	bZip doamin	44.3	1.20E-11
Phvul.008G169000	PF00170.16	CL0018	75	112	74	130	bZIP_1	bZip doamin	36.2	4.10E-09
Phvul.008G247800	PF00170.16	CL0018	166	218	164	225	bZIP_1	bZip doamin	32.4	6.40E-08
Phvul.009G065500	PF00170.16	CL0018	337	386	336	397	bZIP_1	bZip doamin	45.9	3.90E-12

Phvul.010G108800	PF00170.16	CL0018	281	343	281	344	bZIP_1	bZip doamin	71.2	5.00E-20
Phvul.011G035700	PF07716.10	CL0018	87	135	85	148	bZIP_2	bZip doamin	43.8	1.60E-11
Phvul.011G038200	PF00170.16	CL0018	409	467	406	468	bZIP_1	bZip doamin	34.2	1.80E-08
Phvul.011G053500	PF00170.16	CL0018	154	211	152	213	bZIP_1	bZip doamin	31.6	1.20E-07
Phvul.011G093700	PF00170.16	CL0018	185	225	183	231	bZIP_1	bZip doamin	33.7	2.50E-08
Phvul.011G116800	PF07716.10	CL0018	194	244	193	247	bZIP_2	bZip doamin	40.6	1.60E-10
Phvul.011G186800	PF00170.16	CL0018	253	302	249	306	bZIP_1	bZip doamin	50.7	1.30E-13
Phvul.011G203400	PF00170.16	CL0018	180	222	178	228	bZIP_1	bZip doamin	30	3.50E-07
Phvul.001G026700	PF13912.1	CL0361	70	95	70	96	zf-C2H2_6	CCHH domain	43.6	1.70E-11
Phvul.001G093000	PF13894.1	CL0361	71	94	70	94	zf-C2H2_4	CCHH domain	11.2	0.37
Phvul.001G134900	PF13894.1	CL0361	223	244	222	244	zf-C2H2_4	CCHH domain	18.9	0.0013
Phvul.001G136700	PF13894.1	CL0361	185	204	185	208	zf-C2H2_4	CCHH domain	17.4	0.0038
Phvul.001G176200	PF00096.21	CL0361	65	87	65	87	zf-C2H2	CCHH domain	16.2	0.0095
Phvul.001G204700	PF00096.21	CL0361	84	106	84	106	zf-C2H2	CCHH domain	17.1	0.0047
Phvul.002G028100	PF00096.21	CL0361	61	83	61	83	zf-C2H2	CCHH domain	17.1	0.0047
Phvul.002G082200	PF13912.1	CL0361	12	35	11	37	zf-C2H2_6	CCHH domain	22.1	9.60E-05
Phvul.002G168500	PF13912.1	CL0361	6	29	5	31	zf-C2H2_6	CCHH domain	23.2	4.50E-05
Phvul.002G184700	PF00096.21	CL0361	57	79	57	79	zf-C2H2	CCHH domain	18.6	0.0016

Phvul.002G248200	PF13894.1	CL0361	77	98	77	102	zf-C2H2_4	CCHH domain	10.3	0.72
Phvul.002G332500	PF00096.21	CL0361	57	79	57	79	zf-C2H2	CCHH domain	18.8	0.0014
Phvul.003G008100	PF00096.21	CL0361	73	95	73	95	zf-C2H2	CCHH domain	16.3	0.0084
Phvul.003G008300	PF13894.1	CL0361	73	95	73	96	zf-C2H2_4	CCHH domain	14.6	0.029
Phvul.003G027900	PF13894.1	CL0361	125	144	125	148	zf-C2H2_4	CCHH domain	17.9	0.0027
Phvul.003G185100	PF12171.3	CL0361	299	324	298	324	zf-C2H2_jaz	CCHH domain	42.6	4.20E-11
Phvul.003G252400	PF00096.21	CL0361	58	80	58	80	zf-C2H2	CCHH domain	17.2	0.0045
Phvul.004G084300	PF00096.21	CL0361	267	288	266	288	zf-C2H2	CCHH domain	21	0.00027
Phvul.004G145900	PF00096.21	CL0361	57	79	57	79	zf-C2H2	CCHH domain	18.5	0.0017
Phvul.005G020400	PF13894.1	CL0361	163	182	163	186	zf-C2H2_4	CCHH domain	19.2	0.001
Phvul.005G025300	PF00096.21	CL0361	66	87	66	87	zf-C2H2	CCHH domain	18	0.0025
Phvul.005G099800	PF00096.21	CL0361	60	82	60	82	zf-C2H2	CCHH domain	14.7	0.028
Phvul.005G124800	PF13894.1	CL0361	145	164	145	168	zf-C2H2_4	CCHH domain	21.4	0.00019
Phvul.005G130300	PF13912.1	CL0361	111	136	111	137	zf-C2H2_6	CCHH domain	40.1	2.10E-10
Phvul.006G090800	PF00096.21	CL0361	143	165	143	165	zf-C2H2	CCHH domain	17.1	0.005
Phvul.007G020900	PF00096.21	CL0361	71	93	71	93	zf-C2H2	CCHH domain	16.2	0.009
Phvul.007G090500	PF00096.21	CL0361	261	282	260	282	zf-C2H2	CCHH domain	21	0.00027
Phvul.007G099800	PF00096.21	CL0361	66	88	66	88	zf-C2H2	CCHH domain	16.3	0.0084

Phvul.007G139600	PF13912.1	CL0361	145	170	145	171	zf-C2H2_6	CCHH domain	39.8	2.50E-10
Phvul.007G196100	PF00096.21	CL0361	91	113	91	113	zf-C2H2	CCHH domain	16.1	0.01
Phvul.007G221400	PF00096.21	CL0361	64	86	64	86	zf-C2H2	CCHH domain	16.1	0.01
Phvul.007G253000	PF00096.21	CL0361	257	279	256	279	zf-C2H2	CCHH domain	16.7	0.0066
Phvul.007G255500	PF13894.1	CL0361	204	223	204	227	zf-C2H2_4	CCHH domain	17.3	0.004
Phvul.008G122800	PF13894.1	CL0361	103	122	103	126	zf-C2H2_4	CCHH domain	20.3	0.00044
Phvul.008G143900	PF13912.1	CL0361	4	27	3	28	zf-C2H2_6	CCHH domain	24.5	1.70E-05
Phvul.008G270400	PF00096.21	CL0361	63	85	63	85	zf-C2H2	CCHH domain	20.3	0.00045
Phvul.009G070800	PF13912.1	CL0361	99	124	99	125	zf-C2H2_6	CCHH domain	42.5	3.70E-11
Phvul.010G071300	PF13912.1	CL0361	188	212	187	213	zf-C2H2_6	CCHH domain	36.2	3.50E-09
Phvul.010G138200	PF00096.21	CL0361	88	110	88	110	zf-C2H2	CCHH domain	16.2	0.0096
Phvul.011G058600	PF13894.1	CL0361	171	190	171	194	zf-C2H2_4	CCHH domain	20.7	0.00034
Phvul.011G074100	PF00096.21	CL0361	69	91	69	91	zf-C2H2	CCHH domain	15.2	0.02
Phvul.011G093300	PF13894.1	CL0361	158	177	158	181	zf-C2H2_4	CCHH domain	21.4	0.00021
Phvul.011G123200	PF00096.21	CL0361	66	86	64	86	zf-C2H2	CCHH domain	14.6	0.029
Phvul.011G212900	PF13894.1	CL0361	182	201	182	205	zf-C2H2_4	CCHH domain	24.6	2.00E-05
Phvul.L007800	PF00096.21	CL0361	85	107	85	107	zf-C2H2	CCHH domain	17.1	0.005
Phvul.001G141900	PF14608.1	CL0537	48	59	39	60	zf-CCCH_2	CCHH domain	10.6	0.43

