



SCIENCE DISSECTED

Genetically Modified Organisms Model-Evidence Link Diagram (MEL)

Biotechnology is a multifaceted subject that includes many different ethical issues that relate to our growing scientific knowledge. The safety of consumption of genetically modified foods is often debated and scientific information exists that supports both models. The topic also provides insightful guidance through the scientific literature and current events and is ideal for identifying reliable peer reviewed sources.

The term genetically modified organism (GMO) refers to any organism whose genetic material has been altered using genetic engineering techniques generally known as recombinant DNA technology. This issue of Science Dissected provides an instructional resource for teachers to present students with the opportunity to examine several pieces of evidence compiled about genetically modified organisms and critically evaluate two competing views of biotechnology.

Model A: Generically modified organisms are beneficial to society.

Model B: Generically modified organisms are not beneficial to society.

Evidence #1: There are many viruses, fungi and bacteria that cause plant diseases.

Evidence #2: Unexpected frost can destroy sensitive seedlings. An antifreeze gene from cold water fish has been introduced into plants such as tobacco and potato.

Evidence #3: Studies show that pollen from B.t. corn caused high mortality rates in monarch butterfly caterpillars.

Evidence #4: Crop losses from insect pests can be staggering, resulting in devastating financial loss for farmers and starvation in developing countries.

Evidence #5: Medicines and vaccines often are costly to produce and sometimes require special storage conditions not readily available in third world countries.

The following is a suggestion for using this MEL with students:

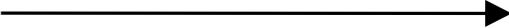
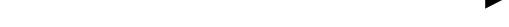
1. Hand out the Genetically Modified Organism Model Evidence Link Diagram (page 1). Instruct students to read the directions, descriptions of Model A and Model B, and the five evidence texts presented.
2. Handout the five evidence text pages (pages 3-9).
3. Instruct students to carefully review the Evidence #1 text page (page 3), then construct two lines from Evidence #1; one to Model A and one to Model B. Remind students that the shape of the arrow they draw indicates their plausibility judgment (potential truthfulness) connection to the model.
4. Repeat for Evidence #2-5 (pages 4-9).
5. Handout page 2 for the students to critically evaluate their links and construct understanding.

Once students have completed page 2, they can then engage in collaborative argumentation as they compare their links and explanations with that of their peers. Students should be given the opportunity to revise the link weighting during the argumentation exercise. If time permits, have students reflect on their understanding of GMO's through a PCR experiment testing various GM foods provided by Bio-rad or by a written reflection argument essay.

Name: _____ Period: _____

Directions: draw two arrows from each evidence box. One to each model. You will draw a total of 10 arrows.

Key:

	The evidence supports the model
	The evidence STRONGLY supports the model
	The evidence contradicts the model (shows its wrong)
	The evidence has nothing to do with the model

Standards: L.12.A.1-2

Standard: L.12.B.3

Standards: N.12.B.1-4

Evidence #1
There are many viruses, fungi and bacteria that cause plant diseases.

Model A
Genetically modified organisms are beneficial to society .

Evidence #3
Studies show that pollen from B.t. corn caused high mortality rates in monarch butterfly caterpillars.

Evidence #2
Unexpected frost can destroy sensitive seedlings. An antifreeze gene from cold water fish has been introduced into plants such as tobacco and potato.

Model B
Genetically modified organisms are not beneficial to society.

Evidence #4
Crop losses from insect pests can be staggering, resulting in devastating financial loss for farmers and starvation in developing countries.

Evidence #5
Medicines and vaccines often are costly to produce and sometimes require special storage conditions not readily available in third world countries.

Provide a reason for three of the arrows you have drawn. **Write your reasons for the three most interesting or important arrows.**

- A. Write the number of the evidence you are writing about.
- B. Circle the appropriate descriptor (**strongly supports** | **supports** | **contradicts** | **has nothing to do with**).
- C. Write the letter of the model you are writing about.
- D. Then write your reason.

