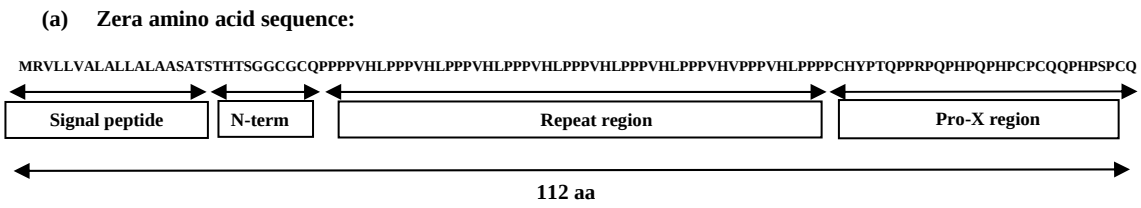
Supplemental data

Figure S1



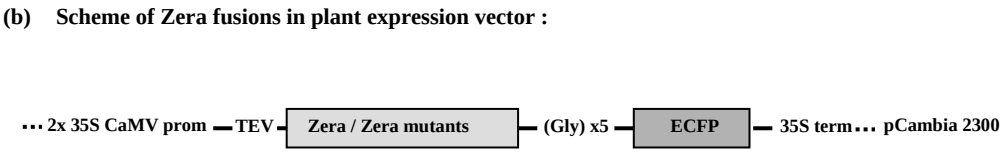


Figure S1. - (a) Amino acid sequence of Zera including a schematic representation of Zera regions (**b**) Scheme of Zera-fusion constructs inserted in the plant expression vector. A linker of five glycines was inserted between Zera and ECFP in all constructs.

