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Cry toxins and CRISPR/Cas technology: How biotechnology advances can contribute to new crops biotech

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Plants are usually sessile organisms, and they must evolve towards a developmental and physiological flexibility to generate adaptation mechanisms against different biotic and abiotic stresses. In an agronomical aspect, these environmental factors impact negatively on plant development, which represents a high economic cost. In this case, Biotechnology actively acts in the development of tools capable of optimizing agricultural production, reducing in an eco-friendly way the negative influence of the environment. Although genetically modified (GM) crops expressing Cry toxins have been worldwide applied for insect resistance, no commercial GM cotton have been successfully developed to the cotton boll weevil (CBW) control, one of the most critical cotton primary insect pest in Brazil. Recently advances showed that the transgenic expression of a Cry10 toxin conferred to GM cotton high resistance to CBW. The entomotoxic effect to CBW was maintained in T_2 plants as the Cry toxin expression levels remained high in both tissues, ranging up to 19.0 μg g^{-1} fresh tissue, and the CBW mortality rate remained around 100%.On the other hand, the development of water stress tolerance without agricultural penalties is a great challenge to Biotechnology. Genetic manipulation of plant genomes can overcome some of these difficulties. The type II CRISPR/Cas system has been adapted to plants to control the genetic modification in a more targeted and precise procedure. The catalytically inactive Cas9 (dCas9) fused to activators has already been used to regulate transcription in transformed tobacco leaves and *Arabidopsis thaliana* plants. To validate the CRISPR/Cas system, we have used the dCas9 fused to the tripartite activator VPR, as well as two Arabidopsis epigenetic modification domains (the Acetyltransferase domain from AtHAC1 - *AT domain* and the methyltransferase domain from *Curly Leaf* (CLF) gene - *SET domain*). The strategy was tested to control the endogen Arabidopsis promoter of the transcriptional factor At AREB1, known to regulate key genes in response to drought stress. The AtAREB1 transcript expression was increased in plants expressing the dCas9-VPR and dCas9-AT fusions, and these plants showed a better tolerance to drought stress. On the other hand, the results with the SET domain variated from one line to another, displaying activation and inhibition of AtAREB1 expression, with opposed phenotypes when submitted to water withdrawal. These data demonstrated that it is entirely possible to modulate gene expression in plants of agronomic interest (such as soybean and cotton) using CRISPR/Cas technology and thereby express a particular phenotype, for example, drought tolerance.Thus, the association of both technologies, Cry toxins and CRISPR/Cas system (dCas9), can be considered important biotech approaches to develop crops efficiently more resistant to insect pests. Several studies have shown that elevated levels of Cry toxins expressed in transgenic plants can reduce or prevent the emergence of toxin-resistant insect populations. In this way, it is perfectly possible to prevent possible populations of CBW resistant to Cry toxin through increased expression of the toxin mediated by dCas9-VPR/AT. Finally, the engineered transgenic plants could be introduced in breeding programs, such as pyramidalization, in which the combination of two or more characteristics could improve resistance/tolerance to both biotic and abiotic stresses. In this way, the recent biotechnology approaches can be considered to increment the world production of food and other bioproducts, which can be used to supply the needs of growing human population sustainably.

Biography

M.Fatima Grossi-de-Sa is a Plant Biotechnology research group leader at EMBRAPA Genetic Resources and Biotechnology, professor at the Genomic Sciences and Biotechnology Graduation Program at Brasilia Catholic University, Brasilia-DF, Brazil and President of the Brazilian Society of Biotechnology (SBBIOTEC). She is a full member of the Brazilian Academy of Sciences (member elected in 2011) and full fellow of the World Academy of Science -TWAS (member elected in 2014). Her current research includes Plant Pests interaction, Genetic manipulation of crop plants, Gene prospection and functional studies of plant promoters, functional genomics of plants and insect-pests, and Molecular strategies to the phytonematodes and insect pests control.

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