ΠΡΟΓΡΑΜΜΑ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗΣ ΑΕΙ ΓΙΑ ΤΗΝ ΕΠΙΚΑΙΡΟΠΟΙΗΣΗ ΓΝΩΣΕΩΝ ΑΠΟΦΟΙΤΩΝ ΑΕΙ (ΠΕΓΑ)

«Οι σύγχρονες τεχνικές βιο-ανάλυσης στην υγεία, τη γεωργία, το περιβάλλον και τη διατροφή»

Ευρωπαϊκή Ένωση
Ευρωπαϊκό Κοινωνικό Ταμείο

ΕΠΙΧΕΙΡΗΣΙΑΚΟ ΠΡΟΓΡΑΜΜΑ
ΕΚΠΑΙΔΕΥΣΗ ΚΑΙ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗ
επένδυση στην κοινωνία της γνώσης
ΥΠΟΥΡΓΕΙΟ ΠΑΙΔΕΙΑΣ ΚΑΙ ΘΡΗΣΚΕΥΜΑΤΩΝ
ΕΙΔΙΚΗ ΥΠΗΡΕΣΙΑ ΔΙΑΧΕΙΡΙΣΗΣ

ΕΣΠΑ 2007-2013
πρόγραμμα για την ανάπτυξη
ΕΥΡΩΠΑΪΚΟ ΚΟΙΝΩΝΙΚΟ ΤΑΜΕΙΟ

Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

ΓΕΝΕΤΙΚΑ ΤΡΟΠΟΠΟΙΗΜΕΝΟ ΚΑΛΑΜΠΟΚΙ ΠΙΘΑΝΟΙ ΚΙΝΔΥΝΟΙ ΓΙΑ ΤΗ ΓΕΩΡΓΙΑ ΚΑΙ ΤΟ ΠΕΡΙΒΑΛΛΟΝ

Κουρέτας Δημήτριος

Καθηγητής

Φυσιολογίας Ζωϊκών Οργανισμών

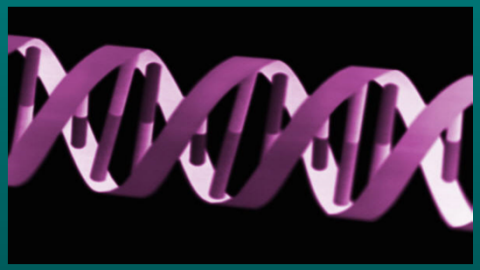
Πανεπιστήμιο Θεσσαλίας

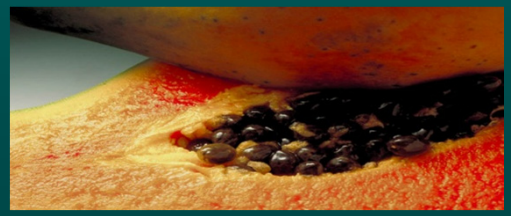
Τμήμα Βιοχημείας & Βιοτεχνολογίας

Εργαστήριο Φυσιολογίας Ζωικών Οργανισμών

ΤΙ ΕΙΝΑΙ ΓΕΝΕΤΙΚΑ ΤΡΟΠΟΠΙΗΜΕΝΑ (ΓΤ) ΟΡΓΑΝΙΣΜΟΙ (GENETICALLY MODIFIED ή GENETICALLY ENGINEERED)

- Γενετικά τροποποιημένοι είναι οι οργανισμοί στους οποίους έχει γίνει **εισαγωγή γονιδίων** ενός άλλου οργανισμού.

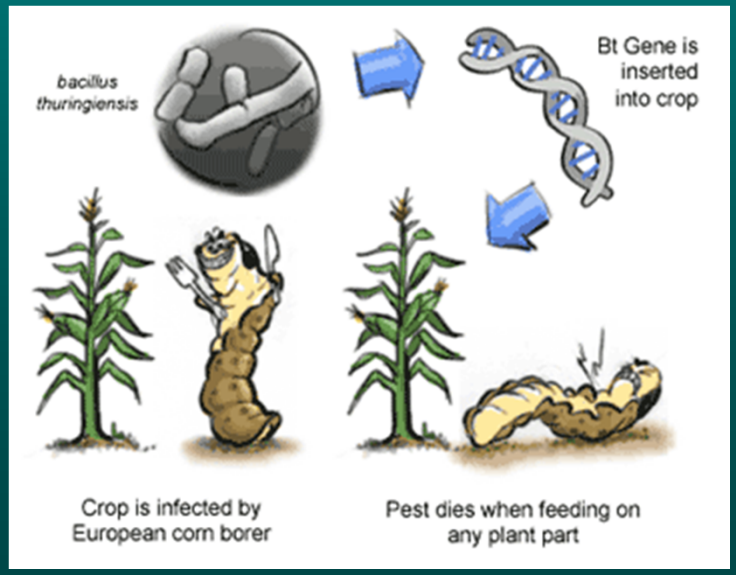




ΤΥΠΟΙ ΓΕΝΕΤΙΚΑ ΤΡΟΠΟΠΟΙΗΜΕΝΟΥ ΚΑΛΑΜΠΟΚΙΟΥ

- Όλοι οι ΓΤ ποικιλίες καλαμποκιού έχουν τα εξής χαρακτηριστικά:
- Ανθεκτικότητα στα ζιζανιοκτόνα (π.χ. Monsanto's Roundup Ready).
- Ανθεκτικότητα στα έντομα (π.χ. Monsanto's MON810, Syngenta's Bt11, Pioneer's 1507).

- Οι ποικιλίες MON810, Bt11 και 1507 έχουν τροποποιηθεί γενετικά με την εισαγωγή ενός γονιδίου που ονομάζεται Bt, από ένα βακτήριο του εδάφους το *Bacillus thuringiensis*.
- Το γονίδιο *Bt* φέρει την πληροφορία για την σύνθεση μιας πρωτεΐνης (τοξίνης) της Bt.
- Τα φυτά στα οποία έχει γίνει εισαγωγή του γονιδίου παράγουν την τοξίνη Bt.



ΤΥΠΟΙ ΓΕΝΕΤΙΚΑ ΤΡΟΠΟΠΟΙΗΜΕΝΟΥ ΚΑΛΑΜΠΟΚΙΟΥ

- **Η ποικιλία MON810 έχει πάρει την έγκριση της Ε.Ε. για καλλιέργεια αλλά υπόκειται σε απαγορεύσεις από κράτη μέλη της Ε.Ε. και έτσι καλλιεργείται μόνο στο 0.2% του εδάφους της Ε.Ε. και κυρίως στην Ισπανία, την Δημοκρατία της Τσεχίας, την Γερμανία, την Σλοβακία, την Πορτογαλία, την Ρουμανία και την Πολωνία.**

- **Οι ποικιλίες Bt11 και 1507 είναι στο τελικό στάδιο έγκρισης από την Ε.Ε.**

- Ήδη από τη δεκαετία του ’50, είχαν χρησιμοποιηθεί εντομοκτόνα σε μορφή σπρέϋ που περιείχαν Bt και τα οποία δεν προκαλούσαν βλάβες σε άλλα έντομα ή στην άγρια χλωρίδα και πανίδα.
- Οι τοξίνες Bt όμως που παράγονται από το ΓΤ καλαμπόκι διαφέρουν από αυτή που περιείχαν τα σπρέϋ.
- Πρόκειται για μια τοξίνη που είναι μικρότερη σε μέγεθος και έχει λιγότερο επιλεκτική δράση από ότι τα σπρέϋ Bt και συνεπώς μπορεί να προκαλεί βλάβες και σε άλλα έντομα εκτός από τα έντομα-στόχους.

ΤΟΞΙΚΗ ΕΠΙΔΡΑΣΗ ΤΟΥ ΓΤ ΚΑΛΑΜΠΟΚΙΟΥ ΣΕ ΟΡΓΑΝΙΣΜΟΥΣ ΠΟΥ ΔΕΝ ΚΑΤΑΣΤΡΕΦΟΥΝ ΤΑ ΦΥΤΑ ΚΑΛΑΜΠΟΚΙΟΥ

- Τα ΓΤ καλαμπόκια παράγουν την Bt τοξίνη που είναι τοξική σε ορισμένα είδη λεπιδόπτερων και πεταλούδων που καταστρέφουν τα φυτά του καλαμποκιού.
- Όμως, προνύμφες από λεπιδόπτερα και πεταλούδες που δεν είναι βλαβερά για το καλαμπόκι μπορεί να καταναλώσουν την Bt τοξίνη από φυτά που μεγαλώνουν κοντά σε καλλιέργειες καλαμποκιού.

Ενήλικο άτομο λεπιδόπτερου και κάμπια σε φύλλα καλαμποκιού

ΤΟΞΙΚΗ ΕΠΙΔΡΑΣΗ ΤΟΥ ΓΤ ΚΑΛΑΜΠΟΚΙΟΥ ΣΕ ΟΡΓΑΝΙΣΜΟΥΣ ΠΟΥ ΔΕΝ ΚΑΤΑΣΤΡΕΦΟΥΝ ΤΑ ΦΥΤΑ ΚΑΛΑΜΠΟΚΙΟΥ

- Για παράδειγμα, οι προνύμφες της βασιλικής πεταλούδας της Βορείου Αμερικής (που δεν είναι βλαβερές για το καλαμπόκι) είναι ιδιαίτερα ευαίσθητες στην τοξίνη Bt του ΓΤ καλαμποκιού. Χρόνια έκθεση στην τοξίνη Bt μείωσε την επιβίωση των προνυμφών της βασιλικής πεταλούδας έως την ενήλικη μορφή.
- Το παραπάνω παράδειγμα δείχνει πως οργανισμοί που δεν είναι βλαβεροί για το καλαμπόκι μπορεί να επηρεαστούν από την τοξίνη Bt.
- Επίσης, το παράδειγμα της βασιλικής πεταλούδας δείχνει πως μικρής διάρκειας αλλά και μακράς διάρκειας μελέτες είναι αναγκαίες. Στην περίπτωση της βασιλικής πεταλούδας, μικρής διάρκειας μελέτες (4-5 μέρες) δεν έδειξαν καμία επίδραση της τοξίνης Bt του ΓΤ καλαμποκιού, ενώ μόνο μακράς διάρκειας μελέτες (2 χρόνια) έδειξαν επίδραση.

Βασιλική πεταλούδα (monarch butterfly)

ΤΟΞΙΚΗ ΔΡΑΣΗ ΣΕ ΩΦΕΛΙΜΑ ΕΝΤΟΜΑ

- Η τοξίνη Bt του ΓΤ καλαμποκιού θα μπορούσε να επιδράσει σε έντομα που είναι ωφέλιμα για τον φυσικό έλεγχο των εντόμων που καταστρέφουν το καλαμπόκι.
- Για παράδειγμα, η τοξίνη Bt έχει **έμμεση τοξική δράση** στις **πράσινες ψείρες** (green lacewing) μέσω των εντόμων που αποτελούν λεία για αυτές, τα οποία με τη σειρά τους τρώνε το ΓΤ καλαμπόκι.
- Έτσι, η τοξίνη Bt από το ΓΤ καλαμπόκι μπορεί να σκοτώσει έντομα που δεν είναι βλαβερά για το καλαμπόκι και να περάσει **σε ανώτερα τροφικά επίπεδα μέσω της τροφικής αλυσίδας**.
- **Η έμμεση τοξικότητα** δυσχεραίνει τον προσδιορισμό των δυσμενών επιδράσεων της τοξίνης Bt του ΓΤ καλαμποκιού.