Phvul.002G306100	PF00642.19	CL0537	125	145	121	146	zf-CCCH	C3H domain	23.3	3.50E-05
Phvul.003G102600	PF00642.19	CL0537	48	73	47	73	zf-CCCH	C3H domain	36.5	2.50E-09
Phvul.003G207900	PF00642.19	CL0537	143	165	140	166	zf-CCCH	C3H domain	31.6	8.70E-08
Phvul.003G284200	PF00642.19	CL0537	87	111	87	112	zf-CCCH	C3H domain	24.8	1.20E-05
Phvul.004G122600	PF00642.19	CL0537	100	121	97	121	zf-CCCH	C3H domain	28.7	7.10E-07
Phvul.004G173600	PF00642.19	CL0537	128	149	125	150	zf-CCCH	C3H domain	25.7	6.40E-06
Phvul.005G019900	PF00642.19	CL0537	34	53	32	55	zf-CCCH	C3H domain	26.2	4.30E-06
Phvul.005G020000	PF00642.19	CL0537	4	24	3	26	zf-CCCH	C3H domain	20.7	0.00023
Phvul.005G022000	PF00642.19	CL0537	213	233	212	234	zf-CCCH	C3H domain	21.7	0.00011
Phvul.005G101500	PF00642.19	CL0537	122	142	118	143	zf-CCCH	C3H domain	24	2.10E-05
Phvul.007G033200	PF00642.19	CL0537	281	306	281	307	zf-CCCH	C3H domain	47.5	9.40E-13
Phvul.007G118500	PF00642.19	CL0537	53	79	53	79	zf-CCCH	C3H domain	35.6	5.00E-09
Phvul.007G165200	PF00642.19	CL0537	49	75	49	75	zf-CCCH	C3H domain	35.7	4.60E-09
Phvul.007G254100	PF00642.19	CL0537	276	296	275	297	zf-CCCH	C3H domain	22.3	7.20E-05
Phvul.007G259300	PF14608.1	CL0537	47	59	39	60	zf-CCCH_2	C3H domain	11.8	0.18
Phvul.008G054800	PF00642.19	CL0537	39	65	39	65	zf-CCCH	C3H domain	35.6	4.80E-09
Phvul.008G260900	PF00642.19	CL0537	272	292	271	293	zf-CCCH	C3H domain	22.1	8.20E-05
Phvul.009G169600	PF00642.19	CL0537	159	181	156	182	zf-CCCH	C3H domain	31.8	7.80E-08

Phvul.010G084000	PF00642.19	CL0537	193	218	193	219	zf-CCCH	C3H domain	38.2	7.80E-10
Phvul.011G018600	PF00642.19	CL0537	3	24	2	25	zf-CCCH	C3H domain	31.6	8.70E-08
Phvul.011G078600	PF00642.19	CL0537	67	94	67	94	zf-CCCH	C3H domain	25.7	6.40E-06
Phvul.011G180700	PF00642.19	CL0537	130	154	129	155	zf-CCCH	C3H domain	33.8	1.80E-08
Phvul.011G211700	PF00642.19	CL0537	279	299	278	300	zf-CCCH	C3H domain	22.3	7.30E-05
Phvul.001G009700	PF00643.19	CL0389	44	83	41	87	zf-B_box	Zf B domain	22.1	9.90E-05
Phvul.002G111400	PF00643.19	CL0389	4	33	2	46	zf-B_box	Zf B domain	22.7	6.40E-05
Phvul.003G260100	PF00643.19	CL0389	13	43	11	56	zf-B_box	Zf B domain	22.3	8.80E-05
Phvul.005G076700	PF00643.19	CL0389	54	94	52	99	zf-B_box	Zf B domain	26.5	4.20E-06
Phvul.005G153500	PF00643.19	CL0389	53	86	52	98	zf-B_box	Zf B domain	30.2	3.00E-07
Phvul.009G014100	PF00643.19	CL0389	4	43	2	47	zf-B_box	Zf B domain	22.3	8.30E-05
Phvul.011G038900	PF00643.19	CL0389	4	33	2	46	zf-B_box	Zf B domain	22	0.0001
Phvul.011G053700	PF00643.19	CL0389	4	44	2	47	zf-B_box	Zf B domain	22.6	7.10E-05
Phvul.011G168500	PF00643.19	CL0389	54	94	52	99	zf-B_box	Zf B domain	24.3	2.00E-05
Phvul.002G056300	PF04618.7	CL0389	1	101	1	102	HD-ZIP_N	Zf B domain	96.8	1.10E-27
Phvul.003G223200	PF04618.7	CL0389	1	108	1	110	HD-ZIP_N	Zf B domain	109.8	9.30E-32
Phvul.008G045100	PF04618.7	CL0389	1	98	1	101	HD-ZIP_N	Zf B domain	57.9	1.20E-15
Phvul.010G052200	PF04618.7	CL0389	1	114	1	115	HD-ZIP_N	Zf B domain	69.7	2.60E-19

Phvul.001G080800	PF02701.10	CL0389	101	160	100	162	zf-Dof	Dof domain	122.9	3.30E-36
Phvul.002G170600	PF02701.10	CL0389	122	181	121	183	zf-Dof	Dof domain	126.3	3.00E-37
Phvul.003G182100	PF02701.10	CL0389	39	99	38	100	zf-Dof	Dof domain	123.9	1.70E-36
Phvul.003G189300	PF02701.10	CL0389	118	178	117	179	zf-Dof	Dof domain	125	7.40E-37
Phvul.003G200600	PF02701.10	CL0389	52	111	51	113	zf-Dof	Dof domain	127	1.80E-37
Phvul.003G248500	PF02701.10	CL0389	26	86	25	87	zf-Dof	Dof domain	121.1	1.30E-35
Phvul.006G114900	PF02701.10	CL0389	20	80	19	81	zf-Dof	Dof domain	125.4	5.80E-37
Phvul.008G037000	PF02701.10	CL0389	137	197	136	198	zf-Dof	Dof domain	126.5	2.50E-37
Phvul.008G099400	PF02701.10	CL0389	81	139	79	141	zf-Dof	Dof domain	121.7	8.10E-36
Phvul.009G047500	PF02701.10	CL0389	33	92	32	94	zf-Dof	Dof domain	125.9	3.90E-37
Phvul.009G178300	PF02701.10	CL0389	53	113	52	114	zf-Dof	Dof domain	127.2	1.50E-37
Phvul.009G186600	PF02701.10	CL0389	144	204	143	205	zf-Dof	Dof domain	125.6	4.80E-37
Phvul.009G204000	PF02701.10	CL0389	21	81	20	82	zf-Dof	Dof domain	123.2	2.80E-36
Phvul.010G013500	PF02701.10	CL0389	76	135	74	136	zf-Dof	Dof domain	120.3	2.20E-35
Phvul.011G064800	PF02701.10	CL0389	67	127	66	128	zf-Dof	Dof domain	123.5	2.30E-36
Phvul.011G071900	PF02701.10	CL0389	35	96	34	96	zf-Dof	Dof domain	129	4.20E-38
Phvul.002G039800	PF02319.15	CL0123	91	173	90	173	E2F_TDP	E2F domain	87.3	3.40E-25
Phvul.007G269500	PF02319.15	CL0123	147	212	147	212	E2F_TDP	E2F domain	84.8	2.10E-24