Figure S2

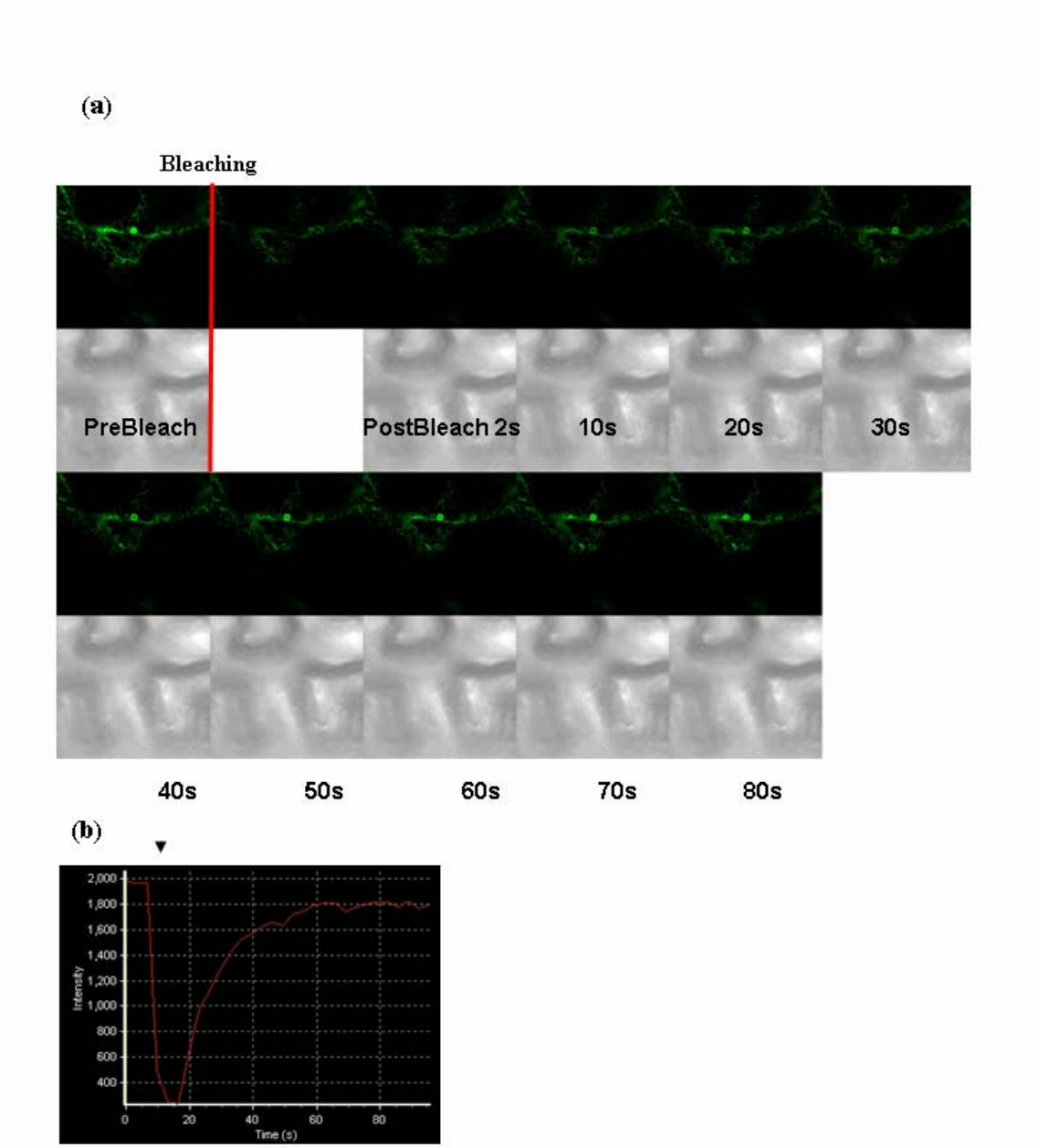


Figure S2. FRAP studies. (a) Spot photobleaching recovery measurements were performed on a Leica TCS SP (Heidelberg, Germany) confocal microscope. A small area of interest (10µm^2) containing an unlabeled Zera-stop induced PB surrounded by YFP-KDEL was selected and the fluorescence of YFP was bleached using a using a high intensity