Green lacewing

ΤΟΞΙΚΗ ΔΡΑΣΗ ΣΕ ΩΦΕΛΙΜΑ ΕΝΤΟΜΑ

- Επίσης, μελέτες έχουν δείξει ότι η τοξίνη Bt του ΓΤ καλαμποκιού θα μπορούσε να επιδράσει τη διαδικασία μάθησης των μελισσών οι οποίες είναι σημαντικοί επικονιαστές.
- Ο κανονισμός της Ε.Ε. για τον προσδιορισμό του κινδύνου από την τοξίνη Bt περιλαμβάνει μικρής διάρκειας μελέτες θνησιμότητας σε ένα μόνο είδος. Τέτοιου είδους μελέτες δεν μπορούν να ανιχνεύσουν δράση έμμεσης τοξικότητας ή επίδραση στην συμπεριφορά ενός οργανισμού.
- Η επίδραση της τοξίνης Bt πρέπει να μελετάται σε πολλαπλά επίπεδα της τροφικής αλυσίδας.

ΠΙΘΑΝΗ ΒΛΑΒΗ ΜΑΚΡΑΣ ΔΙΑΡΚΕΙΑΣ ΣΤΑ ΕΔΑΦΗ ΑΠΟ ΤΗΝ ΤΟΞΙΝΗ Bt ΤΟΥ ΓΤ ΚΑΛΑΜΠΟΚΙΟΥ

- Η τοξίνη Bt του ΓΤ καλαμποκιού μπορεί να παραμείνει στο έδαφος σε **βιολογικά ενεργή μορφή.**
- Μελέτες έχουν δείξει ότι η τοξίνη Bt του ΓΤ καλαμποκιού **αποικοδομείται πιο αργά** σε σύγκριση με αυτή των μη ΓΤ ποικιλιών.
- Η μειωμένη αποικοδόμηση της τοξίνης Bt του ΓΤ καλαμποκιού ίσως να οφείλεται στο ότι οι ΓΤ ποικιλίες περιέχουν **υψηλότερα επίπεδα λιγνίνης** από ότι οι μη ΓΤ ποικιλίες.
- Η **λιγνίνη** επηρεάζει την διαδικασία της πέψης στα φυτοφάγα ζώα και θα μπορούσε να καθυστερήσει την αποικοδόμηση των υπολειμμάτων της τοξίνης Bt στο έδαφος.

ΠΙΘΑΝΗ ΒΛΑΒΗ ΜΑΚΡΑΣ ΔΙΑΡΚΕΙΑΣ ΣΤΑ ΕΔΑΦΗ ΑΠΟ ΤΗΝ ΤΟΞΙΝΗ Bt ΤΟΥ ΓΤ ΚΑΛΑΜΠΟΚΙΟΥ

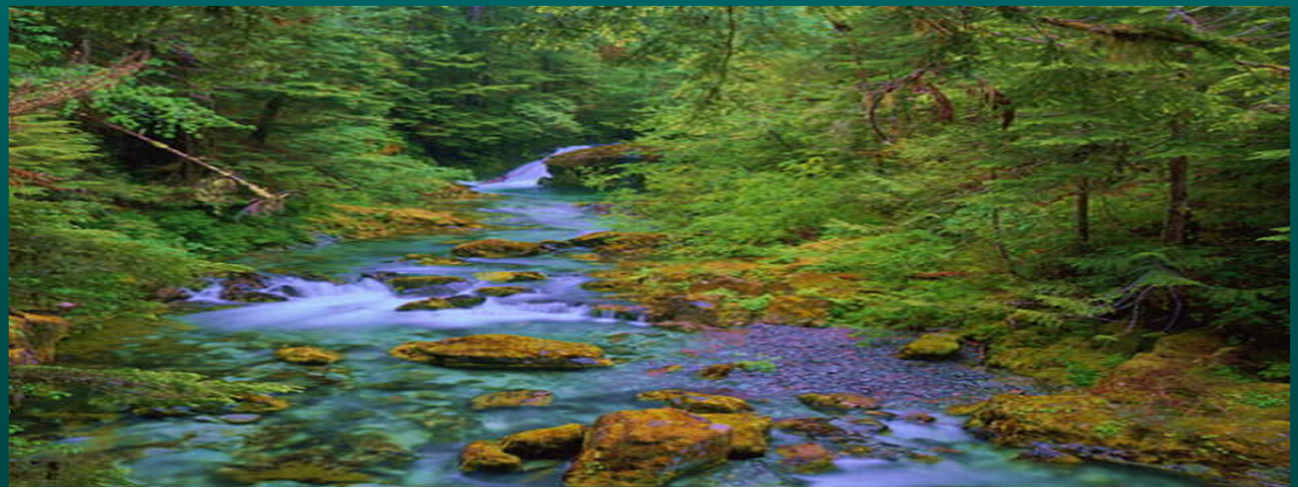
- Αν και τα εδάφη είναι οικοσυστήματα ζωτικής σημασίας για την γεωργία και την βιοποικιλότητα, ωστόσο οι γνώσεις μας για την οικολογία των εδαφών είναι περιορισμένες.
- Η τοξίνη Bt θα μπορούσε να είναι τοξική για οργανισμούς του εδάφους όπως οι γαιοσκώληκες και τα νηματώδη.
- Ιδιαίτερα, πρέπει να μελετηθούν οι μακροχρόνιες επιπτώσεις των ΓΤ καλαμποκιών στο έδαφος.

γαιοσκώληκας

νηματώδες

ΕΠΙΔΡΑΣΗ ΤΗΣ ΤΟΞΙΝΗΣ Bt ΤΟΥ ΓΤ ΚΑΛΑΜΠΟΚΙΟΥ ΣΤΑ ΥΔΑΤΙΚΑ ΟΙΚΟΣΥΣΤΗΜΑΤΑ

- Η τοξίνη Bt του ΓΤ καλαμποκιού που είτε απελευθερώνεται από τις ρίζες είτε από τα υπολείμματα του φυτού που παραμένουν στο χωράφι μπορεί να εισέλθει σε χείμαρρους και να είναι τοξική για υδάτινους μικροοργανισμούς με πιθανές επιπτώσεις ακόμα και σε επίπεδο οικοσυστήματος.
- Τεστ οικοτοξικότητας με οργανισμούς (π.χ. Daphnia magna) που χρησιμοποιούνται ως δείκτες για την ποιότητα του νερού έδειξαν σημαντικά μειωμένη ικανότητα προσαρμοστικότητας όταν τους δινότανε ως τροφή μια ποικιλία ΓΤ καλαμποκιού. Αυτό αποτελεί ένδειξη τοξικότητας.
- Αν και μελέτες έχουν δείξει ότι οι υδατικοί μικροοργανισμοί δεν επηρεάζονται, θα μπορούσε να υπάρχουν επιπτώσεις σε έντομα που ζουν στο νερό και είναι συγγενικά ως προς τα λεπιδόπτερα και τις πεταλούδες.



ΕΠΙΔΡΑΣΗ ΤΗΣ ΤΟΞΙΝΗΣ Bt ΤΟΥ ΓΤ ΚΑΛΑΜΠΟΚΙΟΥ ΣΤΑ ΥΔΑΤΙΚΑ ΟΙΚΟΣΥΣΤΗΜΑΤΑ

- **Το γονίδιο που φέρει τη γενετική πληροφορία για την τοξίνη Bt του ΓΤ καλαμποκιού μπορεί να διατηρείται σε υδάτινα περιβάλλοντα και έχει βρεθεί σε ιστούς μυδιών σε περιοχές όπου καλλιεργούνται ΓΤ καλαμπόκια. Η τοξίνη Bt συσσωρεύεται σε οργανισμούς που καταναλώνονται από τα μύδια.**
- **Οι επιπτώσεις από την παρουσία του γονιδίου Bt σε ζωϊκούς οργανισμούς δεν είναι γνωστές, και ούτε είναι γνωστό αν το γονίδιο μπορεί να εκφραστεί σε ζωϊκούς ιστούς.**
- **Ο κανονισμός της Ε.Ε. για τον προσδιορισμό του κινδύνου από την τοξίνη Bt του ΓΤ καλαμποκιού δεν προβλέπει μελέτη των πιθανών επιπτώσεων στα υδάτινα οικοσυστήματα.**

ΑΥΞΗΜΕΝΗ ΑΝΘΕΚΤΙΚΟΤΗΤΑ ΤΩΝ ΕΝΤΟΜΩΝ ΣΤΗΝ ΤΟΞΙΝΗ Bt ΤΟΥ ΓΤ ΚΑΛΑΜΠΟΚΙΟΥ

- Στις Η.Π.Α. υπάρχουν κανονισμοί που υποχρεώνουν τη δημιουργία περιοχών με μη ΓΤ καλαμπόκια μέσα στις καλλιέργειες ΓΤ καλαμποκιού με σκοπό να εμποδιστεί η ανάπτυξη εντόμων με ανθεκτικότητα στην τοξίνη Bt.

- Αυτές όμως οι περιοχές με μη ΓΤ καλαμπόκια μπορεί να έχουν αποτέλεσμα στις μεγάλες φάρμες των Η.Π.Α., αλλά ίσως να μην έχουν πρακτική χρησιμότητα στις μικρές φάρμες της Ευρώπης λόγω της διασταύρωσης των μη ΓΤ καλαμποκιών με τα ΓΤ καλαμπόκια με αποτέλεσμα τα έντομα να εκτίθενται στην τοξίνη Bt και στις περιοχές των μη ΓΤ καλαμποκιών.

- Επίσης, η διαφορά στην έκφραση της τοξίνης Bt μπορεί να επηρεάσει την ταχύτητα με την οποία αναπτύσσεται ανθεκτικότητα.

- Αν τα επίπεδα έκφρασης είναι χαμηλά η τοξίνη μπορεί να μην σκοτώνει τα έντομα αλλά αντίθετα να τα επιτρέπει να επιβιώνουν υποβοηθώντας την ανάπτυξη ανθεκτικότητας.

ΑΥΞΗΜΕΝΗ ΑΝΘΕΚΤΙΚΟΤΗΤΑ ΤΩΝ ΕΝΤΟΜΩΝ ΣΤΗΝ ΤΟΞΙΝΗ Bt ΤΟΥ ΓΤ ΚΑΛΑΜΠΟΚΙΟΥ

- Υπάρχουν ποικιλίες ΓΤ καλαμποκιού που παρουσιάζουν διαφορές στα επίπεδα έκφρασης της τοξίνης Bt από φυτό σε φυτό. Αυτή η διακύμανση στην έκφραση της τοξίνης Bt αυξάνεται με τη χρήση αζωτούχων λιπασμάτων.

- Υπάρχει πληθώρα επιστημονικών δεδομένων που δείχνουν ότι εάν εμφανιστεί ανθεκτικότητα των εντόμων στην τοξίνη Bt σε μεγάλο βαθμό η ιδιότητα των ΓΤ καλαμποκιών να ανθίστανται στα έντομα θα μειωθεί σημαντικά. Έτσι, η χρησιμοποίηση νέων και ίσως περισσότερο τοξικών εντομοκτόνων θα είναι αναπόφευκτη.

- Υπάρχουν στοιχεία που δείχνουν ότι στις Η.Π.Α. έχουν εμφανιστεί έντομα ανθεκτικά στα ΓΤ φυτά βαμβακιού. Αν και η εμφάνιση ανθεκτικότητας δεν έχει οδηγήσει σε καταστροφές καλλιεργειών γιατί οι αγρότες χρησιμοποιούν εντομοκτόνα και στις καλλιέργειες με ΓΤ φυτά.