1. Evidence # ____ **strongly supports** | **supports** | **contradicts** | **has nothing to do with** Model ____ because:

2. Evidence # ____ **strongly supports** | **supports** | **contradicts** | **has nothing to do with** Model ____ because:

3. Evidence # ____ **strongly supports** | **supports** | **contradicts** | **has nothing to do with** Model ____ because:

4. Circle the plausibility of each model. [Make two circles. One for each model.]

	Greatly implausible (or even impossible)										Highly Plausible
Model A	1	2	3	4	5	6	7	8	9	10	
Model B	1	2	3	4	5	6	7	8	9	10	

5. Circle the model which you think is correct. [Only circle one choice below.]

Very certain that Model A is correct	Somewhat certain that Model A is correct	Uncertain if Model A or B is correct	Somewhat certain that Model B is correct	Very certain that Model B is correct
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Evidence #1: There are many viruses, fungi and bacteria that cause plant diseases**A fruitful outcome to the papaya genome project**Fusheng Wei¹ and Rod A Wing¹¹Department of Plant Sciences and the Arizona Genomics Institute, University of Arizona, Tucson, AZ 85721, USAPublished: 6 June 2008
Genome Biology 2008, 9:227

The nice thing about working with some plant genomes is that at the end of the day you can eat the fruits of your work. Originating from Central and South America, the papaya (*Carica papaya*) bears highly nutritious and delicious fruit and is also a source of papain - a protease used for centuries to tenderize meat. Papaya trees can grow 5-10 meters tall, with large leaves 50-70 cm in diameter and fruits 15-45 cm long and 10-30 cm in diameter (Figure 1). The papaya is grown as a crop in tropical and subtropical regions, but its cultivation has been severely hampered by the papaya ringspot virus (PRSV) (Figure 2). In Hawaii, papaya cultivation was almost completely destroyed by the virus until the introduction of virus-resistant transgenic lines in 1998. Now 80% of the Hawaiian papaya crop is transgenic. A draft genome sequence and analysis of the transgenic papaya variety 'SunUp' has now been published by Ray Ming and co-workers.

In regard to genomics, the papaya is an ideal and interesting species to work with. It has a very small diploid genome of 372 Mb, slightly smaller

than rice and six times smaller than maize. The papaya belongs to the order Brassicales, which includes the model plant *Arabidopsis* as well as the cabbage family; it shared a common ancestor with *Arabidopsis* approximately 72 million years ago. The papaya can also be easily transformed and has a generation time of 9-15 months. Also of interest is its primitive sex-chromosome system, which has interested evolutionary biologists for years.

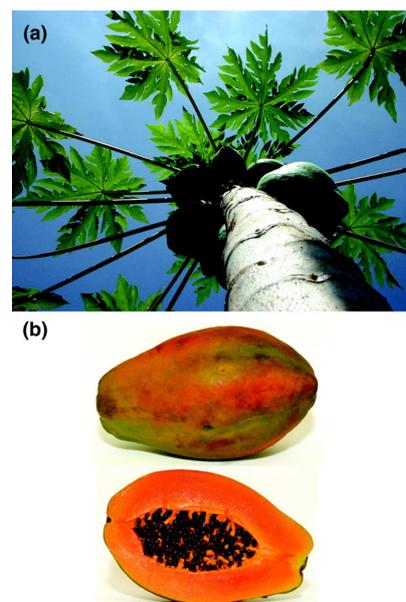


Figure 1. Papaya plant and mature fruit. **(a)** A papaya plant heavy with fruit. **(b)** Mature papaya fruit. (a) Photo courtesy of Wikicommons. (b) Photo courtesy Jayson Talag, University of Arizona.



Figure 2. Field trial of transgenic papaya. The disease free transgenic papaya plants (the right side) and the severely infected and stunted non-transgenic papaya plants (the left side) growing in adjoining plots. Image courtesy of Stephen A Ferreira.

Evidence #2: Unexpected frost can destroy sensitive seedlings. An antifreeze gene from cold water fish has been introduced into plants such as tobacco and potato.

Introduction of Insect Antifreeze Protein Genes into the Model Plant *Arabidopsis thaliana*.

