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Cry1Ac expression in transgenic *Bt* cotton hybrids is influenced by soil moisture and depth

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Cry1Ac toxin concentration was assessed in leaves of *Bt* transgenic cotton hybrid grown on shallow (<60 cm) and deep (>90 cm) black soils of Nagpur, Maharashtra, India. Cry toxin concentration increased up to 80 days after sowing followed by a steep decline. In general, toxin concentration was greater on the deep black soils than the shallow soil. This was because of greater water-holding capacity of the deep soils. Cry toxin concentration was closely related to the soil water content. Beyond (excess moisture) and below (moisture deficit) field capacity, toxin concentration declined. A cubic polynomial best described the relationship between Cry toxin concentration and soil moisture content (R^2 = 0.95).

Keywords: *Bt* cotton hybrid, black and shallow soil, Cry toxin, soil moisture.

COTTON cultivation, in India, was transformed after the introduction of *Bt* cotton hybrids. At present, almost the entire cotton acreage is planted under *Bt* transgenic hybrids. Consequently, productivity in the post-*Bt* era increased from 303 kg/ha in 2001–02 to 526 kg lint/ha in 2008–09 (ref. 1). Compared to the world average, however, productivity levels are still low mainly because of the abiotic constraints[2]. Most of the cotton grown in the country is rain-dependent and the crop experiences moisture stress. Furthermore, cotton is grown on soils of varying depths, and it has been observed that productivity is better on deep Vertisols compared to the shallow soils because the former has a better water-holding capacity[3]. Apart from productivity being affected, Cry toxin expression may also be affected. Water stress has been reported to affect expression of transgenes in transgenic crops such as maize[4], peas[5] and cotton[6–8]. This has serious implications: (i) ineffective pest control; (ii) pest becoming resistant to the *Bt* toxin, and (iii) high pesticide use. Kranthi *et al.*[9] demonstrated that the toxin expression declined with crop age in all the *Bt* hybrids tested. Under rainfed conditions of central India, rains cease early in September. Thus, the crops grown in deep Vertisols are less likely to experience moisture stress than those grown on shallow soils. However, the impact on the Cry toxin production is less known. To address this issue field studies were conducted to assess the effect of soil depth on Cry toxin expression.

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Field studies were conducted on soils of varying depth (shallow and deep soils) on the experimental fields of the Central Institute for Cotton Research, Nagpur, during the rainy season of 2006–07 and 2007–08. The shallow soils were classified as fine, hyperthermic, Vertic Ustopepts and their depth was less than 60 cm. The deep Vertisols were classified as very fine, montmorillonitic, hyperthermic and Typic Haplusterts, with soil depth >90 cm. Ankur-651 *Bt* was grown during both the years at a spacing of 60 cm between rows and 60 cm within rows. In 2007–08, Bunny *Bt* was grown in addition to Ankur-651 *Bt* with an inter-row spacing of 90 cm and intra-row spacing of 60 cm. Both the hybrids contained *Cry1Ac* gene (Mon 531 of Monsanto). Recommended dose of fertilizers (90 kg N, 19 kg P and 37 kg K) was applied and appropriate agronomic measures were adopted, such as pest control and weeding. Soil samples in the 0–0.15, 0.15–0.30 and 0.30–0.45 m soil depth were collected from each site (triplicate soil samples) during the crop growth period of 2006–07 and soil moisture content was determined gravimetrically. During 2007–08, soil samples were collected twice, coinciding with the peak Cry toxin expression.

For the Cry toxin analysis, leaf samples were collected from three plants of 21 plots of each soil type. Thus, there were 63 samples each for the shallow and deep soils. The third leaf from the top was sampled periodically during crop growth and immediately brought to the laboratory in ice chambers to be analysed for Cry toxin content[10]. Leaf discs were cut with Eppendorf vials, weighed and the samples were crushed in Eppendorf vials. Buffer solution was added and the Cry1Ac toxin content was determined using ELISA reader. Simultaneously, standards were also run and the data tabulated on a fresh weight basis as μg/g after dilution factors were accounted for. Scatter plot was made for the soil moisture content (average values for the soil depths sampled) and Cry toxin concentration, and curve fitting was done following the least square approach. The data for the period of August and September were utilized for the scatter plot and curve fitting because this period coincided with the maximum Cry toxin production.

