Multi-Omics Integration reveals an overlapping candidate region harboring Auxin responsive factor involve in Flesh Firmness of Watermelon

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INTRODUCTION

Flesh firmness of watermelon (*Citrullus lanatus*) is an important quality trait for commercial fruit values, including fruit storability, transportability, and shelf life. To date, knowledge of the gene controlling this trait is still limited. Herein, we used 214 watermelon accessions representing worldwide distribution, both wild and cultivated species; *Citrullus mucasospermus* (CM), *Citrullus colocynthis* (CC), *Citrullus amarus* (CA), edible seeds (CL-ES), landraces (CL-LR), and improved (CL-IM) varieties for the generation of SNP's, by association analysis we locate a significant SNP on chromosome 6 with high stinginess with flesh firmness trait of watermelon. Consecutively, we developed the F2 population from a cross between a low flesh firmness variety Zhongguojingxin and a high flesh firmness wild variety PI595203 and identified a significant QTL on chromosome 6. Using the Combined approach of GWAS and BSR-Seq analysis, We selected the conserve overlapping interval of 192.48 kb. The candidate genes were screened by the Virus-induced gene silencing (VIGS) method and validated by Transit-overexpression. Our results supported the auxin-responsive gene is the primary candidate gene controlling the flesh firmness trait of watermelon fruit flesh.
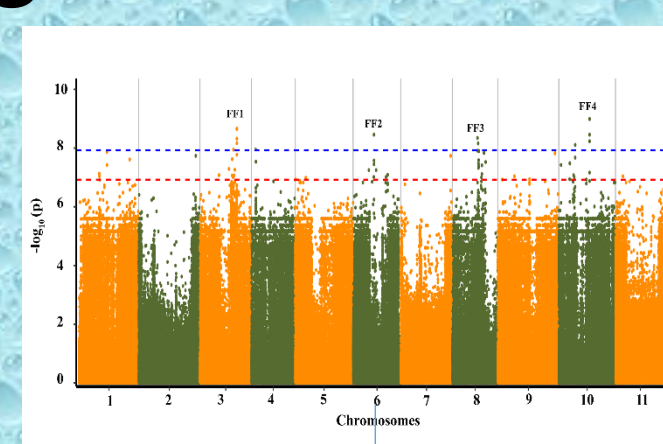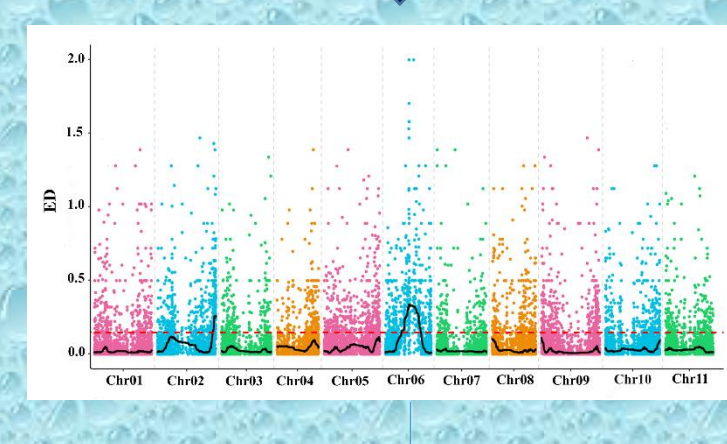
MATERIAL AND METHODS
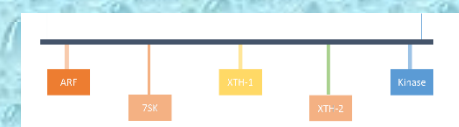
Material
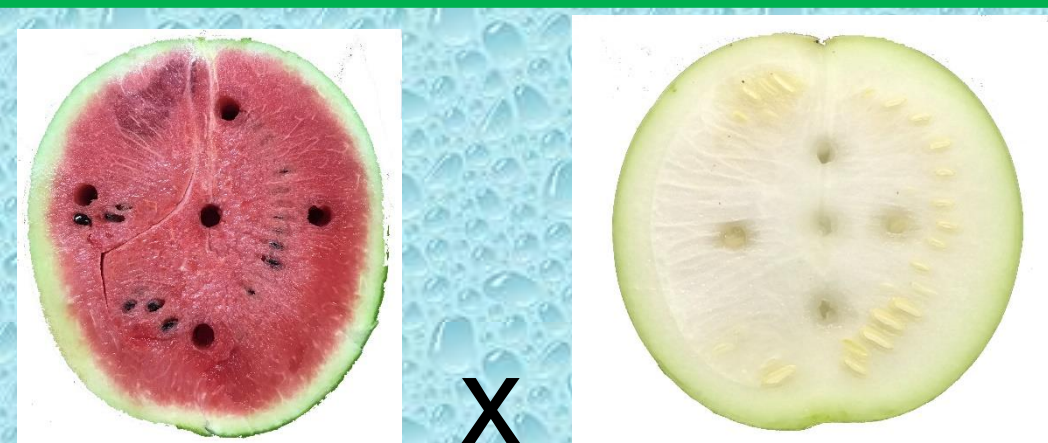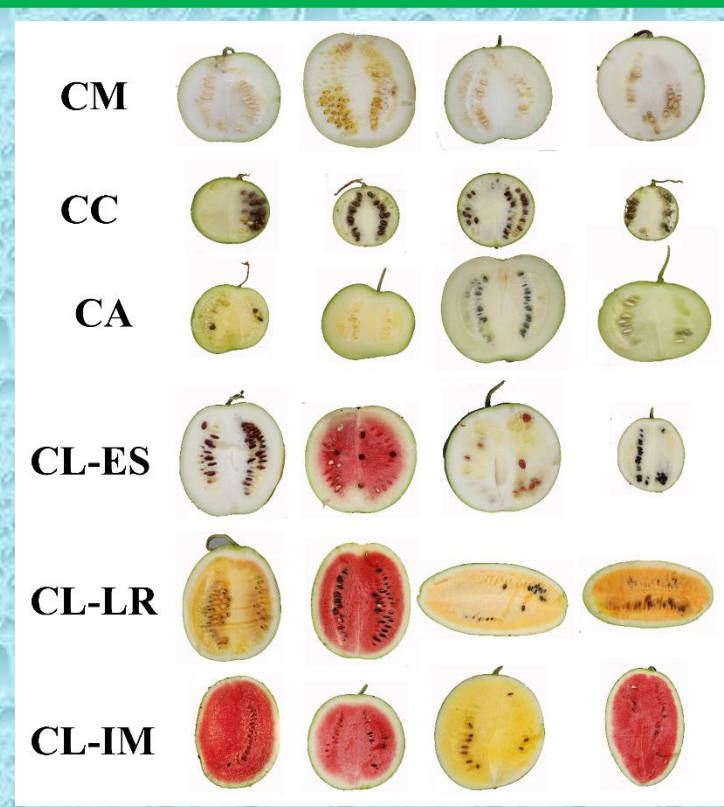
Methods