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Faculty Sponsor: Michael Abler, Ph.D., Department of Biology

ABSTRACT

Injury inflicted by frost and freezing is found in all plants exposed to such damaging temperatures. Antifreeze proteins protect against these injurious temperatures by providing freeze resistance to organisms in which they occur. The expression of antifreeze proteins in a plant is a possible means of increasing the frost resistance and freeze tolerance of plants. Freeze tolerance curves of non-cold acclimated and cold acclimated *A. thaliana* were generated in untransformed *A. thaliana*. Research was conducted to obtain organisms with highly efficient antifreeze proteins. The *Tenebrio molitor* and *Choristoneura fumiferana* cDNA clones were acquired from A/F Proteins Inc. through a material transfer agreement. The antifreeze proteinencoding cDNA fragments will be cloned into an expression vector and transformed into *Arabidopsis thaliana* to determine if expression of antifreeze proteins will increase the freeze tolerance of *A. thaliana*.

INTRODUCTION

Freezing temperatures are responsible for more crop losses worldwide than any other single cause. Annual losses total several billion dollars in the United States alone. For example, a single freeze in California in December of 1998 resulted in an estimated \$591 million in damages to citrus crops (CDFA, 1998).

The process of freezing in plants and the resulting damage to plant cells has been studied intensively (Steponkus and Webb, 1992; Wallis et al., 1997). As the temperature decreases iceformation begins on the epidermal surface. This is followed by ice formation within the plant tissue. At about -1°C, the extracytoplasmic space, where solute concentration is the lowest, begins to freeze and as a result withdraws water from the cytoplasm, dehydrating the cells and withering the cell membranes (Steponkus and Webb, 1992). The plant cells have defenses against freezing-induced dehydration, but if the temperature decreases in the tissues to about -10°C, ice crystals penetrate the cytoplasm, resulting in the destruction of the cell membrane and disorganization of the sub-cellular space (Steponkus and Weist, 1988).

Plants have developed a variety of defenses against low temperatures. Frost-hardy plants produce colligative cryoprotectants such as sucrose and proline to reduce the dehydration of the cell (Wallis et al., 1997). However, some cold-hardy plants produce specific antifreeze proteins (Griffith et al., 1994).

Antifreeze proteins (AFPs) share a common function in providing freeze resistance to organisms in which they occur. These proteins reduce freezing injury by thermal hysteresis and inhibition of ice recrystallization. Thermal hysteresis is the process of lowering the freezing point of a liquid below the melting point. Antifreeze proteins bring about thermal hysteresis by inhibiting ice crystal growth through adsorption to the surface of the crystal (Davies and Sykes, 1997; Yeh and Feeney, 1996). Once the ice crystals melt, ice recrystallization (growth of larger ice crystals at the expense of smaller ones) is inhibited by the adsorption of AFPs to the crystal surface.

Thermal hysteresis has been detected in at least 26 species of higher plants and some candidate proteins have been purified. The values of these extracted thermal hysteresis proteins, however, are low, from 0.2 to 0.6°C when purified from plants encountering low environmental temperatures (Worrall et al., 1998). Most freeze inhibitors found in plants are polysaccharides, not proteins. Polysaccharides modify ice structure after ice nucleation has occurred. They apparently do not inhibit the initiation of freezing (Urrutia et al., 1992).

Antifreeze proteins have also been discovered in certain marine teleosts (fish) that survive in seawater temperatures below the freezing point of their blood sera by producing antifreeze proteins (Kenward et al., 1993). The maximum level of thermal hysteresis shown by fish antifreeze proteins is approximately 1.5°C. Recently, the isolation, cloning, and expression of a thermal hysteresis protein from an insect has demonstrated that some insect antifreeze proteins are 10 to 30 times more active than any known fish antifreeze protein (Tyshenko et al., 1997), or more than 60 times more active than any known plant protein. The discovery of AFPs in two insects represents a major advance in cryobiology. Genes encoding AFPs have recently been cloned from the spruce budworm (*Choristoneura fumiferana*) and the common mealworm beetle (*Tenebrio molitor*). During the winter months, the spruce budworm can be exposed to temperatures approaching -30°C, but it still resists freezing (Hew, 1997). The production of antifreeze proteins is an important part of the spruce budworm's survival strategy. Taking into account the conditions of its environment, it is not startling that this insect has evolved an AFP that is the most active known. The vastly superior insect antifreeze proteins may find wider applications in the cryoprotection of cells and tissues.

