

BIOTECH PEANUT WHITE PAPER

“Benefits and Issues”

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CONTRIBUTORS

Elizabeth Grabau, Virginia Tech
Peggy Ozias-Akins, University of Georgia
Victor Nwosu, M&M Mars
Howard Valentine, Peanut Foundation
Rich Wilson, USDA-ARS
Maria Gallo, University of Florida
Kelly Chenault, USDA-ARS, Stillwater
Tom Stalker, North Carolina State University
Art Weissinger, North Carolina State University
Pat Donahue, Kraft
Marshall Lamb, USDA-ARS, Dawson
Emory Murphy, Georgia Peanut Commission

I. Introduction

The peanut industry has attempted to use all available methods to reduce cost of production and improve quality of US peanuts. They have funded work on bioengineered peanuts since 1989 but at lower levels than other crops in the US. The industry is conducting a review of their position on this area of research with the anticipation of revising their timetable and funding efforts.

The industry feels that with proper funding bioengineered peanuts could be commercially available in 5-7 years. Many of the competing nations (i.e., China, India) are developing bioengineered peanuts and will be releasing them in the next 3-5 years. Improvements in cost of production, nutrition and overall quality can be enhanced through this technology. Additionally a potential to reduce the allergenicity of peanuts is possible. This technology would also allow the industry to bring new traits to the market more rapidly.

II. Executive Summary

Several biotech peanuts have been developed since 1996. Many are now at the point of release in the next 5-7 years as commercial peanuts with proper industry and government support. Peanuts with resistance to Lesser Cornstalk Borers, TSWV, Sclerotinia, White Mold, Leaf Spot, and even A flavus. We have attempted in this White Paper to develop a summary of the cost to get these developed varieties to market.

Regulatory approval and licensing agreements for each variety will likely cost in the \$500,000- \$1,000,000 range. Therefore we must be sure the payback for the grower and the rest of the industry will justify this type of expenditure.

The other issue is time. It takes about 10 years from start to finish to deliver a biotech peanut. We have several varieties further down the pipeline but if these are found to not have good agronomic traits then we will have to start at the beginning. Some of these traits are in Runner type peanuts and some are in Virginia type. Currently there is no herbicide peanut in development. This is certainly a trait grower want.

What sort of traits are consumers and manufacturers looking for now and in the next 10 years. We have identified some key potential consumer needs. Nutritional enhancements needs consumers want such as more folate, more flavanoids, sterols, Vitamin A, arginine, more Vitamin E, and Protein. While peanut has most of these, increasing their quantities will make a value added product. There are other issues of course with this kind of work. We must continue to maintain our flavor and shelflife.

Additionally we have identified traits needed by the rest of the industry. Traits that will open up new markets such as biodiesel. Cost saving traits that would reduce the amount of pesticides needed to produce the crop. Finally using traits as a way to make peanuts safe for everyone with reductions in allergenicity and aflatoxin.

What are the costs of the technology needed to develop the new varieties of peanuts we will need. Some of the technology and equipment is already there in universities, ARS, and private industry but a much larger commitment is needed to help peanut keep up with other crops and other origins. A minimum of \$1,000,000 over 2 years plus \$75,000/trait/year is needed to develop the molecular markers to aid in peanut breeding. We can transform peanuts genetically now but to bring this technology up to speed with other crops another \$1,000,000 over 3-year period is required.

Developing a system and inventory of mutated peanuts to remove unwanted traits will require \$500,00 over 3 years plus \$150,000/trait/year. Completing the necessary mapping of the gene rich regions of peanut and determining their functions will cost a minimum of \$3,000,000 over 5-year period. And finally establishing computer systems (bioinformatics) to catalog all this information and make it readily usable by breeders and bioengineers will cost \$100,000.

This investment will have big paybacks over the next decade. Look at the cost savings achieved in other commodities. We have outlined the cost/benefit data that must be developed quickly to evaluate each of these traits. Additionally we have attempted to outline the identity preserved system that the industry would need to adopt to satisfy our customers around the world.

This is just the beginning of the process. Many researchers have been working for decades to get our biotechnology to where we are today with very limited funding. The industry must decide if the issues of cost and consumer acceptance are hurdles we want to cross and if biotech peanuts are our future.