GWAS

BSR-Seq

F2 [3:1]

Overlapping Candidate region

Functional validations

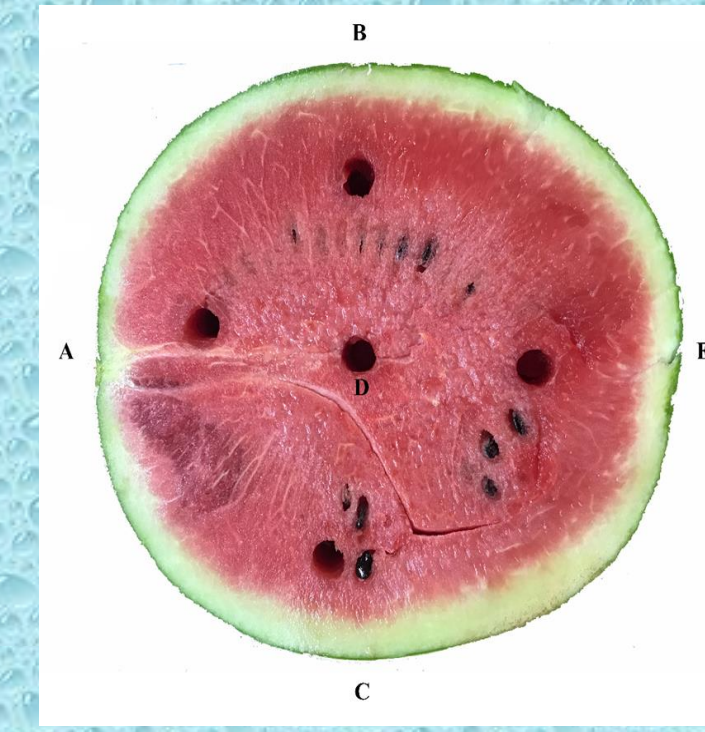## RESULTS

Genome-Wide Association study

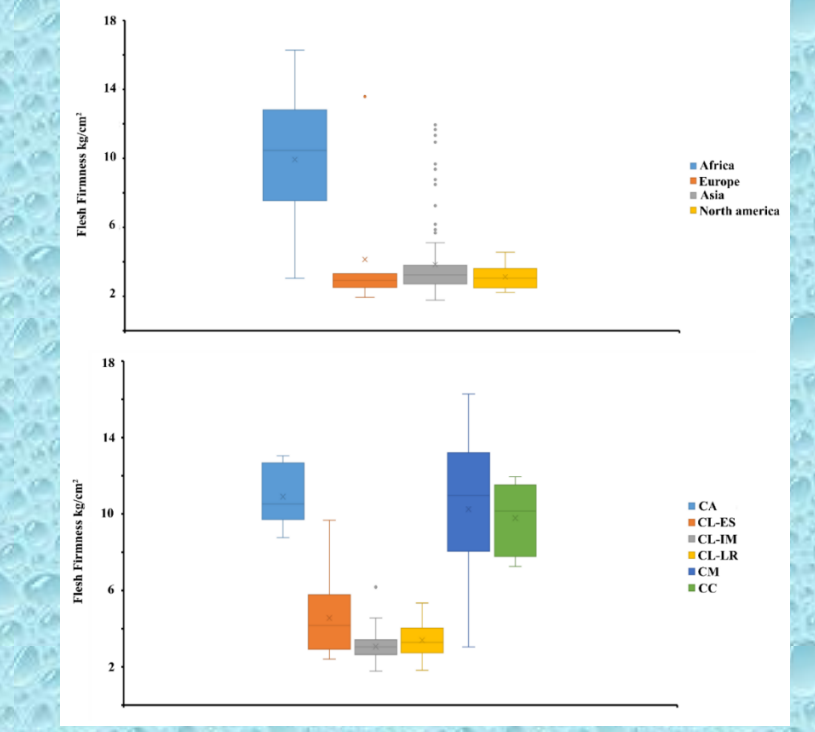**Figure 1.