A look at GMOs, GAO and GE plants as they apply to horticulture©

H.W. Barnes[a]

Barnes Horticultural Services LLc, 2319 Evergreen Ave., Warrington, Pennsylvania 18976, USA.

The fundamental question presented to this panel discussion is: what is the role of GMO (genetically modified organism), GAO (genetically altered organism), and GE (genetically engineered) plants in horticulture. To make sense of that assessment some background information and definitions as to what these acronyms mean is in order. The science of horticulture means plants grown for food, ornament, or function that is different from those plants that are grown for agricultural purposes. In brief, tomatoes are horticultural, wheat generally is not except (Australian Office of Gene Technology, 2005) where it is grown for ornamental purposes, same thing applies to corn and *Panicum* sometimes yes and sometimes no, forest trees and lawn trees are horticultural, cotton is horticultural but soybeans are not, all fruit trees and fruits of any kind on woody or herbaceous plants apart from the grains are horticultural. Seaweed for consumption is agricultural but seaweed grown for the aquarium industry is horticultural. The use of any kind of plant for medical purposes brings it into the fold of horticulture. Definitions and clarity are the rule of the day when considering complex issues such as this.

Approximately 100 years ago plant pathologists discovered that *Agrobacterium tumifacens*, a naturally occurring plant pathogen, was capable of causing a genetic transformation in infected plant tissue (Newhouse et al., 2010). This development showed in later years that specific gene transfer can occur with either the natural infection of *Agrobacterium* or by an artificial infection of the bacteria. Geneticists and plant pathologist then learned that the *Agrobacterium* mechanism can be tailored to introduce foreign genes into plant tissues. The race to use this new technology was on (Wikipedia, 2016).

In today's world we are barraged by the use of the acronym GMO and in some cases GE when referring to plants and other organisms that have foreign gene components. However, the technologies of today are vastly more complex than a simple gene transfer from *Agrobacterium* or by biological application (read gene gun). As the situation becomes more diverse and complicated than the use of terms such as GMO become part of the common vernacular but as it has done so the specific definition of the GMO moniker has lost its meaning.

A study of the history of plant production over the ages demonstrates that even people with limited plant genetic understanding could and did encounter hybrids of a range of plants, with the likes Mendel and Darwin coming to mind. With the increasing advent of human transportation of plants, seed and scion wood dating back to the activities of the Chinese in Asia to the European explorers to the agents of Kew from England, trafficking in plants ultimately lead to wholesale trading of bizarre genetics in hybrids. Plants from India or China naturally had no interaction with those of Western Europe or the Americas and vice-versa, but ease of transportation and scientific inquiry lead to a great mix-up of plant genetics (Azad et al., 2013). Explorers of all kinds paved the way from massive genetic interactions that prior to the ages of advanced transport simply could not and did not happen. The modern corn of agriculture is but one example of a significant diversion from natural genetic compositions (Morroni et al., 2008). So what is the correct nomenclature of a modern corn plant, *Zea mays* as compared to tenosite (*Zea diploperennis*, *Z. perennis*, *Z. nicaraguensis*, *Z. mays* subsp. *huehuetenangensis*, *Z. mays* subsp. *parviglumis*, and *Z. mays* subsp. *mexicana* (Wikipedia, 2016). Is this complex we know as corn, a hybrid? Surely by any definition it is substantially genetically altered from the starting species. Would it be prudent then to attach the label genetically altered organism, GAO, to described the transformation of

[a]E-mail: bhsx17@outlook.com

this as well as thousands of other modern day plants such as the New Guinea impatiens (Dan et al., 2010).

New techniques have come to the forefront and well away from the initial work with *Agrobacterium* and biologistical (gene gun methodologies) interventions. Relatively new technologies are coming to us wholesale with the advent of CRISPR-Cas 9, ZFN, zinc-fringe nuclease, and TALEN transcription activator—like affector nuclease(Bortesi and Fischer, 2015; Dan et al., 2010). What sets these advances apart from simple gene transfer which generally applies to the moniker, GMO (National Academies of Sciences, Engineering, and Medicine, 2016) is that they do not use gene transfer as a mode of action, rather they used gene manipulation. The US Department of Agriculture, APHIS (Waltz, 2016) and the European Conference on Plant Breeding (Lusser et al., 2011) do not consider plants developed from gene manipulation from within the plant by means such as CRISPR to be GMOs. Since that is the case then a more suitable designation is required so that a better understanding of what these plants are, a case can be made for the use of a GAO or a GEO which better describes the status of a particular plant that has been altered but not infused with a gene or genetics from a foreign species but rather a rearrangement of an existing genome or the inclusion of a closely related species. The Europeans go further in stipulating that if a foreign gene is indeed introduced the designation of a GMO resides upon the origin of the introduced gene. For instance a gene from *Lolium* (rye) inserted into a *Triticum* (wheat) is NOT a GMO due to the family connections of the two genera, whereas a newly developed American chestnut (*Castanea dentata*) which contains a gene from *Triticum* is a GMO (Bortesi and Fischer, 2015; Ohlemeir, 2015; Powell, 2016).