III. Cost and Regulatory Approval Needed to Release Biotech Peanut

As we near the end of 2006, more than a decade after the introduction of the first genetically modified crops, several challenges remain for the development and entry into the marketplace of bioengineered peanuts. Transgenic peanuts must be evaluated and approved by governmental agencies charged with oversight of the safety of agricultural products for agriculture, humans, and the environment. Licensing agreements must be obtained for the traits and processes that are protected by patents and necessary for the development of these improved crops. Governmental approval could cost as much as \$100,000 for each variety submitted. Licensing would involve several companies that would include gene owners and transforming technology owners. This would probably involve an upfront cost plus royalties on each pound of seed sold.

Approval or “deregulation” of transgenic peanuts falls under the purview of the Coordinated Framework for Regulation of Biotechnology. This regulatory process involves three agencies: the U. S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). <http://usbiotechreg.nbio.gov/index.asp>

The approval required depends on the trait that has been introduced. Types of modification likely for peanut include:

- Pest Resistance: Pests include viruses, fungi, bacteria, nematodes and insects. The most famous examples of pest resistance genes are the Bt toxin genes from the bacterium *Bacillus thuringiensis*. Bt genes have been introduced into commercial cultivars of cotton and corn to deter insect feeding.
- Chemical Tolerance: This category includes resistance to herbicides such as glyphosate or glufosinate. The best example is Roundup Ready® soybean.
- Abiotic stress tolerance: These traits would allow plants to tolerate high salt, drought, ozone and other environmental stresses. There are no examples for this category on the market.
- Nutritional Enhancement: These transgenes benefit the consumer directly and include improved protein content, altered fatty acid profiles, enhanced nutrient content such as vitamins. An example on the market is soybean with high oleic acid content.

Of the three regulatory agencies, APHIS is charged with protecting America's agricultural industry from diseases and pests. Transgenic plants are considered “regulated articles” and APHIS oversees handling, disposal, movement and release of all transgenic plants. Before a transgenic plant can be commercially grown, it must be “deregulated”. An example determination of non-regulated status can be found at http://www.aphis.usda.gov/brs/aphisdocs2/01_13701p_com.pdf.

FDA is responsible for proper labeling and safety of all foods and animal feeds, including those produced through biotechnology. Consultation through the FDA is voluntary, but no transgenic crops meant for human or animal consumption have foregone the process. The consultation ensures that the product meets all of the standards set forth by the Federal Food, Drug, and Cosmetic Act so that there is no problems once the crops reach the market. Only peanuts judged to contain “food additives” would need to be labeled. According to the FDA, food additives constitute any additions to food excluding the following: 1) dietary supplements such as vitamins, 2) pesticides and 3) products deemed safe by either scientific study or long term use in foods. A sample consultation summary can be found at: <http://www.cfsan.fda.gov/~rdb/bnfm075.html>.

EPA regulates the sale and distribution of all pesticides. Transgenic plants deemed 'pest resistant' fall under their jurisdiction and are referred to as containing 'Plant Incorporated Protectants (PIP). A PIP is any substance introduced into a plant through breeding or biotechnology, which is intended to “prevent, destroy, repel or mitigate any pest.” An example assessment summary for EPA can be found at http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_006484.htm.

To obtain deregulated status, researchers must submit a petition that is reviewed for compliance with guidelines set by these three agencies. The petition must include the following types of data:

- A detailed description of the biology of the crop (in this case peanut), the origin and characteristics of the transgene, the method for introducing the gene, and any other materials used.
- Genetic analysis showing the number of genes inserted into the plant and their structure.
- Characterization of any proteins produced from the transgene, including the amount and stability of the protein, as well as its similarity to other proteins.
- Data on seed composition, where the only difference between transgenic and non-transgenic should be the introduced trait.
- Assessment of allergenicity potential for the transgene product, including a comparison to known allergens and analysis of glycosylation.
- Data on the presence of aflatoxin, which should not exceed levels found in non-transgenic peanuts.
- Effects of transgene product on non-target organisms, such as pollinators, beneficial microorganisms and other pests or pathogens.
- Potential of a 'pesticide' to enter and persist in the water supply.
- Potential for transgenic peanuts to become weeds.
- Possibility of transfer to wild relatives, which should not be a concern in the United States where no wild relatives of peanut are located.

