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Identification of two novel broccoli genes useful for the development of salt- and drought-resistant plants

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1 INTRODUCTION

Climate change presents an imminent global challenge. The increasing occurrence of soil salinization and drought pose substantial threats to agricultural productivity and crop yields. Consequently, exploring the potential of salt- and drought-resistant plants becomes crucial, as they can thrive in harsh environments characterized by limited water availability and high salt concentrations.

Broccoli (*Brassica oleracea var. italica*) is rich in vitamins A, B6, B9, C, K, fibre, and minerals, such as iron, calcium and potassium. It also contains glucosinolates and flavonoids, with antioxidant and anti-inflammatory properties.

The aim of this study is to identify novel broccoli genes that can be used to develop transgenic broccoli plants capable of withstanding salt and drought stress.

2 GENE LIBRARY AND SCREENING

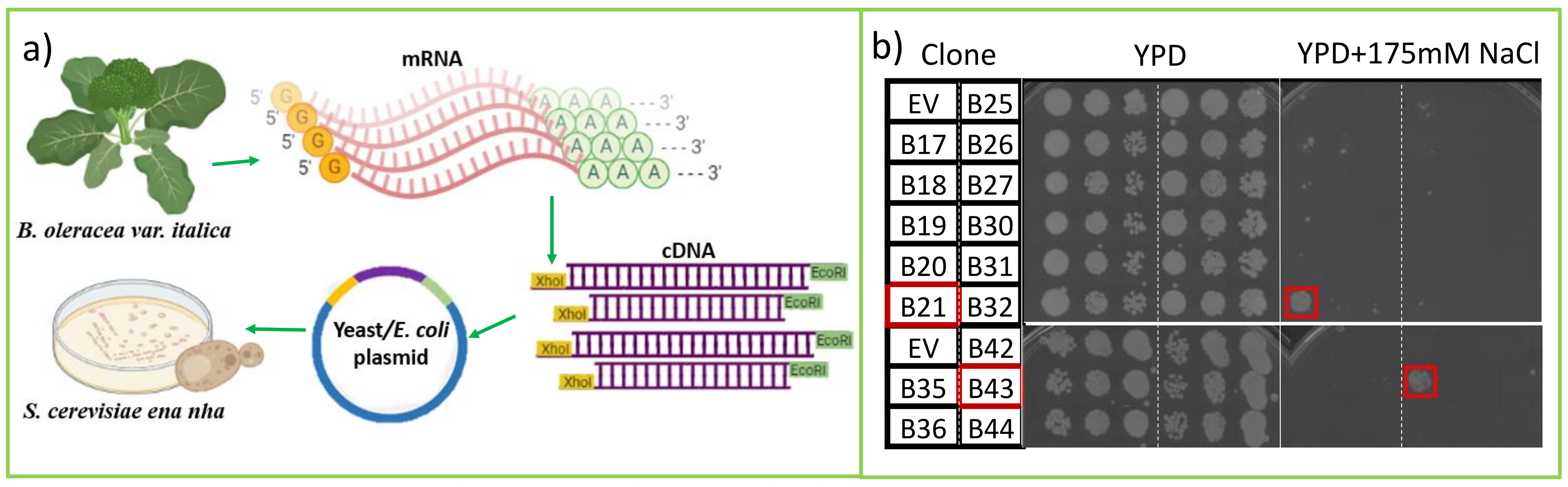


Figure 1. Gene library and novel gene identification. a) Schematic representation of the workflow followed for the gene identification using a broccoli cDNA library. b) Growth assays showing the phenotype of two positive clones in selective medium: B21 and B43 correspond to Candidate1 (340) and Candidate2 (470), respectively.