With this understanding of the nature of the various acronyms for genetically modified, genetically altered, and Genetically engineered plants we now can tackle their roles in horticulture.

Horticultural uses of plants abound but the bulk of plants that are genetically modified, altered, or engineered usually fall into two groups. One is the fruit and vegetable world and the other is the medicinal plant world. A third category that is gaining prominence is trees for lumber, pulp and other industrial purposes. Ornamentals or environmental plants make up the fourth and smallest category. It is a short list, carnations (*Dianthus*), *Gladiolus*, *Lilium*, *Petunia*, *Rosa* (roses) and cotton (mainly agricultural but techniques can carry over to ornamental forms). For greenhouse operations plants such as cucurbits (squash, cucumbers, watermelons, etc.), *Citrus*, *Musa* (banana), eggplant, tomato, are in the offering.

A closer looks (Table 1) shows the plants being researched and for what purpose. Plants for the nursery and greenhouse trade are very limited and perhaps rightfully so. The economic engine that would drive genetic alteration is simply not there because the markets involved are not big enough to push ornamentals (Bruening and Lyons, 2000) or common nursery crops into the genetic modification world. This coupled with much of our products going into reclamation and remediation circumstances automatically disqualifies a genetically altered product. Forestry production nurseries and those propagation nurseries involved in medicinal plant production are of course an exception.

Does the nursery industry as a whole have a future with genetically modified plants? In general no, markets are insufficient and the industry as a whole is resourceful enough so that if *Potentilla fruticosa* A is not adequate the trend is to adjust to *Potentilla* B, rather than systematically trying to improve A. It is years of work and diligence to bring even one non agricultural plant into the market via a genetically altered or improved program and the costs override the benefit of the so called improvement. Genetically altered plants over all have increased layers of complexity that the nursery industry is either unable or inadequate to address. The causes for the lack of adjustment could be either technological or economical. The advent of a unique plant via this route might be a complete flop on the receiving end while the industry might be able to cope with the changes within the plant, the consumer may well not be so fortunate. Without large scale testing of end use the chances of a “design” flaw showing up and rendering a plant useless is significant (Castle, 2009). A plant crashing with a huge magnitude of research dollars behind it could be a serious risk. It seems certain that the standards set by conventional plant breeding via hybridization, chemical, and radiation mutation work will continue to prevail. That being said, the advent of new technologies such as CRISPR could offer new inroads to traditional plant breeding. Recombinate DNA technology will still be viable but used less due to the specificity of CRISPR and similar techniques which is not always the case with recombinate technology. With an increasing distrust by the public towards plants with labels such as GMO and the advent of the CRISPR and related techniques will take precedence as a plant derived from such work is not a GMO and theoretically might be more accepted in the market place. Perhaps the most significant use of CRISPR and related technologies is the induction of sterility into invasive plants that do have significant industry status, such as *Euonymus alatus* 'Compacta' and *Berberis thunbergii*. Novel forms or colors of flowers will probably not make it to the garden party rapidly due to the significant costs involved (Chakravarthy et al., 2014).

Table 1. Genetically altered horticultural plants either available or being researched.