Antifreeze proteins have exceptional properties, and when applied these properties could have beneficial results. The expression of insect antifreeze protein genes in plants is a possible means of increasing the frost resistance and freeze tolerance of plants. The introduction of such insect antifreeze proteins through gene transfer into a model plant may confer increased freeze resistance to the plant. Likewise, increased freezing tolerance may result when such technology is applied to other plants (e.g. citrus trees). The model plant *Arabidopsis thaliana* was used herein because of its short generation period, size, ease of transformation, ease of regulating pollinations, and the vast amount of available information regarding this plant.

Evidence #3: Studies show that pollen from B.t. corn caused high mortality rates in monarch butterfly caterpillars.

Unintended harm to other organisms

Last year a laboratory study was published in *Nature* showing that pollen from B.t. corn caused high mortality rates in monarch butterfly caterpillars. Monarch caterpillars consume milkweed plants, not corn, but the fear is that if pollen from B.t. corn is blown by the wind onto milkweed plants in neighboring fields, the caterpillars could eat the pollen and perish. Although the *Nature* study was not conducted under natural field conditions, the results seemed to support this viewpoint. Unfortunately, B.t. toxins kill many species of insect larvae indiscriminately; it is not possible to design a B.t. toxin that would only kill crop-damaging pests and remain harmless to all other insects. This study is being reexamined by the USDA, the U.S. Environmental Protection Agency (EPA) and other non-government research groups, and preliminary data from new studies suggests that the original study may have been flawed. This topic is the subject of acrimonious debate, and both sides of the argument are defending their data vigorously. Currently, there is no agreement about the results of these studies, and the potential risk of harm to non-target organisms will need to be evaluated further.

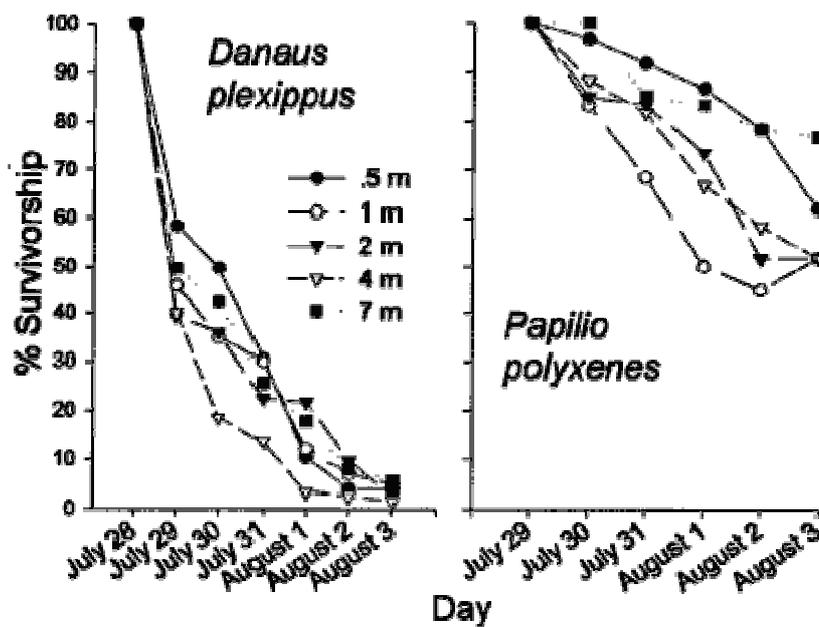


Fig. 2. Survivorship of monarch (*D. plexippus*) and black swallowtail (*P. polyxenes*) larvae as a function of distance from a field of event 176 Bt corn.