Phvul.009G016300	PF02319.15	CL0123	253	318	253	318	E2F_TDP	E2F domain	84.1	3.60E-24
Phvul.011G020500	PF02319.15	CL0123	70	152	69	152	E2F_TDP	E2F domain	87.9	2.40E-25
Phvul.011G097700	PF02319.15	CL0123	144	209	144	209	E2F_TDP	E2F domain	87	4.30E-25
Phvul.002G253900	PF04873.8	CL0123	28	358	27	359	EIN3	E1N3 doamin	398.4	3.00E-119
Phvul.005G150100	PF04873.8	CL0123	38	355	38	421	EIN3	E1N3 doamin	433.2	7.80E-130
Phvul.006G011200	PF04873.8	CL0123	49	425	49	426	EIN3	E1N3 doamin	518.2	1.10E-155
Phvul.006G011300	PF04873.8	CL0123	50	427	50	428	EIN3	E1N3 doamin	516	5.10E-155
Phvul.008G207000	PF04873.8	CL0123	48	425	48	426	EIN3	E1N3 doamin	514.3	1.70E-154
Phvul.001G022900	PF00447.12	CL0123	25	117	24	118	HSF_DNA-bind	HAF domain	114.3	2.50E-33
Phvul.001G037000	PF00447.12	CL0123	45	136	44	138	HSF_DNA-bind	HAF domain	117.1	3.30E-34
Phvul.001G154700	PF00447.12	CL0123	49	140	48	142	HSF_DNA-bind	HAF domain	110.9	2.80E-32
Phvul.002G019100	PF00447.12	CL0123	10	101	9	103	HSF_DNA-bind	HAF domain	117.4	2.60E-34
Phvul.002G138500	PF00447.12	CL0123	21	112	20	114	HSF_DNA-bind	HAF domain	117.2	3.00E-34
Phvul.002G155300	PF00447.12	CL0123	25	115	24	117	HSF_DNA-bind	HAF domain	114.3	2.60E-33
Phvul.002G242000	PF00447.12	CL0123	15	106	14	108	HSF_DNA-bind	HAF domain	106.6	6.10E-31
Phvul.003G283300	PF00447.12	CL0123	28	119	27	121	HSF_DNA-bind	HAF domain	114.9	1.60E-33
Phvul.004G119600	PF00447.12	CL0123	31	121	30	123	HSF_DNA-bind	HAF domain	113	6.10E-33
Phvul.004G163300	PF00447.12	CL0123	17	108	16	110	HSF_DNA-bind	HAF domain	105.5	1.40E-30

Phvul.006G034200	PF00447.12	CL0123	21	111	20	114	HSF_DNA-bind	HAF domain	112.9	6.60E-33
Phvul.006G171700	PF00447.12	CL0123	15	106	14	108	HSF_DNA-bind	HAF domain	109.8	6.40E-32
Phvul.007G061800	PF00447.12	CL0123	45	137	44	138	HSF_DNA-bind	HAF domain	113.2	5.60E-33
Phvul.007G067800	PF00447.12	CL0123	24	114	23	117	HSF_DNA-bind	HAF domain	115.4	1.10E-33
Phvul.007G174800	PF00447.12	CL0123	44	135	43	137	HSF_DNA-bind	HAF domain	110.9	2.80E-32
Phvul.007G223400	PF00447.12	CL0123	23	115	22	116	HSF_DNA-bind	HAF domain	108.2	1.90E-31
Phvul.007G278200	PF00447.12	CL0123	37	129	36	130	HSF_DNA-bind	HAF domain	112.5	8.80E-33
Phvul.009G065800	PF00447.12	CL0123	25	117	24	118	HSF_DNA-bind	HAF domain	114.7	1.90E-33
Phvul.009G078300	PF00447.12	CL0123	46	137	45	139	HSF_DNA-bind	HAF domain	114.2	2.70E-33
Phvul.001G025200	PF13921.1	CL0123	15	73	15	75	Myb_DNA-bind_6	MYB doamin	57.8	8.40E-16
Phvul.001G061200	PF13921.1	CL0123	40	98	40	98	Myb_DNA-bind_6	MYB doamin	56.7	1.80E-15
Phvul.001G071000	PF00249.26	CL0123	55	102	55	102	Myb_DNA-binding	MYB doamin	53.9	1.30E-14
Phvul.001G107600	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	55.2	5.10E-15
Phvul.001G161000	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	52.4	4.00E-14
Phvul.001G179400	PF00249.26	CL0123	14	62	14	62	Myb_DNA-binding	MYB doamin	56.2	2.40E-15
Phvul.001G211700	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	49.1	4.10E-13
Phvul.001G215100	PF00249.26	CL0123	25	72	25	72	Myb_DNA-binding	MYB doamin	55.7	3.60E-15
Phvul.001G215200	PF00249.26	CL0123	22	69	22	69	Myb_DNA-binding	MYB doamin	58	7.00E-16

Phvul.001G219000	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	54.6	8.10E-15
Phvul.001G221500	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	58.1	6.30E-16
Phvul.001G229900	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	48.6	5.80E-13
Phvul.002G008800	PF00249.26	CL0123	37	77	37	77	Myb_DNA-binding	MYB doamin	34.4	1.70E-08
Phvul.002G015100	PF13921.1	CL0123	8	66	8	67	Myb_DNA-bind_6	MYB doamin	64.2	8.50E-18
Phvul.002G040900	PF00249.26	CL0123	13	60	13	60	Myb_DNA-binding	MYB doamin	60.1	1.50E-16
Phvul.002G060500	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	50	2.10E-13
Phvul.002G083800	PF13921.1	CL0123	142	202	142	202	Myb_DNA-bind_6	MYB doamin	57.9	7.80E-16
Phvul.002G088900	PF13921.1	CL0123	29	89	29	89	Myb_DNA-bind_6	MYB doamin	54	1.30E-14
Phvul.002G139500	PF00249.26	CL0123	15	62	15	62	Myb_DNA-binding	MYB doamin	56.9	1.50E-15
Phvul.002G159700	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	54.6	7.60E-15
Phvul.002G170000	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	52.3	4.00E-14
Phvul.002G184600	PF00249.26	CL0123	76	123	76	123	Myb_DNA-binding	MYB doamin	53.8	1.40E-14
Phvul.002G221000	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	58.4	5.10E-16
Phvul.002G279000	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	49.9	2.40E-13
Phvul.002G292600	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	54.7	7.50E-15
Phvul.002G317000	PF00249.26	CL0123	19	66	19	66	Myb_DNA-binding	MYB doamin	55.6	3.90E-15
Phvul.003G013600	PF00249.26	CL0123	11	55	9	58	Myb_DNA-binding	MYB doamin	33.9	2.40E-08

Phvul.003G036400	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	54.2	1.10E-14
Phvul.003G054100	PF00249.26	CL0123	11	55	9	58	Myb_DNA-binding	MYB doamin	41.1	1.30E-10
Phvul.003G067800	PF13921.1	CL0123	51	111	51	111	Myb_DNA-bind_6	MYB doamin	51.1	1.00E-13
Phvul.003G148200	PF00249.26	CL0123	14	62	14	62	Myb_DNA-binding	MYB doamin	48.2	7.60E-13
Phvul.003G176800	PF00249.26	CL0123	8	55	6	56	Myb_DNA-binding	MYB doamin	27.1	3.00E-06
Phvul.003G190400	PF00249.26	CL0123	17	64	17	64	Myb_DNA-binding	MYB doamin	52.3	4.10E-14
Phvul.003G203900	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	52	5.00E-14
Phvul.003G214300	PF00249.26	CL0123	63	110	63	110	Myb_DNA-binding	MYB doamin	49.6	2.80E-13
Phvul.003G232300	PF00249.26	CL0123	14	62	14	62	Myb_DNA-binding	MYB doamin	51.2	8.90E-14
Phvul.003G240200	PF13921.1	CL0123	16	74	16	74	Myb_DNA-bind_6	MYB doamin	63.2	1.70E-17
Phvul.003G284000	PF00249.26	CL0123	16	63	16	63	Myb_DNA-binding	MYB doamin	60.6	1.00E-16
Phvul.004G012000	PF00249.26	CL0123	44	91	44	91	Myb_DNA-binding	MYB doamin	54.1	1.20E-14
Phvul.004G024200	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	58.4	5.00E-16
Phvul.004G028500	PF00249.26	CL0123	13	60	13	60	Myb_DNA-binding	MYB doamin	55.8	3.40E-15
Phvul.004G029800	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	56.1	2.60E-15
Phvul.004G046000	PF00249.26	CL0123	36	83	36	83	Myb_DNA-binding	MYB doamin	49.6	2.80E-13
Phvul.004G057800	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	42	7.00E-11
Phvul.004G090900	PF13921.1	CL0123	10	70	10	70	Myb_DNA-bind_6	MYB doamin	50.9	1.20E-13