- Η εμφάνιση εντόμων ανθεκτικών στην τοξίνη Bt θέτει σοβαρές απειλές για τις αειφόρους και οικολογικές καλλιεργητικές μεθόδους.

ΜΠΟΡΕΙ ΤΟ ΓΤ ΚΑΛΑΜΠΟΚΙ ΝΑ ΟΔΗΓΗΣΕΙ ΣΤΗΝ ΑΝΑΠΤΥΞΗ ΒΛΑΒΕΡΩΝ ΕΝΤΟΜΩΝ

- Αρκετές μελέτες έχουν δείξει ότι άλλα έντομα που προκαλούν βλάβες στο καλαμπόκι μπορούν να αντικαταστήσουν το κενό που δημιουργείται λόγω της καταπολέμησης των εντόμων που αποτελούν στόχους της τοξίνης Bt.

- Για παράδειγμα, στο 42% των καλλιεργειών με ΓΤ πατάτες που συνέθεταν την τοξίνη Bt αυξήθηκαν οι πληθυσμοί εντόμων που προκαλούν βλάβες στις πατάτες αν και δεν ανήκουν στους βασικούς εχθρούς της πατάτας. Το αποτέλεσμα ήταν να παρεμποδίζεται η ανάπτυξη αυτών των ΓΤ φυτών πατάτας οι οποίες δεν είναι πλέον εμπορικά διαθέσιμες.

ΑΥΞΗΜΕΝΗ ΑΝΘΕΚΤΙΚΟΤΗΤΑ ΤΩΝ ΕΝΤΟΜΩΝ ΣΤΗΝ ΤΟΞΙΝΗ Bt ΤΟΥ ΓΤ ΚΑΛΑΜΠΟΚΙΟΥ

- Στην Κίνα και σε άλλες χώρες οι αγρότες έπρεπε να ψεκάζουν περισσότερα εντομοκτόνα μετά από κάποια χρόνια **καλλιέργειας ΓΤ βαμβακιού** που παρήγαγε την τοξίνη Bt για την καταπολέμηση εντόμων που δεν ανήκουν στους κύριους εχθρούς του βαμβακιού.
- Αυτοί οι δευτερεύοντες εχθροί του βαμβακιού ελέγχονταν στο παρελθόν με τη χρήση **κοινών εντομοκτόνων** που χρησιμοποιούνταν στις καλλιέργειες βαμβακιού.
- Έτσι, η χρήση του ΓΤ βαμβακιού αρχικά μείωσε τα εντομοκτόνα που χρησιμοποιούνταν στις παραδοσιακές καλλιέργειες βαμβακιού αλλά είχε ως αποτέλεσμα την ανάπτυξη των δευτερευόντων εντόμων που είναι εχθροί του βαμβακιού, με αποτέλεσμα η χρήση εντομοκτόνων για αυτούς τους δευτερεύοντες εχθρούς να αυξηθεί σε πολλαπλάσιο βαθμό.

ΤΟ ΓΤ ΚΑΛΑΜΠΟΚΙ ΜΠΟΡΕΙ ΝΑ ΟΔΗΓΗΣΕΙ ΣΤΗΝ ΑΝΑΠΤΥΞΗ ΒΛΑΒΕΡΩΝ ΕΝΤΟΜΩΝ

- Στις καλλιέργειες ΓΤ καλαμποκιού στις Η.Π.Α. έχει αυξηθεί ο αριθμός ενός εντόμου, του western bean cutworm, σε σύγκριση με τις συμβατικές καλλιέργειες.
- Επίσης, σε ΓΤ καλλιέργειες καλαμποκιού έχει παρατηρηθεί αύξηση του αριθμού μιας αφίδας που προσβάλει τα φύλλα του καλαμποκιού. Η αύξηση των αφίδων έχει οδηγήσει και σε αύξηση ενός παρασίτου που τρέφεται με τα αυγά των αφίδων.
- Ο λόγος για τον οποίο αυξήθηκε ο αριθμός των αφίδων πιστεύεται ότι σχετίζεται με τα υψηλά επίπεδα κάποιων αμινοξέων στον χυμό του φυτού από τον οποία τρέφονται οι αφίδες. Δεν είναι όμως γνωστό τι προκάλεσε τη μεταβολή στη χημική σύσταση του φυτικού χυμού. Πιθανώς η απροσδόκητη επίδραση οφείλονταν στη διαδικασία της γενετικής μηχανικής για την εισαγωγή του γονιδίου Bt στο φυτό.

Western bean cutworm

ΜΠΟΡΕΙ ΤΟ ΓΤ ΚΑΛΑΜΠΟΚΙ ΝΑ ΟΔΗΓΗΣΕΙ ΣΤΗΝ ΑΝΑΠΤΥΞΗ ΒΛΑΒΕΡΩΝ ΕΝΤΟΜΩΝ

- **Κατά συνέπεια οι αλληλεπιδράσεις μεταξύ των εντόμων που τρέφονται από τα φυτά είναι πολύπλοκες.**

- **Μπορεί με τα ΓΤ φυτά να ελέγχεται ο πληθυσμός ενός εντόμου αλλά αυτό θα αντικατασταθεί από ένα άλλο. Έτσι, η μείωση της χρήσης των εντομοκτόνων στις ΓΤ καλλιέργειες θα είναι παροδική.**

Η ΣΥΝΥΠΑΡΞΗ ΓΤ ΠΟΙΚΙΛΙΩΝ ΚΑΙ ΣΥΜΒΑΤΙΚΩΝ ΔΕΝ ΕΙΝΑΙ ΔΥΝΑΤΗ

- Υπάρχουν πολλές μελέτες που δείχνουν ότι η διασταύρωση μεταξύ ΓΤ ποικιλιών καλαμποκιού είναι δυνατή **σε απόσταση 1km**.

- Σε όλες τις εκθέσεις της Ε.Ε. σχετικά με τον έλεγχο εξάπλωσης των ΓΤ φυτών αναφέρεται ότι το **ΓΤ καλαμπόκι είναι το πιο δύσκολο να ελεγχθεί** λόγω της μεγάλης απόστασης στην οποία μπορούν να συμβούν διασταυρώσεις.

- Για παράδειγμα στην Ισπανία, πολλές **οργανικές καλλιέργειες** καλαμποκιού είχαν επιμολυνθεί με ΓΤ καλαμπόκι αλλά οι καλλιεργητές δεν το ανάφεραν για οικονομικούς λόγους και από φόβο να μην χάσουν το πιστοποιητικό της οργανικής καλλιέργειας.
- Επίσης, μια από τις συνέπειες ήταν να μειωθούν οι εκτάσεις των οργανικών καλλιεργειών **κατά 75%** στην Αραγονία, την περιοχή της Ισπανίας με τις περισσότερες καλλιέργειες ΓΤ καλαμποκιού.

Η ΣΥΝΥΠΑΡΞΗ ΓΤ ΠΟΙΚΙΛΙΩΝ ΚΑΙ ΣΥΜΒΑΤΙΚΩΝ ΔΕΝ ΕΙΝΑΙ ΔΥΝΑΤΗ

- Επίσης, υπάρχει το ενδεχόμενο το ΓΤ καλαμπόκι να επιβιώσει τον χειμώνα στη Μεσογειακή Ευρώπη και έτσι θα μπορεί να επιμολύνει μη ΓΤ καλλιέργειες. ΓΤ φυτά καλαμποκιού έχουν επιβιώσει κατά τη διάρκεια του χειμώνα στην Αγγλία που έχει σχετικά ψυχρό κλίμα σε σχέση με άλλες Ευρωπαϊκές χώρες.

- Φυτά ΓΤ καλαμποκιού έχουν παρατηρηθεί σε χέρσα χωράφια και στις άκρες δρόμων τη χρονιά που ακολουθούσε την παραγωγή ΓΤ καλαμποκιού, προφανώς από σπόρους που είχαν διασπαρεί τυχαία. Τέτοια ΓΤ φυτά θα μπορούσαν να επιμολύνουν γενετικά μη ΓΤ καλλιέργειες καλαμποκιού

- Η συνύπαρξη ΓΤ και μη ΓΤ ποικιλιών καλαμποκιού είναι αδύνατη. Στην Ε.Ε. δεν υπάρχει νομοθεσία για την αποζημίωση των αγροτών των οποίων οι συμβατικές καλλιέργειες καλαμποκιού θα επιμολύνονταν με ΓΤ.

ΣΥΜΠΕΡΑΣΜΑΤΑ

- Όλες οι ποικιλίες ΓΤ καλαμποκιού μπορεί να προκαλέσουν δυσμενείς επιπτώσεις στο φυσικό περιβάλλον και στην γεωργία, όπως:
- Επιδράσεις σε οργανισμούς που δεν αποτελούν εχθρούς του καλαμποκιού, όπως με έμμεση τοξικότητα και μακροχρόνιες επιδράσεις.
- Επιδράσεις, ιδιαίτερα μακροχρόνιες, στην οικολογία των εδαφών.
- Συσσώρευση και διατήρηση της τοξίνης Bt σε υδάτινα περιβάλλοντα.
- Εμφάνιση εντόμων που είναι ανθεκτικά στην τοξίνη Bt.
- Αύξηση στους πληθυσμούς άλλων εντόμων που δεν είναι οι κύριοι εχθροί του καλαμποκιού.
- Επιδράσεις στις μεθόδους της αειφόρου καλλιέργειας.

ΣΥΜΠΕΡΑΣΜΑΤΑ

- **Η διαδικασία που έχει τεθεί από την Ε.Ε. για τον προσδιορισμό του κινδύνου από το ΓΤ καλαμπόκι είναι ανεπαρκής γιατί δεν περιλαμβάνει τον προσδιορισμό επιδράσεων που δεν είναι θανατηφόρες ή είναι μακροχρόνιες, καθώς και την έμμεση τοξικότητα.**

- **Η απελευθέρωση των ΓΤ οργανισμών στο περιβάλλον είναι μη αντιστρεπτή. Συγκεκριμένα, η εξάπλωση τυο ΓΤ καλαμπόκι είναι ανεξέλεγκτη λόγω του μεγάλου βαθμού επιμειξιών με συμβατικές ποικιλίες και των μεγάλων αποστάσεων τις οποίες μπορεί να διανύσει η γύρη αυτών των φυτών.**

- **Η συνύπαρξη ποικιλιών ΓΤ καλαμποκιού και συμβατικών ποικιλιών είναι αδύνατη. Η καλλιέργεια ΓΤ καλαμποκιού θα αποτρέψει τους αγρότες να αρνηθούν την καλλιέργεια του, και κατά συνέπεια τους καταναλωτές να μπορούν να επιλέξουν την αποφυγή ΓΤ τροφών.**

ΣΥΜΠΕΡΑΣΜΑΤΑ

- **Το μέλλον της γεωργίας βασίζεται όχι στα ΓΤ φυτά αλλά στην οικολογική γεωργία που δημιουργεί νέες θέσεις εργασίας, προωθεί την ανάπτυξη των αγροτικών περιοχών και προάγει τη βιοποικιλότητα με το να προστατεύει το έδαφος, το νερό και το κλίμα.**

- **Τα οικολογικά συστήματα εξασφαλίζουν ασφαλή διατροφή για σήμερα και για το μέλλον και δεν επιμολύνουν το περιβάλλον με χημικές ενώσεις ή με γενετικά τροποποιημένους οργανισμούς.**

Box 1-1). This 'one-door-one key' regulatory strategy is in line with the European Commission's effort to integrate food and feed regulation across sectors and to enhance the efficiency and coherence of the review system. It is also hoped that this strategy will help to avoid fiascos such as the finding of Aventis' Starlink™ insect-protected corn in the food chain, although it had only been approved for local cultivation and feed use in the US.

