Analysing an Agricultural Research Project

Contents

Syllabus outcome*:*

H 4.1 Applies appropriate experimental techniques, technologies, research methods, data presentation and analysis in relation to agricultural problems and situations.

Content:

- Analyse a research study of the development and/or implementation of ONE recent Agricultural technology in terms of:
  -design of the study
  - methodology of the study
  - collection of data for the study
  - presentation of data
  - analysis of the data
  - conclusions and recommendations

Specifically, students learn about research methodology and presentation of research by analysing a study of an innovative technology or practice that is advancing productivity in a production system (NSW Board of Studies).

Project Title : The resistance to **Vip3Aa** in *Helicoverpa species* in cotton

Background information:

- Crops expressing genes from *Bacillus thuringiensis* (Bt crops) are among the most successful technologies developed for the control of pests BUT the evolution of **resistance** to them remains a challenge.[1]
- *Helicoverpa armigera* is a pest feeding on more than 180 species of plants of which the most economically valuable are cotton, corn and grain sorghum. *Helicoverpa armigera* is known for its high level and rapid development of resistance to synthetic insecticides.[2]
- For better control of this pest, Bt crops expressing insecticidal proteins from Bacillus thuringiensis have been commercialised globally.[3]
- Bt cotton , marketed under **Ingard** (introduced into Australia in 1996) contained the gene Cry1Ac . **Bollgard II** (introduced into Australia in 2004) had dual toxin present as Cry1Ac and Cry2Ab. Even before the release of this dual toxin cotton in Australia, research showed higher than expected levels of resistance in alleles in *H armigera* and *Helicoverpa punctigera* to one of the Bt proteins (Cry2Ab) and resistance risk remains a critical concern.[4] The significant threat to Bt-based insect control is the continued potential development of insect resistance that could jeopardize their long term success.
- Vip3A is a vegetative insecticidal protein which has an intestine specific virulence factor that attacks the gut of the larvae of *H armigera* and *H punctigera* . Once ingested the proteins are processed by the insect midgut proteases.

  The mechanisms underlying resistance to the Cry proteins have been studied in some field populations and laboratory selected populations. In many cases the gene and mutation responsible has been identified.[5] The first resistance to the Vip3Aa in field populations of Australian H armigera occurred in 2009.

Project Aim:

- **Utilising vegetative insecticidal proteins (Vips) to overcome field resistance to BT cotton**

Experimental Design:

1 Collecting the Vip3A

- A Vip3A clone in *E coli* was used as a source of the toxin. The Vip3A was extracted from small trial plots grown in Australia between 2001 and 2003.
- Eggs were collected at random from commercial cotton farms in northern NSW and southern Qld. Permission was granted by individual land holders to access and collect insects

[1] Downes, Sharon, Tom Walsh, and Wee Tek Tay. "*Bt resistance in Australian insect pest species*". Current Opinion in Insect Science 15 (2016): 78-83.
[2] Ibid
[3] Ibid
[4] Ibid
[5] Ibid

at random from any cotton plants in their fields. None of the assessed commercial farms were protected in any way.

- *E coli* cells were extracted in a laboratory at 37° C in a shaking incubator.
- Vip3A was extracted and concentrated and induced into cells that were further cultivated at 28° C overnight.
- Cells were harvested by centrifugation for 15 mins.
- Cells were then partitioned into aliquots and frozen.
- Purified Vip3A was prepared from the frozen cells and examined for purity and stability.[6]

2 Testing the Vip3A

- Contamination bioassays using purified toxins were performed. Responses to increasing doses of Vip3A toxin were tested in a Bt susceptible colony of H armigera and H punctigera to establish appropriate concentrations of Vip3A to use.[7]
- To examine the features of any Vip3A resistance, H punctigera were collected as eggs from cotton grown in the Gwyder Valley in NSW in 2011. Further samples of eggs were collected from non BT cotton from St George in Qld and the Gwyder Valley, NSW in 2012.[8]

3 Experimental Design and Setup

- The majority of tests involved surface treatments of the eggs and egg larvae with the Vip3A toxin.

Experiment 1.

- The treatment was **REPLICATED** three times.
- Each treatment had a 45 standard well assay plate which was contaminated with a solution of the extracted toxin and allowed to air dry.
- When dry, an individual egg larvae was added to each well and sealed in place with a perforated lid.
- After incubation at 26° C for 7 days a count was done.
- The effectiveness of the Vip3A toxin was assessed by counting the number of alive and dead larvae.
- The experiment was carried out a number of times using both H armigera and H punctigera.[9]

Experiment 2.

- The experiment was **MODIFIED** and **REPLICATED** three times.
- Several doses of Vip3A toxin was mixed into a diet.

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[6] Mahon, R.J., Downes, S.J., James, B., 2012. Vip3A resistance alleles exist at high levels in Australian targets before release of cotton expressing this toxin. PLoS ONE 7 (6), e39192. http://dx.doi.org/10.137/journal.pone.0039192.