3 ALPHAPHOLD2 3D STRUCTURE

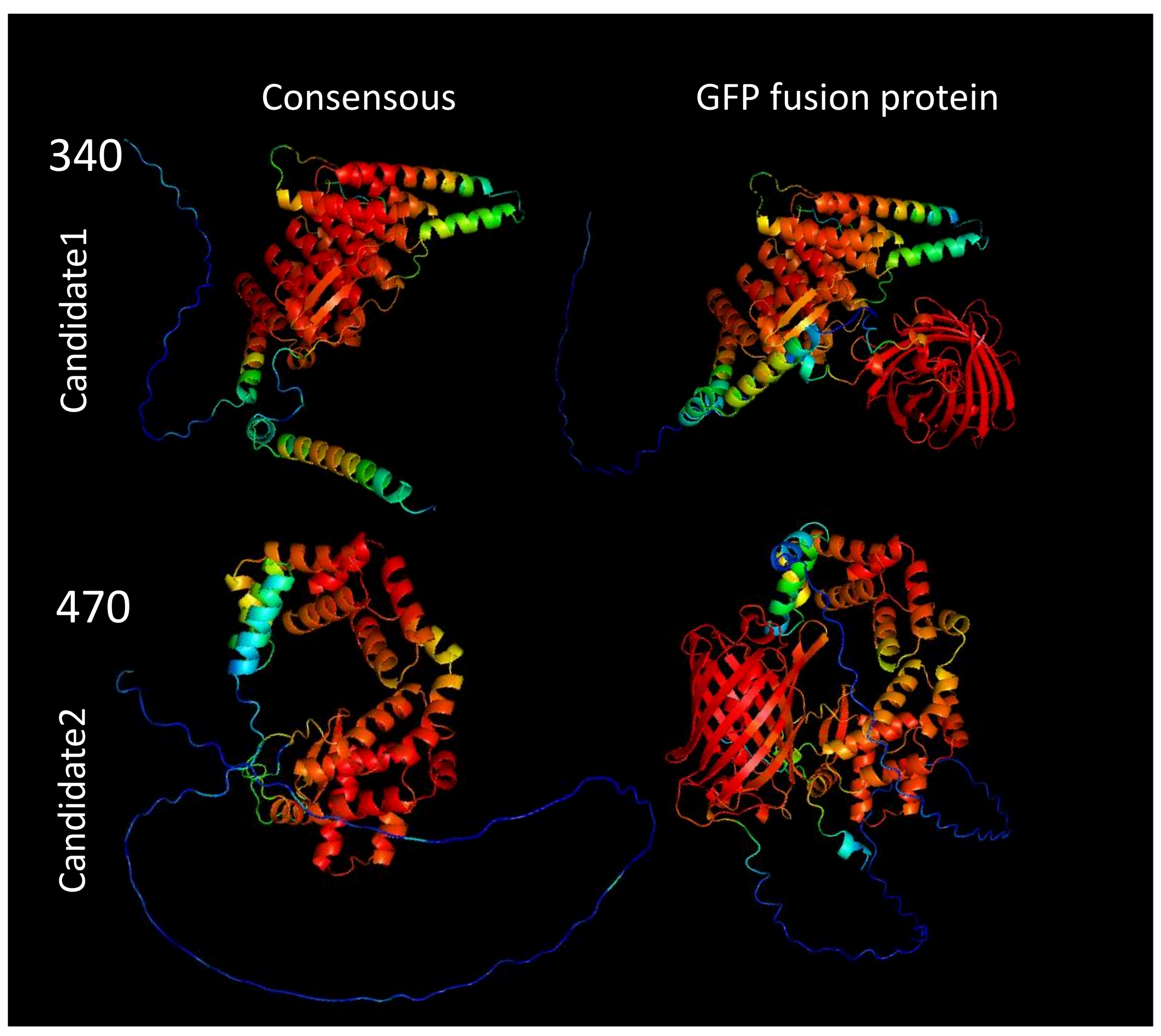
