

## SCREENING OF NATIVE BACILLUS THURINGIENSIS (BT) ISOLATES FOR THE PRESENCE OF CRY 1 AB & VIP 3A

Sunita Kumari Meena\*, Sarvjeet Kaur and B.L. Meena

ICAR – National Research Centre on Plant Biotechnology,  
IARI Campus, New Delhi 110012, India.

Email: Sunita kumari meena (email: meenasb\_bt08@yahoo.com)

Received-03.03.2017, Revised-15.03.2017

**Abstract:** Insecticidal *cry* and *vip* genes from *Bacillus thuringiensis* (Bt) have been used for control of lepidopteran insects in transgenic crops. However, novel genes are required for gene pyramiding to delay evolution of resistance to the currently deployed genes. PCR-based techniques were employed for screening of *cry1Ab* type genes in 96 Bt isolates from diverse habitats in India and 8 known Bt strains. 96 native Bt isolates, recovered from different locations in India and 8 known Bt strains were screened for the presence of *cry1Ab*, *cry1Ac*, *Cry3A* & *vip3A* for Isolation of plasmid DNA from native Bt isolates of *Bacillus thuringiensis*, Screening for the presence of *cry1Ab*, *cry1Ac*, *cry3A* & *vip3A* gene using PCR amplification and Cloning of partial *cry1Ab* & *vip3A* gene using different sets of primers. *Cry1Ab* type genes were more prevalent than *cry1Aa*- and *cry1Ac* type genes. Correlation between source of isolates and abundance of *cry1*-type genes was not observed..

**Keywords:** *Bacillus thuringiensis*, *Cry1Ab* genes, *Cry1Ac*, *Cry3A*, *Vip3A*, *Helicoverpa armigera*, Insecticidal genes

### REFERENCES

- Adang, M.J., Crickmore, N. and Jurat-Fuentes, J.L. (2014). Diversity of *Bacillus thuringiensis* crystal toxins and mechanism of action. In *Advances in insect physiology*. Vol. 47. Edited by T.S. Dhadialla and S.S. Gill. Academic Press, Oxford. pp. 39–87.
- Akhurst, R.J., James, W., Bird, L.J. and Beard, C. (2003). Resistance to the *Cry1Ac* - endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 96: 1290–1299.
- Beard, C.E., Court, L., Mourant, R.G., James, B., Van Rie, J., Masson, L. and Akhurst, R.J. (2008). Use of a *Cry1Ac*-resistant line of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to detect novel insecticidal toxin genes in *Bacillus thuringiensis*. *Curr. Microbiol.* 57:175–180.
- Carozzi, N.B., Kramer, V.C., Warren, G.W., Evola, S. and Koziel, M.G. (1991). Prediction of insecticidal activity of *Bacillus thuringiensis* strains by polymerase chain reaction product profiles. *Appl. Environ. Microbiol.* 57: 3057–3061.
- Carriere, Y., Crickmore, N. and Tabashnik, B.E. (2015). Optimizing pyramided transgenic Bt crops for sustainable pest management. *Nat. Biotechnol.* 33: 161–168.
- Fabrick, J.A., Ponnuraj, J., Singh, A., Tanwar, R.K., Unnithan, G.C., Yelich, A.J. *et al.* (2014). Alternative splicing and highly variable cadherin transcripts associated with field-evolved resistance of pink bollworm to Bt cotton in India. *PLoS ONE*, 9: e97900.
- Gouffon, C., Van Vliet, A., Van Rie, J., Jansens, S. and Jurat-Fuentes, J.L. (2011). Binding sites for *Bacillus thuringiensis* *Cry2Ae* toxin on heliothine brush border membrane vesicles are not shared with *Cry1A*, *Cry1F*, or *Vip3A* Toxin. *Appl. Environ. Microbiol.* 77: 3182–3188.
- Ibargutxi, M., Muñoz, D., Escudero, I.R. and Caballero, P. (2008). Interactions between *Cry1Ac*, *Cry2Ab*, and *Cry1Fa* *Bacillus thuringiensis* toxins in the cotton pests *Helicoverpa armigera* (Hübner) and *Earias insulana* (Boisduval). *Biol. Control*, 47: 89–96
- James, C. (2013). Global status of commercialized biotech/GM crops: 2013. ISAAA Brief No. 46. International Service for the Acquisition of Agri-biotech Applications, Ithaca, New York.
- Jurat-Fuentes, J.L., Gould, F.L. and Adang, M.J. (2003). Dual resistance to *Bacillus thuringiensis* *Cry1Ac* and *Cry2Aa* toxins in *Heliothis virescens* suggests multiple mechanisms of resistance. *Appl. Environ. Microbiol.* 69: 5898–5906.
- Katara, J.L., Deshmukh, R., Singh, N.K. and Kaur, S. (2012). Molecular typing of native *Bacillus thuringiensis* isolates from diverse habitats in India using REP-PCR and ERIC-PCR analysis. *J. Gen. Appl. Microbiol.* 58: 83–94.
- Kaur, S. (2000). Molecular approaches towards development of novel *Bacillus thuringiensis* biopesticides. *World J. Microbiol. Biotechnol.* 16: 781–793.
- Kaur, S. (2006). Molecular approaches for identification and construction of novel insecticidal genes for crop protection. *World J. Microbiol. Biotechnol.* 22: 233–253.
- Kaur, S. (2012). Risk assessment of Bt transgenic crops. In *Bacillus thuringiensis* biotechnology. Edited by E. Sansinenea. Dordrecht, the Netherlands. Springer Publishers, Heidelberg. pp.41–86.