Phvul.004G116500	PF00249.26	CL0123	15	62	15	62	Myb_DNA-binding	MYB doamin	45.5	5.50E-12
Phvul.004G144900	PF13921.1	CL0123	7	67	7	67	Myb_DNA-bind_6	MYB doamin	65.4	3.40E-18
Phvul.005G047400	PF00249.26	CL0123	13	60	13	60	Myb_DNA-binding	MYB doamin	58.1	6.50E-16
Phvul.005G087400	PF00249.26	CL0123	13	60	13	60	Myb_DNA-binding	MYB doamin	65.1	4.00E-18
Phvul.005G109100	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	50.9	1.10E-13
Phvul.005G115500	PF00249.26	CL0123	35	82	35	82	Myb_DNA-binding	MYB doamin	53.2	2.10E-14
Phvul.005G131300	PF00249.26	CL0123	14	62	14	62	Myb_DNA-binding	MYB doamin	51	1.00E-13
Phvul.005G141200	PF00249.26	CL0123	8	55	8	55	Myb_DNA-binding	MYB doamin	51.4	7.80E-14
Phvul.005G147500	PF00249.26	CL0123	16	63	16	63	Myb_DNA-binding	MYB doamin	46.4	2.80E-12
Phvul.005G157600	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	56.8	1.70E-15
Phvul.006G045300	PF00249.26	CL0123	43	90	43	90	Myb_DNA-binding	MYB doamin	55.3	4.70E-15
Phvul.006G061600	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	59	3.40E-16
Phvul.006G065700	PF00249.26	CL0123	30	74	28	76	Myb_DNA-binding	MYB doamin	25	1.40E-05
Phvul.006G120800	PF00249.26	CL0123	25	72	25	72	Myb_DNA-binding	MYB doamin	56	2.80E-15
Phvul.006G192900	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	62.1	3.60E-17
Phvul.006G217200	PF00249.26	CL0123	463	504	462	506	Myb_DNA-binding	MYB doamin	25.3	1.10E-05
Phvul.007G023600	PF00249.26	CL0123	123	167	121	168	Myb_DNA-binding	MYB doamin	44	1.70E-11
Phvul.007G069400	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	62.2	3.40E-17

Phvul.007G108500	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	51.6	6.90E-14
Phvul.007G122400	PF13921.1	CL0123	10	70	10	70	Myb_DNA-bind_6	MYB doamin	50.9	1.20E-13
Phvul.007G132300	PF00249.26	CL0123	22	69	22	69	Myb_DNA-binding	MYB doamin	61.1	7.20E-17
Phvul.007G147600	PF00249.26	CL0123	14	62	14	62	Myb_DNA-binding	MYB doamin	49.7	2.70E-13
Phvul.007G192900	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	48.7	5.50E-13
Phvul.007G206200	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	58.6	4.50E-16
Phvul.007G208400	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	54.4	9.20E-15
Phvul.007G215800	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	51.8	5.70E-14
Phvul.007G231800	PF00249.26	CL0123	51	98	51	98	Myb_DNA-binding	MYB doamin	60.3	1.30E-16
Phvul.007G273400	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	57.6	8.90E-16
Phvul.008G038000	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	55.9	3.20E-15
Phvul.008G038200	PF00249.26	CL0123	10	57	10	57	Myb_DNA-binding	MYB doamin	53.2	2.10E-14
Phvul.008G041500	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	55.8	3.30E-15
Phvul.008G067300	PF00249.26	CL0123	14	62	14	62	Myb_DNA-binding	MYB doamin	52.7	3.10E-14
Phvul.008G102000	PF00249.26	CL0123	60	106	60	106	Myb_DNA-binding	MYB doamin	49.8	2.50E-13
Phvul.008G102300	PF13921.1	CL0123	7	68	7	70	Myb_DNA-bind_6	MYB doamin	51.7	6.40E-14
Phvul.008G146900	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	42.4	5.20E-11
Phvul.008G155700	PF00249.26	CL0123	16	63	16	63	Myb_DNA-binding	MYB doamin	54.2	1.00E-14

Phvul.008G155900	PF00249.26	CL0123	16	63	16	63	Myb_DNA-binding	MYB doamin	54.9	6.10E-15
Phvul.008G226600	PF00249.26	CL0123	126	170	124	171	Myb_DNA-binding	MYB doamin	42.2	5.80E-11
Phvul.008G236500	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	46.6	2.40E-12
Phvul.009G031200	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	59.9	1.80E-16
Phvul.009G068000	PF13921.1	CL0123	14	72	14	74	Myb_DNA-bind_6	MYB doamin	60	1.70E-16
Phvul.009G075000	PF00249.26	CL0123	14	62	14	62	Myb_DNA-binding	MYB doamin	53.8	1.40E-14
Phvul.009G119900	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	53.1	2.30E-14
Phvul.009G158200	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	56.7	1.70E-15
Phvul.009G174900	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	52	5.00E-14
Phvul.009G187700	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	54.2	1.00E-14
Phvul.010G009800	PF13921.1	CL0123	7	68	7	70	Myb_DNA-bind_6	MYB doamin	53.1	2.50E-14
Phvul.010G009900	PF13921.1	CL0123	7	69	7	70	Myb_DNA-bind_6	MYB doamin	48.6	6.10E-13
Phvul.010G012500	PF00249.26	CL0123	58	103	57	103	Myb_DNA-binding	MYB doamin	44.7	9.60E-12
Phvul.010G053200	PF13921.1	CL0123	16	75	16	77	Myb_DNA-bind_6	MYB doamin	47	1.90E-12
Phvul.010G096400	PF00249.26	CL0123	14	62	14	62	Myb_DNA-binding	MYB doamin	52.1	4.90E-14
Phvul.011G019200	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	61.9	4.10E-17
Phvul.011G034900	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	52.8	2.90E-14
Phvul.011G059800	PF00249.26	CL0123	20	67	20	67	Myb_DNA-binding	MYB doamin	43.8	1.90E-11