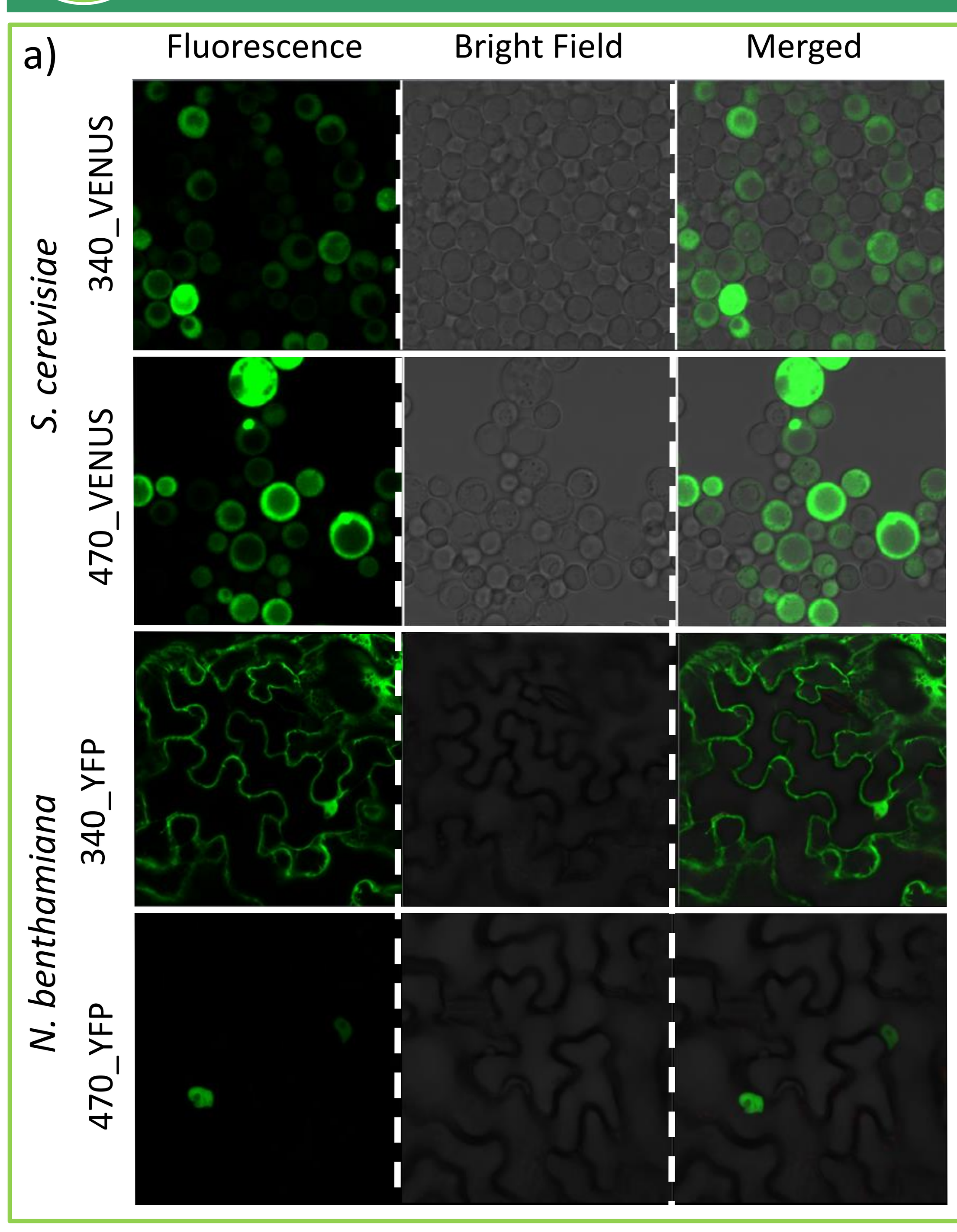
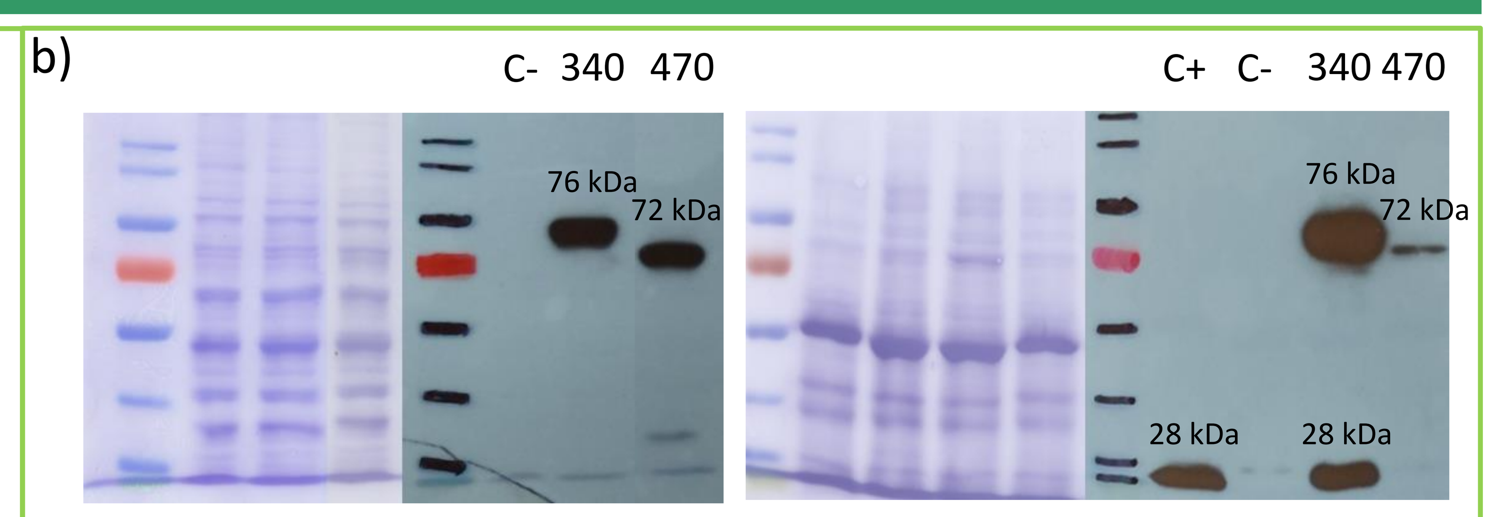