BOX 1-1

EU LEGISLATION ON GM FOODS

Environmental release
Directive 2001/18/EC on the deliberate release into the environment of GMOs and repealing Council Directive 90/220/EEC

GM food and feed
Regulation (EC) No 1829/2003 on GM food and feed

Contained use of GM micro-organisms
Council Directive 90/219/EEC on the contained use of GM micro-organisms
Council Directive 98/81/EC amending Directive 90/219/EEC on the contained use of GM micro-organisms

Labelling of GM food and feed
Council Regulation (EC) No 1139/98 concerning the compulsory indication of the labelling of certain foodstuffs produced from GMOs of particulars other than those provided for in Directive 79/112/EEC
Commission Regulation (EC) No 49/2000 amending Council Regulation (EC) No 1139/98 concerning the compulsory indication on the labelling of certain foodstuffs produced from GMOs of particulars other than those provided for in Directive 79/112/EEC
Commission Regulation (EC) No 50/2000 on the labelling of foodstuffs and food ingredients containing additives and flavourings that have been genetically modified or have been produced from GMOs
Regulation (EC) No 1830/2003 concerning the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs and amending Directive 2001/18/EC

Labelling, traceability, and consumer choice

The European Commission's role in consumer protection has grown together with its increasingly political remit since entry into force in 1987 of the Single European Act. This priority has been further highlighted in the Treaty of Amsterdam. The website of the European Commission DG SANCO spells out that provision of consumer information and choice through labelling and product information is considered an important element required to re-establish consumer trust in the EU food safety analysis and control system after the BSE and other food safety crises. Information should be provided on health-related issues and on salient matters related to food production processes.

US firms 'tried to lie' over GM crops, says EU

By Michael McCarthy, Environment Editor

14 October 2003

American biotech companies tried to lie to Europe in an attempt to force genetically modified crops upon them, Margot Wallström, the European environment commissioner, said yesterday.

Far from developing GM crops to solve the problem of starvation in the world, as they claimed, the biotech companies did so to "solve starvation amongst their shareholders", said the European Union's leading green politician.

Speaking to journalists in London, the 49-year-old Swede followed her broadside over GM with an attack on the US over the so-called ghost fleet of rusting and polluted American ships being sent to Britain for dismantling, saying they should be kept in America.

She further suggested that the US government had been putting pressure on Russia not to ratify the Kyoto protocol.

GM crops unlikely to remain contained, study shows

By Danielle Demetriou

14 October 2003

Genetically modified crops may not remain self-contained, according to a Government study published yesterday which revealed bees are able to carry pollen up to 16 miles away.

Stringent regulations are likely to be enforced on future GM crop trials, after the study discovered that pollen could travel eight times further than previously thought.

A second report found that after GM oilseed rape was grown, it would take 16 years before conventional crops could be grown without fear of breaching the maximum 1 per cent contamination threshold.

The findings, published by the Department for Environment, Food and Rural Affairs, coincided with protests from about 1,000 anti-GM campaigners who marched through central London.

The first study, conducted by the Scottish Crops Research Institute, raises question about previous research in Canada and the UK which claimed the pollen of GM crops could travel only 2.5 miles.

Using "bait plants", it found that contamination could take place as far afield as 16 miles from the original site. While long distance transfer was described as rare and resulted in a dilution gene flow, the study revealed that bees were the key culprits. They carried the pollen back to the hive then swapping it with other pollen, resulting in the fertilisation of plants.

The second study revealed that unless weeds from GM crops were stringently controlled, there was a risk of contaminating conventional crops for up to 16 years. To ensure crops were not contaminated, weedkiller would have to be sprayed regularly.

Study heightens fears over GM superweeds

By Steve Connor Science Editor

10 October 2003

Cross-pollination between GM plants and their wild relatives is inevitable and could create hybrid superweeds resistant to the most powerful weedkillers, according to the first national study of how genes pass from crops to weeds.

Its findings will raise concerns about the impact of GM crops. Next week the results will be published of farm-scale trials which have studied the impact on the countryside of three types of crop.

The government-funded scientists said the latest findings "contrast" with previous assessments of gene flow between farm crops and weeds. They had suggested that the danger of hybridisation - where two types of plant cross-pollinate to create another, for example a superweed - was limited. Superweeds are considered to be a threat because, in some cases, they might absorb resistance to weedkillers from GM crops engineered to be herbicide-tolerant.

But the results of the research, which involved analysing satellite images of the British countryside and patrolling 180 miles of river banks, reveal that hybridisation is both more widespread and frequent than previously anticipated.

Mike Wilkinson of Reading University, who led the study published today in the journal Science, said physical barriers such as isolation distances - buffer zones designed to stop pollen spreading from GM crops into the wild - would have only a limited impact on preventing hybridisation.

"This [study] shows that isolation distances will reduce hybrid numbers but not prevent hybridisation. It depends on what level of hybridisation you deem acceptable but if you want to absolutely prevent hybrids then isolation distances will not do so," Dr Wilkinson said. "Hybridisation is more or less inevitable in the UK context," he added.

The study concentrated on non-GM oilseed rape and assessed how easily it cross-bred with a near-relative in the wild called bargeman's cabbage, also known as wild turnip, which typically grows along river banks. Although the research was based on conventional oilseed rape, Dr Wilkinson said the conclusions applied to any flow of genes that could be expected from the GM varieties of oilseed rape that were undergoing farm-scale trials.

Eosinophilia–myalgia syndrome and tryptophan production: a cautionary tale

Arthur N. Mayeno and Gerald J. Gleich

An epidemic of a new disease, termed eosinophilia–myalgia syndrome, occurred in the USA in 1989. This syndrome was linked to the consumption of L-tryptophan manufactured by a single company utilizing a fermentation process. All the findings indicate that the illness was probably triggered by an impurity formed when the manufacturing conditions were modified. This outbreak highlights the need for close monitoring of the chemical purity of biotechnology-derived products, and for rigorous testing of such products following any significant changes to the manufacturing process.

During the fall of 1989, an epidemic of a new disease occurred in the USA. The illness was characterized by blood eosinophilia (raised numbers of a type of white blood cell) and myalgia (severe muscle pain), and was termed the eosinophilia–myalgia syndrome (EMS) (Refs 1–4). It was initially recognized in October 1989 when physicians in New Mexico identified three women with similar clinical findings; all three had consumed L-tryptophan prior to the onset of their illness[3]. The physicians' findings were publicized by the local news media and, soon after, additional cases of the same illness were identified throughout the USA and in several other countries.

Epidemiological studies initiated in early November 1989 by the health departments of the states of New Mexico and Minnesota demonstrated a strong association between the consumption of tryptophan and the onset of EMS (Refs 6,7). A national surveillance program was initiated by the US Centers for Disease Control (CDC) to investigate the new disease. On 11 November 1989, the US Food and Drug Administration (FDA) issued a nationwide warning advising consumers to discontinue the use of tryptophan food supplements. The agency subsequently requested a nationwide recall of all tryptophan sold over-the-counter.

With the removal of tryptophan from the consumer markets, the number of new cases of EMS diminished rapidly. Nevertheless, >1500 people were affected by the illness, and 37 deaths have so far been attributed to EMS. Many patients are still in a chronic phase of the disease. The toll would certainly have been higher were it not for the alertness of physicians who linked the new disease to tryptophan, the epidemiological investigations by the state health departments and the CDC, and the prompt recall, by the FDA, of products containing L-tryptophan. While epidemiological and chemical investigations indicate that the epidemic of EMS was caused by contaminated L-tryptophan, the precise contaminant(s) causing the disease is still unknown.

A. N. Mayeno and G. J. Gleich are at the Allergic Disease Research Laboratory, and G. J. Gleich is also at the Department of Immunology, Mayo Clinic and Mayo Foundation, Rochester, MN 55905, USA.

Prevalence and reasons for tryptophan use

In 1989, the use of L-tryptophan was widespread in the USA. In Oregon and Minnesota, ~2% of the household members surveyed had used tryptophan at sometime between 1980 and 1989 (Refs 7,8). The most common reasons for using tryptophan were insomnia, premenstrual syndrome and depression. Although most consumers purchased tryptophan for therapeutic use, it was marketed as a food supplement and was widely available in the USA without a prescription. The manufacturers of the product did not make any claims regarding its therapeutic efficacy and the product was not regulated or approved by the FDA. L-tryptophan is an essential amino acid; however, sufficient quantities are present in the diet of most US citizens without the need for supplements.

Eosinophilia–myalgia syndrome

National surveillance data

As of June 1993, 1511 cases of EMS have been reported to the CDC, including 37 deaths[9]. The case definition included: (1) an eosinophil count

Table 1. Genetic modifications of the different strains of *Bacillus amyloliquefaciens* used to manufacture L-tryptophan

Strain[a]	Modification
I	Original strain of *B. amyloliquefaciens* IAM 1521
II	The tryptophan operon (coding for all enzymes catalyzing reactions from chorismate to L-tryptophan, as well as for those involved in the biosynthesis of serine and PRPP) of strain I was duplicated by chromosomal integration
III	The isolated tryptophan operon was attached to a more efficient promoter prior to integration into the chromosomal DNA of strain II
IV	The *ser A* gene (coding for phosphoglycerate dehydrogenase) was amplified using a plasmid vector with strain III
V	The *prs* gene (coding for ribose phosphate pyrophosphokinase) was isolated and integrated into the chromosome of strain IV

[a]Strains II to V were derived by successive modifications of strain I.

bacterium *Bacillus amyloliquefaciens*[7]. The biosynthetic pathway of L-tryptophan is shown in Fig. 1. Several new strains of *B. amyloliquefaciens* (I–V), each modified slightly to increase the biosynthesis of tryptophan, were introduced sequentially during the years preceding the outbreak of EMS (Table 1).

In December 1988, the company introduced a new strain of *B. amyloliquefaciens* (strain V), which had been genetically modified to increase the synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP), an intermediate in the biosynthesis of tryptophan (see Fig. 1). After fermentation, the tryptophan was extracted from the broth and purified using a series of filtration, crystallization, and separation processes. The purification procedures included contact with powdered activated carbon and then granulated activated carbon. The amount of powdered activated carbon in each batch was usually ≥20 kg during 1988. In 1989, the amount of powdered activated carbon used to purify some batches of tryptophan was reduced to 10 kg. From October 1988 to June 1989, a proportion of some of the fermentation batches also bypassed a filtration step that employed a reverse-osmosis–membrane (ROM) filter to remove chemicals with a molecular weight >1000 Da. According to the company, these changes did not significantly alter the purity of the tryptophan powder, which was maintained at 99.6% or greater.