Phvul.011G084500	PF00249.26	CL0123	14	62	14	62	Myb_DNA-binding	MYB doamin	53.9	1.30E-14
Phvul.011G110500	PF00249.26	CL0123	14	61	14	61	Myb_DNA-binding	MYB doamin	57.4	1.00E-15
Phvul.011G212000	PF13921.1	CL0123	25	84	25	86	Myb_DNA-bind_6	MYB doamin	50.1	2.00E-13
Phvul.002G078600	PF00249.26	CL0123	93	137	91	138	Myb_DNA-binding	MYB doamin	44.2	1.40E-11
Phvul.003G195700	PF00249.26	CL0123	88	132	86	133	Myb_DNA-binding	MYB doamin	44.4	1.20E-11
Phvul.003G222900	PF00249.26	CL0123	99	143	97	144	Myb_DNA-binding	MYB doamin	38.4	8.80E-10
Phvul.003G261500	PF00249.26	CL0123	54	96	53	97	Myb_DNA-binding	MYB doamin	24.5	2.00E-05
Phvul.004G143400	PF00249.26	CL0123	53	97	53	98	Myb_DNA-binding	MYB doamin	53.3	2.00E-14
Phvul.005G039500	PF00249.26	CL0123	23	72	22	73	Myb_DNA-binding	MYB doamin	31.8	1.10E-07
Phvul.005G166300	PF00249.26	CL0123	111	155	109	156	Myb_DNA-binding	MYB doamin	41.5	9.50E-11
Phvul.006G123700	PF00249.26	CL0123	5	56	5	56	Myb_DNA-binding	MYB doamin	35.6	6.60E-09
Phvul.007G154900	PF13921.1	CL0123	453	511	453	511	Myb_DNA-bind_6	MYB doamin	38.6	8.40E-10
Phvul.008G240700	PF00249.26	CL0123	60	104	60	105	Myb_DNA-binding	MYB doamin	51.4	7.80E-14
Phvul.009G183300	PF00249.26	CL0123	129	173	128	174	Myb_DNA-binding	MYB doamin	44	1.60E-11
Phvul.001G059900	PF02365.10	CL0123	30	157	30	158	NAC	NAC domain	177.7	9.90E-53
Phvul.001G072200	PF02365.10	CL0123	9	132	8	133	NAC	NAC domain	167.2	1.80E-49
Phvul.001G100200	PF02365.10	CL0123	8	139	8	140	NAC	NAC domain	172.1	5.30E-51
Phvul.001G100500	PF02365.10	CL0123	16	143	16	143	NAC	NAC domain	186.4	2.00E-55

Phvul.002G061000	PF02365.10	CL0123	7	136	7	136	NAC	NAC domain	180	1.90E-53
Phvul.002G110900	PF02365.10	CL0123	96	225	96	225	NAC	NAC domain	177.2	1.40E-52
Phvul.002G170200	PF02365.10	CL0123	9	134	9	135	NAC	NAC domain	177.7	9.80E-53
Phvul.002G206300	PF02365.10	CL0123	8	127	8	128	NAC	NAC domain	113.2	8.50E-33
Phvul.002G224900	PF02365.10	CL0123	15	142	15	143	NAC	NAC domain	173.2	2.50E-51
Phvul.002G275000	PF02365.10	CL0123	7	131	7	132	NAC	NAC domain	164	1.70E-48
Phvul.002G283600	PF02365.10	CL0123	14	141	14	142	NAC	NAC domain	152.1	8.20E-45
Phvul.002G307000	PF02365.10	CL0123	47	189	47	190	NAC	NAC domain	114.9	2.60E-33
Phvul.002G316500	PF02365.10	CL0123	5	130	5	131	NAC	NAC domain	172.5	4.10E-51
Phvul.002G328700	PF02365.10	CL0123	63	203	63	204	NAC	NAC domain	105.7	1.80E-30
Phvul.003G027100	PF02365.10	CL0123	58	197	58	198	NAC	NAC domain	108.5	2.50E-31
Phvul.003G045600	PF02365.10	CL0123	9	134	9	135	NAC	NAC domain	176.1	3.10E-52
Phvul.003G189000	PF02365.10	CL0123	16	142	16	142	NAC	NAC domain	179	4.00E-53
Phvul.003G217600	PF02365.10	CL0123	7	136	7	136	NAC	NAC domain	179.1	3.70E-53
Phvul.003G229600	PF02365.10	CL0123	6	136	6	137	NAC	NAC domain	169.2	4.30E-50
Phvul.003G260500	PF02365.10	CL0123	6	135	6	135	NAC	NAC domain	178.1	7.40E-53
Phvul.004G029900	PF02365.10	CL0123	20	146	20	146	NAC	NAC domain	180.4	1.40E-53
Phvul.004G076900	PF02365.10	CL0123	17	144	17	144	NAC	NAC domain	181.7	5.60E-54

Phvul.004G077400	PF02365.10	CL0123	9	139	9	140	NAC	NAC domain	171.5	8.20E-51
Phvul.004G143100	PF02365.10	CL0123	6	133	6	134	NAC	NAC domain	176.2	2.90E-52
Phvul.004G156700	PF02365.10	CL0123	8	134	8	134	NAC	NAC domain	176.8	1.90E-52
Phvul.004G174000	PF02365.10	CL0123	47	189	47	190	NAC	NAC domain	115.6	1.60E-33
Phvul.005G007900	PF02365.10	CL0123	7	136	7	136	NAC	NAC domain	180.8	1.10E-53
Phvul.005G044600	PF02365.10	CL0123	10	139	10	140	NAC	NAC domain	175.2	5.80E-52
Phvul.005G047300	PF02365.10	CL0123	6	133	6	134	NAC	NAC domain	181.8	5.20E-54
Phvul.005G055400	PF02365.10	CL0123	11	134	11	135	NAC	NAC domain	165.3	6.70E-49
Phvul.005G059000	PF02365.10	CL0123	25	153	23	154	NAC	NAC domain	158.7	7.50E-47
Phvul.005G074500	PF02365.10	CL0123	15	143	15	143	NAC	NAC domain	179.4	2.90E-53
Phvul.005G084500	PF02365.10	CL0123	14	139	14	140	NAC	NAC domain	165.9	4.50E-49
Phvul.005G084600	PF02365.10	CL0123	16	143	16	144	NAC	NAC domain	171.3	9.70E-51
Phvul.005G100100	PF02365.10	CL0123	47	187	47	188	NAC	NAC domain	103.9	6.60E-30
Phvul.005G122500	PF02365.10	CL0123	4	134	4	136	NAC	NAC domain	154.7	1.30E-45
Phvul.006G023100	PF02365.10	CL0123	19	146	18	147	NAC	NAC domain	161.6	9.50E-48
Phvul.006G087000	PF02365.10	CL0123	8	135	8	136	NAC	NAC domain	178.8	4.60E-53
Phvul.006G181500	PF02365.10	CL0123	63	204	63	205	NAC	NAC domain	109.2	1.60E-31
Phvul.006G188900	PF02365.10	CL0123	18	145	18	146	NAC	NAC domain	172.1	5.30E-51

Phvul.006G208600	PF02365.10	CL0123	8	127	8	128	NAC	NAC domain	124	3.90E-36
Phvul.007G085600	PF02365.10	CL0123	20	146	19	147	NAC	NAC domain	167.9	1.00E-49
Phvul.007G089600	PF02365.10	CL0123	18	146	18	147	NAC	NAC domain	173.7	1.70E-51
Phvul.007G140300	PF02365.10	CL0123	32	159	32	160	NAC	NAC domain	172.4	4.20E-51
Phvul.008G001000	PF02365.10	CL0123	14	138	14	139	NAC	NAC domain	167	2.10E-49
Phvul.008G037900	PF02365.10	CL0123	16	142	16	142	NAC	NAC domain	173.7	1.70E-51
Phvul.008G194600	PF02365.10	CL0123	21	148	20	149	NAC	NAC domain	162	7.20E-48
Phvul.008G241200	PF02365.10	CL0123	5	132	5	133	NAC	NAC domain	183.1	2.10E-54
Phvul.009G104400	PF02365.10	CL0123	27	154	27	155	NAC	NAC domain	175.2	5.70E-52
Phvul.009G125900	PF02365.10	CL0123	7	131	7	132	NAC	NAC domain	168.7	6.00E-50
Phvul.009G152800	PF02365.10	CL0123	7	131	7	132	NAC	NAC domain	170.3	1.90E-50
Phvul.009G152900	PF02365.10	CL0123	7	131	7	132	NAC	NAC domain	169.9	2.50E-50
Phvul.009G156300	PF02365.10	CL0123	8	127	8	128	NAC	NAC domain	102.4	1.90E-29
Phvul.009G186000	PF02365.10	CL0123	16	142	16	142	NAC	NAC domain	181.4	7.00E-54
Phvul.010G118700	PF02365.10	CL0123	15	143	15	144	NAC	NAC domain	168.9	5.40E-50
Phvul.011G003900	PF02365.10	CL0123	8	137	8	137	NAC	NAC domain	173.3	2.20E-51
Phvul.011G005700	PF02365.10	CL0123	7	136	7	136	NAC	NAC domain	180.5	1.40E-53
Phvul.011G024700	PF02365.10	CL0123	15	142	14	143	NAC	NAC domain	151.9	9.40E-45

