Pyramiding *CRY* and *VIP* genes, a sustainable approach to mitigate Lepidopetrans in cotton

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The insecticidal *cry* proteins of *Bacillus thuringiensis* are crystalline in nature and are widely used in controlling lepidopteran pests. Unfortunately, they had lost efficacy against some pests due to their extensive use and development of field evolved resistance in the pests. In recent years, Pink Bollworm has also developed resistance to most widely deployed Bt toxins, Cry1Ab and Cry2 Ab, thus becoming a challenge to control it. One way to handle this is to engineer more effective Bt toxins against pests that are resistant to previously deployed toxins. For instance, *Cry1AbMod* and *Cry1AcMod* are genetically modified Bt toxins effective against some strains of six species of lepidopteran pests which developed resistance against native toxins *Cry1Ab* and *Cry1Ac*. (Soberon *et al*., 2007, Franklin *et al*., 2009; Munoz-Garay *et a*., 2009). Though these modified toxins are not commercialized yet, transgenic tobacco plants producing *Cry1AbMod* killed larvae of *Manduca sexta* showing resistance to *Cry1Ab* [Porta et al., 2011]. Tabashnik *et al.* (2013) suggested that the modified toxins might be useful in pyramids with native Bt toxins. In principle, such pyramids would be more effective than many current multi-toxin Bt plants that include a toxin to which pests have already evolved resistance in the field.

An alternative approach is the use of Vegetative insecticidal proteins against resistant strains of pests. Vip proteins are secreted into the culture medium by entomopathogenic bacteria and are divided into four families according to their amino acid identity. To begin with, Vip1 and Vip2 proteins act as binary toxins and are toxic to some members of the Coleoptera and Hemiptera. Vip3 proteins are toxic to a wide variety of members of Lepidoptera, whereas, Vip4 family has no target insects found yet. Vip 3 toxins do not show sequence similarity to Vip1 or Vip2 and are the most extensively studied family of Vip toxins. Its mode of action has been shown to resemble that of the Cry proteins in terms of proteolytic activation, binding to the midgut epithelial membrane, and pore formation, although Vip3A proteins do not share binding sites with Cry proteins. Vip3A proteins are good candidates to be combined with Cry proteins in transgenic plants to prevent or delay insect resistance and to broaden the insecticidal spectrum. For example, VipCot and AgriSure Viptera were registered in US in 2008 and 2009, respectively (Syngenta Seeds, Inc.). VipCot produces Vip3Aa19 protein whereas AgriSure Viptera produces the Vip3Aa20 protein. Both events were pyramided with cry1Ab (VipCot: Vip3Aa plus mCry1Ab and Agrisure Viptera: Vip3Aa plus Cry1Ab) and later with cry1Fa (VipCot: Vip3Aa plus Cry1Ac plus Cry1Fa and Agrisure Viptera: Vip3Aa plus Cry1Ab plus Cry1Fa) to confer wider and more robust protection against Lepidoptera (Kurtz et al., 2005; Adamczyk et al., 2008; Burkness et al., 2010). In a three year study, Vip3Aa protein was highly efficacious against *H.armigera* early in the season but progressively declined thereafter although not so drastically as Cry1Ac in Bollgard or Ingard cotton (Llewellyn et al., 2007). In 2015, the first modified Vip3A protein, with improved toxicity, was introduced into tobacco, conferring almost total protection against *H. armigera, A. ipsilon, and S. littoralis* (Gayen et al., 2015). Cotton has also been transformed with a synthetic *vip3A* gene fused to a chloroplast transit peptide coding sequence (Wu et al. 2011). The Vip3A protein accumulated in chloroplasts, and its concentration in plants was higher than that in plants transformed with just the synthetic gene. Transformed plants provoked 100% mortality in larvae of *S. frugiperda*, *S. exigua*, and *H. zea*. Screening and validating most effective Bt protein combinations of native and modified *CRY* genes along with VIP3 genes would be an excellent approach to achieve sustainable resistance in Indian cotton.

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