laser pulse. The YFP fluorescence was recorded before the bleaching event and during 1min after the bleaching event by time-lapse microscopy. (b) Quantitative analysis of fluorescence intensity collected during

the FRAP experiment. Baseline fluorescence intensity is collected before the photobleaching occurs (*arrow*).

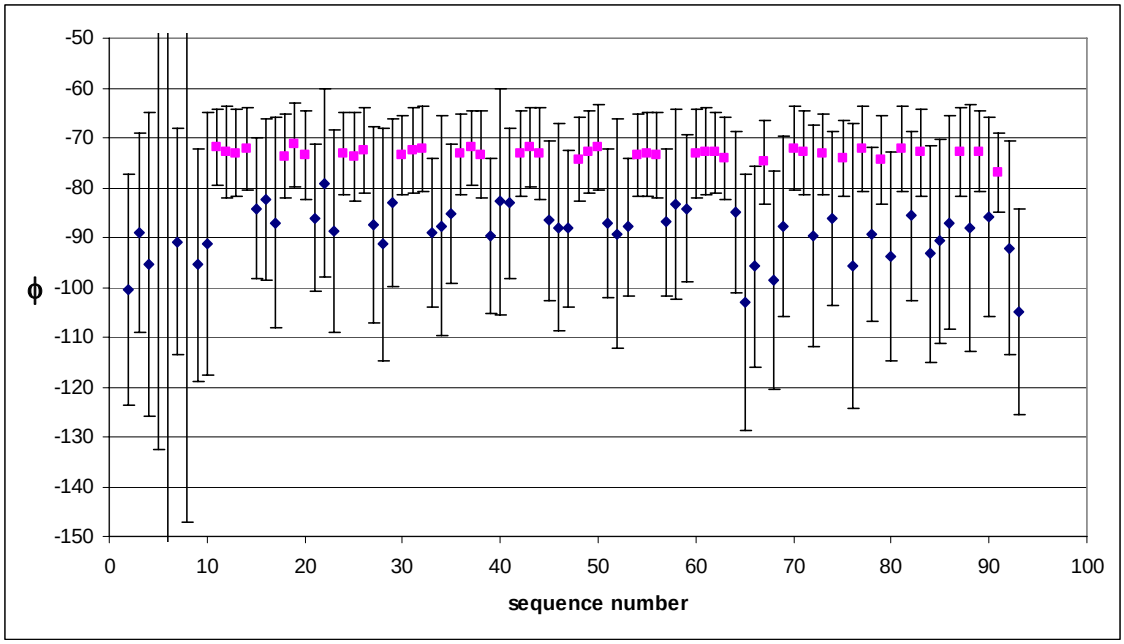
MOLECULAR MODELING (MD)