Plant	Technique	Research goals	GMO/GAO	Reference
Agaricus bisporus	Crispr-CAS 9	Diminished oxidative browning	GMA	Waltz, 2016
Castanea dentata	Gene insertion from wheat	Chestnut blight immunity	GMO	Newhouse et al., 2010; Powell, 2016
Catharanthus roseus	*Agrobacterium* transfer	Increase in rooting and pharmaceutical production	GMO	Zarate and Verpoorte, 2007
Camellia sinensis	*Agrobacterium* transfer	Delaying post harvest senescence	GMO	Mohanpuria et al., 2011
Carica papaya	*Agrobacterium* transfer and RNA silencing	Virus resistance	GMO	Azad et al., 2013
Carya illinoinensis	*Agrobacterium* transfer	No specific goals spelled out	GMO	McGranahan et al., 1993
Cichorium intybus	Onion gene transfer	Increase metabolite harvesting	GMO	Matvenna et al., 2011
Citrus species various	Gene insertion from *Arabadopsis*	Citrus greening disease resistance	GMO	Ohlemeir, 2015
Cryptomeria japonica	*Agrobacterium* transfer	No specific goals spelled out	GMO	Taniguchi et al., 2008
Cucurbits various	*Agrobacterium* transfer	Drought and insect resistance	GMO	Morroni et al., 2008
Eucalyptus camaldulensis	*Agrobacterium* transfer	Improvement of wood quality and increase in rooting of cuttings	GMO	Ho et al., 1998
Fragaria	*Agrobacterium* transfer	Disease resistance	GMO	Hanhineva et al., 2009
Gentiana macrophylla	*Agrobacterium* transfer	Increased metabolite production	GMO	Tiwari et al., 2007
Gladiolus species	Antisense reinsertion via CRISPR or similar technology	Virus resistance	GMA	Kamo et al., 2010
Gossypium species	*Agrobacterium* transfer	Herbicide tolerance, insect resistance	GMO	Chakravarthy et al., 2014
Hypericum perforatum	*Agrobacterium* transfer	Improved pharmaceutical production	GMO	Franklin et al., 2009
Impatiens walleriana	*Agrobacterium* transfer	Novel flower colors, disease resistance	GMO	Dan et al., 2010

Table 1. Continued.

Plant	Technique	Research goals	GMO/GAO	Reference
Juglans species	*Agrobacterium* transfer	Disease resistance	GMO	Michler et al., 2006
Liquidambar styraciflua	*Agrobacterium* transfer	Insect resistance	GMO	Dowd et al., 1998
Malus domestica, several	CRISPR and related techniques	Non-browning upon oxidation	GMA	Waltz, 2016
Musa acuminata	CRISPR and similar techniques	Disease resistance	GMA	Castle, 2009
Pinus taedea	*Agrobacterium* transfer	Increased terpinoid production	GMO	Tang and Tian, 2003
Populus species, various	Various techniques	Increased disease resistance, increased growth profile, increased wood production, insect resistance, modified lignin concentrations	GMO	Mathews and Campbell, 2000; Powell and Maynard, 1997
Prunus domestica	Crispr	Disease resistance	GMA	Scorza et al., 2013
Rosa hybrids	*Agrobacterium* transfer	Flower color manipulation	GMO	Australian Office of Gene Technology, 2005
Saussurea medusa	*Agrobacterium* transfer	Increased metabolite production	GMO	Fu et al., 2006
Solanum tuberosum	CRISPR or similar techniques	Non-browning upon oxidation	GMA	National Acadamies of Sciences, Engineering, and Medicine, 2016
Solanum lycopersicum	Antisense reinsertion via CRISPR or similar technology	Flavor enhancement	GMA	Bruening and Lyons, 2000
Nicotiana species	Bacterial gene transfer	Increase uptake of methylmercury from contaminated soils	GMO	Heaton et al., 1998
Tylophora indica	*Agrobacterium* transfer	Improved pharmaceutical production	GMO	Chaudbhuri et al., 2005
Ulmus species	*Agrobacterium* transfer	Dutch elm disease resistance	GMO	Gartland et al., 2003

Literature cited

Australian Office of Gene Technology. (2005). The biology and ecology of *Rosa* × *hybrid* (Rose). Office of the Gene Technology Regulator.

Azad, M.A., Rabbani, M.G., Amin, L., and Sidik, N.M. (2013). Development of transgenic papaya through *Agrobacterium*-mediated transformation. Int. J. Genom., Article ID 235487, 1–5.

Bortesi, L., and Fischer, R. (2015). The CRISPR/Cas9 system for plant genome editing and beyond. Biotechnol. Adv. *33* (*1*), 41–52 https://doi.org/10.1016/j.biotechadv.2014.12.006. PubMed

Bruening, G., and Lyons, J.M. (2000). The case of the Flavr Savr tomato. Cal. Agr. *54* (*4*), 6–7 https://doi.org/10.3733/ca.v054n04p6.

Castle, M. (2009). The unfortunate sex life of the banana. Damned Interesting, article #324 https://www.damninteresting.com/the-unfortunate-sex-life-of-the-banana/.

