



## SILENCING OF THIOREDOXIN TYPE *H1* BASED ON VIGS IN PEPPER PLANTS (*Capsicum annuum* cv ANAHEIM) INFECTED WITH EUMV-YP

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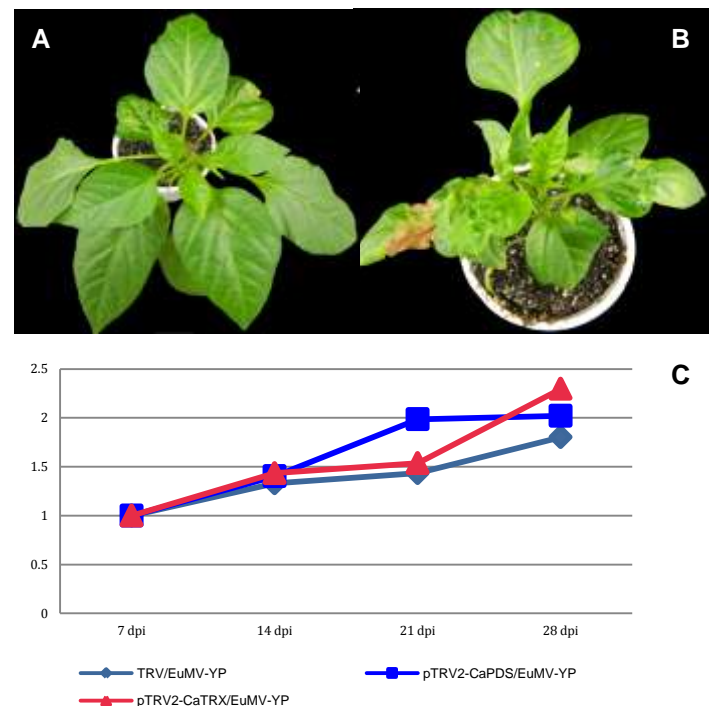
**Key words:** Thioredoxin *h1*, *Euphorbia mosaic virus Yucatan Peninsula*, Non-expressor of PR genes 1.

**Introduction.** Thioredoxins (Trxs) are proteins involved in disulfide bond reduction, with a conserved WCG/PPC motif. In plants, the Trxs appear to play a fundamental role in plant tolerance of oxidative stress, avoiding the oxidative damage by supplying reducing power (1,2). Furthermore, they could act as regulators of scavenging mechanisms, or like components of signaling pathways in the antioxidant network (2,3,4). Based on a previously reported Trx *h* sequence from pepper (5), a set of primers were designed to amplify and clone a 197 pb fragment from *C. annuum* cv Anaheim. The obtained sequence shared 99% of nucleotide identity with the reported Trx *h* (GenBank accession No. EF371503). And was evaluated the expression on leaf, stem and root, showing more expression on leaf tissue. Virus-induced TRX-*h1* gene silencing using the Tobacco rattle virus (TRV) as VIGS vector in pepper plants, and after the same plants were infected with the begomovirus *Euphorbia mosaic virus Yucatan Peninsula* (EuMV-YP). The symptoms in plants with the Trx *h1* silenced are more severe compare with the controls. And the preliminary results evaluating the relative expression of Non-expressor of PR genes 1 (*NPR1*) during a temporal course shows on silencing plants of Trx *h1*, that this expression decrease and the viral replication increase. These results suggest that, *NPR1* and Trx *h1* are correlated during the interaction with EuMV-YP. The main objective of this work is analyze Trxs *h1* role in pepper plants (*Capsicum annuum* (cv Anaheim) during the oxidative stress inflicted in the interaction with EuMV-YP.

**Methods.** Cloning the sequence corresponding to 197 bp of Trx *h1*, we elaborate the protocol that involves agro-infiltration of the tobacco rattle virus-based VIGS vectors carrying the fragment of Trx *h1* gene from *C. annuum* cv Anaheim into seedlings at the two- to four-leaf stage. After two weeks the plants were inoculated by biobalistic method using the begomovirus EuMV-YP as the pathogen. During a temporal course going to be measure the gene expression by Real-Time PCR for Trx *h1* and *NPR1*, and quantify the biochemistry reaction of catalase, peroxidase, super oxide dismutase, salicylic acid and hydroxide peroxide.

**Results.** Evaluating by Real-Time PCR the pattern of expression of Trx *h1* on the different tissues from 30-d-old plants, shows a higher accumulations of the transcripts on leaves, more than in the roots or stem tissue. Also the gene expression for *NPR1*, shows in the temporal course a decrees in the relative expression on the silenced plant

with Trx *h1*, observing a differential behave with the other treatments. The viral replication increase in the treatment with the silenced Trx *h1*. The symptoms on the plants can be related with this viral accumulation, because the silenced plants had severe symptoms compared with the healthy plants or the treatment agroinoculated/inoculated with the TRV VIGS/EuMV-YP.



**Fig. 1** *C. annuum* inoculated with the TRV VIGS/EuMV-YP and viral replication. A) TRV:00/EuMV-YP and B) pTRV2-CaTrx/EuMV-YP. C) Viral replication in a temporal course.

**Conclusions.** This suggest that Trx *h1* maybe is correlated with the defense to the begomovirus EuMV-YP, showing severe symptoms affected on the silenced plants, and the gene expression indicates that could be correlated to *NPR1*.

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