Results indicate that the Cry toxin content was the greatest during August and September (Figures 1 and 2) and declined during October. Such a decline in Cry toxin concentration as the crop ages has been reported earlier[9–12]. This decline coincided with the peak boll formation stage. At this stage, the toxin concentration was 0.48–2.40 μg/g. The toxin concentrations were, in general, less than the critical concentration of 1.90 μg/g (ref. 9). Developing bolls are the largest sinks for N and plant assimilates. Thus, decline in Cry toxin concentration was probably due to a redistribution of N from the leaf tissue to the developing bolls. Consequently, N available for the toxin production would be reduced. A decline in the Cry toxin below the critical limit, within 110 days after sowing, was reported earlier for this location[9] and our results corroborate those findings. Unusually, an increase in toxin concentration was observed from October to November. Such an increase could be due to an interaction of Cry toxin with proanthocyanin[11]. Manjunath *et al.*[12] reported such an increase in Cry1Ac concentration in *Bt* cotton hybrid MRC-7201 between 80 and 150 days after sowing.

The black cotton soils are highly variable in their depth and consequently have different moisture retention capacities. In 2006–07, Cry toxin concentration was significantly better in the *Bt* transgenic hybrids grown on deep Vertisols than in the shallow soils (Figure 1). Toxin concentration was more than fourfold greater in the deep black soils than the shallow soils at the time of boll formation (29 August to 15 September 2006). At later sample dates, Cry toxin concentration was greater in the deep black soils than the shallow soils. The plants grown on the shallow soils had toxin concentration <0.9 μg/g, which was less than the critical level of 1.9 μg/g, from 19 September 2006 (81 days after sowing), which coincided with peak boll formation and boll maturation stages. On the other hand, the *Bt* plants grown on the deep black soils had Cry toxin concentration ranging from 1.29 to 2.04 μg/g, except for the sample collected on 26 September 2006 (88 days after sowing). Interestingly, samples collected during boll maturation and boll opening (1 November 2006 and 22 December 2006) had Cry toxin concentration greater than the critical level. These differences were mainly due to a better moisture-holding capacity of the deep Vertisols. The deep black soils on an average were close to the field capacity on these sample dates, while the shallow soils had moisture content lower than the field capacity. Rochester[8] reported a significant reduction in Cry1Ac protein expression with a single four-day episode of severe drought. Although data on

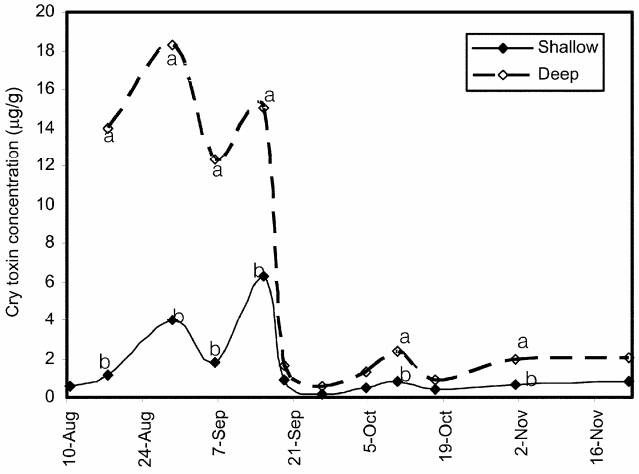

Figure 1. Cry toxin concentration of Ankur-651 *Bt* as affected by soil depth in 2006–07 (values followed by the same letter for a given date are not statistically significant).