Univariate analysis of retail lots of tryptophan consumed by case patients and controls demonstrated an association between the development of EMS and the ingestion of tryptophan processed with 10 kg of powdered carbon per batch (odds ratio, 9.0; 95% confidence interval, 1.1 to 84.6; $p=0.014$) and the use of *B. amyloliquefaciens* strain V (odds ratio, 6.0; 95% confidence interval, 0.8 to 51.8; $p=0.04$) (Ref. 7). Thus, both a reduction in the amount of powdered activated carbon and the use of *B. amyloliquefaciens* (strain V) were significant manufacturing changes, but the independent contribution of each manufacturing change could not be assessed because of the high correlation

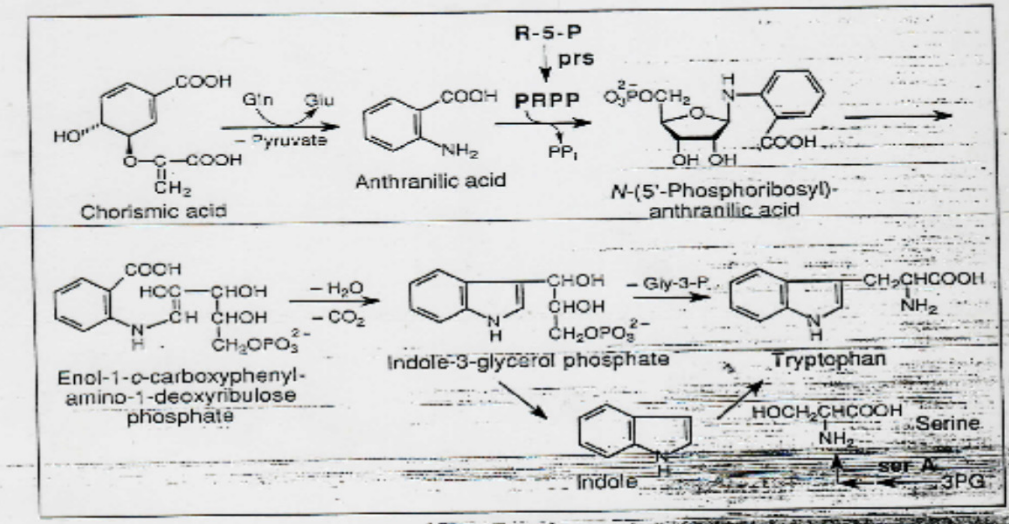


Figure 1.
Biosynthetic pathway of L-tryptophan. (Adapted from Ref. 8.)

between them. Bypass of the ROM filter was not significantly associated with the case lots. Studies carried out by Showa Denko suggested that the 'biochemical and physiological characteristics' of *B. amyloliquefaciens* (strain V) did not differ from those of earlier strains. At present, it is unknown which particular changes in the production process contributed to the formation of the etiological agent(s).

Contaminants associated with EMS

Once the link between EMS and manufactured L-tryptophan had been established, chemical analyses of bulk tryptophan lots were performed by researchers at the Mayo Clinic (Rochester, MN, USA), the FDA (Washington, DC, USA), the CDC (Atlanta, GA, USA), and the Japanese National Institute of Hygienic Sciences (Tokyo, Japan) to determine if any contaminants were associated with EMS (Refs 7, 16–18). High performance liquid chromatography was used to separate the contaminants in the tryptophan and revealed that each manufacturer's tryptophan produced a unique chromatographic pattern, or 'fingerprint', that was distinctive for the product from each company. The chromatographic pattern consisted of multiple peaks, each of which represented a trace chemical constituent other than tryptophan. The chromatogram for Showa Denko tryptophan included five 'signature' peaks that were present in all the tryptophan manufactured by this company (Fig. 2). Initial comparison of individual peaks in case and control lots of tryptophan demonstrated a single peak (called 'peak E' or 'peak 97') that was significantly associated with case lots[7]. The chemical structure of peak E was subsequently determined to be 1,1'-ethylidenebis[L-tryptophan], or EBT (Refs 19, 20) (Fig. 3). Two other contaminants were subsequently reported to be associated with case lots of tryptophan manufactured by Showa Denko[15]. One of the peaks, labeled UV-5, eluted before tryptophan, and was determined to be 3-(phenylamino)-L-alanine (PAA) (Refs 21, 22) (Fig. 3). The other peak (UV-28) eluted much later than EBT and is as yet uncharacterized. Recent HPLC studies reveal 60 trace contaminants in Showa Denko tryptophan, six of which are associated with EMS (Ref. 18). The structures of three of these contaminants are known [EBT, PAA, and 'peak 200' (2[3-indolylmethyl]-L-tryptophan)] (Ref. 18), but the other three have not yet been characterized. One of the uncharacterized contaminants, called 'peak AAA', was the contaminant most significantly associated with EMS and was recommended for characterization[18].

The amount of EBT present in Showa Denko tryptophan varied markedly in the period 1987–1989 (Fig. 4), presumably reflecting alterations in the manufacturing conditions. It is likely that levels of all of the contaminants varied with time. These data are consistent with the hypothesis that a contaminant in tryptophan is responsible for EMS and for the sporadic cases of EP between 1986 and 1988 (Ref. 14). Recent statistical analyses of EBT, adjusted for serial autocorrelation (to take into account that sequential lots

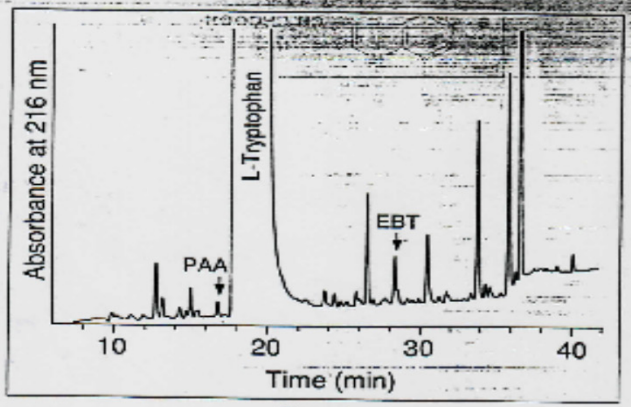


Figure 2
High-performance liquid chromatography (HPLC) chromatogram of eosinophilia–myalgia syndrome (EMS)-associated L-tryptophan

of tryptophan may be related), reveal that higher levels of EBT are still associated with EMS, but the association ($p = 0.120$) did not achieve statistical significance[23]. Nonetheless, the authors caution that the results do not vindicate EBT as a cause of EMS because misclassification of lots as case or control could weaken the association, and the methods used to account for the lack of independence of observations over time probably reduce the power of the statistics.

Connection with toxic oil syndrome

The clinical and pathological findings of EMS bear a striking resemblance to those of the toxic-oil syndrome (TOS) (Ref. 24), an outbreak of which occurred in Spain during 1981. Over 20000 people were affected, and there were 839 deaths[25]. Unlike EMS, respiratory symptoms (cough or dyspnea) were prominent and severe in TOS during the first week of illness. Other early symptoms included fever, malaise, headache, nausea, splenomegaly (abnormal enlargement of the spleen), diffuse adenopathy (enlargement of the lymphatic glands) and pruritic (itchy) rash[24]. In some patients, the disease progressed to an intermediate and chronic phase that resembled EMS more closely. The intermediate phase (two to eight weeks after onset) was characterized by eosinophilia and leukocytosis (raised numbers of leukocytes). Patients whose illness progressed to the late phase developed muscle cramps and severe myalgias, peripheral edema, scleroderma-like skin changes and polyneuropathy. The histopathology of skin, nerve and skeletal muscle is remarkably similar in EMS and TOS (Refs 26, 27).

Epidemiological investigations implicated ingestion of aniline-denatured rapeseed oil that was sold by itinerant salesmen after being reprocessed illegally. Chemical analyses of implicated oil samples and 'control' oil samples demonstrated that [illegible]

How might a manufacturer guard against the possibility that a product could cause EMS or a related disease? First, insistence on chemical purity is important; the L-tryptophan causing EMS contained >60 trace contaminants, some at concentrations of several hundred parts per million. Second, following manufacturing changes in a product intended for human consumption, the product should be tested in animals in an appropriate bridging study, i.e. a study previously demonstrated to produce definitive toxicological endpoints. In the absence of a suitable animal model, however, cautious dose-escalation of the test product to a limited study population and/or human volunteers, with careful evaluation of their responses, particularly changes in the number of peripheral blood eosinophils, should be performed. Third, manufacturing conditions must be carefully regulated and, in the event of any significant process changes, the product should be tested as previously described. Finally, because all manufactured chemicals (produced synthetically or biologically) contain impurities, all new substances produced for human consumption warrant testing. Even with these precautions, variability in the manufacturing process could alter the types and quantities of impurities. The level of EBT contamination varied markedly in lots of tryptophan produced between December 1987 and December 1989 (Fig. 4). Testing of tryptophan produced in December 1988 or September 1989 might well have been negative, even though tryptophan produced in the period January to May 1989 was associated with EMS (Refs 7, 11). Moreover, the quantities of the known EMS-associated contaminants, EBT and PAA, were remarkably small, of the order of 0.01%, and could easily escape detection. Hence, periodic testing of all manufactured products is a reasonable precaution.

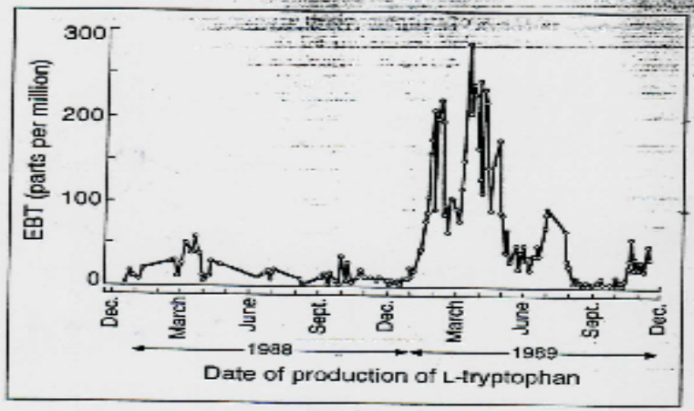


Figure 4
Levels of 1,1'-ethylidenebis[L-tryptophan] (EBT) in lots of L-tryptophan produced by Showa Denko K.K. during 1988 and 1989.

Summary

EMS is an inflammatory disease that resulted from the ingestion of tryptophan manufactured by one company. It is clinically and pathologically similar to eosinophilic fasciitis (EF) and the toxic oil syndrome (TOS). The syndrome is triggered by one or more contaminants in tryptophan. Contaminants that have been studied include 1,1'-ethylidenebis[tryptophan] (EBT) and 3-(phenylamino)alanine (PAA), although other uncharacterized contaminants have recently been discovered. One or more of these chemicals may trigger EMS by an undefined mechanism, or they may be surrogate markers for another unidentified substance that triggers the syndrome. Consumption of high doses of tryptophan and increased age of the consumer have been identified as risk factors. The occurrence of EF during 1986–1988 in patients who ingested tryptophan was probably caused by tryptophan-associated contaminants. Ongoing research is focused on identifying contaminants in implicated tryptophan and on establishing an animal model of the disease. The presence of chemically related aniline derivatives in tryptophan and toxic oil is suggestive of a related etiology. Success in these endeavors would greatly increase our understanding of eosinophilic diseases and prevent the outbreak of future epidemics.

Acknowledgements

We thank Dr Yasushi Torigne of Showa Denko K.K. for Fig. 4 and Table 1.

References

Owing to *TIBTECH*'s policy of short reference lists, the number of references cited in this article has been limited. Some work has, therefore, unfortunately been left unacknowledged.