Phvul.011G095500	PF02365.10	CL0123	9	140	8	142	NAC	NAC domain	136.9	4.00E-40
Phvul.011G123400	PF02365.10	CL0123	49	189	49	190	NAC	NAC domain	106.5	1.00E-30
Phvul.011G147800	PF02365.10	CL0123	14	139	14	140	NAC	NAC domain	166.4	3.10E-49
Phvul.011G148000	PF02365.10	CL0123	17	142	17	143	NAC	NAC domain	173.6	1.90E-51
Phvul.011G160400	PF02365.10	CL0123	22	150	22	150	NAC	NAC domain	177.5	1.10E-52
Phvul.L010000	PF02365.10	CL0123	51	176	50	177	NAC	NAC domain	160.3	2.50E-47
Phvul.002G331600.1	PF01422.12	CL012	388	405	388	406	zf-NF-X1	NF-X1 domain	22.1	0.0001
Phvul.002G246600	PF02045.10	CL0123	111	167	111	167	CBFB_NFYA	NF-X1 domain	102.4	1.30E-29
Phvul.006G062200	PF02045.10	CL0123	175	231	175	231	CBFB_NFYA	NF-X1 domain	102.7	1.00E-29
Phvul.008G283100	PF02045.10	CL0123	150	206	150	206	CBFB_NFYA	NF-X1 domain	103	8.90E-30
Phvul.010G133300	PF02045.10	CL0123	183	239	183	239	CBFB_NFYA	NF-X1 domain	105.2	1.80E-30
Phvul.001G174600	PF00808.18	CL0012	57	121	56	121	CBFD_NFYB_HMF	NF-Y domain	96.8	5.40E-28
Phvul.002G273800	PF00808.18	CL0012	62	126	61	126	CBFD_NFYB_HMF	NF-Y domain	95.4	1.40E-27
Phvul.003G179300	PF00808.18	CL0012	14	79	14	79	CBFD_NFYB_HMF	NF-Y domain	77	8.20E-22
Phvul.006G139400	PF00808.18	CL0012	30	94	29	94	CBFD_NFYB_HMF	NF-Y domain	97.2	4.10E-28
Phvul.007G134000	PF00808.18	CL0012	31	95	30	95	CBFD_NFYB_HMF	NF-Y domain	99.2	9.40E-29
Phvul.007G163100	PF00808.18	CL0012	31	95	30	95	CBFD_NFYB_HMF	NF-Y domain	98.1	2.10E-28
Phvul.008G278900	PF00808.18	CL0012	30	94	29	94	CBFD_NFYB_HMF	NF-Y domain	96.8	5.20E-28

Phvul.009G155500	PF00808.18	CL0012	47	111	46	111	CBFD_NFYB_HMF	NF-Y domain	98.7	1.40E-28
Phvul.009G201500	PF00808.18	CL0012	14	79	14	79	CBFD_NFYB_HMF	NF-Y domain	79	1.90E-22
Phvul.001G141000	PF03110.9	CL0272	69	145	69	147	SBP	SBP domain	137.9	9.30E-41
Phvul.002G286000	PF03110.9	CL0272	94	170	93	171	SBP	SBP domain	132.3	5.20E-39
Phvul.003G008800	PF03110.9	CL0272	88	165	88	166	SBP	SBP domain	129	5.40E-38
Phvul.003G120700	PF03110.9	CL0272	157	234	157	235	SBP	SBP domain	126.2	4.30E-37
Phvul.007G210600	PF03110.9	CL0272	155	232	155	233	SBP	SBP domain	129.1	5.20E-38
Phvul.007G258400	PF03110.9	CL0272	77	153	77	155	SBP	SBP domain	135.4	5.50E-40
Phvul.008G157200	PF03110.9	CL0272	180	257	180	258	SBP	SBP domain	132.8	3.70E-39
Phvul.009G165100	PF03110.9	CL0272	97	173	96	174	SBP	SBP domain	130.1	2.50E-38
Phvul.009G219200	PF03110.9	CL0272	147	224	147	225	SBP	SBP domain	129.7	3.50E-38
Phvul.010G056000	PF03110.9	CL0272	192	269	192	270	SBP	SBP domain	132.9	3.40E-39
Phvul.001G089100	PF03634.8	CL0272	67	149	66	192	TCP	TCP domain	126.1	1.10E-36
Phvul.002G086600	PF03634.8	CL0272	70	194	69	217	TCP	TCP domain	131.9	1.90E-38
Phvul.003G003300	PF03634.8	CL0272	119	254	118	302	TCP	TCP domain	119.4	1.30E-34
Phvul.003G190900	PF03634.8	CL0272	73	244	72	259	TCP	TCP domain	132.9	9.60E-39
Phvul.003G213300	PF03634.8	CL0272	83	187	82	206	TCP	TCP domain	116	1.60E-33
Phvul.006G166600	PF03634.8	CL0272	76	236	75	248	TCP	TCP domain	114.7	3.80E-33

Phvul.006G217800	PF03634.8	CL0272	129	239	127	288	TCP	TCP domain	131.6	2.30E-38
Phvul.007G015000	PF03634.8	CL0272	28	120	27	154	TCP	TCP domain	125.6	1.70E-36
Phvul.007G065900	PF03634.8	CL0272	64	160	63	188	TCP	TCP domain	118.5	2.50E-34
Phvul.009G188100	PF03634.8	CL0272	82	215	81	255	TCP	TCP domain	131.5	2.50E-38
Phvul.011G156900	PF03634.8	CL0272	41	166	40	179	TCP	TCP domain	117.8	4.20E-34
Phvul.001G042200	PF03106.10	CL0274	133	190	132	192	WRKY	WRKY domain	95.5	1.20E-27
Phvul.001G060200	PF03106.10	CL0274	242	299	240	300	WRKY	WRKY domain	98.7	1.10E-28
Phvul.001G173700	PF03106.10	CL0274	196	252	194	252	WRKY	WRKY domain	104	2.60E-30
Phvul.001G214400	PF03106.10	CL0274	160	217	159	218	WRKY	WRKY domain	108.4	1.10E-31
Phvul.001G218500	PF03106.10	CL0274	284	342	283	343	WRKY	WRKY domain	95.7	9.70E-28
Phvul.002G016100	PF03106.10	CL0274	253	310	251	311	WRKY	WRKY domain	99.7	5.60E-29
Phvul.002G081600	PF03106.10	CL0274	143	200	142	201	WRKY	WRKY domain	104.5	1.80E-30
Phvul.002G089700	PF03106.10	CL0274	144	203	144	204	WRKY	WRKY domain	96.5	5.50E-28
Phvul.002G091100	PF03106.10	CL0274	182	238	181	239	WRKY	WRKY domain	101.7	1.30E-29
Phvul.002G103400	PF03106.10	CL0274	170	226	169	227	WRKY	WRKY domain	94.2	3.00E-27
Phvul.002G196800	PF03106.10	CL0274	185	242	184	243	WRKY	WRKY domain	106.2	5.20E-31
Phvul.002G297100	PF03106.10	CL0274	133	193	132	194	WRKY	WRKY domain	93.7	4.20E-27
Phvul.003G139500	PF03106.10	CL0274	243	300	241	301	WRKY	WRKY domain	100.8	2.50E-29