\*Corresponding Author

- Kaur, S. and Allam, U.S.** (2006). PCR-based cloning of a novel cry1Ac gene from a *Bacillus thuringiensis* isolate recovered from stored cottonseeds. *Biopestic. Int.* 2: 120–128.
- Kaur, S. and Singh, A.** (2000). Natural occurrence of *Bacillus thuringiensis* in leguminous phylloplanes in the New Delhi region of India. *World J. Microbiol. Biotechnol.* 16: 679–682.
- Koziel, M.G., Beland, G.L., Bowman, C., Carozzi, N.B., Crenshaw, R., Crossland, L. et al.** (1993). Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Biotechnology (NY)*, 11:194–200.
- Li, H. and Bower, G.** (2014). Evaluation of the synergistic activities of *Bacillus thuringiensis* Cry proteins against *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Invertebr. Pathol.* 121:7–13.
- Liao, C., Heckel, D.G. and Akhurst, R.** (2002). Toxicity of *Bacillus thuringiensis* insecticidal proteins for *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae), major pests of cotton. *J. Invertebr. Pathol.* 80: 55–63.
- Lin, Y., Fang, G. and Cai, F.** (2008). The insecticidal crystal protein Cry2Ab10 from *Bacillus thuringiensis*: cloning, expression and structure simulation. *Biotechnol. Lett.* 30: 513–519.
- Misra, H.S., Khairnar, N.P., Mathur, M., Vijayalakshmi, N., Hire, R.S., Dongre, T.K. and Mahajan, S.K.** (2002). Cloning and characterization of an insecticidal crystal protein gene from *Bacillus thuringiensis* subspecies *kenyae*. *J. Genet.* 81: 5–11.
- Pardo-López, L., Soberón, M. and Bravo, A.** (2013). *Bacillus thuringiensis* insecticidal three-domain Cry toxins: mode of action, insect resistance and consequences for crop protection. *FEMS Microbiol. Rev.* 37: 3–22.
- Porcar, M. and Juarez-Perez, V.M.** (2003). PCR-based identification of *Bacillus thuringiensis* pesticidal crystal genes. *FEMS Microbiol. Rev.* 26: 419–432.
- Sauka, D.H., Cozzi, J.G. and Benintende, G.B.** (2005). Screening of cry2 genes in *Bacillus thuringiensis* isolates from Argentina. *Antonie Van Leeuwenhoek*, 88: 163–165.
- Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R. and Dean, D.H.** (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62: 775–806.
- Shu, C., Zhang, J., Chen, G., Liang, G., He, K., Crickmore, N. et al.** (2013). Use of a pooled clone method to isolate a novel *Bacillus thuringiensis* Cry2A toxin with activity against *Ostrinia furnacalis*. *J. Invertebr. Pathol.* 114: 31–33.
- Somwatcharajit, R., Tiantad, I. and Panbangred, W.** (2014). Coexpression of the silent cry2Ab27 together with cry1 genes in *Bacillus thuringiensis* subsp. *aizawai* SP41 leads to formation of amorphous crystal toxin and enhanced toxicity against *Helicoverpa armigera*. *J. Invertebr. Pathol.* 116: 48–55.
- Subramanian, S. and Mohunkumar, S.** (2006). Genetic variability of the bollworm, *Helicoverpa armigera*, occurring on different host plants. *J. Insect. Sci.* 6: 26–28.
- Tabashnik, B.E., Fabrick, J.A., Unnithan, G.C., Yelich, A.J., Masson, L., Zhang, J. et al.** (2013). Efficacy of genetically modified Bt toxins alone and in combinations against pink boll worm resistant to Cry1Ac and Cry2Ab. *PLoS ONE*, 8: e80496.
- Van Frankenhuyzen, K.** (2013). Cross order and cross-phylum activity of *Bacillus thuringiensis* pesticidal proteins. *J.*