Figure 2. 3D representation of the proteins using Alphafold2 algorithm. Different colors represent different degrees of confidence for the predicted structure (LDDT; red represents 100% confidence and blue is for values below 10%).

4 TRANSIENT TRANSFORMATION IN YEAST, NICOTIANA AND BROCCOLI





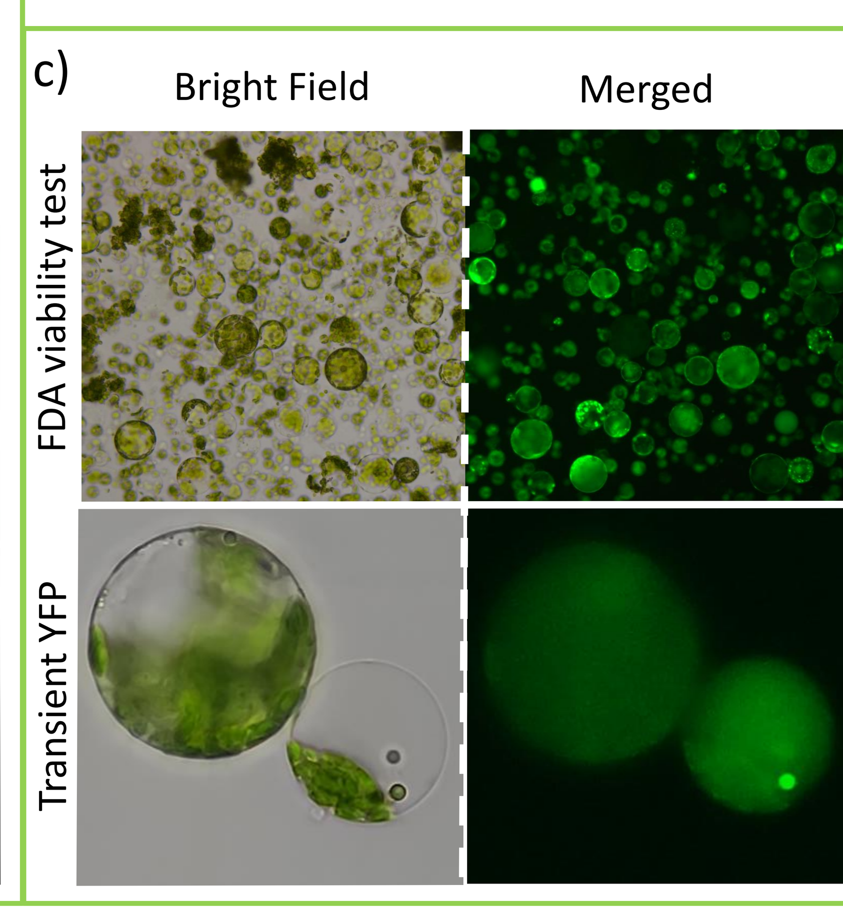


Figure 3. Protein subcellular localization and immunoblotting assays. a) Fluorescence microscopy images of the GFP-fusion proteins in yeast (upper) and plant leaves (lower). b) Immunoblotting using anti-GFP antibody of yeast extracts (left) and plant leaf extracts (right). c) Broccoli protoplast isolation and FDA viability test (upper), and protoplast transient transformation assay for the overexpression of YFP (lower).