Sources

- Transgenic pollen harms monarch larvae (*Nature*, Vol 399, No 6733, p 214, May 1999)
- GM corn poses little threat to monarch (*Nature Biotechnology*, Vol 17, p 1154, Dec 1999)
- Bt and the Monarch Butterfly: Update by Dr. Douglas Powell (*AGCare Update Magazine* <http://www.agcare.org/AGCareUpdate.htm#Monarch>)

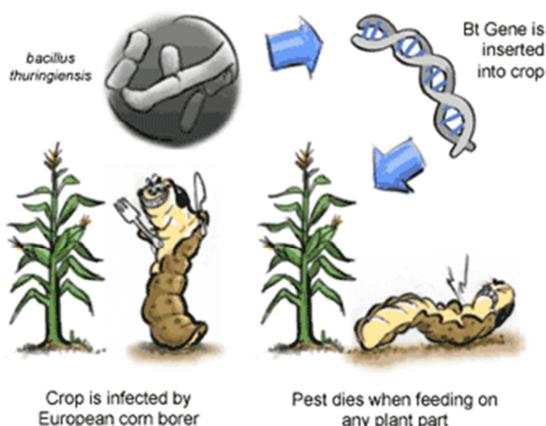
Evidence #4 - Crop losses from insect pests can be staggering, resulting in devastating financial loss for farmers and starvation in developing countries.

Resistance To GM Crops By Gunnar De Winter | July 30th 2011 Large amounts of research and money have been invested in the development of transgenic, or GM (genetically modified) crops. These crops are genetically engineered to withstand drought, excessive rain or other weather conditions, or to improve their yield or increase their rate of development, or to express certain toxins that would limit the amount of insects feeding on them.



Figure 1: The western corn rootworm

While transgenic crops might potentially be a means to increase food production, they have been, and remain to be, quite controversial. There are many reasons why people oppose them, but one of them is that insect resistance is unlikely to last. Now, a study seems to provide some evidence for the occurrence of field-evolved resistance to toxins produced by transgenic maize in the western corn rootworm (or *Diabrotica virgifera virgifera*, don't let the common name fool you, it is, in fact, an insect) (see figure 1).



Bt maize has been engineered to produce toxins derived from the bacterium *Bacillus thuringiensis* (hence Bt) (see figure 2). Bt crops are planted on millions of hectares each year and strongly reduce the need for conventional insecticides, as well as the occurrence of pests. Resistance to the toxins, however, could significantly impact these benefits. More specifically, fields which have been used to grow Cry3Bb1 maize (a type of Bt maize) for several consecutive growing seasons, showed more severe feeding injury from rootworms, indicating higher rootworm survival.

Rootworms from several fields were collected, and their larvae, reared in the laboratory, were introduced to Bt maize. Turns out that the larvae whose parents came from fields with a longer history of growing Cry3Bb1 maize exhibited a higher survival. In fact, the survival correlated nicely with the number of consecutive growing seasons of this type of maize (see figure 3).

Figure 2: The making of Bt maize

history of growing Cry3Bb1 maize exhibited a higher survival. In fact, the survival correlated nicely with the number of consecutive growing seasons of this type of maize (see figure 3).

Gassmann concludes that, to date, the widespread planting of Bt crops has resulted in pest resistance for only a small subset of all pest populations managed by this technology. However, these recent cases suggest a need to develop more integrated management solutions for pests targeted by Bt crops. A common pattern observed among problem fields in this study was the consecutive planting of the same type of Bt maize over several seasons.

As always, further research is required to understand exactly how this resistance evolves, and what the most appropriate means are for preventing this.

Reference

Gassmann, A.J.; Petzold-Maxwell, J.L.; Keweshan, R.S. and Dunbar, M.W. (2011). Field-Evolved Resistance to Bt Maize by Western Corn Rootworm. *PLoS ONE* 6(7): e22629. doi:10.1371/journal.pone.0022629.

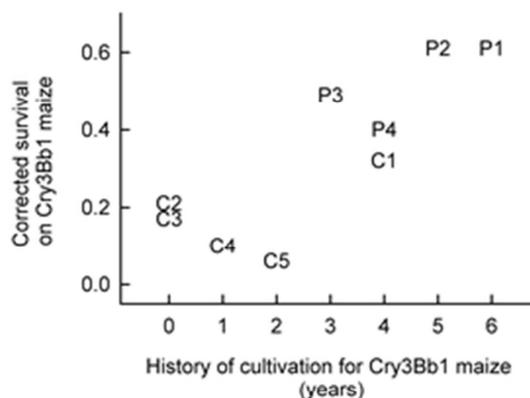


Figure 3: Correlation between the number of years that Cry3Bb1 maize has been grown on a field and rootworm survival (C: control field, P: problem field). Source: Gassmann et al., 2011)

Evidence#5- Medicines and vaccines often are costly to produce and sometimes require special storage conditions not readily available in third world countries.