Phvul.003G192000	PF03106.10	CL0274	282	341	282	342	WRKY	WRKY domain	94.4	2.50E-27
Phvul.003G240900	PF03106.10	CL0274	248	305	246	306	WRKY	WRKY domain	99.3	7.40E-29
Phvul.005G005800	PF03106.10	CL0274	313	370	312	370	WRKY	WRKY domain	100.6	2.90E-29
Phvul.005G116000	PF03106.10	CL0274	361	419	360	420	WRKY	WRKY domain	97	4.10E-28
Phvul.005G181800	PF03106.10	CL0274	156	215	156	216	WRKY	WRKY domain	93.9	3.70E-27
Phvul.006G053300	PF03106.10	CL0274	227	284	226	284	WRKY	WRKY domain	101	2.20E-29
Phvul.006G074600	PF03106.10	CL0274	216	273	215	273	WRKY	WRKY domain	105.8	7.20E-31
Phvul.006G123000	PF03106.10	CL0274	264	322	263	323	WRKY	WRKY domain	97.6	2.60E-28
Phvul.006G147800	PF03106.10	CL0274	356	414	355	415	WRKY	WRKY domain	96.4	6.20E-28
Phvul.007G075400	PF03106.10	CL0274	181	239	180	240	WRKY	WRKY domain	95.8	9.40E-28
Phvul.007G177900	PF03106.10	CL0274	292	350	290	350	WRKY	WRKY domain	99.1	8.40E-29
Phvul.007G212900	PF03106.10	CL0274	166	223	165	224	WRKY	WRKY domain	107.8	1.70E-31
Phvul.008G039900	PF03106.10	CL0274	224	281	223	281	WRKY	WRKY domain	104.2	2.20E-30
Phvul.008G043000	PF03106.10	CL0274	190	249	190	250	WRKY	WRKY domain	94.8	1.90E-27
Phvul.008G048100	PF03106.10	CL0274	149	207	149	208	WRKY	WRKY domain	103.4	4.10E-30
Phvul.008G054100	PF03106.10	CL0274	236	292	235	293	WRKY	WRKY domain	102.8	6.00E-30
Phvul.008G058300	PF03106.10	CL0274	158	216	157	217	WRKY	WRKY domain	97.9	2.00E-28
Phvul.008G090300	PF03106.10	CL0274	200	257	199	257	WRKY	WRKY domain	104	2.60E-30

Phvul.008G192800	PF03106.10	CL0274	179	236	178	237	WRKY	WRKY domain	105.4	9.40E-31
Phvul.008G251300	PF03106.10	CL0274	232	289	231	289	WRKY	WRKY domain	106.1	5.90E-31
Phvul.008G275300	PF03106.10	CL0274	229	286	228	286	WRKY	WRKY domain	101.7	1.30E-29
Phvul.009G101900	PF03106.10	CL0274	218	276	217	277	WRKY	WRKY domain	98.6	1.20E-28
Phvul.009G189700	PF03106.10	CL0274	242	300	241	301	WRKY	WRKY domain	94.7	2.10E-27
Phvul.009G195200	PF03106.10	CL0274	163	219	162	220	WRKY	WRKY domain	76.3	1.20E-21
Phvul.009G233900	PF03106.10	CL0274	230	287	228	288	WRKY	WRKY domain	100.9	2.40E-29
Phvul.010G062500	PF03106.10	CL0274	201	258	200	258	WRKY	WRKY domain	98	1.90E-28
Phvul.010G104200	PF03106.10	CL0274	193	251	192	252	WRKY	WRKY domain	99.9	5.00E-29
Phvul.010G161100	PF03106.10	CL0274	160	219	160	220	WRKY	WRKY domain	94.3	2.70E-27
Phvul.011G101900	PF03106.10	CL0274	335	393	334	394	WRKY	WRKY domain	96.6	5.10E-28
Phvul.003G068800	PF08536.6	CL0106	85	223	85	223	WHIRLY	WHIRLY domain	195	3.30E-58
Phvul.002G069700	PF04690.8	CL0114	45	201	44	204	YABBY	YABBY domain	199	6.10E-59
Phvul.003G039800	PF04690.8	CL0114	10	156	9	160	YABBY	YABBY domain	152.2	1.50E-44
Phvul.003G166100	PF04690.8	CL0114	8	159	5	159	YABBY	YABBY domain	200.4	2.30E-59
Phvul.003G217700	PF04690.8	CL0114	11	179	10	179	YABBY	YABBY domain	233.1	2.00E-69
Phvul.005G117600	PF04690.8	CL0114	6	162	2	162	YABBY	YABBY domain	232	4.40E-69
Phvul.010G079200	PF04690.8	CL0114	12	179	11	179	YABBY	YABBY domain	246.3	1.90E-73

Phvul.011G100300	PF04690.8	CL0114	6	160	2	160	YABBY	YABBY domain	223.5	1.80E-66
Phvul.002G171000	PF04770.7	CL0119	60	118	58	119	ZF-HD_dimer	ZF HD domain	109	8.00E-32
Phvul.003G091100	PF04770.7	CL0119	67	124	66	125	ZF-HD_dimer	ZF HD domain	100.4	4.00E-29
Phvul.003G254200	PF04770.7	CL0119	111	168	110	169	ZF-HD_dimer	ZF HD domain	104.5	2.00E-30
Phvul.004G028600	PF04770.7	CL0119	66	123	64	125	ZF-HD_dimer	ZF HD domain	103.9	3.20E-30
Phvul.008G041300	PF04770.7	CL0119	64	120	62	123	ZF-HD_dimer	ZF HD domain	107.6	2.30E-31
Phvul.008G179200	PF04770.7	CL0119	52	108	50	109	ZF-HD_dimer	ZF HD domain	97.7	2.70E-28
Phvul.009G037900	PF04770.7	CL0119	67	122	65	124	ZF-HD_dimer	ZF HD domain	97.3	3.70E-28
Phvul.002G171000	PF04770.7	CL0119	60	118	58	119	ZF-HD_dimer	ZF HD domain	109	8.00E-32
Phvul.003G091100	PF04770.7	CL0119	67	124	66	125	ZF-HD_dimer	ZF HD domain	100.4	4.00E-29
Phvul.003G254200	PF04770.7	CL0119	111	168	110	169	ZF-HD_dimer	ZF HD domain	104.5	2.10E-30
Phvul.004G028600	PF04770.7	CL0119	66	123	64	125	ZF-HD_dimer	ZF HD domain	103.9	3.20E-30
Phvul.008G041300	PF04770.7	CL0119	64	120	62	123	ZF-HD_dimer	ZF HD domain	107.6	2.30E-31
Phvul.008G179200	PF04770.7	CL0119	52	108	50	109	ZF-HD_dimer	ZF HD domain	97.7	2.70E-28
Phvul.009G037900	PF04770.7	CL0119	67	122	65	124	ZF-HD_dimer	ZF HD domain	97.3	3.70E-28

APPENDIX X

List of RNA loading solutions used in experiment

a) RNA loading dye composition (5X)		
Sl. No.	Components	Volume (µl)
1	Saturated aqueous bromophenol blue solution	16.00
2	EDTA (0.5M; pH: 8.0)	80.00
3	Formaldehyde	720.00
4	Glycerol (100 %)	2000.00
5	Formamide	3048.00
6	10X FA gel buffer	4000.00
7	RNase free water	100.00
	Total	10 ml

b) Ethidium bromide : 10 mg/ml in *RNase* free water

Caution: Stored at 4 °C in a dark bottle.

c) Recipe for 1.2 % formaldehyde agarose gel (60 ml)

- : 720 mg Agarose
- : 1 ml Formaldehyde
- : 3 µl Ethidium bromide (10 mg/ml)
- : 60 ml 1X MOPs buffer

d) 10X MOPs electrophoresis buffer

- : 41.8 g MOPs
- : 20 ml Sodium Acetate (DEPC-treated)
- : 20 ml EDTA (DEPC-treated; pH: 8.0) Total volume 1000 ml with DEPC treated water