** Measurement of Fruit flesh firmness from five different points

Figure 2. Box plot showing variation in fruit Flesh-firmness of different watermelon species.
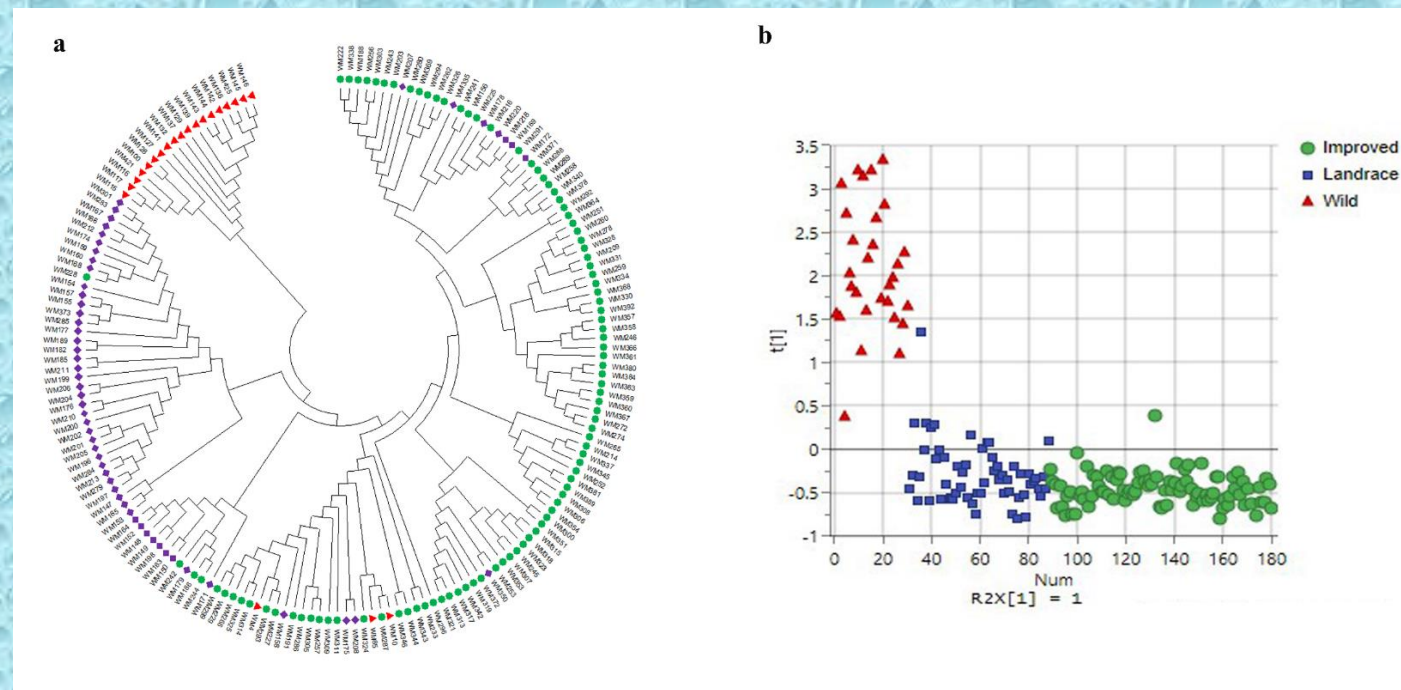