Ethical perception of human gene in transgenic banana

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Transgenic banana has been developed to prevent hepatitis B through vaccination. Its production seems to be an ideal alternative for cheaper vaccines. The objective of this paper is to assess the ethical perception of transgenic banana which involved the transfer of human albumin gene, and to compare their ethical dimensions across several demographic variables. A survey was carried out in the Klang Valley region from August, 2009 till February, 2010 using self constructed multi-dimensional instrument measuring ethical perception of transgenic banana. The respondents (n=434) were stratified according to stakeholder groups which consisted of eleven groups: Producers, scientists, policy makers, non-governmental organisations (NGOs), media, religious scholars (Islamic, Buddhist, Christian, and Hindu scholars), university students and consumers. Results of the study showed that the Malaysian stakeholders were unfamiliar with transgenic banana, and perceived transgenic banana as having moderate risks and marginally beneficial to the Malaysian society and the ethical aspects were moderately acceptable to them as well as from their religious point of view. ANOVAs showed that all the four ethical dimensions: Familiarity, denying benefits, ethical acceptance and perceived risks significantly differed across stakeholders' groups while the last three dimensions also differed significantly across religion. Perceived risks, denying benefits, ethical and religious acceptance further differed significantly across races. However, with respect to ages, only the factor familiarity differed and no significant difference were found across educational level and gender. Although, the idea of producing an edible vaccine through transgenic banana seems to be an ideal alternative for cheaper vaccines, the Malaysian stakeholders were still not ready and have a cautious stance. The research finding is useful to understand the social construct of the ethical acceptance of cross-species gene transfers in developing country. Further research needs to be done to determine the perspectives of various religions on the use of human gene in plants.

Key words: Ethical perception, transgenic banana, Malaysian stakeholder.

INTRODUCTION

Nowadays, with the development of genetic engineering, plants characteristics can be modified for the development of pharmaceutical products. Through genetic engineering, an edible vaccine can be developed as an alternative for cheaper vaccines compared to the typical vaccine that are more expensive due to the storage, transportation and purification cost (Goldstein and Thomas, 2004). Typical vaccines are composed of killed or attenuated disease-causing organisms (Mishra et al., 2008) while transgenic pharmaceutical plants are modified by the introduction of novel gene sequences which drive the production of proteins or peptides that have properties allowing them to be used as precursors in the synthesis of medical compounds (Goldstein and Thomas, 2004).

An edible vaccine has many advantages identified such as edible means of administration, reduced need for medical personnel and sterile

injection conditions, economical in mass production and transportation, therapeutic protein are free of pathogens and toxins, storage near the site of use, heat-stable, eliminating the need for refrigeration, antigen protection through bio-encapsulation, subunit vaccine (not attenuated pathogens) means improve safety, seroconversion in the presence of mucosal immunity, enhanced compliance (specially in children), delivery of multiple antigens, integration with other vaccine approaches, plant derived antigens assemble spontaneously into oligomers and onto virus like particles (Mei et al., 2006). But there is still limitations in producing edible vaccines such as the development of immunotolerance of vaccine peptide or protein, consistency of dosage form fruit to fruit, plant-plant and generation-generation is not similar, stability of the vaccine in fruit is not known, evaluation of the dosage requirement is tedious, selection of the best plant is difficult, certain foods are not eaten raw, and cooking

the food might weaken the medicine present in it (Mei et al., 2006). However, Kong et al. (2001) claimed that they could substantially reduced the immunogenicity of the vaccine by cooking (boiling) the food. They also found that the plant-derived HBsAg which is delivered as food is orally immunogenic in mice and elicit a primary antibody response. Furthermore, the strong secondary response also seen after boosting with rHBsAg represents a true memory response generated from the mice fed by HBsAg transgenic potatoes.