APPENDIX XI

Specific primer pair sequences of french bean RGAs analyzed in response to rust and *MYMV* disease manifestation using qRT-PCR

Sl. no.	Primer name	Primer sequence (5'-3')	Length (bp)	Tm (°C)	GC (%)	Product size (bp)
1	COHFBRGA1_F	ATGCAGGCCTCTGCAGTC	18	60.1	61.1	163
	COHFBRGA1_R	ACCTCGCGAATGCATCTA	18	57.9	50.0	
2	COHFBRGA2_F	GAGTCAGTGAGCGAGGAAGC	20	60.3	60.0	263
	COHFBRGA2_R	AGCTTGGCGTAATCATGGTC	20	60.1	50.0	
3	COHFBRGA3_F	ACCATGATTACGCCAAGCTC	20	60.1	50.0	245
	COHFBRGA3_R	CAGCAGCAGAAGCACAACTC	20	59.9	55.0	
4	COHFBRGA4_F	CAGGCGACGTCGAGATCTAT	20	60.4	55.0	162
	COHFBRGA4_R	GTGCTGCAAGGCGATTAAGT	20	60.4	50.0	
5	COHFBRGA9_F	GAGTCAGTGAGCGAGGAAGC	20	60.3	60.0	263
	COHFBRGA9_R	AGCTTGGCGTAATCATGGTC	20	60.1	50.0	
6	COHFBRGA25_F	GTCGAGGAAATGGCCAAA	18	59.6	50.0	154
	COHFBRGA25_R	CACAGTCCCAGCAGCAGA	18	59.7	61.1	
7	COHFBRGA26_F	CGAGGAAATGGCCAAAAGTA	20	60.1	45.0	179
	COHFBRGA26_R	CGCTGGAAGAAGAGAAATGC	20	60.1	50.0	
8	COHFBRGA27_F	CGAGGAAATGGCCAAAAGTA	20	60.1	45.0	179
	COHFBRGA27_R	CGCTGGAAGAAGAGAAATGC	20	60.1	50.0	
9	COHFBRGA32_F	CTCCGCCTAGGAGTGAGTTG	20	60.0	60.0	217
	COHFBRGA32_R	GCCGTGCCTAAAGACTGAAC	20	59.9	55.0	
10	COHFBRGA38_F	AACGTCGTGACTGGGAAAAC	20	60.0	50.0	145
	COHFBRGA38_R	AATTTCCATTCGCCATTCAG	20	59.9	40.0	

APPENDIX XII

Specific primer pair sequences of french bean TFs analyzed in response to rust and *MYMV* disease manifestation using qRT-PCR

Sl. no	Family	Sequence ID	Primer name	Primer sequence (5'-3')	Length (bp)	Tm (°C)	GC (%)	Product size (bp)
1	AP2-1	>Phvul.003G241900.1	FBTFPHVL1F FBTFPHVL1R	AGGAATCCGAAGGGTGTCT CGTCCTCCTCTTCGTCCTC	20 19	59.9 59.9	50 63.2	156
2	AP2 -2	>Phvul.003G153100.1	FBTFPHVL2F FBTFPHVL2R	ACTCACAGCTCCGAATCCAG TCGGTGCCTAGTGACTCCTC	20 20	60.4 60.4	55 60	161
3	AP2 -3	>Phvul.003G144500.1	FBTFPHVL3F FBTFPHVL3R	GGAGGAGGCATCTTTGTTCA CGTAACCAGCACTGCTCTCA	20 20	60.2 60.2	50 55	151
4	AP2 -4	>Phvul.005G138300.1	FBTFPHVL4F FBTFPHVL4R	CCGCCACAGTACAGGTTTCT AGCCCTTGCAGCTTCTACTTC	20 21	60.2 60.2	55 52.4	154
5	AP2 -5	>Phvul.001G174400.1	FBTFPHVL5F FBTFPHVL5R	CTCGTATGGGGCAGTTTCTG GGGAAATCACCTCCCCTTC	20 19	60.6 60.6	55 57.9	162
6	B3 -1	>Phvul.010G120900.1	FBTFPHVL6F FBTFPHVL6R	CTGAACCGATTGGTGATTCC TCACATAGCTCTGGCTGCTG	20 20	60.3 60.3	50 55	157
7	B3 -2	>Phvul.010G070200.1	FBTFPHVL7F FBTFPHVL7R	CAGGTCTTCATGTGCTGCTC CTGCCAGAGTCACTTGCATC	20 20	59.6 59.6	55 55	189
8	B3 -3	>Phvul.003G011300.1	FBTFPHVL8F FBTFPHVL8R	GGGGAAGGTGATGTCTGTGT GCAAATGAGGGTTCAAGAGC	20 20	59.8 59.8	55 50	166
9	B3 -4	>Phvul.011G050600.1	FBTFPHVL9F FBTFPHVL9R	GCTGCCTTTATAGGGGAGGT ATTCTGCTGCAAAGGAGGAG	20 20	59.6 59.6	55 50	191
10	B3-5	>Phvul.001G193100.1	FBTFPHVL10F FBTFPHVL10R	AGCGCGAAAGGTGTGTTACT GATGGGTTCGGTGTCTGTG	20 19	59.9 59.9	50 57.9	178

11	BHLH-1	>Phvul.010G024800.1	FBTFPHVL11F	GCAGTATCAGCCCCAACACT	20	60.1	55	150
			FBTFPHVL11R	CAGCTGAGAATTCAGCCACA	20	60.1	50	
12	BHLH-2	>Phvul.009G137400.1	FBTFPHVL12F	ACATCAACGAGTTGCAGCAG	20	60.1	50	173
			FBTFPHVL12R	TGGAGTTCTTGGGCTAATGG	20	60.1	50	
13	BHLH-3	>Phvul.011G141300.1	FBTFPHVL13F	ACTAGCTCCAAGGCATGTCG	20	60.4	55	155
			FBTFPHVL13R	CCCCGTAAGTGTGTCACCA	19	60.4	57.9	
14	BHLH-4	>Phvul.011G178100.1	FBTFPHVL14F	CATCCTGCAGTCATCTCCAA	20	59.8	50	168
			FBTFPHVL14R	GCTCTCCCTGAATGATTTGC	20	59.8	50	
15	BHLH-5	>Phvul.L009100.1	FBTFPHVL15F	AGGCAACACCACAAACACTG	20	59.6	50	155
			FBTFPHVL15R	GCTGTGAATGAGGGATGTCA	20	59.6	50	
16	BZIP-1	>Phvul.003G252000.1	FBTFPHVL16F	GAAAGGCAGGCTCAGGTTC	19	59.9	57.6	173
			FBTFPHVL16R	AGAATTGTTCTGGGCATTGG	20	59.9	45	
17	BZIP-2	>Phvul.003G028800.1	FBTFPHVL17F	GGGACCTTCACCATTCTCT	20	60.3	55	151
			FBTFPHVL17R	ATCTCCGAAGACCCAACATTC	21	60.3	47.6	
18	BZIP-3	>Phvul.009G065500.1	FBTFPHVL18F	ATCCACTGGTGGGTCTTCAG	20	60	55	169
			FBTFPHVL18R	GGCATCTGAGCAACTGCATA	20	60	50	
19	BZIP-4	>Phvul.011G093700.1	FBTFPHVL19F	GAGGCTTTTGCTCTGTCAGG	20	60.1	55	166
			FBTFPHVL19R	AATCAGTGCTTTCCCCTCCT	20	60.1	50	
20	BZIP -5	>Phvul.011G053500.1	FBTFPHVL20F	CCGAGCTTGAAAGGAAAGTG	20	60	50	154
			FBTFPHVL20R	TGCAAGTGAACCTGTTGCTC	20	60	50	