USDA APHIS and FDA do not charge fees for their petition and consultation processes. The EPA, however, charges substantial fees for registration of plant incorporated protectants. Fees range from \$100,000 to \$400,000 for registration depending on the exact nature of the PIP. Small businesses are entitled to exemptions from these fees. For the development of products by public institutions, there seems to be an opportunity for negotiation concerning applicable fees.

There is also the possibility of applying for an exemption through the EPA for some transgenes. The agency has the authority to grant exemptions from registration for any PIP or category of PIPs that are deemed to have "a low probability of risk to the environment" even without regulation. Registration may not be required for virus resistant plants.

Many of the gene sequences and tools required for producing transgenic plants are subject to patents owned by industry. These tools may require licensing fees for use. Examples of intellectual property associated with development of transgenic peanuts include:

- The gene gun and *Agrobacterium tumefaciens*, two systems used to transform plants with the desired foreign DNA sequences
- Selectable marker genes such as hygromycin phosphotransferase and neomycin phosphotransferase, which allow researchers to distinguish between tissue containing the transgene and tissue that does not

- Signals for the expression (turning on and off) the introduced gene, known as promoters and terminators
- Genes conferring the traits of interest (e.g. pest resistance, improved oil composition)

To provide alternatives to intellectual property owned by corporations, PIPRA, the Public Intellectual Property Resource for Agriculture is “an organization committed to the strategic management of intellectual property owned by universities and not-for-profit research institutions”. PIPRA maintains a database of relevant intellectual property from member institutions (<http://pipra.m-cam.com/>), although many of these inventions are subject to prior art.

In summary, obtaining funds to provide the data necessary for navigating the regulatory requirements and procuring freedom to operate remain the greatest challenges to releasing transgenic crops. This is particularly true in the public sector and for more diverse and lower acreage crops such as peanut (compared to crops such as soybean and corn). The only public sector transgenic crop that has been released to date is papaya engineered for virus resistance. Peanut represents an opportunity to streamline the regulatory process and realize the benefits of transgenic technology in a crop that is not dominated by large multi-national agricultural companies.

IV. Time Frame Needed to Develop and Deliver Biotech Peanut

The most widely used and genotype-independent method of transforming peanut is through direct DNA delivery using microprojectile bombardment. A major constraint of producing transgenic peanuts via microprojectile bombardment is the length of time needed to generate stable, commercially viable lines. Producing quality somatic embryos at the correct developmental stage for bombardment requires 6 – 9 months. Selection in liquid and then on solid media requires another 3-4 months. Generation of shoot and root systems can take up to an additional 3-4 months. As is the case for any transgenic culture system, a major challenge can exist in maintaining sterile conditions for the period prior to transfer to the soil. Once the primary transgenic or T₀ plant is placed into soil and grown to maturity, another 3-4 months may be required. Conservatively, producing one generation of transgenic peanut via this method involves a minimum time period of 15-19 months.

Once the T₀ generation has been produced, molecular testing is required to determine the number of transgene copies inserted into the peanut genome, expression levels and transgene performance (desired trait acquisition). Additionally, the inheritance of the transgene over several generations must be evaluated for stable performance. To accomplish these objectives, initial laboratory and greenhouse testing is needed which takes generally 2-3 years. This is then followed by 3-6 years of field-testing of the transgenic lines that is necessary to determine the performance of the transgene under “real” conditions. Assuming all government regulations have been met, a transgenic peanut line could be developed and released for commercial production in 7-

10 years, possibly shaving 3-5 years off the time required to develop a peanut cultivar through traditional breeding methods.

V. List of Possible Desired Attributes—Consumer

The American diet is highly fortified with essential nutrients to provide nourishment to the consuming public. However, it is known that certain nutrients are more readily absorbed in natural form than as supplements. On the basis of this information, it is recommended that peanuts be bio-engineered to contain appreciable quantities of the following nutrients:

Folate: This is an essential vitamin that is found to be deficient in most American diets. Most people meet their daily requirements by taking supplements. However, research has shown that natural forms are more readily absorbed than the supplements. Peanut is good source of this vitamin, but further enhancement will be beneficial to consumers.

Flavonoids: Peanut skins contain some amounts of this group of antioxidant compounds. Further enhancement of these tissue-specific compounds will improve the nutritional image and perception of peanuts.

