Functional characterization of resistance gene Rvi 12 (Vb) against apple scab

Yousaf A[1,2], Baldi P.[2], Piazza S. [2], Patocchi A.[3], M Malnoy[2]

[1] University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences, Via delle Scienze 206, 3100 Udine, Italy
[2] Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1, 38010 San Michele all'Adige, TN, Italy
[3] Breeding Research, Research Division Plant Breeding, Agroscope, Mueller-Thurgau-Strasse 29, 8820 10 Waedenswil, Switzerland

Introduction

Apple (*Malus domestica*) is one of the most popular fruits, belongs to the *rosaceae* family and considered to be a major useful food source. It is rich in fiber, antioxidants and phytochemicals that have benign effects for human health (Gosse *et a*l, 2005). Apple scab one of the most damaging disease caused by the ascomycete *venturia inaequalis* (Cova *et al*, 2015) which infects all parts of tree and causes characteristic black spot, light green to olive brown lesions, scabby blotches and in severe cases deformation of the fruits (Gessler *et al,* 2006).

The ecological problems and fungicide resistant pathogens are threatening agriculture that's why identification of unique resistant genes and their functional alleles is the key to develop healthy apple cultivars (Desnoues *et a*l, 2018). Expeditious advancement in biotechnological and genetic engineering such as *Agrobacterium* mediated transformation (Song *et al*, 2019), cisgenesis and genome editing has lessen the time needed for fruit breeding by manipulating or inducing single or multiple resistance genes to get desired phenotype and genotype of apple lines. (*Malnoy et al*, 2010; Krens *et a*l, 2015). The Rvi 12 (vb) has been mapped previously from Siberian crab apple 'Hansen's baccata #2' by using molecular markers and subjected to protein domain analysis and real time PCR which indicated LRR receptor like seriene/threonine kinase for Rvi 12 resistance against scab (Padmarasu *et al*, 2018)

Corresponding transcription factors (TFs) to promoter region of resistance gene are also important factors in the response to fungal infection. A number of families of transcription factors such as ERF, bZIP, bHLH, MYBs, NAC and WRKY which helps to engineer pathogen resistance in plants (Amorim *et al*, 2016).

In this study, the DNA sequence of native promoter region of vb was completed and screened through Insilco analysis. The Vb gene was also expressed under its own promoter and 35S in susceptible Gala plants to assess resistance mechanism against scab.