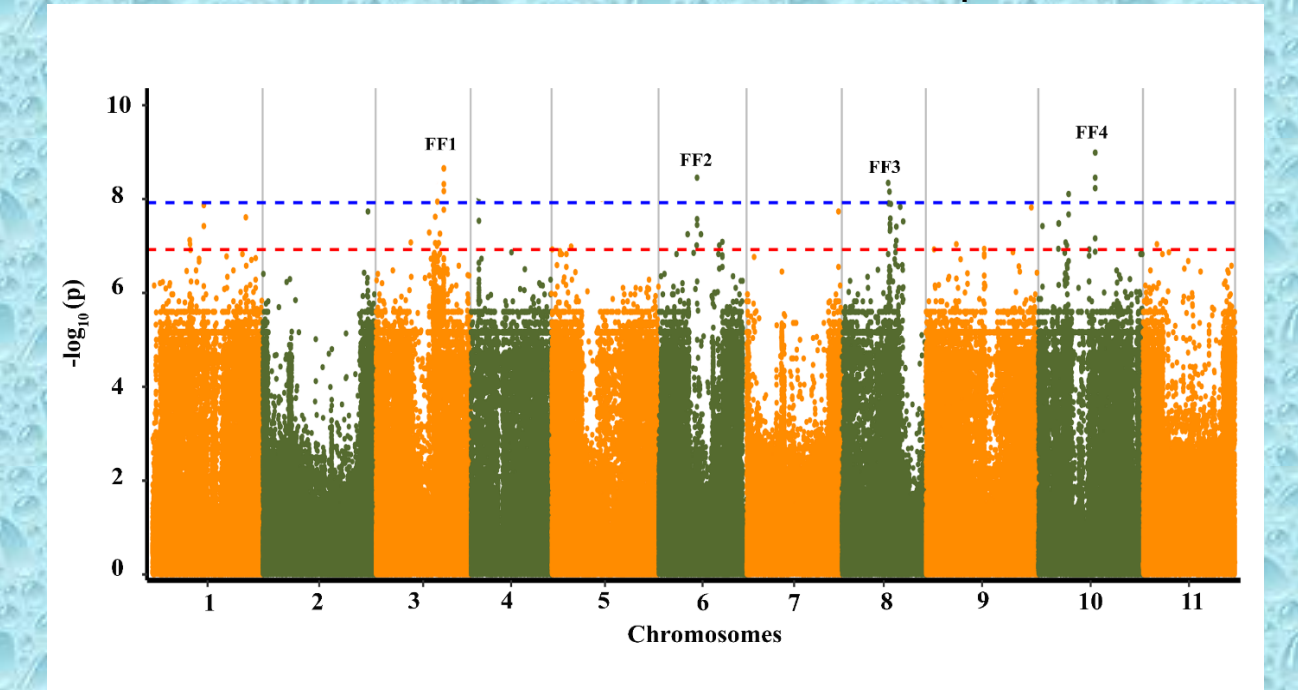**Figure 3.** Neighbor –joining tree on bases of SNP's data sets and PCA/Scattered plot based on phenotype.

