VISVESVARAYA TECHNOLOGICAL UNIVERSITY

BELGAUM

A Project report on

"Demonstration of RNAi technique Using Phytoene Desaturase in Tomato Plant"

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ABSTRACT

RNA interference (RNAi) is a process that inhibits gene expression by double stranded RNA (dsRNA) that can cause the degradation of target messenger RNA (mRNA). RNAi has become one of the most exciting discoveries in biology, for its high specificity, accuracy & hereditability.

The phenomenon of RNAi was firstly reported for its co-suppression of homologous genes in transgenic plants, in which attempts were made to deepen the purple colour of flowers. After introducing a pigment producing gene under the control of a powerful promoter many of the flowers appeared variegated or even white not at all the results expected. This phenomenon was called "Co-suppression". From then on co-suppression has been found to occur in many plant species. It also exists in fungi & in Neurospora crassa where it is known as "quelling" The mechanisms of RNAi consist of two steps. First the ds RNA is processed into small interfering RNA's of 21-23 nucleotides by an RNase iii-like enzyme called Dicer(initiation step) & is then progressively cleaved in an ATP-dependent type. Then the siRNA assemble into an endo-ribonuclease containing complexes known as RNA induced silencing complexes (RISC's). Subsequently the duplex RNA unwinds& leaves the antisense strand to guide the RISC's to complementary mRNA where they cleave & destroy the cognate RNA. Tomato plants contain Phytoene Desaturase (Pds) gene. A modular construct for Pds gene is developed to demonstrate RNAi technology. We isolated Phytoene desaturase gene from tomato plant by using gene specific primers ,then we constructed the vector in such a way that it is having sense as well as anti-sense orientation .To increase the RNAi efficiency we inserted intron between sense exon and anti sense exon and the total construct was cloned in 35s cassette.

35S Promoter	EXON(sense)	INTRON	EXON(antisense)	NOS Terminator

This total cassette introduced into binary vector (pGreen) for plant transformation .From this binary vector is electroporated (transfered) into Agrobacterium for indirect plant transformation.

Key Words: RNAi, co-suppression, quelling, binaryvector, phytoene desaturase.