5 GENE EXPRESSION IN RESPONSE TO SALINITY AND DROUGHT

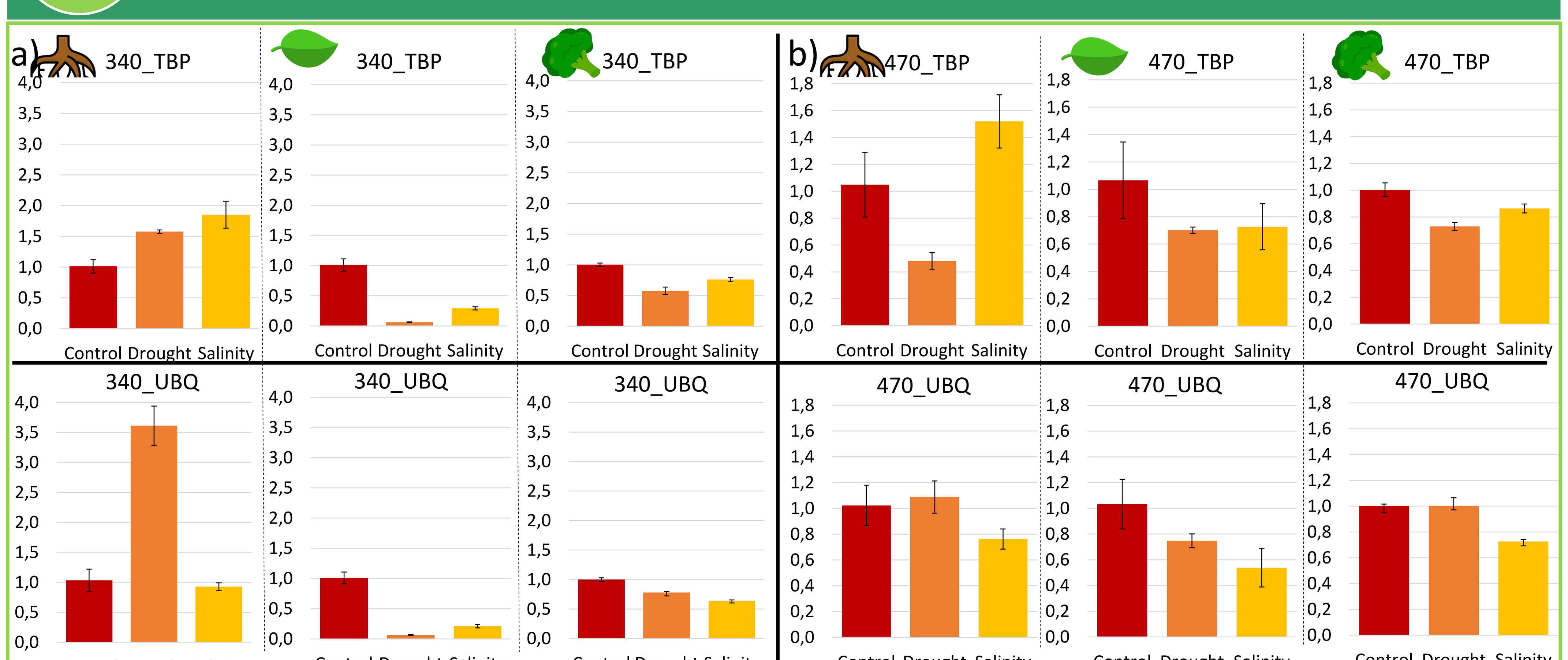


Figure 5. Gene expression profile under saline and drought stress. a) Expression of 340 normalized to the reference genes TBP (upper) and UBQ (lower) in root, leaf and bud. b) Expression of 470 normalized to the reference genes TBP (upper) and UBQ (lower) in root, leaf and bud.

6 CONCLUSIONS AND FUTURE WORK

The aim of this work is to generate stable transgenic broccoli plants with high potential for the resistance to salt and drought stress. To meet this goal, we are now working on:

1.Development of *Arabidopsis thaliana* overexpression lines for phenotyping under salt and drought stress.

2.Complementation of Arabidopsis KO lines lacking the homologous genes.

3.Broccoli protoplast transient transformation.

4.Agrobacterium-mediated stable transformation and design of the necessary vectors for CRISPR-Cas9 edition.

We believe this work could not only set the basis for the study of salinity and drought in broccoli, but also contribute to the creation of resilient crop varieties, thereby mitigating the adverse effects of climate change on agricultural productivity.