1 Kilbourne, E. M. (1992) *Epidemiol. Rev.* 14, 16–36
2 Belongia, E. A., Mayeno, A. N. and Osterholm, M. T. (1992) *Annu. Rev. Nutr.* 12, 235–256
3 Varga, J., Jimenez, S. A. and Uitto, J. (1993) *J. Invest. Dermatol.* 100, 97S–105S
4 Taylor, R. and McNeil, J. J. (1993) *Med. J. Aust.* 158, 51–55
5 Hertzman, P. A., Blevins, W. L., Mayer, J., Greenfield, B., Ting, M. and Gleich, G. J. (1990) *New Engl. J. Med.* 322, 869–873
6 Eidson, M., Philen, R. M., Sewell, C. M., Voorhees, R. and Kilbourne, E. M. (1990) *Lancet* 335, 645–648
7 Belongia, E. A. *et al.* (1990) *New Engl. J. Med.* 323, 357–365
8 Slutsker, L., Hoesly, F. C., Miller, L., Williams, J. P., Watson, J. C. and Fleming, D. W. (1990) *J. Am. Med. Assoc.* 264, 213–217
9 Hsieh, D. L. and Goldman, L. R. (1993) *Arch. Intern. Med.* 153, 2368–2373
10 Swygert, L. A., Maes, E. F., Sewell, L. E., Miller, L., Falk, H. and Kilbourne, E. M. (1990) *J. Am. Med. Assoc.* 264, 1698–1703
11 Beck, E. E., Henning, K. J., Kallenbach, L. R., Brix, K. A., Gunn, R. A. and Melius, J. M. (1993) *J. Rheumatol.* 20, 666–672
12 Kamb, M. L. *et al.* (1992) *J. Am. Med. Assoc.* 267, 77–82
13 Philen, R. M., Eidson, M., Kilbourne, E. M., Sewell, C. M. and Voorhees, R. (1991) *Arch. Intern. Med.* 151, 533–537
14 Martin, R. W., Duffy, J. and Liu, J. T. (1991) *Mayo Clin. Proc.* 66, 892–898
15 Lehninger, A. L., Nelson, D. L. and Cox, M. M. (1993) *In Principles of Biochemistry*, 2nd edn, p. 705, Worth
16 Toyo'oka, T. *et al.* (1991) *Chem. Pharm. Bull.* 39, 820–825
17 Tsuchiya, M. W. (1993) *J. Chromatogr.*

Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine

Stanley W B Ewen, Arpad Pusztai

See Commentaries pages 1314, 1315

Diets containing genetically modified (GM) potatoes expressing the lectin *Galanthus nivalis* agglutinin (GNA) had variable effects on different parts of the rat gastrointestinal tract. Some effects, such as the proliferation of the gastric mucosa, were mainly due to the expression of the GNA transgene. However, other parts of the construct or the genetic transformation (or both) could also have contributed to the overall biological effects of the GNA-GM potatoes, particularly on the small intestine and caecum.

Genetically modified (GM) plant products are becoming increasingly common in the human food-chain, yet in contrast to the general acceptance of the need for the biological testing of novel foods and feedstuffs, few studies have been carried out on the possible effects of GM products on the mammalian ut mucosa. GM potatoes expressing a snowdrop lectin (*Galanthus nivalis* agglutinin [GNA]) under the CaMV35s promoter have been developed to increase insect and nematode resistance.[1] GNA was selected for insertion into potatoes because the initial effect of this mannose-specific lectin on the rat small bowel has been shown to be minimal,[2] and because its binding to mannose present on the epithelial surface of rat jejunal villi is demonstrable only after feeding for 10 days. We compared the histological indices of the gut of rats fed potato diets containing GM potatoes, non-GM potatoes, or non-GM potatoes supplemented with GNA, to find out whether GNA gene insertion had affected the nutritional and physiological impact of potatoes on the mammalian gut.

ELISA analysis confirmed that the expression level of GNA in raw GM potatoes was 25·4 μg/g dry matter; the concentration was decreased to 4·9 μg/g after boiling for 1 h. Six rats were randomly allocated to each group, and were fed diets containing either raw or boiled GNA-GM potatoes, parent potatoes (Desiree), or parent-line potatoes supplemented with 25·4 μg/g GNA for 10 days. All potato diets were isocaloric and contained an average of 6% protein. Histological samples of stomach, jejunum, ileum, caecum, and colon were taken 10 days after the start of feeding. The samples, each 2 cm in length, were opened along the antimesenteric border. The serosal surface was allowed to adhere to card for 3 min and was then fixed in 10% neutral buffered formalin for 18 h at 20°C. Paraffin sections (4 μm) were stained with haematoxylin and eosin, and mucosal thickness (stomach) or crypt length (jejunum, ileum, caecum, and colon) was measured by video-image analysis. Intraepithelial lymphocytes are equally distributed in all parts of the small intestine, and are known to increase when non-specific intestinal damage occurs. Thus, to assess potential damage, intraepithelial lymphocytes were counted in eight jejunal villi from each of the six rats fed diets containing GNA-GM potatoes or parent potatoes, both raw and boiled. No such measurements were made for the group fed parent potatoes spiked with GNA because dietary GNA or other lectins do not induce lymphocyte infiltration. GNA binding to the jejunum and ileum was measured by elution with 0·1 mol/L mannose, followed by ELISA.

	Mean (SD) crypt length (μm) and difference between treatments*						Statistical analysis (p)†			Interaction (p)†	
	Parent	Parent *vs* parent+GNA (p)	Parent+GNA	Parent+GNA *vs* GNA-GM (p)	GNA-GM	Parent *vs* GNA-GM (p)	Effect of GNA	Effect of cooking	Effect of trans-formation	GNA×cook	Trans×cook
Stomach											
Boiled	294 (46)	0·29	347 (42)	0·37	339 (36)	0·02	0·001	0·052	0·868	0·917	0·543
Raw	261 (32)	0·03	312 (32)	0·98	323 (54)	0·07					
p	0·18		0·94		0·35						
Jejunum											
Boiled	75 (19)	0·72	78 (17)	0·97	78 (12)	0·71	0·029	0·171	0·041	0·035	0·037
Raw	57 (8)	0·14	64 (11)	0·01	90 (20)	<0·01					
p	0·06		0·09		0·24						
Ileum											
Boiled	59 (8)	0·20	55 (7)	0·12	63 (13)	0·43	0·221	0·001	0·106	0·209	0·942
Raw	71 (9)	0·24	79 (13)	0·43	87 (25)	0·15					
p	0·02		<0·01		0·06						
Caecum											
Boiled	95 (19)	0·90	98 (21)	0·04	70 (15)	0·05	0·033	0·001	0·566	0·497	0·021
Raw	132 (19)	0·02	104 (17)	0·25	119 (25)	0·35					
p	<0·01		0·55		<0·01						
Colon											
Boiled	146 (15)	0·02	177 (24)	0·02	139 (24)	0·65	0·878	0·002	0·181	0·231	0·001
Raw	192 (34)	0·04	148 (25)	<0·01	215 (34)	0·28					
p	0·02		0·07		<0·01						

Data are the means of six animals calculated from five observations for each. GNA×cook=interaction between GNA and cooking; Trans×cook=interaction between transformation and cooking.
*By Student's *t* test. †By multivariate analysis with Tukey's test.

Table 1: **Effect of raw and cooked parent, parent+GNA, and GNA+GM potatoes on histological indices of rat gut**

et al. [7] is homologous to 6A SP-A cDNA [6]. The nucleotide sequence of oligo 8b is common to both 1A and 6A cDNAs [24].

Following restriction enzyme and Southern blot analysis all three positive clones hybridized with oligo 8b but only clones H3 and H5 hybridized with oligo 7. A 2.5-kb *Acc*I fragment (shown schematically in Fig. 3A) from clones H3 and H5 that hybridized with oligo 7 was subsequently cloned into M13 and sequenced. The sequencing strategy is shown in Fig. 3A and the sequencing data in Fig. 3B. Sequencing data from four clones, two each from H3 and H5, were identical.

Comparison of Rat and Human Sequences. The rat and human sequences (Fig. 2B and Fig. 3B) were compared using dot matrix analysis (DNA STAR). A number of regions showing sequence homology were revealed (Fig. 4). We chose to study the two boxed sequences because they were the longest homologous sequences and because their relative positions within the two 5′ flanking regions were similar, as shown by their positions close to the matrix diagonal. The possibility that other homologous sequences play various roles in SP-A regulation cannot be eliminated at this time. This approach revealed two regions of homology between the two species, depicted in boxes in Fig. 4. The highest degree of homology (~75%) was found between −225/−17 in rats (−156 and +1, in the numbering of Lacaze-Masmonteil et al. [36]) and −226/−36 in humans. Another ho-

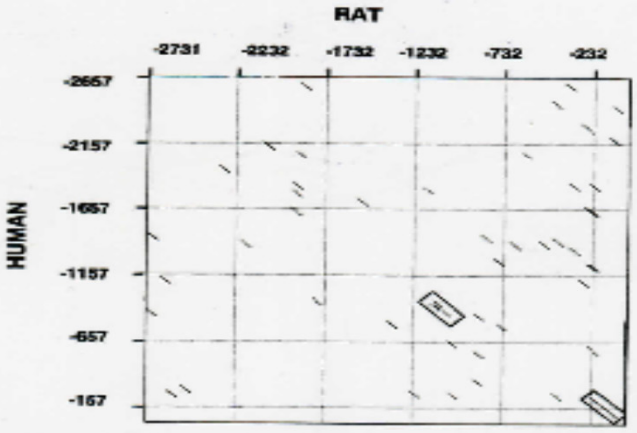


Figure 4 Dot matrix representation of nucleotide homology between rat and human SP-A 5′ flanking sequences. The sequence comparison includes approximately 2.7 kb upstream from the TATAA box of the rat and 2.6 kb of the human SP-A gene. Each dot represents 15 nucleotides (stringency) within a 30-nucleotide stretch (window size) with a repetition of 25 consecutive windows (minimum quality = 25). DNASTAR program (option Compare and DOT PLOT) was used for these studies. The two regions with the highest degree of homology between the two species are boxed.

Reversibility of hepatocyte nuclear modifications in mice fed on genetically modified soybean

M. Malatesta,[1] C. Tiberi,[1,2] B. Baldelli,[1] S. Battistelli,[1] E. Manuali,[2] M. Biggiogera[3]
[1]Istituto di Istologia e Analisi di Laboratorio, University of Urbino "Carlo Bo", Urbino; [2]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia; [3]Laboratorio di Biologia Cellulare e Neurobiologia del Dipartimento di Biologia Animale ed Istituto di Genetica Molecolare del CNR (Sezione di Istochimica e Citometria), University of Pavia, Italy

In the literature, the reports on the effects of a genetically modified (GM) diet are scanty and heterogeneous; in particular, no direct evidence has so far been reported that GM food may affect human or animal health.
Hepatocytes represent a suitable model for monitoring the effects of a GM diet, the liver potentially being a primary target. In a previous study, we demonstrated that some modifications occur in hepatocyte nuclei of mice fed on GM soybean. In order to elucidate whether such modifications can be reversed, in the present study, 3 months old mice fed on GM soybean since their weaning were submitted to a diet containing wild type soybean, for one month. In parallel, to investigate the influence of GM soybean on adult individuals, mice fed on wild type soybean were changed to a GM diet, for the same time. Using immunoelectron microscopy, we demonstrated that a one-month diet reversion can influence some nuclear features in adult mice, restoring typical characteristics of controls in GM-fed animals, and inducing in control mice modifications similar to those observed in animals fed on GM soybean from weaning. This suggests that the modifications related to GM soybean are potentially reversible, but also that some modifications are inducible in adult organisms in a short time.