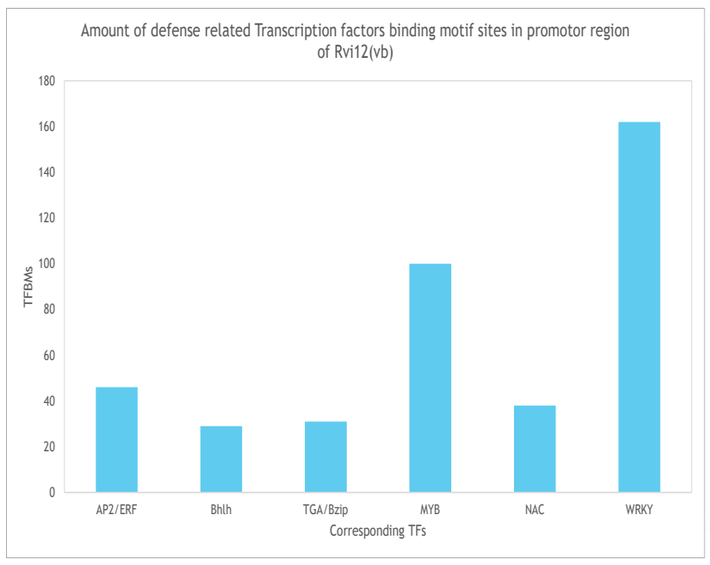

Figure 1: Occurrence of defense related transcription factors binding motifs

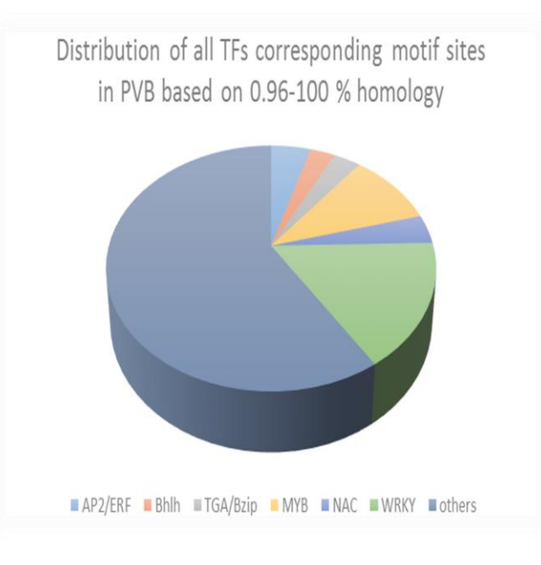

Figure 2: Distribution of all TFs corresponding motifs in promoter

Reference

Dos-Santos, Romulo & Guida-Santos, Mauro & Crovella, Sergio & Iseppon, Ana. (2016). Transcription factors involved in plant resistance to pathogens. Current protein & peptide science. 17. 10.2174/1389203717666160619185308.

Desnous, Elsa, Norelli, J.L., Aldwinckle H.s, Winckle, Michael, Evans, Kate, Melnoy Mickael & Khan Awais (2018) Identification of novel strain specific and environment dependent mirror QTLs lonked to fire blight resistance in apples. Plant molecular biology reporter;36; 10.1007/s11105-018-1076-0.

Jones AL., Aldwinkle HS (1990) Completion of apple and pear diseases. APS Press, St.Paul.

Krens FA, Schaart JG, Van der Burgh AM, Tinnerbroek-Capel IE, Groenwold R, K odde LP, Brogginii GA, Gessler C, Schouten HJ (2015) Cisgenic apple trees; development, characterization and performance. Front Plant Sci; 6:286.

Kost TD, Gessler C, Jänsch M, Flachowsky H, Patocchi A, Broggini GAL (2015) Development of the First Cisgenic Apple with Increased Resistance to Fire Blight. PLoS ONE 10(12): e0143980. https://doi.org/10.1371/journal.pone.0143980.

Melnoy, M., E.E Borejsza-Wysocka, J.L. Norelli, M.A. Flaishmen, D. Gidoni and H.S. Aldwinkle (2010) Genetic transformation of apple (*Malus Domestica*) without use of selectable marker gene. Tree Genet. Genomics; 6:423-433.

Padmarasu S., Sergent D., Patocchi A., Troggio M., Baldi P., & Linsmith, Gareth &poles, Lara &Jansh, M& Kellerhals, Markes & tartarini , S& Velasco, Riccardo (2018) Identification of receptor like serine/threonine kinase as a candidate gene for Rvi 12 (vb) based apple scab resistance. Molecular Breeding; 38.10.1007/s11032-018-0825-y.

Song G-q, Prieto H and Orbovic V (2019) Agrobacterium-Mediated Transformation of Tree Fruit Crops: Methods, Progress, and Challenges. Front. Plant Sci. 10:226. doi: 10.3389/fpls.2019.00226

Contact
Ayesha Yousaf: Ayesha.yousaf@fmach.it
Mickael Malnoy: Mickael.Malnoy@fmach.it

Material and Methods

Promoter Sequence

The 6f11 BAC contig ((Padmarasu *et al*, 2018) from Malus Baccata was used to design set of primers to complete the unknown sequence of promoter region of Rvi 12.

Insilco Analysis of Promoter

Transcription factors, motifs, domains and protein kinase families of promoter reading was performed by using group of different databases online (PlantPan3.0, Blast(n,p), PlantTFDB, UniProt, EMBL-EBI, Pfam, InterPro).

Plant material transformation for cisgenic and transgenic plants

Plant material consisted of in vitro cultivated shoots of susceptible 'Gala' were used for agrobacterium mediated apple Transformation by following Zürich protocol (Kost TD *et al*, 2015).