Hepatitis B is a major global health problem and the most serious type of viral hepatitis spreads through blood transfusion and sexual contact. It is a viral infection that attacks the liver and can cause both acute and chronic disease. It is estimated that two billion people are infected with hepatitis B virus (HBV), and more than 350 million have chronic (long-term) liver infections in the worldwide. Therefore, the production of edible vaccine for hepatitis B through the development of transgenic plants could be an alternative for cheaper vaccine.

Arntzen has developed transgenic banana for the delivery of edible vaccine in developing countries (Prakash, 1996). Banana was considered as an ideal fruit for the production of edible vaccine because of several factors, such as those that easily grows in the tropics (Prakash, 1996) and subtropics (Sunil Kumar et al., 2004), favourite food for small children (Prakash, 1996), digestibility and palatability by the infants (Sunil Kumar et al., 2004) and can be consumed uncooked, thus, eliminating the possibility of protein denaturation due to high temperature (Goldstein and Thomas, 2004).

Since genetic engineering is new and the advancement in these areas have been so rapid, it has been the object of some doubts, fears, concerns, as well as an intense and divisive debate worldwide on the potential risks to human health, to the environment and to the society (Costa-Font and Gil, 2009). The debate was typically seen as a conflict between supporters who envisage the potential benefits and the opposition groups who view Latifah et al. 12487 GM products as tampering with nature (Bloomfield, 2011). Although the benefits of transgenic banana was promising, past studies also showed that genetic engineering has been associated with negative constructs, such as ethical concerns, changing nature, and its possible risks to the environment (Blaine et al., 2002). According to Batalion (2000), the central problem underlying the use of biotechnology is not just its shortterm benefits and long term drawbacks, but the overall attempt to "control" living nature on an erroneous mechanistic view. Humans generally have conscience and religious beliefs and many of these

religious beliefs do not allow unrestricted interference with life, such as, genetic engineering (Epstein, 1998). The pace of discovery in genetic-based biotechnology is very rapid and there is anxiety that a kind of technological compulsion ('if we can do it, let's do it') have been driving developments ahead of proper ethical consideration of their propriety (Polkinghorne, 2000). Furedi (1997) argued that societal and individual risk perceptions are proportional to a system of moral values. Individuals were willing to accept some level of risk if a product was deemed worthy and was not morally objectionable. Of the variables studied, namely, usefulness, perceived risk and morality, it was found that moral acceptability was the strongest predictor of support for biotechnology by the Canadians (Eisendel, 2000). Gaskell et al. (2000) also noticed that moral acceptability appeared to act as a veto for the support of biotechnology among the Europeans. The results of the US public survey (Priest, 2000) also suggested the possibility of the US people using moral reasoning in forming opinions towards six applications of biotechnology.

Basic categories of moral or ethical concerns regarding modern biotechnology fall into two classes: intrinsic and extrinsic (Comstock, 2000). Extrinsic objection refers to the concerns regarding the possible concerns and risks of different application of biotechnology to human health, environment, economy and society (Gott and Monamy, 2004). While, intrinsic objections hold that, the process of modern biotechnology is objectionable in itself. This belief is associated with the claim that the technology is not natural. This technology is perceived as changing nature and is attempting to play "God". Beyond this, other researchers have raised intrinsic issues which include biotechnology being perceived as a threat to the natural order of living things (BABAS, 1999) and whether human has the rights to modify living things for their benefits (Brook 2003). These intrinsic concerns include a religious dimension and concern on the underlying set of religious beliefs and principles concerning the relationships between God, nature and human beings (BABAS, 1999).

Modern biotechnology has been given priority by the Malaysian government to spearhead the country's economy. The future development and commercialization of modern biotechnology products in Malaysia depends heavily on public acceptance. Besides, main decision on agriculture activities are shown to be influenced by the public (Ogunsumi, 2007). This paper will look at the Malaysians views on transgenic banana. Malaysians has been found to be concerned about the moral aspects of several modern biotechnology applications such as genetically modified

food and medicine (ISAAA-UIUC, 2003; Latifah et al., 2008, 2010). Past study has indicated that moral concern was found to be an important determinant of the Malaysians' perceptions in Klang Valley (Latifah et al., 2010). The objective of this paper is to assess the ethical perception of transgenic banana (containing human albumin gene) held by Malaysian stakeholders and to compare their perception across several demographic variables.