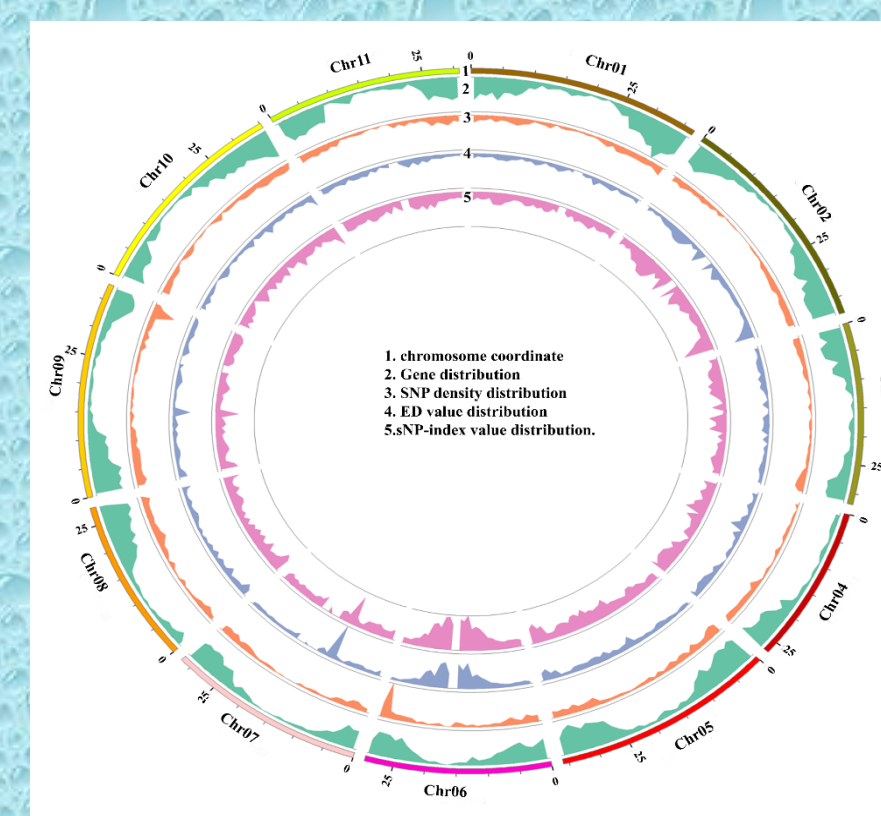**Figure 4.** Association analysis to locate the significant SNP's.

BSR-Seq

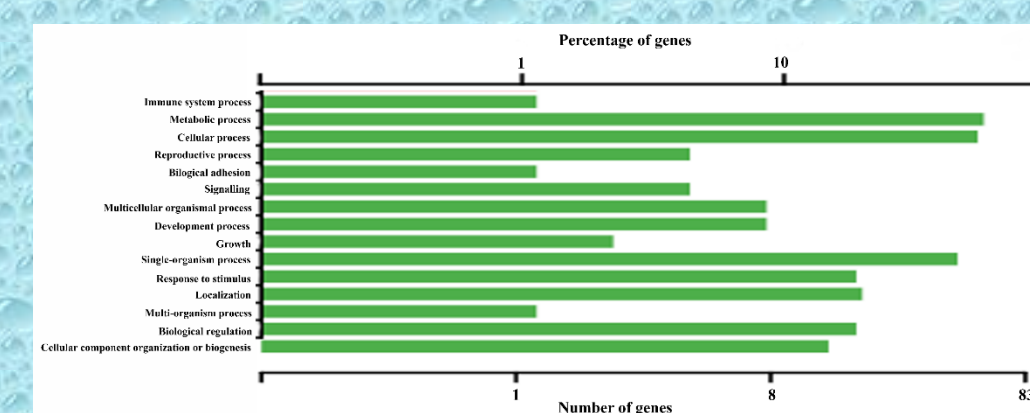**Figure 5 :** CIRCOS plot on the bases of BSR-Seq results.

Figure 6: Significant QTL, physical position, on chromosome 6.

Figure 7: Go analysis of candidate region genes.

Type	Mutation
UPSTREAM	5
SYNONYMOUS_CODING	3
NON_SYNONYMOUS_CODING	7
INTRON	97
INTERGENIC	23
DOWNSTREAM	19
SPLICE_SITE_REGION	2
Other	14

Table 1: candidate region genes mutation types.