Key words: cell nucleus, liver, genetically modified soybean, electron microscopy.

Correspondence: Dr. Manuela Malatesta,
Istituto di Istologia e Analisi di Laboratorio, University of Urbino "Carlo Bo", via Zeppi s.n., 61029 Urbino, Italy
Tel: +39.0722.321473.
Fax: +39.0722.322370.
E-mail: malatesta@uniurb.it

Paper accepted on April 18, 2005

European Journal of Histochemistry
2005; vol. 49 issue 3 (Jul-Sep): 237-242

In the literature, the reports on the effects of a genetically modified (GM) diet are scanty and heterogeneous (e.g., Schubbert *et al.*, 1998; Ewen and Pustzai, 1999; Chiter *et al.*, 2000; Edwards *et al.*, 2000); in particular, no direct evidence has so far been reported that GM food may affect human or animal health, and scarce are the studies on the effects of a diet containing significant amounts of GM plants (Malatesta *et al.*, 2002a,b, 2003; Vecchio *et al.*, 2004). These studies have been obviously focussed on organs and tissues which may be seen as potential targets (either directly or indirectly) of this diet. In this view, the liver is a primary site where the biotransformation of the products of digestion takes place through the degradation and/or detoxification of xenobiotic compounds received from the intestines or from the general circulation; in addition, the liver is involved in the synthesis of many plasmatic protein components and, more generally, in the overall metabolic control of the organism. Hepatocytes may, therefore, represent a suitable model for monitoring –at the cellular level- one of the targets of the diet. In a previous study (Malatesta *et al.*, 2002a), we demonstrated that some modifications occur in hepatocyte nuclei of mice fed on GM soybean: these changes are mainly related to the structural constituents involved in the transcription and splicing processes. In the present investigation, we aimed at elucidating whether such modifications can be reversed: to do this, mice fed on GM soybean from their weaning to the third month of age were submitted to a diet containing wild type soybean, for one month. In parallel, to investigate the influence of GM soybean on adult individuals, mice which had been fed on wild type soybean were administered for one month a GM diet. Morphometrical and immunocytochemical analyses have been carried out on hepatocyte nuclei at electron microscopy,

Ultrastructural analysis of testes from mice fed on genetically modified soybean.

Vecchio L, Cisterna B, Malatesta M, Martin TE, Biggiogera M.

We have considered the possible effects of a diet containing genetically modified (GM) soybean on mouse testis. This organ, in fact, is a well known bioindicator and it has already been utilized, for instance, to monitor pollution by heavy metals. In this preliminary study, we have focussed our attention on Sertoli cells, spermatogonia and spermatocytes by means of immunoelectron microscopy. Our results point out that the immunolabelling for Sm antigen, hnRNPs, SC35 and RNA Polymerase II is decreased in 2 and 5 month-old GM-fed mice, and is restored to normal at 8 months. In GM-fed mice of all ages considered, the number of perichromatin granules is higher and the nuclear pore density lower. Moreover, we found enlargements in the smooth endoplasmic reticulum in GM-fed mice Sertoli cells. A possible role played by traces of the herbicide to which the soybean is resistant is discussed.

PMID: 15718213 [PubMed - indexed for MEDLINE]

ARTICLE IN PRESS

Available online at www.sciencedirect.com

ScienceDirect

Food and Chemical Toxicology xxx (2006) xxx–xxx

www.elsevier.com/locate/foodchemtox

A 90-day safety study in Wistar rats fed genetically modified rice expressing snowdrop lectin *Galanthus nivalis* (GNA)

Morten Poulsen [a,*], Stine Kroghsbo [a], Malene Schrøder [a], Andrea Wilcks [b], Helene Jacobsen [a], Andreas Miller [c], Thomas Frenzel [c], Jürgen Danier [d], Michael Rychlik [e], Qingyao Shu [f], Kaveh Emami [g], Duraialagraja Sudhakar [h], Angharad Gatehouse [g], Karl-Heinz Engel [c], Ib Knudsen [a]

[a] *Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark*
[b] *Department of Microbiological Food Safety, Danish Institute for Food and Veterinary Research, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark*
[c] *Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technical University Munich, Am Forum 2, D-85350 Freising-Weihenstephan, Germany*
[d] *Bioanalytik Weihenstephan, Alte Akademie 10, D-85350, Freising-Weihenstephan, Germany*
[e] *Lehrstuhl für Lebensmittelchemie, Technical University Munich, Lichtenbergstr. 4, D-85748 Garching, Germany*
[f] *Institute of Nuclear Agricultural Sciences, Zhejiang University, Hangzhou 310029, China*
[g] *School of Biology, Institute for Research on Environment and Sustainability, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK*
[h] *Tamil Nadu Agricultural University, Coimbatore 648003, Tamil Nadu, India*

Received 14 October 2005; accepted 1 September 2006

Abstract

Genetically modified plants expressing insecticidal traits offer a new strategy for crop protection, but at the same time present a challenge in terms of food safety assessment. The present 90-day feeding study was designed to assess the safety of a rice variety expressing the snowdrop *Galanthus nivalis* lectin (GNA lectin), and forms part of a EU-funded project where the objective has been to develop and validate sensitive and specific methods to assess the safety of genetically modified foods. Male and female Wistar rats were given a purified diet containing either 60% genetically modified or parental rice for 90 days. This corresponds to a mean daily GNA lectin intake of approximately 58 and 67 mg/kg body weight for males and females, respectively. Prior to the animal study comprehensive analytical characterization of both rice materials was performed. The chemical analyses showed a number of statistically significant differences, with the majority being within the ranges reported in the literature. In the animal study a range of clinical, biological, immunological, microbiological and pathological parameters were examined. A number of significant differences were seen between groups fed the two diets, but none of them were considered to be adverse. In conclusion, the design of the present animal study did not enable us to conclude on the safety of the GM food. Additional group(s) where the expressed gene products have been spiked to the diet should be included in order to be able to distinguish whether the observed effects were due to the GNA lectin *per se* or to secondary changes in the GM rice.

Keywords: Genetically modified rice; Safety assessment; Animal study; GNA lectin; SAFOTEST

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world. Worldwide it comprises approximately 23% of all calories consumed; in some countries more than 60% of the dietary calories are derived from this cereal (Khush, 2003).

The ever-increasing demands on yield are responsible for the development of many different high yielding varieties of rice. However, whilst the extensive cultivation of modern high yielding varieties has on the one hand resulted

* Corresponding author. Tel.: +45 7234 7540; fax: +45 7234 7699.
E-mail address: mop@dfvf.dk (M. Poulsen).

0278-6915/$ - see front matter
doi:10.1016/j.fct.2006.09.002

Table 2
Proximate composition of brown rice material from transgenic line GNA and its corresponding parental line ASD16 (mean ± confidence interval, $n = 4$, $p < 0.05$)

Component (%)	GNA	ASD16	Literature data[a]
Moisture	12.9 ± 0.2	12.8 ± 0.3	9.1–14.1
Starch	63.9 ± 0.7[b]	69.3 ± 1.8	57–77
Fiber	1.8 ± 0.1[b]	1.4 ± 0.3	0.5–3.5
Sugars	0.6 ± 0.1[b]	0.3 ± 0.1	0.6–1.3
Protein	12.6 ± 0.2[b]	10.5 ± 0.3	6.1–9.5
Fat	3.23 ± 0.44	3.47 ± 0.10	1.4–2.9
Ash	2.01 ± 0.03[b]	1.49 ± 0.03	0.9–1.5

[a] Ranges from minimum to maximum reported values (Juliano, 1985; Scherz and Senser, 2000; Møller et al., 2002; USDA, 2004; OECD, 2004).
[b] Statistically significant different from parental line ($p < 0.05$).

Table 3
Amino acid levels in brown rice material from transgenic line GNA and its corresponding parental line ASD16 (g/100 g; mean ± confidence interval, $n = 3$, $p < 0.05$)

Amino acid	GNA	ASD16	Literature data[a]
Alanine	0.75 ± 0.09[b]	0.57 ± 0.05	0.46–0.58
Arginine	1.03 ± 0.04[b]	0.88 ± 0.07	0.44–0.91
Aspartic acid	1.55 ± 0.04[b]	1.39 ± 0.10	0.74–0.87
Cystine	0.33 ± 0.02	0.31 ± 0.01	0.06–0.19
Glutamic acid	2.28 ± 0.21[b]	1.77 ± 0.16	1.52–1.76
Glycine	0.54 ± 0.07	0.60 ± 0.03	0.39–0.49
Histidine	0.50 ± 0.07[b]	0.38 ± 0.04	0.12–0.27
Isoleucine	0.55 ± 0.04[b]	0.44 ± 0.05	0.26–0.57
Leucine	1.06 ± 0.09[b]	0.88 ± 0.02	0.50–0.93
Lysine	0.63 ± 0.05[b]	0.46 ± 0.04	0.10–0.42
Methionine	0.38 ± 0.03	0.35 ± 0.05	0.05–0.31
Phenylalanine	0.77 ± 0.15	0.58 ± 0.07	0.30–0.55
Proline	0.95 ± 0.04[b]	0.45 ± 0.01	0.37–0.40
Serine	0.63 ± 0.05[b]	0.51 ± 0.05	0.41–0.50
Threonine	0.47 ± 0.05[b]	0.35 ± 0.07	0.19–0.62
Tryptophan	0.16 ± 0.00[b]	0.13 ± 0.01	0.03–0.11
Tyrosine	0.72 ± 0.08	0.62 ± 0.15	0.21–0.47
Valine	0.78 ± 0.03[b]	0.67 ± 0.05	0.40–0.76

[a] Ranges from minimum to maximum reported values (Scherz and Senser, 2000; USDA, 2004).
[b] Statistically significant different from parental line ($p < 0.05$).

The difference in protein content was also reflected in the amino acid levels (Table 3). GNA rice exhibited statistically significant higher contents of almost all amino acids. In particular the content of proline was very high in GNA rice compared to the parental rice (+111%). Except for isoleucine, threonine and valine, data for GNA rice exceed data reported in the literature (Scherz and Senser, 2000; USDA, 2004).

Fatty acid distributions of the two lines were similar (Table 4). Minor but statistically significant differences were detected for proportions of myristic acid (+33%) and stearic acid (+26%). Patterns of both lines were in agreement to data reported in literature (Scherz and Senser, 2000; USDA, 2004; OECD, 2004; Kitta et al., 2005).

Mineral compositions are presented in Table 5. No statistically significant differences were observed for contents of calcium, magnesium, molybdenum and zinc. However,

Table 4
Fatty acid distribution in brown rice material from transgenic line GNA and its corresponding parental line ASD16 (mean ± confidence interval, $n = 4$, $p < 0.05$)[a]

Fatty acid	GNA	ASD16	Literature data[b]
Myristic acid	0.4 ± 0.0[c]	0.3 ± 0.0	0.4–3.0
Palmitic acid	20.2 ± 0.2	19.9 ± 0.1	18–31
Stearic acid	2.4 ± 0.0[c]	1.9 ± 0.0	1.6–2.6
Oleic acid	39.0 ± 0.1	39.4 ± 0.1	27–41
Linoleic acid	33.2 ± 0.2	33.2 ± 0.1	31–40
Linolenic acid	1.4 ± 0.0	1.4 ± 0.0	0.9–1.7

[a] Proportions of total fatty acids (%).
[b] Ranges from minimum to maximum reported values (Scherz and Senser, 2000; USDA, 2004; OECD, 2004; Kitta et al., 2005).
[c] Statistically significant different from parental line ($p < 0.05$).