Sterols: Plant sterols (specifically sitosterol) have health benefits, and enhancement of this group of compounds will be useful. These are already present in peanut, and will be viewed negatively if enhanced.

Vitamin A: Peanut contains no Vitamin A but is considered one of the essential vitamins. Many of the plants currently modified to increase their Vitamin A content (Golden Rice) can only supply minimum quantities per serving. High oil content foods would be able to supply much higher quantities per serving.

Arginine: This amino acid is obtained from the digestion of plant and animal protein. Many studies implicate it in improving heart healthiness. Peanuts contain arginine but not in high enough quantities.

Vitamin E: Certainly one of the many attributes of peanuts currently but many tree nuts contain significantly higher levels (almonds, hazelnuts). This is a potent antioxidant with proven health benefits.

Protein: Peanuts are high in protein but much of it is not digestible. Can this be changed by biotechnology?

Enhancement of these compounds in peanuts will greatly improve the nutritional image of peanuts.

VI. List of Possible Desired Attributes—Other

Growers are already benefiting from biotechnology in other crops. New varieties of peanuts are beginning to offer some disease resistant characteristics. Unfortunately

peanuts, even the wild species, don't have resistance to many of the diseases, pest and weeds that cause peanut production cost to soar. Many of the new traits could also open up peanuts for new markets or reduced cost of packaging. Reducing some of the negative market attributes would also help peanut markets. Here are some of the issues that could be addressed through biotechnology:

- Abatement of aflatoxin contamination, field and storage
- Mitigation of peanut allergy via modification/replacement of offending seed proteins
- Sclerotinia resistance (have transgenic cvs now, in OK)
- Increased oil concentration plus higher oleic acid, for biofuel applications
- TSWV, Sclerotinia, and Leafspot resistance
- Herbicide resistant
- LCB, Army worm, Thrips resistant

VII. Gaps in Peanut Researcher's Facilities/Equipment/Manpower

The Strategic Plan for Peanut Genomics Research outlines an aggressive process for obtaining genomics tools and data for plant improvement and to increase the biological knowledge of the species. This section will outline the resources needed to meet the objectives of the different areas of research for peanut based on priorities indicated in the plan. While scientists are working in each of the areas, it must be recognized that most of the investigators are also conducting research with other species and there are few full-time scientists working in the area of peanut molecular biology and genomics. It is beyond the scope of the Peanut Foundation's mandate to employ full time scientists at the faculty level at universities or scientists at comparable professional levels in the USDA or private industry. However, support personnel in terms of research associates, technicians, and graduate students could provide the hands to perform needed research under the direction of program leaders.

To conform with the Strategic Plan, this section will be divided into sections addressing the following areas: (1) Genetic Tools and Breeding Methods, (2) Plant Transformation Technology, (3) Genomic Sequencing & Gene Discovery, (4) Functional Genomics & Proteomics, (5) Immunology of Peanut Proteins in Model Systems, and (6) Bioinformatics. Although equipment will be needed in all laboratories to expand activities, this is believed to be a minor part of the over-all cost as compared to people and supplies to conduct research. Before proceeding, it must be noted that specific names of investigators are being omitted as to avoid minimizing the collective contributions of multiple laboratories.

1. Improving the utility of genetic tools for peanut genomics research by developing useful gene markers.

A goal of developing 150,000 expressed sequence tags was set for completion by 2008. To accomplish this goal, \$600, 000 will be required for technical help, supplies and sequencing. An additional \$300,000 will be required for developing useful SSR and SNP

markers for research associates, graduate students, and supplies. Equipment additions to laboratories will be required for sample storage and DNA analyses of about \$100,000.

Upon completion of the databases, marker assisted programs will be required for each trait of interest. Included in these projects will be population development so testing materials will be available to answer appropriate questions. To conduct a marker-assisted breeding program, a technician will be required for combinations of two or three traits for simply inherited characters, but for complex traits such as allergens, multiple technicians will be needed per trait. To estimate a cost, about \$75,000 /trait/year will be required to conduct plant improvement programs.

**Cost: \$1,000,000 (total) for 2 years + \$75,000/trait/year
after initial databases are developed**

2. Improving technologies for gene manipulation in genomes by developing useful transformation methods for functional genomic research in peanut.