Chakravarthy, V.S., Reddy, T.P., Reddy, V.D., and Rao, K.V. (2014). Current status of genetic engineering in cotton (*Gossypium hirsutum* L): an assessment. Crit. Rev. Biotechnol. *34* (*2*), 144–160 https://doi.org/10.3109/07388551.2012.743502. PubMed

Chaudhuri, K.N., Ghosh, B., Tepfer, D., and Jha, S. (2005). Genetic transformation of *Tylophora indica* with

Agrobacterium rhizogenes A4: growth and tylophorine productivity in different transformed root clones. Plant Cell Rep. *24* (*1*), 25–35 https://doi.org/10.1007/s00299-004-0904-x. PubMed

Dan, Y., Baxter, A., Zhang, S., Pantazis, C.J., and Veilleux, R.E. (2010). Development of efficient plant regeneration and transformation system for *impatiens* using *Agrobacterium tumefaciens* and multiple bud cultures as explants. BMC Plant Biol. *10* (*1*), 165 https://doi.org/10.1186/1471-2229-10-165. PubMed

Dowd, P.F., Lagrimini, L.M., and Herms, D.A. (1998). Differential leaf resistance to insects of transgenic sweetgum (*Liquidambar styraciflua*) expressing tobacco anionic peroxidase. Cell. Mol. Life Sci. *54* (*7*), 712–720 https://doi.org/10.1007/s000180050198. PubMed

Franklin, G., Oliveira, M.M., and Dias, A.C. (2009). Transgenic *Hypericum perforatum.* Methods Mol. Biol. *547*, 217–234 https://doi.org/10.1007/978-1-60327-287-2_18. PubMed

Fu, C.X., Xu, Y.J., Zhao, D.X., and Ma, F.S. (2006). A comparison between hairy root cultures and wild plants of *Saussurea involucrata* in phenylpropanoids production. Plant Cell Rep. *24* (*12*), 750–754 https://doi.org/10.1007/s00299-005-0049-6. PubMed

Gartland, K., Robert, M.A., Crow, M., Fenning, T.M., and Gartland, J.S. (2003). Genetically modified trees: production, properties, and potential. J. Arboric. *29* (*5*), 259–266.

Hanhineva, K., Kokko, H., Siljanen, H., Rogachev, I., Aharoni, A., and Kärenlampi, S.O. (2009). Stilbene synthase gene transfer caused alterations in the phenylpropanoid metabolism of transgenic strawberry (*Fragaria x ananassa*). J. Exp. Bot. *60* (*7*), 2093–2106 https://doi.org/10.1093/jxb/erp085. PubMed

Heaton, C.P.A., Rugh, C.L., Wang, N., and Meagher, R.B. (1998). Phytoremediation of mercury- and methylmercury-polluted soils using genetically engineered plants. J.Soil Contamination *7* (*4*), 497–509 https://doi.org/10.1080/10588339891334384.

Ho, C.-K., Chang, S.-H., Tsay, J.-Y., Tsai, C.-J., Chiang, V.L., and Chen, Z.-Z. (1998). *Agrobacterium tumefaciens* -mediated transformation of *Eucalyptus camaldulensis* and production of transgenic plants. Plant Cell Rep. *17* (*9*), 675–680 https://doi.org/10.1007/s002990050464.

Kamo, K., Jordan, R., Guaragna, M.A., Hsu, H.T., and Ueng, P. (2010). Resistance to Cucumber mosaic virus in *Gladiolus* plants transformed with either a defective replicase or coat protein subgroup II gene from Cucumber mosaic virus. Plant Cell Rep. *29* (*7*), 695–704 https://doi.org/10.1007/s00299-010-0855-3. PubMed

Lusser, M., Parisi, C., Plan, D., and Rodríguez-Cerezo, E. (2011). New plant breeding techniques State-of-the-art and prospects for commercial development, JRC Scientific and Technical Reports, JRC European Commission Institute for Health and Consumer Protection, EUR24760EN-2011

Mangelsdorf, P.C. (1974). Corn Its Origin, Evolution and Improvement (79 Garden Street, Cambridge, Massachusetts 02138 USA: Harvard University Press).

Mathews, J.H., and Campbell, M.M. (2000). The advantages and disadvantages of the application of genetic engineering to forest trees: a discussion. Forestry *73* (*4*), 371–380 https://doi.org/10.1093/forestry/73.4.371.

Matvenna, N.A, Kishchenoko, E.M., Shakhovskii, A.M., Potrokov, A.A., Kuchak, N.V. (2011). Abstract. Regeneration of transgenic plants from *Cichorium intybus* L. var. *foliosum* Heji hairy roots. Tsitol. Genetics.