Table 5
Contents of minerals in brown rice material from transgenic line GNA and its corresponding parental line ASD16 (mean ± confidence interval, $n = 4$, $p < 0.05$)

Mineral	GNA	ASD16	Literature data[a]
Calcium (g/kg)	0.3 ± 0.0	0.2 ± 0.0	0.1–0.5
Copper (mg/kg)	2.5 ± 0.2[b]	3.1 ± 0.1	1–6
Iron (mg/kg)	44 ± 4[b]	18 ± 2	2–52
Magnesium (g/kg)	1.6 ± 0.0	1.5 ± 0.0	0.2–1.7
Manganese (mg/kg)	24.6 ± 0.3[b]	21.4 ± 0.5	2–37
Molybdenum (mg/kg)	1.3 ± 0.1	1.2 ± 0.1	0.3–1.0
Phosphorous (g/kg)	4.3 ± 0.0[b]	3.6 ± 0.0	1.7–4.4
Potassium (g/kg)	3.4 ± 0.1[b]	2.6 ± 0.1	0.6–2.8
Zinc (mg/kg)	24.5 ± 11.2	28.0 ± 0.3	6–28

[a] Ranges from minimum to maximum reported values (Juliano, 1985; Scherz and Senser, 2000; Møller et al., 2002; USDA, 2004).
[b] Statistically significant different from parental line ($p < 0.05$).

GNA rice exhibited statistically significant higher contents of iron (+144%), manganese (+15%), phosphorous (+19%) and potassium (+31%), and a statistically significant lower copper content (−19%). Given the very large variation previously reported for mineral levels in brown rice (Juliano, 1985; Scherz and Senser, 2000; Møller et al., 2002; USDA, 2004), these differences observed between GNA and ASD16 were considered as low.

Statistically significant differences were found for important vitamins of the B-complex (Table 6). GNA rice exhibited higher contents of vitamin B_1 (+28%) and B_6 (+50%). No statistically significant difference was observed for the niacin content. Whereas the content of total pantothenic acid was higher in GNA rice (+52%), the content of total folic acid was higher in the parental rice (+129%). In both lines 5-methyl-H_4 folate was the major folate vitamer. Vitamin contents for both lines were in agreement with data reported for brown rice (Juliano, 1985; Scherz and Senser, 2000; Møller et al., 2002; USDA, 2004).

γ-Oryzanol comprises a mixture of steryl ferulates found in rice (Xu and Godber, 1999). They exhibit antioxidative (Xu et al., 2001) and cholesterol-lowering properties (Rong et al., 1997). No statistically significant difference was observed for the γ-oryzanol contents (Table 7). Data from

Table 14
Absolute and relative organ weights for rats fed on GNA rice diet and control rice diet

	Males		Females	
	GNA rice	Control	GNA rice	Control
Absolute weight				
Body weight	422 ± 33	417 ± 40	244 ± 22	257 ± 16
Adrenals	0.0576 ± 0.007	0.0600 ± 0.012	0.0759 ± 0.015[a]	0.0666 ± 0.008
Brains	2.02 ± 0.07	2.00 ± 0.09	1.88 ± 0.08	1.86 ± 0.10
Epididymis	1.176 ± 0.10	1.178 ± 0.16	–	–
Heart	1.14 ± 0.11	1.12 ± 0.09	0.805 ± 0.07	0.818 ± 0.08
Kidneys	2.40 ± 0.28	2.32 ± 0.25	1.52 ± 0.16	1.57 ± 0.16
Liver	12.7 ± 1.4	12.7 ± 1.7	7.54 ± 0.88	7.78 ± 0.72
Mesenterial ln.	0.109 ± 0.03	0.108 ± 0.03	0.092 ± 0.03[b]	0.131 ± 0.04
Ovaries	–	–	0.133 ± 0.03	0.122 ± 0.03
Pancreas	1.379 ± 0.43	1.284 ± 0.36	1.050 ± 0.23	1.047 ± 0.14
Small intestine	8.05 ± 1.05	8.07 ± 1.03	6.14 ± 0.84	5.91 ± 0.59
Spleen	0.776 ± 0.07	0.762 ± 0.10	0.555 ± 0.079	0.552 ± 0.051
Testes	3.91 ± 0.34	3.92 ± 0.34	–	–
Thymus	0.393 ± 0.08	0.385 ± 0.06	0.334 ± 0.078	0.365 ± 0.075
Uterus	–	–	0.500 ± 0.12	0.482 ± 0.14
Length small int.	112.8 ± 7.2	111.5 ± 8.0	100.8 ± 4.4	100.8 ± 3.1
Relative weight				
Adrenals	0.0137 ± 0.002	0.0145 ± 0.003	0.0313 ± 0.006[a]	0.0261 ± 0.003
Brains	0.481 ± 0.03	0.491 ± 0.04	0.777 ± 0.06	0.727 ± 0.04
Epididymidis	0.280 ± 0.02	0.284 ± 0.05	–	–
Heart	0.270 ± 0.02	0.269 ± 0.02	0.331 ± 0.02	0.319 ± 0.03
Kidneys	0.569 ± 0.04	0.557 ± 0.03	0.625 ± 0.04	0.614 ± 0.06
Liver	3.00 ± 0.19	3.04 ± 0.22	3.09 ± 0.26	3.04 ± 0.30
Mesenterisln.	0.0258 ± 0.006	0.0260 ± 0.009	0.0379 ± 0.011[b]	0.0509 ± 0.013
Ovaries	–	–	0.0547 ± 0.013	0.0478 ± 0.011
Pancreas	0.324 ± 0.10	0.310 ± 0.09	0.433 ± 0.09	0.410 ± 0.06
Small intestine	1.95 ± 0.25	1.91 ± 0.26	2.52 ± 0.34[a]	2.30 ± 0.20
Spleen	0.185 ± 0.02	0.183 ± 0.02	0.228 ± 0.027	0.215 ± 0.019
Testis	0.931 ± 0.09	0.945 ± 0.10	–	–
Thymus	0.093 ± 0.02	0.093 ± 0.01	0.137 ± 0.028	0.142 ± 0.024
Uterus	–	–	0.206 ± 0.05	0.189 ± 0.05
Length small int.	0.269 ± 0.018	0.259 ± 0.023	0.416 ± 0.036	0.395 ± 0.026

Relative organ weights expressed as g/100 g body weight. Small intestinal length and relative length is expressed in cm and cm/g body weight. Data is presented as group mean values ± SD.
[a] Statistically significant different from control group ($p < 0.05$).
[b] Statistically significant different from control group ($p < 0.01$).

(+14% and +20%, respectively). Furthermore, this group had a significantly reduced absolute (−30%) and relative (−26%) weight of the mesenterial lymph node compared with the female control group. No macroscopic or histological findings were observed.

4. Discussion

Even though the two rice varieties were grown under almost identical environmental conditions, chemical analyses revealed a number of statistically significant differences between transgenic and parental rice. Differences were detected for proximates (starch, fiber, sugars, protein, and ash), amino acids, minerals (copper, iron, manganese, phosphorous, and potassium) and vitamins (B_1, B_6, pantothenic acid, folic acid). Minor, but statistically significant differences were also observed for distributions of fatty acids and steryl ferulates. Additional field trials would be necessary to determine whether the differences detected are due to the genetic modification or due to biological variability in the field.

The compositional data for transgenic rice were within the ranges reported in the literature except for protein, amino acids, ash and potassium. One has to keep in mind that existing food composition databases do not necessarily reflect the complete natural variation (Burlingame, 2004). In the present case, for example, protein contents exceed literature data for both the transgenic and the parental line. To assess the overall relevance of statistically significant differences in the light of natural variability within species, more comprehensive databases for the different plant species are necessary, which include samples with different genetic and/or environmental backgrounds. Recently, the International Life Science Institute released a comprehensive crop composition database that provides information on the natural variability in compositions of maize, soybean and cotton (Ridley et al., 2004). The intended extension of the database to other crops including rice will assist

ELSEVIER

FEMS Microbiology Letters 200 (2001) 163–167

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Transformation of an oral bacterium via chromosomal integration of free DNA in the presence of human saliva

Derry K. Mercer [a,*], Karen P. Scott [a], Claire M. Melville [a], L. Anne Glover [b], Harry J. Flint [a]

[a] *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK*
[b] *Department of Molecular and Cell Biology, University of Aberdeen, IMS, Foresterhill, Aberdeen AB25 2ZD, UK*

Received 9 March 2001; received in revised form 23 April 2001; accepted 27 April 2001

First published online 30 May 2001

Abstract

Transformation of *Streptococcus gordonii* DL1 by free DNA was studied in human saliva. Competent *S. gordonii* could be transformed in vitro with plasmid DNA that had been taken into the human mouth. Transformation also occurred with a plasmid that cannot replicate in *S. gordonii*, but that has a region of chromosomal homology, by integration into the bacterial chromosome, although linearised plasmid DNA gave no transformants. Linear chromosomal DNA fragments did however transform *S. gordonii*Tn*916* efficiently in saliva when regions of homology with the recipient chromosome flanked the marker gene. These findings are discussed in relation to the potential for acquisition of DNA sequences, including genetically modified DNA, by gut and oral bacteria.

Keywords: Transformation; Homologous recombination; DNA survival; Genetically modified DNA; Chromosomal integration; *Streptococcus*

1. Introduction

As genetically modified (GM) plants and microorganisms are increasingly being considered as components of animal feed and human food, it is important to address concerns over the possible acquisition of GM DNA sequences by bacteria in the gastrointestinal tract [1,2]. While conjugal transfer and transduction are undoubtedly important in gene transfer between bacteria, natural transformation [3] represents potentially the most general mechanism for acquisition by gut bacteria of foreign DNA fragments that may be released from food. It has been shown that fragments of bacteriophage M13 DNA can survive passage through the mouse gut [4,5] and also that transformation of bacteria such as *Bacillus subtilis* and *Escherichia coli* can occur in foodstuffs [6,7]. Additionally, recent evidence suggests that only quite extensive processing of plant material in foodstuffs will prevent the survival of large DNA fragments [8].

Previous work showed that the oral bacterium *Streptococcus gordonii* DL1 could be transformed in vitro by free plasmid DNA in the presence of human saliva [9], and this finding has some implications for the use of plasmids in GM bacteria intended for applications in food or in the environment. However, the great majority of GM constructs, particularly in plants, involve chromosomally integrated sequences. Nevertheless, non-self replicating DNA fragments still have the potential to become integrated into the chromosomes of gut and oral bacteria during transformation. Assuming that insertion sequences and transposable elements are excluded from modified DNA intended for food use, this possibility will depend mainly on the presence or absence of significant regions of sequence homology that allow homologous recombination [3]. Potential regions of homology between GM plants and gut bacteria include antibiotic resistance genes, polylinker sequences, promoters and terminators [10].

Here we examine the potential for sequences of bacterial origin present in GM DNA [11], in particular antibiotic resistance genes [12], to facilitate acquisition of foreign DNA by oral bacteria. Antibiotic resistance genes are widely used as selectable markers in genetic modification

* Corresponding author. Tel.: +44 (1224) 716624; Fax: +44 (1224) 716687; E-mail: d.mercer@rri.sari.ac.uk

PII: S0378-1097(01)00220-8