Although plant transformation techniques have been developed for peanut, procedures are cumbersome and time-consuming. To make the available systems practical for plant improvement, incubators, sterile hoods, freezers, greenhouses, and misc. small equipment will be required to develop a high-through-put transformation laboratory with an initial cost of \$500,000. Developing improved technologies will require additional funds of at least \$500,000 for labor and supplies over a two to three year period. Continuing funding for plant improvement projects will then be required.

Cost: \$1,000,000 over 3-year period

3. Building a framework for assembling the peanut genome by identifying and integrating the positions of expressed genes on genetic, transcript and physical maps.

After expressed sequence tag (EST) libraries and markers are developed, genetic maps can be developed. Because the peanut has two genomes, maps will likely be required for the cultivated species and at least one of the diploid progenitors. A research associate and technician will be required to develop the maps and the work will take three to five years. Thus, labor will be at least \$500,000, plus supplies (\$150,000) and equipment (\$100,000).

Research to develop physical maps using BAC libraries will require a significant amount of DNA sequencing and labor. Although about 180,000 BACs exist for peanut, to utilize this library will require additional research associates, technicians, supplies, and misc. equipment at a cost of \$250,000/year for annotations for three to five years. To solve specific problems, such as those related to allergens, additional research associates will be needed (\$200,000/year).

Cost: \$3,000,000 over 5-year period

4. Improving knowledge of gene identification and regulation by providing baseline data and tools that facilitate the association of DNA-sequences in gene-rich regions of the peanut genome with a biological function.

Application of ‘reverse genetics’ to explore gene function will require developing specific populations (TILLING populations). Developing and maintaining an adequate population will require about \$500,000 for research associates, technicians, supplies, and sequencing expenses.

Gene discovery from the TILLING population, for example for allergen identification or regulation of seed composition and quality, will require at least a research associate per project. Microarrays also will need to be developed for each trait of interest.

Cost: \$500,000 over 3-year period + \$150,000/trait/year for 3-5 years

5. Understanding and then overcoming immunological problems associated with peanut proteins.

Making progress in this area will require good genomic databases to be assembled from which proteomic research can be conducted. Construction of proteomic maps and determining the effect of gene knockouts and substitutions for allergen genes on peanut allergy and seed composition will require an additional \$500,000 for labor, equipment, and supplies.

To make significant progress to solve allergen problems, a model system will be needed for testing human serum. The National Soybean Board and USDA are funding a project to develop a model pig population for soybean allergens, and in large part because of the efforts of Drs. Richard Wilson and Niels Nielson, the same population is being used for peanut. The cost of the project will be at least \$2 million. To be useful for peanut research after the inbred populations have been developed, additional test animals will be needed for challenging human serum. The cost of maintaining and utilizing the pig populations for peanut improvement is anticipated to be \$500,000 over a multi-year period for animal care, challenging with serum, and genetic testing.

Cost: \$1,000,000 over 3-4 year period

6. Provide bioinformatic management of peanut biological information resources by establishing an interactive system for public distribution of data and information.

The USDA is funding an *Arachis* database at the National Center for Genomic Research, Santa Fe, NM. To utilize this database on a continuing basis will require funding a research associate’s salary and support, totaling about \$100,000/year.

Cost: \$100,000/year

VIII. Elements of System to Identity Preserve Biotech Peanuts

Presently none of the peanut varieties being grown in the US are GMO derived varieties. With competition from other crops for farmer acres, the need of competitive peanut varieties is essential. One of the possible ways to improve competitiveness is to allow the use of some GMO genes to lower production costs. Many in Europe and other places are opposed to GMO and in order to keep those customers, the successful segregation of GMO and Non-GMO material must be maintained.

Recent industry mixes of GMO grains in Rice and Corn have cost those industries millions of dollars in both sales and remedial programs. The peanut industry method of segregation of peanuts through the bulk handling systems has come under pressure for change. There are presently a number of cases where varieties with specific desirable qualities are being grown and kept separate in the bulk handling system by means other than visual identification. The purpose of this paper is to discuss issues around non-visual segregation and propose a framework for an identity preserved (or IP) system of handling both large and small volume segregations. The purpose is to stimulate discussion around how IP systems could be set up, not to necessarily endorse these systems.