McGranahan, G.H., Leslie, C.A., Dandekar, A.M., Uratsu, S.L., and Yates, I.E. (1993). Transformation of pecan and regeneration of transgenic plants. Plant Cell Rep. *12* (*11*), 634–638 https://doi.org/10.1007/BF00232814. PubMed

Michler, C.H., Pijut, P.M., Jacobs, D.F., Meilan, R., Woeste, K.E., and Ostry, M.E. (2006). Improving disease resistance of butternut (*Juglans cinerea*), a threatened fine hardwood: a case for single-tree selection through genetic improvement and deployment. Tree Physiol. *26* (*1*), 121–128 https://doi.org/10.1093/treephys/26.1.121. PubMed

Mohanpuria, P., Kumar, V., Ahuja, P.S., and Yadav, S.K. (2011). *Agrobacterium*-mediated silencing of caffeine synthesis through root transformation in *Camellia sinensis* L. Mol. Biotechnol. *48* (*3*), 235–243 https://doi.org/10.1007/s12033-010-9364-4. PubMed

Morroni, M., Thompson, J.R., and Tepfer, M. (2008). Twenty years of transgenic plants resistant to Cucumber mosaic virus. Mol. Plant Microbe Interact. *21* (*6*), 675–684 https://doi.org/10.1094/MPMI-21-6-0675. PubMed

Nester, E. 2008. *Agrobacterium:* the natural genetic engineer 100 years later. Online. APSnet Features.

Newhouse, A.E., Zhang, A.B., Nothern, L., Maynard, C.A., and Powell, W.A. (2010). Analysis of transgenic American chestnut. Phytophath. *100* (*6*), 1–24.

Ohlemeir, D. (2015). GMO citrus resistant to greening disease successfully grown in Florida. https://www.geneticliteracyproject.org/2015/08/13/gmo-citrus-resistant-to-greening-disease-successfully-grown-in-florida/

Powell, W.A. (2016). New genetically engineered American chestnut will help restore the decimated, iconic tree. http://theconversation.com/new-genetically-engineered-american-chestnut-will-help-restore-the-decimated-iconic-tree-52191.

Powell, W.A., and Maynard, C.A. (1997). Designing small antimicrobial peptides and their encoding genes. In Micropropagation, Genetic Engineering, and Molecular Biology of *Populus*. Gen. Tech. Rep. RM-GTR-297, N.B. Klopfenstein, Y.W. Chun, M.S. Kim, and M.R. Ahuja, eds. (Ft. Collins, Colorado: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station), p.165–172.

Scorza, R., Kriss, A.B., Callahan, A.M., Webb, K., Demuth, M., and Gottwald, T. (2013). Spatial and temporal assessment of pollen- and seed-mediated gene flow from genetically engineered plum *Prunus domestica.* PLoS ONE *8* (*10*), e75291 https://doi.org/10.1371/journal.pone.0075291. PubMed

Tang, W., and Tian, Y. (2003). Transgenic loblolly pine (*Pinus taeda* L.) plants expressing a modified delta-endotoxin gene of *Bacillus thuringiensis* with enhanced resistance to *Dendrolimus punctatus* Walker and *Crypyothelea formosicola* Staud. J. Exp. Bot. *54* (*383*), 835–844 https://doi.org/10.1093/jxb/erg071. PubMed

Taniguchi, T., Ohmiya, Y., Kurita, M., Tsubomura, M., and Kondo, T. (2008). Regeneration of transgenic *Cryptomeria japonica* D. Don after *Agrobacterium tumefaciens*-mediated transformation of embryogenic tissue. Plant Cell Rep. *27* (*9*), 1461–1466 https://doi.org/10.1007/s00299-008-0569-y. PubMed

National Academies of Sciences, Engineering, and Medicine. (2016). Genetically Engineered Crops: Experience and Prospects.

Tiwari, R.K., Trivedi, M., Guang, Z.C., Guo, G.Q., and Zheng, G.C. (2007). Genetic transformation of *Gentiana macrophylla* with *Agrobacterium rhizogenes*: growth and production of secoiridoid glucoside gentiopicroside in transformed hairy root cultures. Plant Cell Rep. *26* (*2*), 199–210 https://doi.org/10.1007/s00299-006-0236-0. PubMed

Waltz, E. (2016). Gene-edited CRISPR mushroom escapes US regulation. Nature *532* (*7599*), 293 https://doi.org/10.1038/nature.2016.19754. PubMed

Wikipedia. Origin of maize and interaction with teosintes. https://en.wikipedia.org/wiki/Zea_(plant)#Origin_of_maize_and_interaction_with_teosintes (accessed 2016).

Zarate, R., and Verpoorte, R. (2007). Strategies for the genetic modification of the medicinal plant *Catharanthus roseus* (L.) G. Don. Phytochem. Rev. *6* (*2*), 475–491 https://doi.org/10.1007/s11101-006-9020-6.