[7] Idib
[8] Ibid
[9] Ibid

- 6 mm³ of diet was placed in the wells of the standard well assay plate.
- Once larvae were placed in each well it was then heat sealed.
- After incubation at 26° C for 7 days a count was done.
- The effectiveness of the Vip3A toxin was assessed by counting the number of alive and dead larvae.[10]

Results:

- Research also showed that the larvae resistant to Vip3A are not cross resistant to Cry1Ac or Cry2Ab. This means that the combination of the three toxins has an increasing effect upon the larvae of H armigera and H punctigera. This is because each of those toxins has a different mode of action, or kills the larvae in three different ways. So if one of the toxins hasn't quite killed the larvae once it has bitten the leaf, the other two toxins are there to make sure that the larvae dies.

Discussion:

- In Australia monitoring for resistance to the Vip3A in field populations of H armigera has been ongoing since 2009 which enable isolation of resistant alleles and development of colonies with these genes in the laboratory. The frequency of Vip3A resistance alleles in Australian populations of H armigera has not increased significantly in the six seasons that monitering has taken place.
- This third toxin Vip3A will help increase the longevity of the technology.
- Currently resistance to Bollgard II is managed through restricting planting windows, pupae busting and planting refuge crops.
- Bollgard III will provide a more robust resistance management tool for Australian cotton growers. The three proteins, Cry1Ac, Cry2Ab and Vip3A will increase the longevity of the technology.[11]

Recommendations for use of Bt cotton

- Over a 15 year period to 2012 the use of Bt cotton has been responsible for an additional 12.5 million tonnes of cotton lint, with the insect resistant traits accounting for almost all of the additional production of these crops.[12]
- The evolution of Bt cotton will continue. As more toxins are added to the profile the reliance upon chemical usage to control the boll moth will decrease. Until **Ingard** was released there were no Bt cotton crops grown in Australia.

[10] Ibid

[11] Monsanto. "Cotton Research and Development ". http://www.monsanto.com/global/au/products/pages/cotton-research-and-development.aspx (accessed June 6, 2016).

[12] Cotton Australia, "Biotechnology FactSheet", http://cottonaustralia.com.au/uploads/factsheets/CA_FACT_BIOTECHNOLOGY.pdf (accessed June 6, 2016).

- Increases yield of cotton – biotech cotton areas have grown to over 60% of the world cotton area by 2010.[13] 50% more cotton is produced worldwide today using the same amount of land as compared to 40 years ago. [14] Today in Australia, more than 99% of planted cotton uses biotechnology.
- Increases in farm income – since 2010 the total farm income gain derived by Australian cotton farmers from using this technology has been $395 million, an average of about $180/ha.[15]
- Reduction in pesticide use – in the cultivation of Bt cotton , Australian cotton farmers have reduced their insecticide use by 89% in the last 10 years with some crops not sprayed for insects at all.[16]
- Reductions in the costs of cultivations leading to improved soil quality.[17]
- A decrease in labour and fuel usage[18]
- Reduction in environmental pollution due to reduced pesticide usage and run-off. Bt cotton exhibits genetic resistance or inbuilt resistance which is a permanent type of resistance and not affected by environmental factors
- Improved farm workers and neighbour safety[19]
- Increased opportunities to grow cotton in areas of high pest infestation
- Improved fibre quality, maturity, less water and/or drought tolerant
- Bt cotton is environmentally sustainable as it does not have adverse effects on natural parasites (ladybirds, lacewings)/ predators/ beneficial insecticides and organisms present in the soil. It promotes multiplication of parasites and predators which help in controlling the bollworms by feeding on the larvae and eggs of the bollworm.
- Bt cotton is early maturing when compared to non Bt cotton

Disadvantages of Bt Cotton

- High cost of Bt cotton seeds as compared to non Bt cotton seed
- Effectiveness up to 120 days, after that the toxin producing efficiency of the Bt gene rapidly reduces though even reduced, it is still an effective control
- Ineffective against sucking pests such as jassids, aphids, whitefly.[20]

[13] Ibid
[14] Ibid
[15] Ibid
[16] Ibid
[17] Ibid
[18] Ibid
[19] Ibid

Conclusion:

- Bollgard technology is genetically modified cotton that is resistant to pests such as the cotton boll worm
- This new technology will be released in the 2016/17 cotton growing season
- Monsanto's 3rd generation Bt cotton will be sold as **Genuity Bollgard® III**. It will be especially valuable in Australia where Bollgard® II is used on at least 90% of cotton farms.[21]
- In the USA, Dow chemical company will utilise Vip3A in addition to Cry1Ac and Cry1F toxins in its chemical "Widespread". Bayer chemical company plans to licence from Syngenta a Bt cotton containing Cry1B and Vip3A that will trade as "Vipcot".[22]

Bibliography

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21 Monsanto, "Genuity Bollgard II Cotton" http://www.monsanto.com/products/pages/genuity-bollgardii-cotton.aspx (accessed 6 June, 2016)

22 Op. cit. Mahon, R.J et. al.