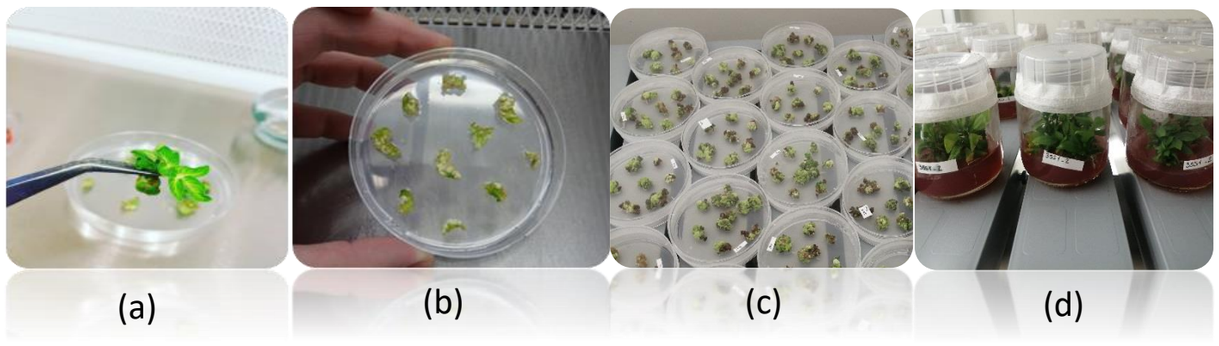

Figure 2: (a) Cutting of 3-4 weeks old shoots of Gala leaves (b) Incubation of at co-culture media in the dark at 25°C for 3 days (c) explants on regeneration media for several weeks (d) Micro-propagation

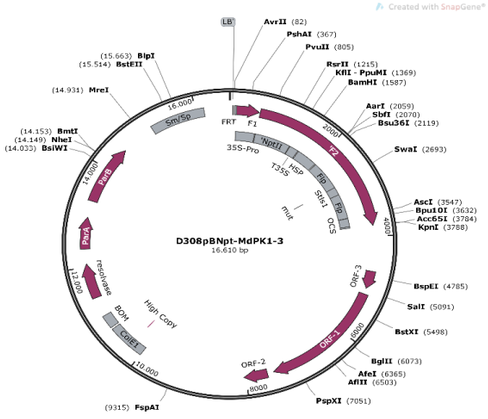

Figure 3a: Vector construct carrying R gene (Vb) with 35S promoter

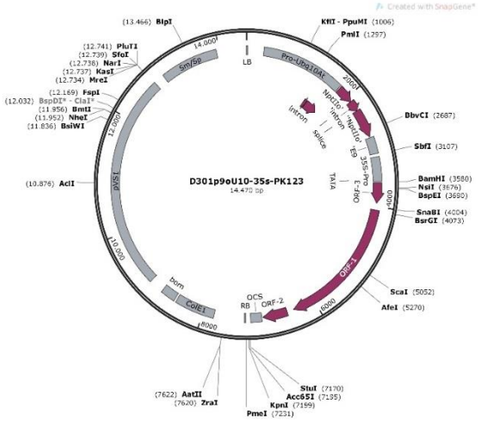

Figure 3b: Vector construct carrying R gene (Vb) with native promoter

Type of transformation	Number of regeneration media plates	Number of successful growth in propagation media jars	DNA extraction and confirmation of positive lines
Transgenic Gala plants (35s)	80	62	32 lines and others in progress
Cisgenic Gala plants (Vb)	80	60	in progress

Table 1: Total number of transformed Gala plants , their regeneration efficiency and confirmed transformed lines

Results and Conclusion

The In-silico analysis of native Promoter sequence of Rvi 12 (Vb) showed resemblance to six major defense related transcription factor binding sites (Fig:2). The highest amount of WRKY motifs were found which are plays essential role in plants defensive mechanisms against biotic and abiotic factors (Fig1). The presence of resistance related proteins indicates the importance of native promoter region in Rvi 12 scab resistance.

Several Gala plants expressed under 35 S promoter or under Vb native promoter have been obtained and are growing in vitro (Fig 3 a,b).

We will undertake the analysis of the copy number of insertion in the genome of the 60 transgenic and cisgenic lines obtained (Table1). Currently, confirmed cisgenic and transgenic lines are being checked to see the copy number of T-DNA and insertion sites by RT-PCR amplifications. The genotypic and phenotypic analysis will be performed to see expression level of gene against scab incidence. Another aspect of this project is to get deep understanding of gene function at target sites and protein-protein interaction between R gene and fungus under transcriptomics and proteomics approaches.