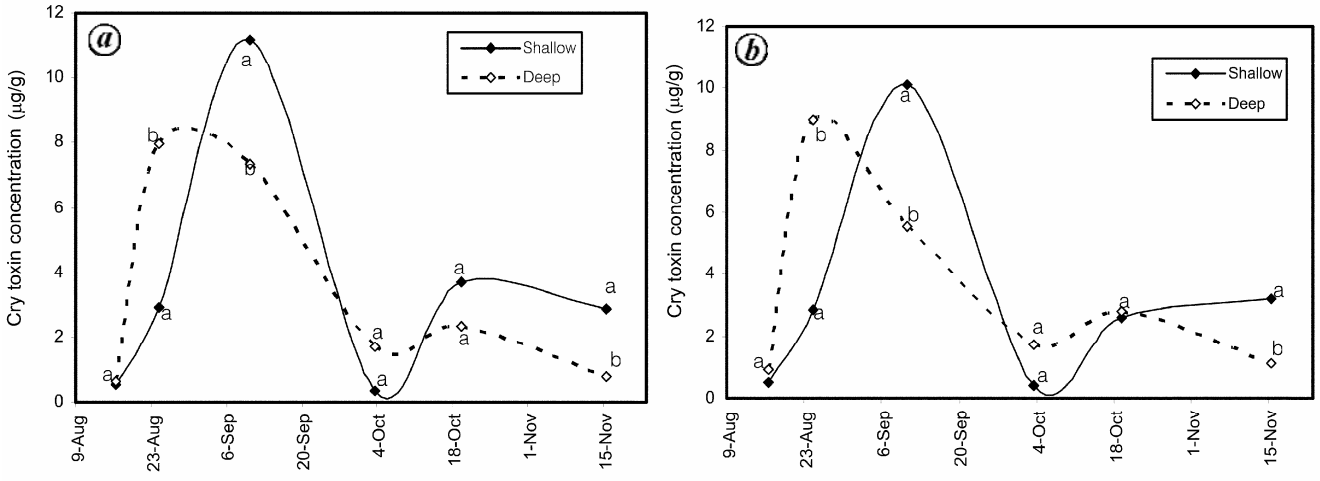

Figure 2. Effect of soil depth on Cry toxin expression of (***a***) Ankur-651 *Bt* and (***b***) Bunny *Bt* in 2007–08 (values followed by the same letter for a given date are not statistically significant).

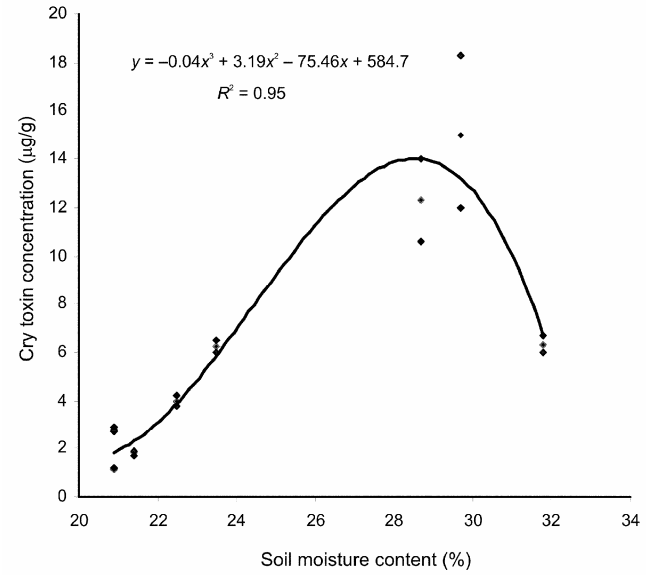

Figure 3. Correlation of Cry toxin concentration and soil moisture content.

bollworm damage were not collected, there is a likelihood of some bollworms developing resistance[13] to the Cry toxin because of low toxin concentrations in plants grown on shallow soils. However, the field reality is that bollworm resistance to *Bt* cotton has not occurred in the last 15 years since its commercial cultivation in several countries from 1996 and in India from 2002, but for rare reports in certain parts of Gujarat in 2009.