A Peanut IP system likely would include these elements:

- Seed should be of an approved registered variety, with genetic markers for variety identification specified.
- Producers contracted to grow the grain should use seed acceptable to the contractor (normally either Certified seed or “verified” as the specified variety).
- Contractors who market the grain should have production contracts with producers and be responsible for finding alternative markets for the grain if it does not meet specifications.
- Movement of the grain through the handling and transportation system should be accompanied by a paper trail, with samples taken and kept at every link in the chain where accountability shifts from one party to another.
- Accountability will rest with the facility that loads the transport conveyance.
- An independent testing facility should test grain shipments to confirm that the grain meets contract specifications.
- If shipments do not meet specifications; the samples may be tested down the chain to determine where the problem occurred and thus where financial accountability lies.

A major problem with IP systems is how to identify problems and assign liability in cases where the non-IP grain is contaminated with grain of the IP varieties. This issue requires further work to be resolved.

IX. Cost/Benefit for Industry from Biotech Peanut

Currently no bioengineered (Genetically Modified (GM)) peanuts are available for commercial production in the United States. However, the majority of cotton, corn, and soybeans produced in the US are GM commodities. Research has shown that GM technology has had a positive impact on farm income that resulted from a combination of increased crop productivity and efficiency gains (Brookes and Barfoot, 2006). Commercial use of these GM crops has been available since 1985 and the continual improvements in these crops are increasingly placing peanuts at a competitive disadvantage. The increase in crop value and rates of return on GM crops is positive and varies with different levels of technology utilized (for example, herbicide tolerance only or stacked genes with herbicide tolerance and insect resistance).

Quantifying the cost and benefits in peanuts is difficult because of the differing production problems faced by US producers within specific production regions and across the peanut belt as a whole. Additionally, there are certain technologies that should be avoided in peanuts because of farm management problems that would result (example, the majority of herbicide tolerant GM crops are glyphosate resistant and a glyphosate resistant peanut would cause significant problems in rotation systems and glyphosate tolerant weeds have emerged and are quickly spreading indicating that a change in herbicide chemistry with less dependence on glyphosate is needed). In the Southeast and portions of the Southwest, genetic modifications to minimize losses to Tomato Spotted Wilt Virus (either through insect resistance to thrips or other methods that directly impact the virus) could offer arguably the highest cost savings followed by rhizoctonia, white mold, and leaf spot. In the Virginia/Carolina area and other portions of the Southwest, minimizing losses that occur due to sclerotinia blight and other pathogens.

However, for the US peanut industry as a whole, the highest payback for a GM peanut would probably be in the development of improved drought tolerance (and salt tolerance). In all US peanut producing regions, water to adequately irrigate peanuts is either not available or becoming increasingly burdened by high energy cost, urban expansion increasing demand for non-agricultural water use, or gradually depleting aquifers. Various approaches have been tested to produce stress tolerant plants (not necessarily peanuts) using classical genetic methods that had limited success (Flowers and Yeo, 1995). However, genetic engineering has allowed the introduction of new pathways of various compatible pathways into plants resulting in the production of transgenic plants with documented improvements in stress tolerance (Chen and Murata, 2002).

The purpose of this paper is not to draw definitive conclusions about the cost/benefits of a GM peanut. With the current state of GM technology in peanuts, any conclusions drawn at this time would be speculative at best and potentially more harmful than beneficial. Instead, the purpose of this paper is to stimulate open discussion about:

What are the major economic problems facing the US peanut producer that can be addressed by GM technologies?

What resources are currently available to start the process of developing GM technologies for peanuts?

What improved GM technologies could be used if additional resources become available?

If funding and resources were not an issue, what is the timeline for the development and release of a GM peanut that would reduce the impact of #1? (This is an important because GM technologies for commodities that are competing with peanuts will continue to improve. Can we catch up?)

X. Glossary

Transgene – a DNA sequence or gene that is introduced into an organism from another source (foreign DNA)

Transformation – the process of introducing transgenes into the genome of an organism

Transgenic – an organism containing a foreign DNA or transgene

Genetically modified (GM) – as commonly used, is the equivalent of transgenic, although formally any modification, transgenic or not, would qualify. For example, traditional plant breeding also modifies the genetic composition of a crop plant