Overlapping-Candidate region

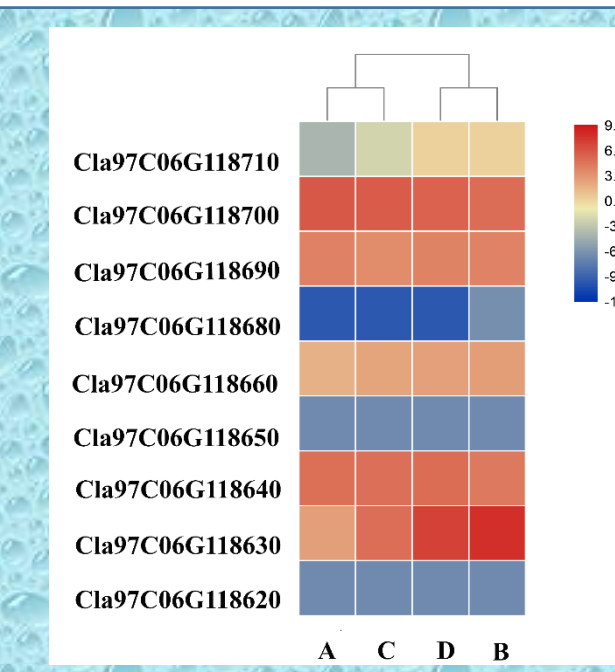**Figure 9** . Expression of candidate genes A) Zhongguojingxin, C) Soft-pool, B) PI595203 D) Hard-pool.
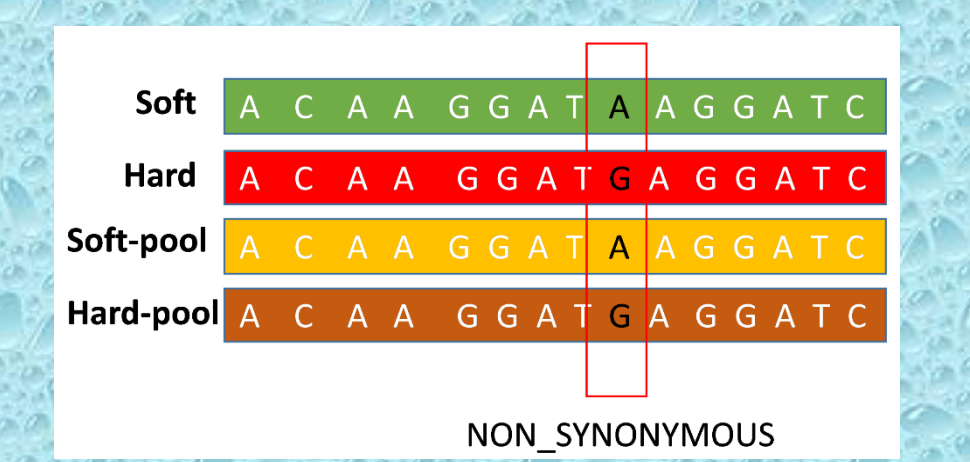
Figure 10. Mutation in candidate gene

Figure 8: Overlapping conserve region between GWAS and BSR-Seq.