Under rainfed conditions, crops experience not only moisture deficit but also excess moisture condition which was the case in 2007. Leaves of the plant samples, collected on 24 August 2007 (flowering stage) from deep Vertisols, had nearly threefold greater Cry toxin concentration than those of the shallow soils, and these differences were significant (Figure 2 *a* and *b*). At this time the soils were near field capacity. At later sample dates, a reverse trend was observed. As is evident from Figure 2, Cry toxin concentration was adversely affected in the deep soils which got saturated following heavy rains compared to the shallow soils which were well drained. Excess soil moisture content in the deep Vertisols compared to field capacity in the shallow soils, resulted in a decline in the toxin concentration to an extent of 35–45%. Field observations in China suggest that waterlogging has a much more damaging effect on the expression of *Bt* toxins compared to drought[7]. Decline in toxin concentration was greater in the Bunny *Bt* (Figure 2 *b*) compared to the Ankur-651 *Bt* (Figure 2 *a*), which was probably due to differences in the growth pattern of the hybrids. Ankur-651 *Bt* is of a shorter duration with a compact growth habit compared to the Bunny *Bt*, which is of a longer duration. Differences in the Cry toxin production were reported to vary among cultivars[9,14].

Cubic polynomial best described the relationship between soil water content and the leaf Cry toxin concentration (Figure 3). The data indicate that the toxin concentration follows a diminishing pattern beyond field capacity. Toxin concentration declined whenever the soil was saturated or waterlogged. Luo *et al.*[7] reported reduced *Bt* protein content by 38–50% following waterlogging. Toxin concentration was also low at low soil moisture content and is in agreement with studies conducted on Vertisols in Australia[8].

Our studies suggest that the Cry toxin concentration is affected by soil depth mainly due to the differences in soil water-holding capacity. Toxin concentration was optimal when the soils were close to field capacity. Soil moisture stress (excess as well as deficit) had an adverse effect on the toxin production.

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Yield, soil health and economics of aonla (*Emblica officinalis* Gaertn.)-based agri-horticultural systems in eastern India

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An intercropping trial was conducted during 2007–2010 on 6-year-old aonla (*Emblica officinalis* Gaertn.; cv. NA-7) orchard planted at 6 m × 6 m spacing and growing under rainfed calciorthent soil, to identify the suitable and profitable intercrops. The intercrops grown were turmeric, ginger and arbi. The results indicated that the production of fruits significantly increased due to intercrops and it was maximum in aonla in association with turmeric (13.30 tonnes/ha) followed by arbi (11.71 tonnes/ha). On the other hand, reduction in yield of intercrops was 7.5–12.0% for turmeric, 12.2–19.3% for ginger and 15.7–25.3% for arbi compared to the yield in open area without trees. It was confirmed that aonla-based agri-horticultural systems were effective in bringing about improvement in the soil properties as reflected by the significant increase in organic carbon, available nitrogen and phosphorus. Economic analysis of the systems in terms of benefits : cost ratio revealed that 'aonla + turmeric' gave a higher value (6.29) followed by 'aonla + ginger' (3.44) and 'aonla + arbi' (3.20). The interspaces of the aonla orchard in calcareous belt of eastern India could be utilized for growing various intercrops to generate substantial additional income without adverse effect on the soil fertility and productivity of the main crop.

Keywords: Aonla, economic analysis, intercrops, soil fertility.

RESOURCE degradation leading to an unsustainable production system has demanded our attention for sustainable practices to assure continued production. In this context, aonla or Indian gooseberry (*Emblica officinalis* Gaertn.)-based agri-horticultural system has immense potential to utilize and conserve rainfed area for betterment of poor farmers. Aonla being a deep-rooted deciduous tree species has a wide range of adaptability to grow in any type of soil. It is considered a highly tolerant and potential fruit species suitable for growing under salt-affected and wasteland/ravine lands. Aonla provides higher economic returns with little investments in plantation establishment and its management. Cultivation of agricultural crops

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