Study of PPII conformation of Zera by MD calculations

MD simulations were performed to analyze the stability of the PPII helix conformation of Zera molecule lacking signal peptide. In particular, the differential conformational behavior of the two different regions of Zera molecule, R8 repeat and PX, was studied. The average values of ϕ and ψ dihedral angles for each residue were obtained from the MD calculations of the Zera monomer (Figure S3) and the Zera dimer (Figure S4). In both cases, we observe that almost all residues are stable in PPII helix conformation (i.e., ϕ and ψ mean values fall in the PPII region with small error bars for both values). Only terminal residues are flexible, being the N-terminal region of Zera (first 8 residues) the greatest part of Zera molecule that loses the initial PPII helix conformation in all the simulations. From the comparison of ϕ and ψ mean values of the residues corresponding to the R8 repeat and the PX, we see not significant differences between both regions. In contrast, differences in ϕ and ψ values are observed between proline and non-proline residues of both regions. Proline residues have a ϕ value about -73 degrees and a ψ value about 165 degrees, for the R8 repeat and the PX. However, non-proline residues have a ϕ value about -88 degrees and a ψ value about 149 degrees. As expected, proline residues are the most rigid since they are the residues having the smallest error bars.

Figure S3

(a)



(b)

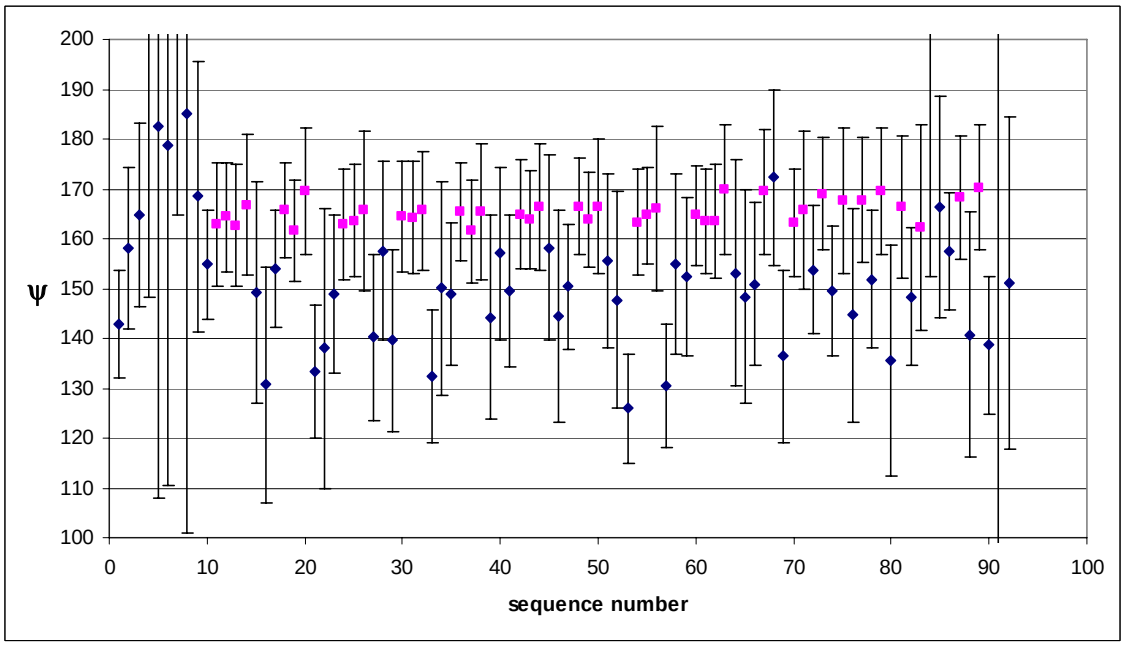
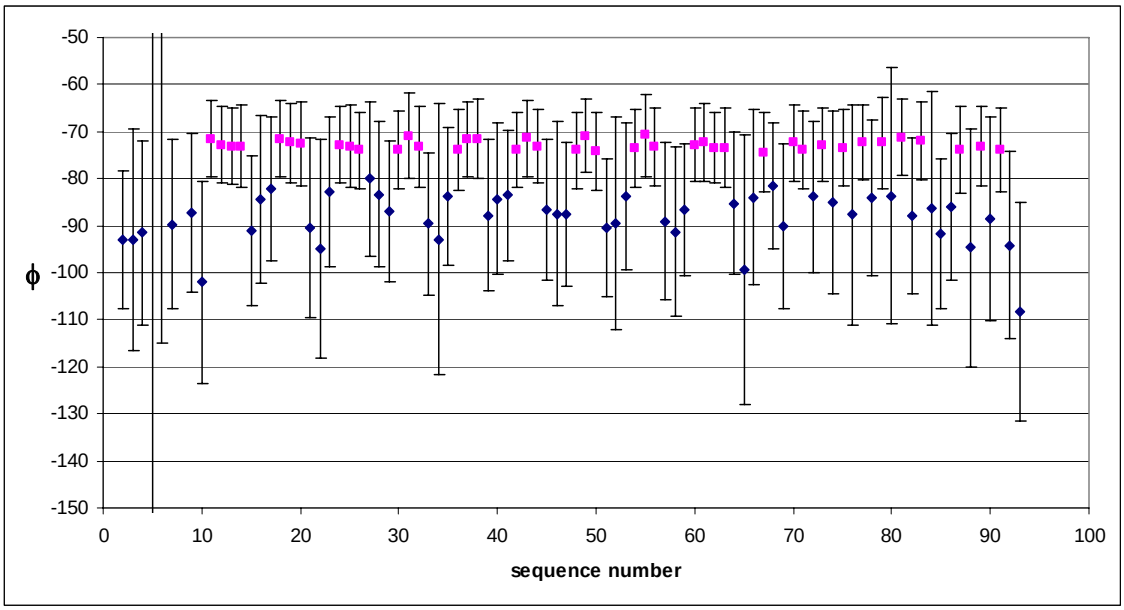


Figure S3. - (**a**) ϕ and (**b**) ψ mean values of each residue of the Zera molecule in the simulations of the Zera monomer in water. Errors indicate standard deviation values. Proline residues in squares and non-proline residues in diamonds.

Figure S4

(**a**)



(**b**)

