FICHA DE PROJETO

Acrónimo:	-
Designação do projeto (PT/EN):	VIRUSREST - COMPETE2030-FEDER-00693100
Código do projeto:	COMPETE2030-FEDER-00693100
Objetivo principal:	-
Entidade financiadora/Programa de financiamento:	Compete 2030
Região de intervenção:	NUTS II - Alentejo
Investimento Total Elegível:	170.856,00 €
Custo total elegível (IPSantarém):	170.856,00 €
Apoio financeiro da União Europeia:	145.227,60 €
Apoio financeiro público nacional/regional:	25.628,40 €
Taxas de financiamento:	85,00 %
Entidade beneficiária:	Instituto Politécnico de Santarém
Investigador Responsável:	Carla Marisa Reis Varanda
Parceiros:	-
Equipa:	Maria do Céu Costa Godinho; Ana Paula Martins Farinha Resende
Data da aprovação:	2025/04/22
Data de início:	2025/01/01
Data da conclusão:	2027/12/31
Domínio científico e subárea científica:	-
Resumo (objetivos, atividades e resultados esperados) - em PT e/ou EN:	Tomato is one of the agronomically most important food crops consumed worldwide (http://faostat.fao.org). Adapting the cultivation of tomato to climate changes has not been an easy task for growers, the requirements in terms of colour and Brix are increasingly tight and agronomists are permanently working for the high quality standards imposed by the industry. In 2018, the surfaces dedicated to tomato have fallen to their lowest level since 2013 (26% decrease), mostly due to the difficulties that growers found in 2017 in terms of plant health issues, originating quality issues such as colour defects and consequently a lower value crop, which put off a large number of growers. Mitigating the impact of climate change, such as the prevention and control of emerging diseases, under a One Health and Food Safety approach is one of the main challenges in agriculture worldwide. One of such emerging diseases is caused by Tomato brown rugose fruit virus (ToBRFV), a virus responsible for extremely significant economic losses in tomato. ToBRFV has entered to the European and Mediterranean Plant Protection Organization (EPPO) alert list in 2019 to achieve early warning and draw attention to its many risks. At present, control of the vírus rely on preventive sanitary measures and on the very few ToBRFV-resistant varieties that are slowly arising greatly dependent on the labor-intensive, cumbersome and time-consuming traditional breeding techniques. At present,

	producers are forced to choose between a ToBRV-resistant variety or a variety they desire in terms of colour, brix, production cycle, cost, etc. In addition, the continuous resurgence and spread of ToBRFV makes essential a rapid, timely and efficient mode of control. Recently, genomics-assisted breeding has accelerated tomato improvement [1,2] and, together with genome editing techniques such as CRISPR/Cas, have opened many opportunities for plant breeding in many crops [3,4]. Due to their simplicity and specificity, these systems have been used as genome-editing platforms simplifying the field of genome engineering and revolutionizing plant genetic manipulation [3,4]. In this project, we intend to develop ToBRFV-resistant tomato plants, by developing a strategy based on Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins. CRISPR-Cas are natural immunity systems existent in bacteria that protect them from invading viruses [5]. Most systems consist of two components: a Cas endonuclease and a guide RNA (gRNA); these systems guide Cas to target and cleave a specific homologous nucleic acid sequence. Here, we will use a Cas endonuclease and we will design synthetic RNAs, to target and cleave tomato ToBRFV-susceptible genes without compromising tomato growth and development. Susceptible genes provide essential assistance at different stages of virus life cycle and their mutation prevent virus replication. Among these proteins, TObamovirus Multiplication proteins TOM1 and TOM3 and the small GTP- binding protein ARL8 play essential roles in the viral replication complex [6,7]. There are two main issues on the use of gene editing techniques: the occurence of off target effects and the generation of transgenic plants, as most delivery strategies use recombinant DNA at least in an intermediate step, making possible the integration of DNA into the host genome. This raises regulatory issues and hamper their use in agriculture. In this proposal, we intend to use Ribonucleoproteins (RNPs) for the delivery of the CRISPR Cas system in plants. This way we will reduce off target effects and develop an efficient transgene DNA free plant editing technique. We will preassemble RNP complexes of purified Cas protein and guide RNA (gRNA) in vitro and deliver them into plant protoplasts via PEG-mediated. As expected intermediate results, we intend to obtain RNP complexes, carrying optimized CRISPR systems against ToBRFV (containing Cas9 together with combinations of several sgRNA fragments, complement to specific regions of specific susceptible tomato genes), that can be introduced into tomato plants, conferring them resistance to the virus. Despite not being the main objective of this work, selected RNP complexes will also be tested for their ability to cause resistance to other tomato Tobamoviruses. The development of such technology is particularly important in an era that is facing continuous emergent diseases demanding efficient and rapid responses, not only at plant level, but also in other organisms, as seen by the human COVID-19.

	Production of plants through gene edited techniques, ensuring DNA free plants is beyond the reach of genetically modified organism legislation in several countries. In EU, plants produced by specific new genomic techniques (NGTs) will be soon out of GMOs legislation ?umbrella?, after the recent vote in favor of the proposal of NGTs by the EU parliament on 7th February 2024. This means that it will be established a regulatory framework for gene edited plants that are indistinguishable from natural mutations or conventional breeding, meaning that for these plants no risk assessment has to be made and they can be labelled in the same way as conventional plants. In this way, ToBRFV-resistant tomato lines established as in this proposal could soon be cultivated globally without any restrictions. The technology and results from this project will contribute to many benefits to both society and business sectors, increasing the technological intensification and modernization of tomato production, and consequently increase the economic value of one of the most important crops in the world. The development and implementation of this technology will allow the production of products free from toxic chemicals, essential for food quality and safety and fits in goals 2,3, 9, 12 and 13 of 2030 Agenda. This work will be performed in facilities belonging to Escola Superior Agrária de Santarém (ESAS -IPS) and Mediterranean Institute for Agriculture, Environment and Development (MED) and the objectives are integrated in the main aims of the research institutes, whose main concerns are the development of plants adapted to different growing conditions and the sustainability of the agrosystems. The team involved in this project has the motivation, abilities and skills to execute the proposed tasks. The team has been working on plant viruses for over 20 years, in terms of epidemiology, biology, genetics and plant protection and their expertise has been internationally recognized by several organizations.
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Outras informações:	-