CONCLUSION

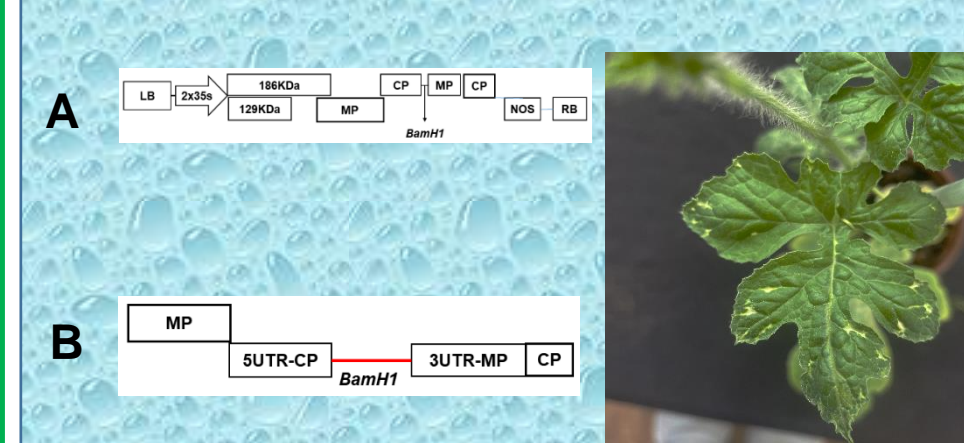**Virus-induced gene silencing (VIGS)**

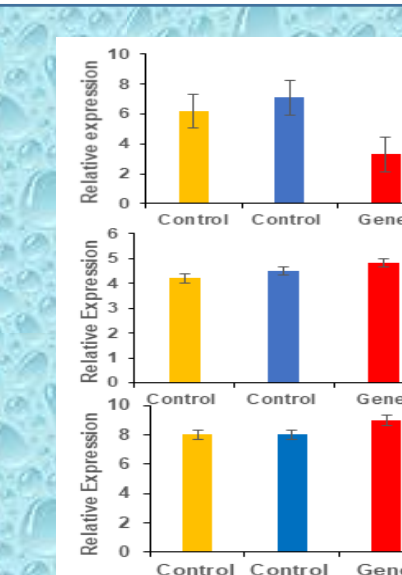
Figure 11: VIGS vectors

Figure 12: Effectiveness of vector

Figure 13: Inoculated in seedlings
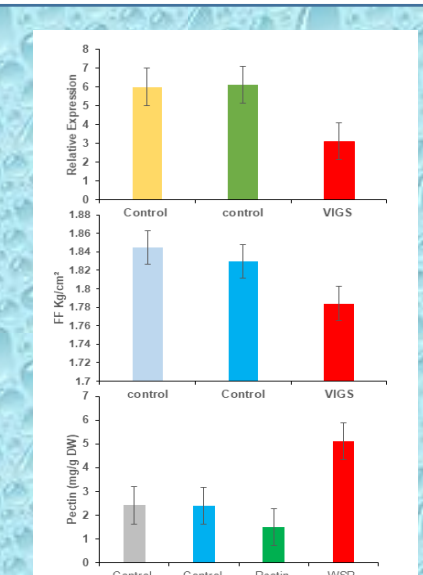
Figure 14: Relative expression of genes
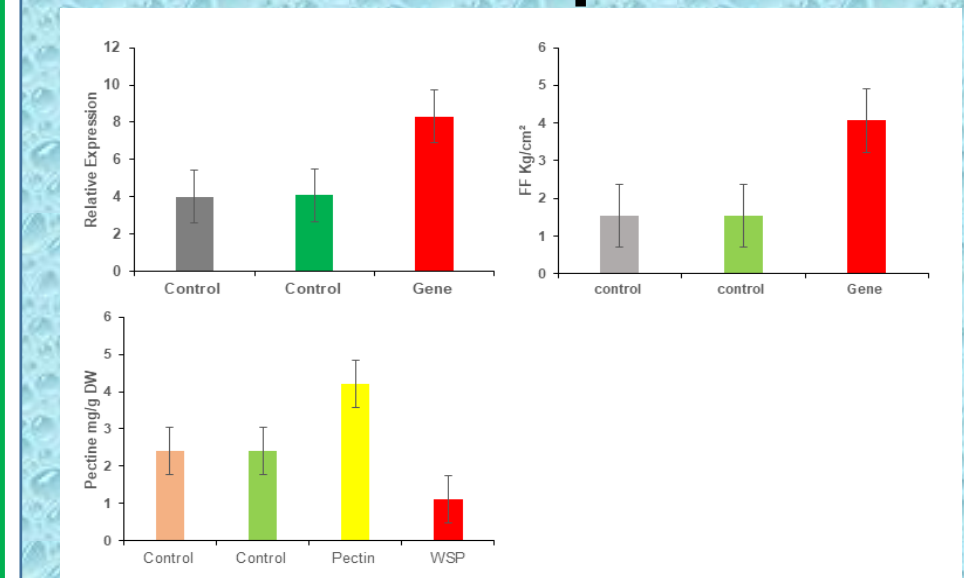
Figure 15: Inoculation of candidate gene

Figure 16: Affect of inoculation on fruits

Transit-Overexpression

Figure 17: Relative expression and affect of overexpression of gene

➢ The candidate gene are selected and screened by Virus-induced gene silencing method.
➢ From the results we select the ARX gene as candidate gene.
➢ The gene affects the content metabolites of cell wall; total pectin and WSP
➢ The results showed relatedness of this gene with total pectin, high expression inactive the sensitiveness of fruits to ethylene and Flesh firmness is high.
➢ Low expression boost the pectin degradation and increased WSP, Flesh firmness decreased.