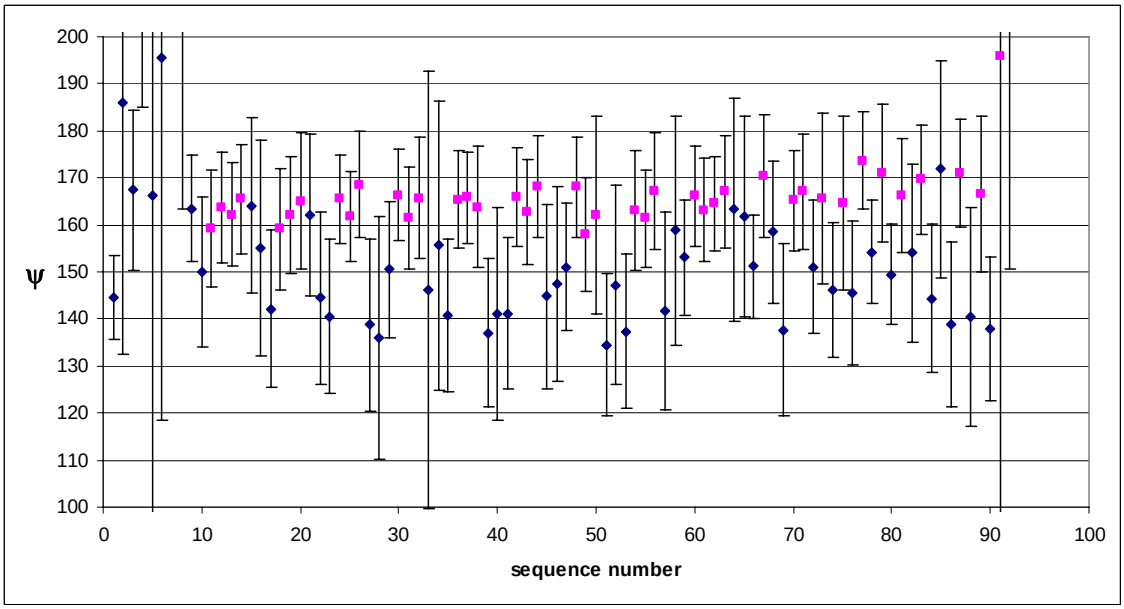


Figure S4. - (**a**) ϕ and (**b**) ψ mean values of each residue of the Zera molecule in the simulations of the Zera dimer in water. Errors indicate standard deviation values. Proline residues in squares and non-proline residues in diamonds.

Figure S5

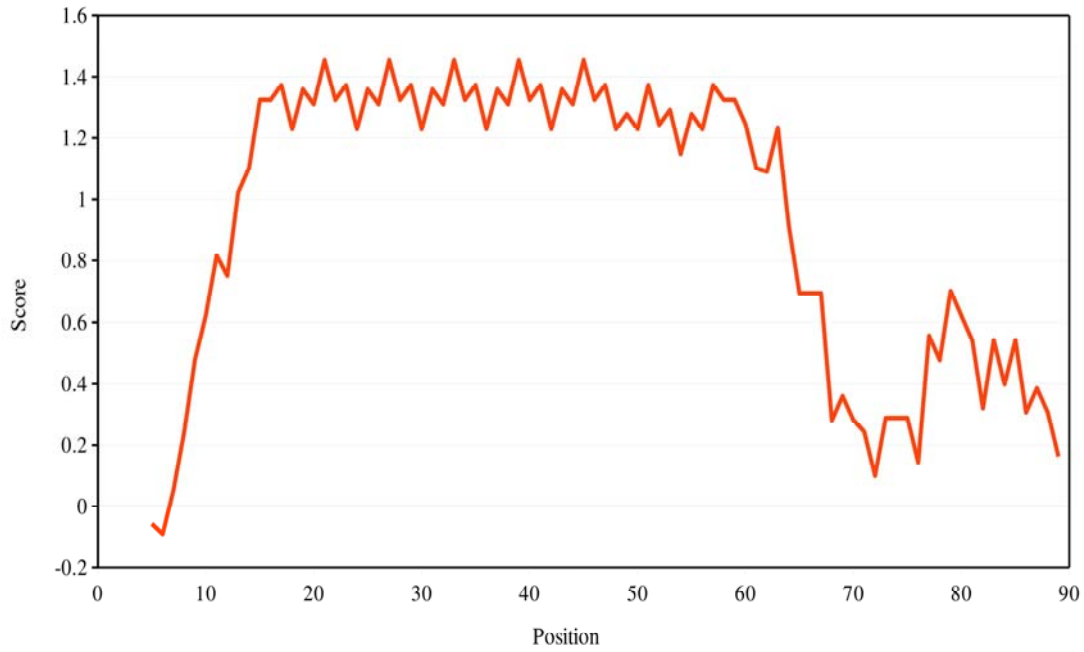


Figure S5. - Hydrophobicity profile of Zera sequence calculated by means of expasy server using the hydrophobicity scale of Abraham and Leo (Abraham DJ and Leo AJ (1987) Extension of the fragment method to calculate amino acid zwitterion and side chain partition coefficients. Proteins: Structure, Function and Genetics ***2*:** 130-152).